Pyrethroid Resistance in Association with the Use of Insecticide Impregnated Bed Nets

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Abstract

There has been concern about whether pyrethroid tolerance or resistance will be selected in anophelines and other mosquitoes through the use of pyrethroid-impregnated bed nets. Pieces of nylon netting impregnated with different concentrations of lambda-cyhalothrin and permethrin were used for mosquito selection, to investigate the development of pyrethroid tolerance or resistance in adult *Aedes aegypti* and *Anopheles maculatus*. The WHO susceptibility test kits were lined inside with insecticide-impregnated nylon netting and mosquitoes were exposed for periods of 30 seconds to 4 minutes. Mortality was scored after a 24-hour observation period. The LT_{50} values and the resistance ratio or increased tolerance in each generation were determined. The exposure of 9 generations of *Ae. aegypti* to 0.015 g/m^2 lambda-cyhalothrin, and 8 generations of *Ae. aegypti* to 0.15 g/m^2 permethrin produced 2.6-fold and 2.8-fold tolerance, respectively, whereas 5 generations of *An. maculatus* exposed to 0.1 g/m^2 permethrin showed 1.4-fold tolerance. The pattern of increased tolerance levels to lambda-cyhalothrin and permethrin by both mosquito species indicated no evidence for the development of pyrethroid resistance.

Keywords: pyrethroid-impregnated bed nets, pyrethroid tolerance, *Aedes aegypti*, *Anopheles maculatus*

Introduction

Insecticides remain the primary control tools in the majority of vector and pest control programs throughout the world. The advent of synthetic insecticides has proved so successful that they are still the main weapon used against the vector species involved in disease transmission. At present, the most promising new insecticide-based method of malaria control is the use of mosquito nets and curtains impregnated with synthetic pyrethroids, and this method has been extensively employed in many malarious regions of the world [1]. Entomological studies have shown that insecticide impregnated bed nets reduce man-vector contact and diminish mosquito populations [2-6]. However, widespread use of insecticides has accelerated insecticide resistance in arthropods. Accordingly, it is necessary to determine the levels of susceptibility and provide the information needed to decide whether a particular insecticide should continue in use or be replaced by an alternative insecticide. The current study investigates the tendency to develop pyrethroid tolerance or resistance in adult anopheline and culicine mosquitos after exposure to insecticide-impregnated nets for many generations.

Materials and methods

Mosquitoes

The colonies of *Ae. aegypti* and *An. maculatus* were derived originally from eggs pooled from females collected in the field. They were then continuously reared and maintained in an insectarium at 26 ± 2°C and 85 ± 5% relative humidity in the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Insecticides

Two pyrethroids, *viz* permethrin and lambda-cyhalothrin, which are commonly used for bed net impregnation, were used in this study. Permethrin: [(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2, dimethyl cyclopropane carboxylate] cis:trans isomeric ratio 25:75; 10% w/v, emulsifiable concentrate (Wellcome Singapore, Pte Ltd). Lambda-cyhalothrin: [(+)-cyano-3-phenoxybenzyl(=)cis-trans-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] 2.5% w/v, emulsifiable concentrate (ZENECA Agrochemicals, Yalding, Maidstone, Kent, UK).

Impregnation of netting

A piece of 12 × 15 cm^2 nylon net was soaked individually and thoroughly in a polythene bag with insecticide solution. The amount of insecticide solution was calculated against the area of each piece of netting in order to apply the desired concentration onto the net. Deposit concentrations for permethrin of 0.10 g/m^2, and 0.15 g/m^2, and for lambda-cyhalothrin 0.015 g/m^2, were used in this study.

Mosquito test procedure

Susceptibility tests of *Ae. aegypti* and *An. maculatus* to permethrin and lambda-cyhalothrin were conducted by WHO test kit method. Instead of insecticide impregnated paper, the insides of the test kits were lined with insecticide-impregnated nylon netting. Twenty, 3-5 days old, non-bloodfed female mosquitoes were exposed to the insecticide-impregnated net for 0.5, 1, 2 and 4 min. At the end of each exposure time, the mosquitoes were transferred to the holding tube and kept for 24 hr with a piece of cotton wool soaked with 10% sugar solution attached to the mesh screen end of the holding tube. Mortality counts were made at the end of the 24-hr observation period. Four replicate tests were performed with each exposure time and controls were run with untreated pieces of netting. The mosquitoes that survived after the 24-hr observation period were separated and reared to the next progeny. The testing procedure mentioned above was repeated in each generation. Mortality data were analyzed for susceptibility using the computer program Probit Analysis by Raymond [7] based on Finney [8]. The resistance ratio or increased tolerance pattern in each generation was determined by the ratio of LT_{50} value for the parent strain and successive generations.

