Original Article

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Insecticide Resistance of *Aedes albopictus* in Zhejiang Province, China

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SUMMARY From 2003 until 2018, a total of 12 outbreaks with 1,654 confirmed dengue cases have been reported in Zhejiang Province. The emergence of insecticide resistance in mosquitoes will affect the control of dengue. Our study aims to investigate the current situation of insecticide resistance of *Ae. albopictus* in Zhejiang Province and compares it with the situation in 2016. *Ae. albopictus* were collected from 12 Zhejiang Province cities in 2019. Resistance to three major categories of insecticides, including 8 commonly used insecticides, was evaluated according to the tube test protocol recommended by China CDC. *Ae. albopictus* in all cities, except Hangzhou, Wenzhou, Lishui and Shaoxing, showed decreased susceptibility to beta-cypermethrin, deltamethrin and permethrin. For malathion, 3 cities *Ae. albopictus* have developed resistance, 3 cities *Ae. albopictus* have decreased susceptibility. For propoxur, in 3 cities *Ae. albopictus* showed decreased susceptibility with mortality ranging from 94.24% to 96.67%. The resistance to alpha-cypermethrin, lambda-cyhalothrin and fenitrothion is rare in *Ae. albopictus* in that only Zhoushan's mosquitoes showed decreased susceptibility to alpha-cypermethrin. The resistance to beta-cypermethrin, deltamethrin and permethrin was significantly correlated with each other. Compared to the situation in 2016, the insecticide resistance of *Ae. albopictus* in Zhejiang Province has become more common in 2019. In the emergency preparedness for future mosquito-borne diseases, two things should be done: 1) the selection of insecticides should be made based on information from insecticide resistance surveillance 2) the use of insecticide should follow scientific guidance.

Keywords *Aedes albopictus*, insecticide resistance, mosquito-borne disease, dengue fever, Zhejiang Province

1. Introduction

Aedes albopictus, also known as Asian tiger mosquito, originates from South-East Asia and spreads to all continents except Antarctica, making it the most invasive mosquito in the world (*1*). It is widely distributed across different regions in China in that cases have been reported from warm south areas, such as Hainan Island and Guangdong Province, as well as cold north regions, like Tibet plateau and Shenyang Province(*2*). Zhejiang Province, located on the southeast coast of China, has a subtropical monsoon climate. The local natural resources, annual average temperature of 15~18°C, and abundant rainfall make it an ideal environment for mosquito's growth and reproduction. Although *Aedes aegypti* has not been discovered yet, *Ae. albopictus* has been discovered in many places in Zhejiang Province.

Ae. albopictus, as the secondary vector, plays an important role in transmission of dengue virus and Chikungunya virus (*3-5*). It may also be a potential vector of Zika virus (*6-8*). Of note, *Ae. albopictus* is the main vector of Aedes-borne disease in China and the sole vector in Zhejiang Province (*9,10*). With the expansion of *Ae. albopictus* distribution, the global health burden caused by Aedes-borne diseases is increasing. Taking dengue fever as an example, it is estimated that 50 million population in over 100 countries contract this disease every year, and about half of the world's population are at risk for contracting dengue virus (*11-13*). In China, dengue fever first emerged in the southeast coast of China (Fujian Province and Guangdong Province), and then spread to the southwest and central area (including Yunnan Province, Henan Province and Shandong Province) (*14-16*). It is estimated that dengue fever has influence on one billion Chinese residents' health (*16*).

Dengue fever also severely threatens the population's health in Zhejiang Province and impedes social and economic development. From 2014 to now, autochthonous dengue outbreak happens in Zhejiang Province every year (*17,18*). In 2017, there was a large-scale dengue outbreak in Hangzhou City with 1,136 cases confirmed (*18*). Also, in that same year there was a autochthonous Chikungunya outbreak in Zhejiang Province (*19*).

At present, for most mosquito-borne diseases, there is no effective vaccine or specific therapeutic drug available. Therefore, controlling mosquito vectors has become a paramount measure of the global mosquito-borne disease control strategy (*20*). Because of high efficacy and low cost for manufacture, chemical insecticides are used as the major tool in mosquito control (especially for mosquito-borne disease control). For example, during the outbreak of dengue in Guangzhou in 2014, a large amount of pyrethroids and temephos were used to control *Ae. albopictus* (*21*). Although the use of chemical insecticides can quickly reduce mosquito density, the improper use of insecticides (*i.e.*, used at high frequency, excessive amount of insecticide used per time, and other incorrect use behaviors) will lead to selection pressure on mosquitoes, which induces insecticide resistance in the survivors (*22*). The emergence of insecticide resistance will make this mosquito control strategy ineffective and eventually lead to the resurgence of mosquito-borne diseases (*23*).

