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Extensive Resistance of *Anopheles sinensis* to Insecticides in Malaria-Endemic Areas of Hainan Province, China

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Abstract. *Anopheles sinensis* is one of the major malaria vectors and among the dominant species in Hainan Province, China. The resistance of *An. sinensis* to insecticides is an important threat to malaria control. However, few reports on insecticide resistance of *An. sinensis* were reported in this area. Eight districts in Hainan Province were selected as the study areas. Insecticide susceptibility bioassays were tested on wild-caught female mosquitoes of *An. sinensis* to 4% dichlorodiphenyltrichloroethane (DDT), 0.05% deltamethrin, and 5% malathion by using the World Health Organization standard resistance tube assay procedure. All the tested *An. sinensis* mosquitoes demonstrated resistance to 4% DDT, with less than 72% mortality in the standard assay. The populations from Baisha and Qiongzhong demonstrated possible resistance to 0.05% deltamethrin, with 94–95% mortality, whereas the populations from other districts demonstrated resistance to 0.05% deltamethrin in the standard assay. The populations from Baisha, Qiongzhong, and Dongfang demonstrated susceptibility to 5% malathion, but the populations from other districts demonstrated resistance. These results facilitate the improvement of effective control strategies for malaria vector mosquitoes in Hainan.

INTRODUCTION

Anopheles sinensis, *Anopheles fluviatilis*, *Anopheles candidiensis*, *Anopheles philippinensis*, *Anopheles minimus*, and *Anopheles dirus* are the main malaria vectors in Hainan, a tropic island that had the highest morbidity of malaria in China before 2002.[1] One of the most widely distributed malaria vectors in this area is *An. sinensis*.[2] Vector control is one of the key measures used to control malaria in endemic areas.[3] The use of insecticide-treated bed nets and indoor residual spraying (IRS) were the main methods to control anopheline mosquitoes. Malaria was successfully under control after the implementation of these control strategies. The population of malaria vectors was decreased[2] and no locally acquired malaria cases were reported from 2012 to 2014 in Hainan Province.[4] In 2010, a plan of action for the elimination of malaria was launched by the Chinese government and the government decided to achieve this goal across China by 2020.

Organochlorines, such as dichlorodiphenyltrichloroethane (DDT), have been widely used for malaria vector control since the 1950s in China.[5] In the 1980s, DDT was replaced by more specific and less toxic chemicals, such as pyrethroids.[6] With the approval of the World Health Organization (WHO), pyrethroids, such as deltamethrin, have been extensively used for IRS and impregnated bed nets for malaria vector control.[7] However, abusive application of chemical insecticides not only leads to environmental pollution, but also to emergence of resistance in malaria vector.

Numbers of reports about the resistance of *An. sinensis* to insecticides were reported in China, but the information on malaria vector resistance in recent years lacked of comprehensive and systematic standards, especially in the malaria-endemic areas of Hainan Province.

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MATERIALS AND METHODS

Study area. One sentinel site in each of eight cities or counties from different malaria-endemic areas was chosen for this study (Figure 1). The selected sites were chosen in highly endemic malaria villages where insecticides were used for malaria vectors, and near the rice fields or small streams, where it is suitable for breeding of *An. sinensis*.

Sampling collection. Wild-caught female *An. sinensis* mosquitoes were used for the bioassays directly. From 2011 to 2014, female mosquitoes were attracted to and fed on cattle in the sentinel sites. All wild-caught female mosquitoes were fed sugar and identified morphologically using taxonomic keys.[8] The next day, the identified female mosquitoes of *An. sinensis* were used for resistance tests.