Results

The percentage mortality in each generation of *Ae. aegypti,* with lambda-cyhalothrin (0.015 g/m^2) are summarized in Table 1. The resistance ratios or increased tolerance levels during 9 generations of selection with lambda-cyhalothrin (0.015 g/m^2) are also shown in Table 1. It was observed that the LT_{50} value (1.99 min) in generation F_2 increased by 2.4 times over that of the parent strain. However, it should be noted that the increased tolerance in generation F_2 was not sustained during the succeeding generations, F_3 to F_6. An appreciable increase in LT_{50} value (2.06 and 2.14 min) was noticed in generations F_8 and F_9, respectively. The increased tolerance in generation F_9 was 2.6 fold higher than that of the parent strain, indicating the development of decreased susceptibility in *Ae. aegypti* after continued exposure to lambda-cyhalothrin (0.015 g/m^2) for 9 generations. The same pattern of response was also observed (Table 2) during 8 generations of *Ae. aegypti* exposed to permethrin (0.15 g/m^2), with a tolerance level of 1.5 fold (LT_{50} = 1.03 min) in F_1 compared with the parent strain (LT_{50} = 0.69 min). The increased tolerance levels in F_6, F_7 and F_8 generations were 1.7, 2.5 and 2.8 fold, respectively, compared with the parent strain. Continued exposure of *An. maculatus* for 5 generations to 0.1 g/m^2 permethrin exhibited very low level tolerance (Table 3), compared with *Ae. aegypti,* to permethrin and lambda-cyhalothrin. A slight increase in tolerance was observed in F_4 and F_5 generations (1.2 and 1.4 fold, respectively) over that of the parent strain.

Discussion

The effects of pyrethroid-impregnated mosquito nets and curtains have been studied in

Table 1 **Exposure time-mortality results, LT_{50} and resistance ratio of different generations of *Aedes aegypti* exposed to lambda-cyhalothrin (0.015 g/m²) treated nylon netting.**

Exposure time (min)	Mean mortality (%)									
	P	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
0.5	27.7	47.5	16.2	26.2	21.2	13.7	36.2	13.7	15.2	21.2
1	67.5	50.0	21.2	37.5	46.2	47.5	40.0	31.2	20.8	27.5
2	75.0	58.7	47.5	42.5	77.5	73.7	58.7	47.5	48.6	45.0
4	91.2	78.7	76.2	95.0	82.5	95.0	91.2	77.5	73.5	68.7
LT_{50}	0.82	0.79	1.99	1.38	1.12	1.14	1.08	1.84	2.06	2.14
RR	-	-	2.4	1.7	1.4	1.4	1.3	2.3	2.5	2.6

LT_{50} = Lethal time that is required to kill 50% of the tested mosquitoes.
RR = Resistance ratio is the ratio of LT_{50} value of the parent and successive generations.

Table 2 **Exposure time-mortality results, LT_{50} and resistance ratio of different generations of *Aedes aegypti* exposed to permethrin (0.15 g/m²) treated nylon netting.**

Exposure time (min)	Mean mortality (%)								
	P	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0.5	36.2	27.5	31.2	30.0	28.7	23.7	18.7	16.2	12.5
1	70.0	51.2	53.7	57.5	55.0	48.7	45.0	32.5	28.7
2	76.2	68.7	71.2	73.7	75.0	66.2	67.5	47.5	43.7
4	93.7	87.5	95.0	91.2	92.5	88.7	90.0	82.5	77.5
LT_{50}	0.69	1.03	0.91	0.89	0.91	1.10	1.18	1.69	1.96
RR	-	1.5	1.3	1.2	1.3	1.6	1.7	2.5	2.8

LT_{50} = Lethal time that is required to kill 50% of the tested mosquitoes.
RR = Resistance ratio is the ratio of LT_{50} value of the parent and successive generations.

Table 3 Exposure time-mortality results, LT_{50} and resistance ratio of different generations of *Anopheles maculatus* exposed to permethrin (0.1 g/m^2) treated nylon netting.