Therefore, to maximize the control effect on mosquitoes and delay their insecticide resistance development, it is crucial to guide people to use insecticides properly. And the guidance should be made on the basis of monitoring and evaluating the situation of insecticide resistance in mosquito species in Zhejiang Province. In this study, *Ae. Albopictus* were collected from 11 prefecture-level cities and Yiwu City of Zhejiang Province in 2019 and eight commonly used insecticides were included in insecticide resistance testing.

2. Materials and Methods

2.1. Mosquito collection and feeding

Ae. albopictus were collected from 11 prefecture-level cities (including Hangzhou, Ningbo, Quzhou, Wenzhou, Lishui, Jinhua, Taizhou, Huzhou, Zhoushan, Jiaxing and Shaoxing) and Yiwu City in Zhejiang Province. In order to obtain the resistance level of the whole city using a multi-point sampling method, the larvae of *Ae. albopictus* were collected from at least three localities in each city (Figure 1). Then larvae were raised with dechlorinated tap water and fed with mouse feed. The adults were identified as species by morphology. Adult mosquitoes were fed with 10% glucose water. *Ae. albopictus* were maintained in a controlled laboratory, where temperature was 27 ± 2˚C, relative humidity was 75 ± 10%, and light: dark cycle was 14 h:10 h.

2.2. Insecticide-impregnated paper

According to frequency of use, three major categories of insecticides were selected for resistance bioassays (pyrethroids: beta-cypermethrin, deltamethrin, permethrin, alpha-cypermethrin, lambda-cyhalothrin; organophosphates: malathion, fenitrothion; carbamate: propoxur.). The discriminating doses used in this study were offered by the staff of China CDC, based on the laboratory susceptible strain of *Ae. Albopictus*. They also provided the insecticide-impregnated papers (0.4% beta-cypermethrin, 0.1% deltamethrin, 3% permethrin, 0.5% malathion, 0.05% propoxur, 1.4% alpha-cypermethrin, 0.2% fenitrothion, 0.5% lambda-cyhalothrin) and control papers for this study.

2.3. Adult resistance bioassays

The bioassays were carried out in 2019, according to the tube test protocol recommended by China CDC. The temperature, humidity and light in the testing room were the same as those in the feeding room. Both test group and control group consisted of the F1 generation of non-blood fed female mosquitoes (3~5days old). For each insecticide test, the test group was exposed to insecticide-impregnated paper for one hour with three replicates. There were 12 control groups, each of which was repeated three times. After exposure, the mosquitoes were fed with 10% glucose water.

Mortality counts were conducted 24 hours after the end of the bioassay. Mosquitoes, which were unable to fly when they were given mechanical stimulation were considered dead. If the control mortality is ≥ 5% and < 20%, the mortality should be corrected by Abbott's formula, as follows:

$$\text{Corrected mortality} = \frac{(\%\text{observed mortality} - \%\text{control mortality})}{\text{Corrected mortality}} \times 100$$

If the mortality of control group was ≥ 20%, the bioassay should be redone. Resistant status was classified into three categories by mortality rate: resistance if mortality < 90%, probable resistance if mortality was between 90 and 98%, and susceptibility if mortality > 98%.

2.4. Statistical Analysis

The correlation of the mortality between different insecticides was analyzed by Pearson correlation. Statistical analyses were performed using SPSS (version 20.0) software and a value of < 0.05 was considered to be statistically significant.

3. Results

The mosquitoes in all cities, except Hangzhou and Wenzhou, showed a decreased susceptibility to the