Resistance tests. Three insecticides were tested at discriminating concentrations recommended by the WHO: 0.05% deltamethrin, 5% malathion, and 4% DDT. Insecticide-impregnated papers were obtained from the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention. At least 100 female mosquitoes (4–5 replicates of approximately 25 mosquitoes) were exposed to the insecticide-impregnated paper for 60 minutes. Mortality was recorded 24 hours postexposure. A minimum of 50 mosquitoes (two replicates of approximately 25 mosquitoes) were used as controls, and exposed to papers impregnated with carrier oil only in a single bioassay test. The control mosquitoes were tested at the same time under the same conditions to estimate a base mortality during the test. The observed mortality was corrected using the Abbott formula when control mortality was between 5% and 20%. The bioassay results were categorized in three resistance levels as defined by WHO[9]: susceptible (mortality between 98% and 100%), possible resistant (mortality between 90% and 98%), and resistant (mortality lower than 90%).

RESULTS

No correction was required as less than 3.92% mortality was observed in control groups.

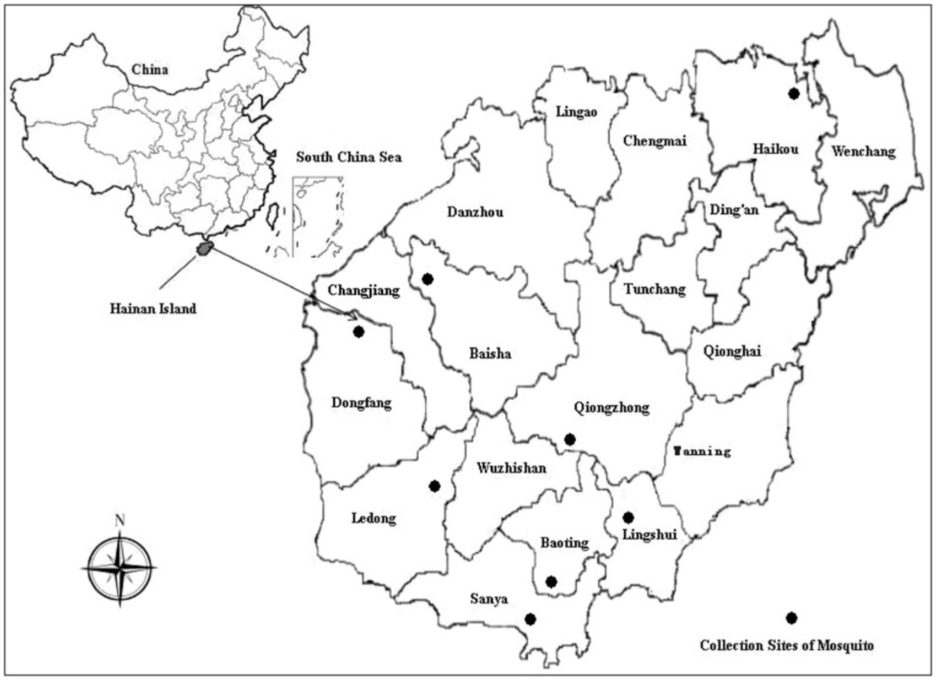

FIGURE 1. The location of the mosquito collection in Hainan Province, China. The black circles in the right map indicate the eight collection sites.

Wild-caught female *An. sinensis* mosquitoes in Sanya, Lingshui, Baoting, Dongfang, Ledong, and Haikou demonstrated resistance to 0.05% deltamethrin, with mortality ranging from 17.00% to 68.93%, whereas mosquitoes tested in the other two sites demonstrated possible resistance to deltamethrin, with mortality ranging from 94.12% to 95.00% in the standard assay. The mortality was significantly different among different areas, and the highest percentage of mosquitoes surviving the WHO diagnostic doses was observed in the agricultural districts of Lingshui, whereas the lowest was observed in Qiongzhong.

A higher frequency of resistance was observed with all the tested *An. sinensis* exposed to 4% DDT, with 24% (in Lingshui) to 72% (in Baisha) mortality in the standard assay.

In the standard assay, *An. sinensis* populations in Qiongzhong, Dongfang, and Baisha were susceptible when exposed to 5% malathion, with higher than 98.00% mortality, whereas the populations from Sanya, Lingshui, Baoting, Ledong, and Haikou were obviously resistant, with 16.00–73.00% mortality in the standard assay.

Our results showed that *An. sinensis* mosquitoes in Hainan Province demonstrated resistance or possible resistance to these common insecticides (Table 1), especially to DDT.