Exposure time (min)	Mean mortality (%)					
	P	F_1	F_2	F_3	F_4	F_5
0.5	36.0	34.7	29.1	34.6	31.9	30.4
1	69.4	66.1	63.8	74.9	58.3	51.3
2	79.1	81.9	83.3	80.5	77.7	69.4
4	94.4	95.8	93.0	90.2	93.0	88.8
LT_{50}	0.69	0.72	0.79	0.65	0.82	0.96
RR	-	1.0	1.1	-	1.2	1.4

LT_{50} = Lethal time that is required to kill 50% of the tested mosquitoes.
RR = Resistance ratio is the ratio of LT_{50} value of the parent and successive generations.

the laboratory, experimental huts and village scale trials against anophelines and culicines in various parts of the world. Hossain and Curtis [9] found a considerable difference in susceptibility of *Ae. aegypti, An. gambiae* and *Cx. quinquefasciatus* to permethrin-impregnated nets. They reported lower susceptibility in *An. gambiae* than *Ae. aegypti*, however, *Cx. quinquefasciatus* was found to have greater tolerance. In resistant and susceptible strains of *An. stephensi,* Hodjati and Curtis [10] found 100% mortality with permethrin (0.2 g/m^2) treated bed nets and Loong *et al* [11] also reported 100% mortality for *An. maculatus* with permethrin (0.2 g/m^2) treated bed nets. The bioassay results of *An. gambiae* and *An. funestus* with permethrin (0.1 g/m^2) treated cotton nets showed 100% mortality at 8 min exposure time [12].

A few comparable published data are available regarding pyrethroid tolerance or resistance in mosquitoes through the use of insecticide-treated nets. This study confirms the finding of Vulule *et al* [13], who reported a 2.5-fold increase in LT_{50} for *An. gambiae* after one year of permethrin-impregnated mosquito net use in Kenyan villages. They also reported a 1.5-fold increase in LT_{50} of *An. gambiae* after two generations of selection. However, in the same study, permethrin tolerance did not increase during the next two years, despite the ongoing use of permethrin-impregnated nets and curtains, indicating insufficient selection pressure from impregnated nets for permethrin tolerance in *An. gambiae* [14].

Similar results have been reported for other anophelines. Cheng *et al* [15] found no evidence for the development of resistance in *An. anthropophagus* and *An. sinensis,* despite prolonged and extensive use of deltamethrin-impregnated bed nets in Sichuan Province, China. Similarly, Curtis *et al* [16] reported that permethrin resistance remained unchanged after seven generations of selection for female *An. stephensi* with permethrin-impregnated nets. However, in the same experiment, the resistance pattern increased when selection pressure was applied to both sexes.

The emergence of pyrethroid resistance in adult culicine and anopheline mosquitoes has been demonstrated in laboratory selection experiments [17-19]. Similarly, several laboratory selection experiments on larvae of culicines and anophelines have also shown pyrethroid resistance [20-23].

The development of resistance to any insecticide within a population of mosquito vectors depends on whether the genetic determinant for resistance mechanism is present within the gene pool of the population. If the gene is absent, resistance will not develop unless a mutation occurs within that population during the course of exposure to the insecticide. Conversely, if the gene for resistance to that insecticide is present within the population, resistance can develop. The speed of selection for resistance to any insecticide depends on several factors, including the size of the population, the frequency of the gene in the population, the dominance relationship of the gene and the intensity with which the population is exposed to the insecticide. In the present study, there was no indication of any high tolerance or resistance by *Ae. aegypti* to lambda-cyhalothrin or permethrin, even after exposure for nine generations. The low levels of pyrethroid tolerance observed in this study were presumably due to inadequate selection pressure in each generation and the small size of the population tested. On the other hand, it is usually assumed that the frequency of an unselected gene for resistance is initially extremely low. Hence, the larger the population the more likely that the population will contain individuals carrying the resistant gene. The speed with which that gene increases in frequency depends heavily on the level of exposure to the insecticide and the dominance relationship of the resistant gene. If the population is only occasionally exposed to the insecticide, then the frequency of the gene may change little at all. However, if the population is routinely exposed, individuals carrying the resistant gene should have an advantage.

When the resistant gene is recessive, it is often incompletely recessive, so that heterozygotes have a slight advantage, allowing at least some of them to survive exposure to levels of insecticide that kill homologous susceptible individuals. In this case, it is expected that resistance will develop more slowly and it would take longer for sufficient numbers of heterozygotes to accumulate in the population, to the point that they mate and produce some homozygous resistant individuals. This only happens when a high level of resistance becomes apparent. Moreover, even with a powerful resistance mechanism, the proportions of resistant genotypes in the population may increase slowly, so that the full effects may not have been able to be observed during the present study.

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