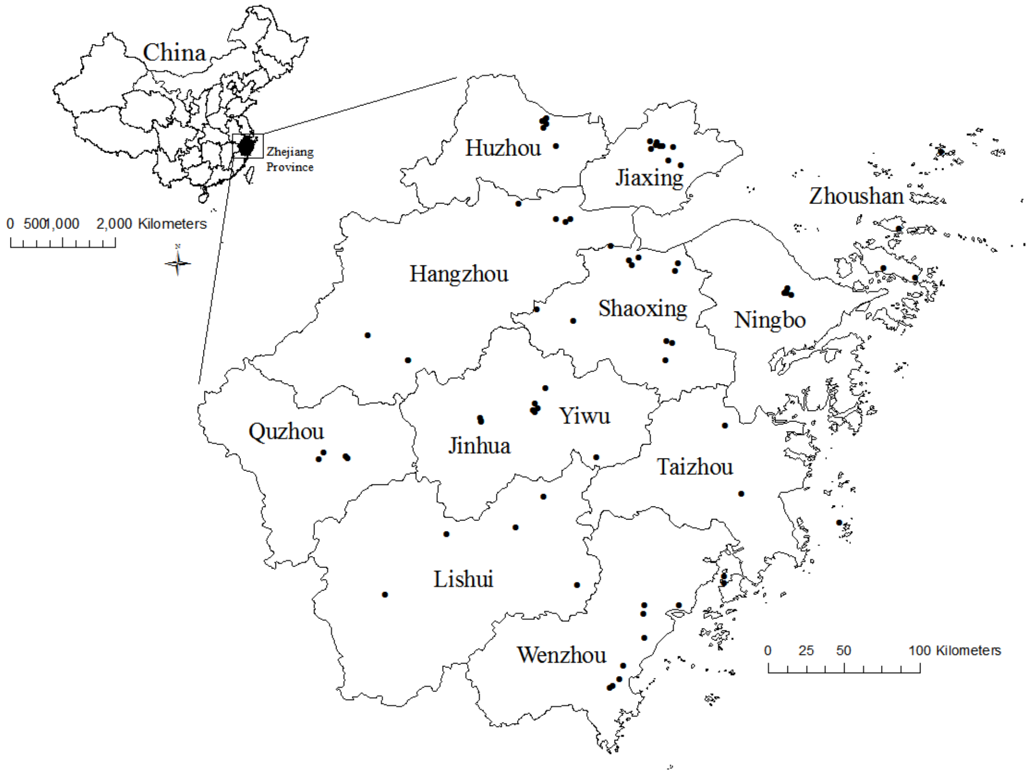

Figure 1. The sampling sites of *Ae. albopictus* in Zhejiang Province, China. The sampling sites of *Ae. albopictus* in 11 prefecture-level cities of Zhejiang Province.

three major categories of insecticides tested (Table 1, Figure 2). Mosquitoes in Ningbo and Huzhou showed a probable resistance to one of the pyrethroids; mosquitoes in Lishui showed a probable resistance to malathion; mosquitoes in Jinhua showed a probable resistance to one of the pyrethroids and propoxur; mosquitoes in Jiaxing showed a probable resistance to two of the pyrethroids and malathion; mosquitoes in Taizhou showed a probable resistance and resistance to three pyrethroids and malathion; mosquitoes in Quzhou showed a resistance to deltamethrin, permethrin and malathion, with a mortality of 82.73-88.68%, and showed to be probably resistant to beta-cypermethrin and propoxur. The city with most serious insecticide resistance problem is Yiwu, in which the mosquitoes showed a resistance to the pyrethroids tested, with a mortality of 61.15-80.00%, and showed a probable resistance to propoxur.

For beta-cypermetrin, mosquitoes in Yiwu and Taizhou had developed resistance, while mosquitoes in Quzhou, Jinhua and Jiaxing had developed probable resistance. For deltamethrin, mosquitoes in Yiwu, Quzhou and Zhoushan had developed resistance, while mosquitoes in Ningbo, Taizhou and Jiaxing had developed probable resistance. For permethrin, mosquitoes in Yiwu and Quzhou had developed resistance, while mosquitoes in Zhoushan, Taizhou and Huzhou had probable resistance. For malathion, mosquitoes in Shaoxing, Quzhou and Taizhou had developed resistance, while mosquitoes in Zhoushan, Jiaxing and Lishui have probable resistance. For propoxur, mosquitoes in Quzhou, Yiwu and Jinhua had probable resistance. For alpha-cypermethrin, lambda-cyhalothrin and fenitrothion, only mosquitoes in Zhoushan had developed probable resistance, while mosquitoes in other cities are all susceptible.

Table 2 shows the correlation of the mortality between different insecticides. The mortality of beta-cypermethrin was related with that of deltamethrin and permethrin, and the r was 0.834 ($P < 0.001$) and 0.864 ($P < 0.001$), respectively. The mortality of deltamethrin was significantly associated with that of permethrin and the r of 0.960 ($P < 0.0001$).