DISCUSSION

The results from this study show that field populations of *An. sinensis* females from Hainan Province developed high resistance to the tested insecticides, especially to DDT. The resistance to DDT is extremely prevalent, as less than 72% mortality was observed in the standard assay, and in some cases up to 76% of the mosquitoes survived at the diagnostic dose. Obviously the resistance to DDT still exists in

TABLE 1
Resistance of wild-caught female mosquitoes of *Anopheles sinensis* to the tested insecticides in Hainan Province

	Deltamethrin (0.05% w/v)*					DDT (4% w/v)*					Malathion (5% w/v)*				
				Control					Control					Control	
City/county	Total	(%) Mortality	Resistance status†	Total	(%) Mortality	Total	(%) Mortality	Resistance status†	Total	(%) Mortality	Total	(%) Mortality	Resistance status†	Total	(%) Mortality
Sanya	101	25.74	R	50	0	100	27.00	R	51	0	100	16.00	R	52	0
Lingshui	100	17.00	R	51	1.96	100	24.00	R	50	2	100	41.00	R	50	0
Qiongzhong	100	95.00	M	50	0	105	60.95	R	50	0	121	100.00	S	51	3.92
Baoting	103	68.93	R	52	3.85	100	53.00	R	52	1.92	100	73.00	R	51	1.96
Dongfang	100	49.00	R	50	2.00	100	31.00	R	50	0	101	99.01	S	50	0
Baisha	102	94.12	M	50	2.00	100	72.00	R	51	0	103	99.03	S	50	0
Ledong	100	38.00	R	51	3.92	100	41.00	R	53	1.89	100	56.00	R	52	1.92
Haikou	100	35.00	R	50	0	100	36.00	R	50	2.00	100	39.00	R	51	0

DDT = dichlorodiphenyltrichloroethane.
* World Health Organization diagnostic concentration.
† S = susceptible; M = possible resistant; R = resistant.

An. sinensis populations, although they have not been treated with DDT for more than 30 years in Hainan.

Pyrethroid insecticides are neurotoxins and share many characteristics with DDT, they both target insect sodium channels, have a negative temperature coefficient, and have a rapid knockdown effect followed by a lethal effect.[10] Also, resistance to one is likely to create cross-resistance to the other. In our study, we observed an obvious correlation between deltamethrin and DDT in mortality rates of *An. sinensis* (Figure 2). The higher mortality to deltamethrin with a higher mortality to DDT indicated a positive correlation between the levels of resistance to both insecticides in the tested population. Similar results were also reported on *Anopheles gambiae* populations from Cameroon,[11–13] *Anopheles arabiensis* populations from Sudan,[14] and *An. sinensis* populations from other provinces in China.[15] The mode of action of malathion to mosquitoes is different from DDT and deltamethrin, as it is an inhibitor of acetylcholinesterase activity.[16] No correlation was observed between malathion and DDT/deltamethrin in this study.

Anopheles sinensis prefers large bodies of water, such as rice fields. As a consequence, insecticides used in agriculture will put a selective pressure on the larvae of *An. sinensis*.[17] Reports from Diabate and others[18] showed that the resistance of *An. gambiae* to insecticides was related to the use of insecticides in agronomy. No information on pesticide use at the household or communal level was collected in our study. However, about 8.3×10^7 kg of pyrethroids insecticide per year has been used in China since 1997.[19] Extensive abuse of pyrethroids in agriculture and public health probably lead to the extensive resistance in *An. sinensis*.

Reduced target site sensitivity and increased metabolic detoxification are two proven insecticide resistance mechanisms in mosquitoes. Mutations in the para-type sodium channel gene can decrease the affinity of these insecticides to insect sodium channels and cause target site resistance to DDT and deltamethrin.[20] Target resistance to malathion was different from DDT and deltamethrin because it was a mutation of the acetylcholinesterase (*ace-1*) gene.[16] Metabolic resistance to insecticides is caused by the increased activity of detoxification enzymes, including cytochrome P450 monooxygenases (P450s), glutathione S-transferases, and carboxylesterases.[16] A report from Qin and others[21] showed that target-site insensitivity and metabolic resistance both play important roles in insecticide resistance in *An. sinensis* in Hainan Island.

