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Widespread multiple insecticide resistance in the major dengue vector *Aedes albopictus* in Hainan Province, China

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Abstract

BACKGROUND: *Aedes albopictus* is a highly invasive mosquito and has become a potential vector of dengue, chikungunya and Zika viruses. Insecticide-based mosquito interventions are the main tools for vector-borne disease control. However, mosquito resistance to insecticides is a major threat to effective prevention and control. Five *Ae. albopictus* populations across Hainan Province, China were investigated for susceptibility to multiple insecticide and resistance mechanisms.

RESULTS: Larval bioassays indicated that resistance to pyrethroids was common in all larval populations. Adult bioassays revealed all populations were either resistant or highly resistant to at least four of the six synthetic insecticides (deltamethrin, permethrin, cyfluthrin, propoxur, malathion, and DDT) tested. Pre-exposure of mosquitoes to the synergistic agent piperonyl butoxide (PBO) increased mosquito mortality by 2.4–43.3% in bioassays to DDT, malathion, and permethrin and rendered mosquito sensitive to deltamethrin, cyfluthrin, and propoxur. The frequency of knockdown resistance (*kdr*) mutations (F1534S and F1534C) ranged from 69.8% to 89.3% and from 38.1% to 87.0% in field-resistant and sensitive populations, respectively. F1534S mutation was significantly associated with pyrethroid resistance. No mutation was detected in the acetylcholinesterase (*ace-1*) gene in the two examined populations.

CONCLUSION: This study provides evidence of widespread resistance to multiple insecticides in *Ae. albopictus* in Hainan Province, China. Both *kdr* mutations and metabolic detoxification were potential causes of insecticide resistance for *Ae. albopictus*. Our findings highlight the need for insecticide resistance management and mosquito control measures that do not entirely depend on synthetic insecticides.
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Supporting information may be found in the online version of this article.

Keywords: *Aedes albopictus*; insecticide resistance; knockdown resistance (*kdr*); piperonyl butoxide; Hainan Province

1 BACKGROUND

Aedes albopictus (Skuse) is a potential vector of at least 20 arboviruses with public health importance in tropical and subtropical regions, including yellow fever virus, dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV).[1–7] It is listed among the most dangerous invasive mosquito species globally.[8] Furthermore, *Ae. albopictus* was responsible for the vast majority of dengue and chikungunya outbreaks in China during the past two decades.[9–12] The species is widely distributed in China from the southern tropical area of Hainan Province to the temperate climate Liaoning Province in the north.[13,14]

So far, there is no effective drug or vaccine for most *Ae. albopictus* transmitted diseases except the vaccine for yellow fever, therefore mosquito control is the most important method for preventing *Aedes* mosquito-borne infectious diseases.[1–4] Adult *Aedes*

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mosquito control depends largely on outdoor and indoor insecticide spraying, while larval control relies on either chemical (e.g. temephos) or biological (e.g. *bti*) insecticides and community engagement for habitat management.[15–17] In China, the primary tool for mosquito control is the use of chemical insecticides.[18,19] However, *Ae. albopictus* resistance to all four main classes of insecticides (carbamates, organochlorines, organophosphates, and pyrethroids) has been detected in the Americas, Africa, and Asia in recent years.[15,20,21]

Hainan Province, located in southern China, is a dengue fever epidemic area. Dengue fever outbreaks have occurred several times since the late 1970s. The 1979–1982 dengue pandemic on the island caused a total of 604 854 cases and 475 deaths.[22,23] The most recent Dengue outbreaks occurred on this island in 2019.[24] As an international tourist destination and now a free-trade area, mosquito-borne infectious disease risk will likely grow due to the increasing influx of goods and human travel. This threat is underscored by the presence of dengue fever throughout the neighboring southeastern countries.[25] Furthermore, the tropical climate of the island makes it particularly suitable for the development and survival of vector mosquitoes. *Ae. albopictus* is widely distributed on the island, and the species is now almost the sole vector of dengue fever in Hainan.[26,27] Previously, *Ae. aegypti* was the major dengue vector from the 1970s to the 1990s, but *Ae. aegypti* has not been found on the island during the past 10 years.[23,28,29]

In the past 40 years, Hainan Province has used a large quantity of insecticides to help control vector-borne diseases, including malaria and dengue.[23] However, there is limited information available on the current status of insecticide resistance in *Ae. albopictus*. A previous study in Haikou city in Hainan indicated that *Ae. albopictus* had developed resistance to deltamethrin, permethrin, and beta-cypermethrin.[22] Thus, monitoring the development of

insecticide resistance in *Aedes* mosquitoes has become a local key task for dengue control.[7]

Due to massive agricultural and public health use of insecticides, systematic studies on *Ae. albopictus* resistance to insecticides are critically needed for the prevention and control of mosquito-borne diseases. In the present study, we studied the insecticide resistance profiles of larval and adult *Ae. albopictus* in five areas across Hainan Province during 2017–2018. We implemented synergist and molecular assays to identify putative resistance mechanisms in *Ae. albopictus* adults.

2 MATERIALS AND METHODS

2.1 Ethics statement
No permits were required for the described field studies. Mosquito collections in breeding sites were consented orally by the owners at each location. For the insecticide usage survey, oral consent was obtained from all participating households after explanation of the study aim and design. This study did not involve collection of any human-related samples or personal information such as participants' names, addresses, phone numbers, and GPS location of their homes etc.

2.2 Study sites and mosquito collections
Immature *Ae. albopictus* mosquitoes were collected from five study sites in Hainan Province, southern China between 2017 and 2018 (Fig. 1 and Table 1). The five sites represent different ecological settings on the island (Table 1). Annual mean temperature ranges from 23.1 to 25.9 °C, and annual precipitation ranges from 1300 to 2300 mm (Table 1).