4. Discussion

As the increasing health burden caused by arboviral diseases, mosquito-borne disease has become a major international public health concern. Mosquito control is the key part of the global strategy for mosquito-borne disease prevention, and insecticides are the most critical component of this work. In the past, the use of insecticides (such as long-lasting insecticidal nets; indoor residual spraying; space spraying) has effectively reduced the incidence of mosquito-borne diseases and has saved millions of people (*24*). However, with the overuse of chemical insecticides, mosquitoes have developed resistance to protect their lives. The first case

Table 1. The mortalities of *Ae.albopictus* in twelve cities exposed to discriminating does of eight insecticides

Cities	Beta-cypermethrin		Deltamethrin		Permethrin		Malathion		Propoxur		Alpha-cypermethrin		Lambda-cyhalothrin		Fenitrothion	
	No.	M (%)	No.	M (%)	No.	M (%)	No.	M (%)	No.	M (%)	No.	M (%)	No.	M (%)	No.	M (%)
Hangzhou	80	100.00	82	98.78	80	100.00	82	100.00	76	100.00	83	100.00	80	98.75	72	100.00
Ningbo*	90	98.75	90	91.25	90	98.75	90	98.75	90	100.00	90	98.75	-	-	-	-
Quzhou*	60	90.57	59	82.73	60	88.68	60	86.79	59	94.24	-	-	-	-	-	-
Yiwu	66	65.15	60	61.67	60	80.00	70	98.57	60	96.67	-	-	-	-	-	-
Wenzhou	78	100.00	81	100.00	-	-	79	100.00	84	100.00	-	-	80	100.00	-	-
Lishui	90	100.00	84	100.00	87	100.00	83	95.18	86	100.00	-	-	-	-	-	-
Jinhua	90	92.22	90	100.00	90	100.00	90	100.00	90	96.67	-	-	-	-	-	-
Taizhou*	66	82.37	66	94.71	79	94.11	67	89.58	63	100.00	-	-	-	-	-	-
Huzhou*	69	100.00	67	98.41	68	96.87	73	100.00	65	100.00	-	-	-	-	-	-
Zhoushan	91	98.90	92	89.13	90	94.44	91	91.21	95	100.00	92	94.57	-	-	-	-
Jiaxing	75	96.00	75	97.33	75	98.67	75	92.00	75	98.67	-	-	-	-	-	-
Shaoxing	60	100.00	60	100.00	60	100.00	60	85.00	60	100.00	-	-	-	-	-	-

No.: number of *Ae.albopictus*; M: mortality; *The mortality corrected by Abbott's formula.