Historically, DDT was widely used for IRS to control malaria vector in Hainan Province since the 1950s. Since the 1980s, pyrethroid insecticides, such as deltamethrin and cyfluthrin, have been widely used for insecticide-treated nets or long-lasting insecticide-treated nets for malaria vector control.[22] Resistance to deltamethrin in our study was related to the long-term use of these insecticides. Although DDT had not been used for malaria vector control for many years, vector resistance to DDT still exists. Maybe DDT and pyrethroid insecticides have similar mechanisms of action in malaria vectors, which could explain the observed cross-resistance between DDT and pyrethroid insecticides in our study. The larvae of *An. sinensis* prefer to develop in large bodies of water, such as rice fields. Organophosphorus insecticides, such as malathion, are not the main insecticides used for malaria vector control in Hainan Province, but they are widely used for rice pest control. Selective pressure from pesticide exposure will inevitably result in resistance to malathion in the larvae of *An. sinensis*.

Resistance of malaria vector to insecticides is a common threat to public health all over the world, especially resistance to pyrethroids. For this reason, the choice of insecticides to control mosquitoes is limited. Fortunately, chemicals are not the only choice. Integrated vector management is a sustainable way to control malaria vectors. Effective and economical interventions should be used for mosquito control, such as environmental manipulation or management,[23,24] house modification,[25] biopesticides,[26,27] and paratransgenesis.[28]

In our study, we detected that different levels of insecticide resistance in *An. sinensis* populations in Hainan Province demonstrated possible resistance or resistance to deltamethrin, resistance to DDT, and sensitivity or resistance to

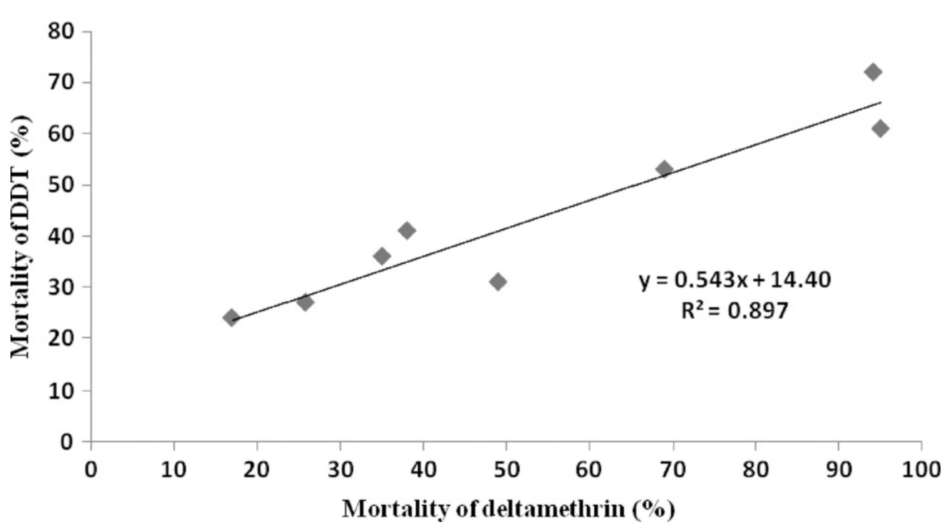

FIGURE 2. The relationship between the mortality of dichlorodiphenyltrichloroethane (DDT) and that of deltamethrin in *Anopheles sinensis* population. The equation ($Y = 0.543x + 14.40$) is the linear correlation equation of mortalities of *An. sinensis* to DDT and deltamethrin. R^2 is the square of correlation coefficient.

malathion. For effective and economical use of insecticides in malaria vector control, it is necessary to set up networks to systematically and continuously monitor the resistance levels of malaria vectors to insecticides. More research should be done to clarify the resistance mechanisms and screen more effective insecticides.

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