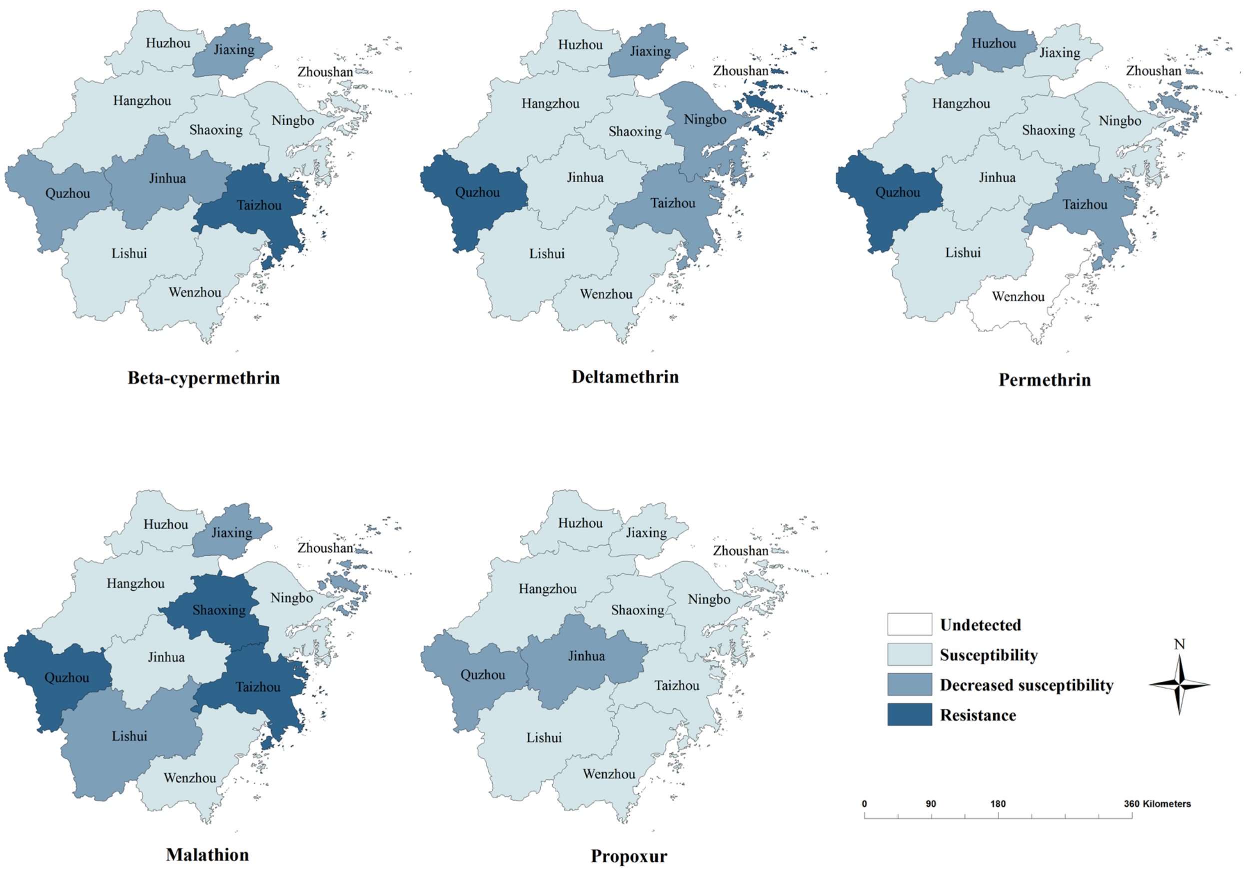

Figure 2. The resistance to five insecticides in Zhejiang Province. Beta--cypermethrin: The resistance level of *Ae. albopictus* to beta--cypermethrin in 11 prefecture-level cities. **Deltamethrin**: The resistance level of *Ae. albopictus* to deltamethrin in 11 prefecture-level cities. **Permethrin**: The resistance level of *Ae. albopictus* to permethrin in 11 prefecture-level cities. **Malathion**: The resistance level of *Ae. albopictus* to malathion in 11 prefecture-level cities. **Propoxur**: The resistance level of *Ae. albopictus* to propoxur in 11 prefecture-level cities.

of insecticide resistance in mosquitoes was reported in 1952, and later on similar cases were reported across the world (*25-29*). The emergence of resistance negatively influenced the control of mosquito-borne diseases (*30-32*) and promoted scientists to develop new insecticide-based methods (*33*). For example, insect-attractive targeted sugar baits are being developed to control mosquitoes (*34-36*). However, such methods will undergo the same pattern as previous tools did in that the method works out at the beginning, but the

Table 2. Correlation of mortality between different insecticides

	Beta-cypermethrin		Deltamethrin		Permethrin		Malathion		Propoxur	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Beta-cypermethrin	1	-								
Deltamethrin	0.834	0.001*	1	-						
Permethrin	0.864	0.001*	0.960	0.000*	1	-				
Malathion	0.004	0.990	0.022	0.947	0.066	0.847	1	-		
Propoxur	0.511	0.089	0.552	0.063	0.582	0.060	0.162	0.615	1	-

*$p < 0.05$

effectiveness would decrease in a short time because mosquitoes develop resistance (*33*).

We investigated the *Ae. albopictus* adults' resistance to five commonly used insecticides in Zhejiang Province in 2016 (*37*). Generally, after insecticide exposure, a population with a mortality < 90% is considered to be resistant, a population with a mortality > 90% and < 98% is considered to be probably resistant, and a population with mortality > 98% is considered to be susceptible. According to this, for the three pyrethroids, only Shaoxing showed resistance for beta-cypermethrin in 2016. However, the number of cities in Zhejiang Province where mosquitoes are resistant to the three pyrethroids increased in 2019. For beta-cypermathrin tested for mosquitoes in Ningbo, Quzhou, Yiwu, Jinhua, Taizhou, and Jiaxiang, the mortality in 2019 is lower than the rate in 2016, suggesting a decrease in mosquitoes' susceptibility to beta-cypermathrin. For deltamethrin tested for mosquitoes in Hangzhou, Ningbo, Quzhou, Yiwu, Taizhou, Huzhou, Zhoushan and Jiaxing, the morality in 2019 is lower than the rate in 2016. In addition, for permethrin tested for mosquitoes in Quzhou, Yiwu, Taizhou, Huzhou, Zhoushan, the morality in 2019 is lower than the rate in 2016.

In 2019, mosquitoes in Yiwu and Taizhou newly developed resistance to beta-cypermetrin; mosquitoes in Yiwu, Quzhou and Zhoushan newly developed resistance to deltamethrin; and mosquitoes in Yiwu and Quzhou have developed resistance to permethrin. These results informed us that there are more cities in Zhejiang province in which mosquitoes develop insecticide resistance.

Different from pyrethroids resistance, malathion resistance in Zhejiang Province in 2019 has been mitigated, by comparison with the situation in 2016, only for Shaoxing, Taizhou and Quzhou, mosquitoes in these cities still show resistance to malathion. The resistance status of propoxur was similar to the status in 2016 that mosquitoes in most cities are still susceptible.

Notably, a relatively intense resistance to beta-cypermetrin, deltamethrin and permethrin was observed in mosquitoes in Yiwu and the mortality was 65.15%, 61.67% and 80%, respectively in 2019. Yiwu is the largest wholesale market for small commodities in the world in that about 15,000 foreign businessmen from more than 100 countries and regions reside here. Our surveillance data shows that the number of imported cases of dengue fever in Yiwu is the largest in Zhejiang Province. The annual number of imported cases from 2016 to 2019 was 9, 12, 12 and 35 respectively. The local government attaches great importance to mosquito-borne diseases. The government exterminates mosquitoes in each place where any mosquito-related cases were identified, and regularly organizes large-scale mosquito control campaigns. Beta cypermetrin, deltamethrin and permethrin are often used to kill mosquitoes and flies. Large scale and high frequency use of insecticides can lead to development of resistance, which may be the explanation for resistance of adult mosquitoes in Yiwu city.

In recent years, not only has *Ae. albopictus* in Zhejiang Province developed resistance to pyrethroids, but also has the indigenous house fly. We studied the resistance of house flies in 2011, 2014 and 2017, and found that the resistance to pyrethroids was very common (*38*). The increase of vector resistance may be attributed to large-scale use of chemical insecticides. With the increasing challenge of mosquito-borne diseases prevention and control, the frequency of using pyrethroids increased. Wei *et al.* investigated the resistance of *Ae. albopictus* in the early and late stages of emergency dengue fever control in Hangzhou in 2017, and found that after 3 months of wide-ranging use of insecticides, the resistance of *Ae. albopictus* to pyrethroids increased (*39*). Correlations among beta-cypermethrin, deltamethrin and permethrin suggested that cross resistance may exist between them.

Through this study, we found that the resistance of *Ae. Albopictus* in Zhejiang Province has increased significantly in the past three years, and there are several implications from the study results: 1) the local government and healthcare facilities should highlight the use of insecticides resistance surveillance; 2) the insecticides should be used in a more strategic and sustainable way to reduce insecticide resistance 3) scientific management of infectious disease vectors should be developed. Guo *et al.* constructed "mosquito-free village" in rural areas mainly by cultivating the health literacy of villagers to reduce mosquito breeding areas (*40*). The success of the "mosquito-free village" construction suggests that we can control mosquito density at a lower level with less or no insecticides. Therefore, we are calling on the government and health institutions to use the environment management and environment modification method to create an environment not conducive to mosquitoes' survival. This activity should be affiliated with other measures, such as an anti-mosquito education program to prevent future mosquito-borne disease in Zhejiang Province.

In summary, our study found that mosquitoes in most cities had resistance or probable resistance to the three major categories of tested insecticides. For beta-cypermetrin and permethrin, 2 cities' mosquitoes have developed resistance, and 3 cities' mosquitoes have decreased susceptibility. For deltamethrin and malathion, 3 cities' mosquitoes have developed resistance, and 3 cities' mosquitoes have decreased susceptibility. Few cities' mosquitoes had resistance to alpha-cypermethrin, lambda-cyhalothrin and fenitrothion. In the future daily mosquito control, the government and health institutions should adopt a comprehensive management of mosquito control, which involves environment management, biocontrol, physical control, and chemical control. To more efficiently reduce the density of mosquitoes and lower the risk of mosquito-borne disease in Zhejiang Province, the next objective is to strengthen activities in eliminating mosquito breeding sites within this province. To achieve this, the government and health institutions should launch anti-mosquito campaigns to raise citizen awareness of mosquito control strategies and guide healthcare workers to use insecticides properly. In the emergency preparedness for future mosquito-borne diseases, two things should be done: 1) the selection of insecticides should be made based on information obtained from insecticide resistance surveillance 2) the use of insecticide should strictly follow scientific guidance.

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