Mosquito larvae were collected from at least 50 habitats in each study site, which included both urban and suburban areas. Larvae were brought back to the insectary located at Hainan Medical
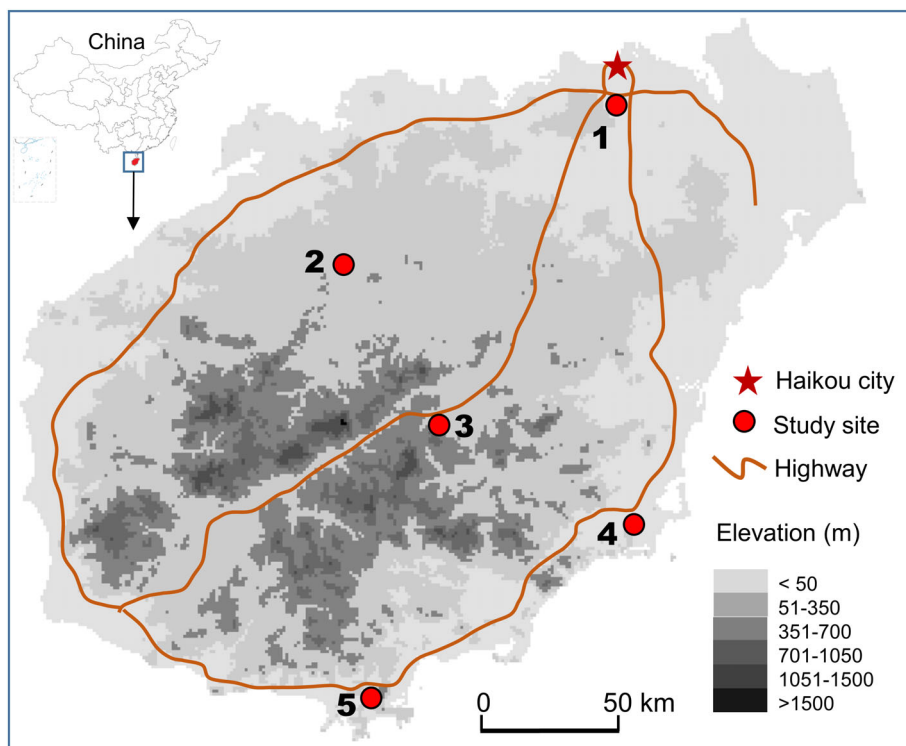

Figure 1. Map of study area in Hainan Province, China.

Table 1. Description of *Aedes albopictus* mosquito population collection sites in Hainan Province, China

| Study area | Landscape | Coordinates | | Altitude (m) |
		Latitude (N)	Longitude (E)	
Haikou	Densely populated port city	110°20′29″	20°04′39″	4
Wanning	Plain, agriculture area	110°23′15″	18°48′10″	13
Sanya	Tourist destination	109°30′28″	18°17′32″	26
Qiongzhong	Heavily forested area	109°50′47″	19°03′03″	267
Danzhou	Major agricultural area	109°33′35″	19°31′30″	184

University. Larvae were reared at 27 ± 2 °C, 70% ± 10% relative humidity (RH) and a 12:12 h light:dark period, and fed with yeast. Emerged adults were fed with 10% sugar solution at 27 ± 2 °C, 70% ± 10% RH.

2.3 Insecticide resistance bioassays

2.3.1 Larval bioassays

Eight insecticides were used to examine larval mortality. Seven of the insecticides were obtained from the Chinese Center for Disease Control and Prevention (China CDC). Three of them were pyrethroid insecticides: deltamethrin (technical grade 95.95%), permethrin (99%), and beta-cypermethrin (92.00%). The other chemical insecticides included were propoxur (95.56%), malathion (95%), temephos (87.40%), and DDT (94.00%). Lastly, one microbial larvicide was evaluated: *Bacillus thuringiensis israelensis* (*Bti*) (7000 ITU/mg, Wuhan Nature's Favour Bioengineering Co., Ltd, Wuhan, China). All chemical insecticides were diluted in acetone, and *Bti* was diluted in water to the required dosage following WHO guidelines.[30] We included adulticides for larval resistance tests because in China pyrethroid and other adulticides have been widely used for agricultural pest control and for public health use such as control mosquitoes inside rainwater drain basins. The residue of these insecticides in the water may causes larval resistance after long-term use.

The larvae of F1-2 generations were used for larval bioassays after species identification. In each test, 25 third- and fourth-instar larvae were incubated in a 100 mL solution consisting of 99 mL of distilled water and 1 mL of insecticide solution at the specified concentration using the standard 250 mL plastic cup.[30] Control treatments for the chemical insecticides were incubated in 99 mL of distilled water and 1 mL of acetone. Control treatments for *Bti* were incubated in 100 mL of water only. Three to five replicates were tested for each concentration. Each bioassay comprised one control group. Five to nine concentrations, providing a range of mortalities between 0% and 100%, were used to determine the 50% mortality lethal concentration (LC_{50}). Mosquitoes of the Foshan strain, which have been reared in the laboratory since 1981 without insecticide exposure, were used as a control. Larval mortality was recorded after 24 h of exposure. Bioassays were conducted at 27 ± 2 °C, 70% ± 10% RH, and a 12: 12 h light: dark period.

2.3.2 Adult resistance bioassays

The adults from F1-2 of field-collected *Ae. albopictus* larvae were tested using the standard WHO insecticide susceptibility tube test.[31,32] The mosquitoes were subjected to insecticide susceptibility tests against pyrethroids (0.15% cyfluthrin, 0.03% deltamethrin, and 0.25% permethrin), organochlorine (4% DDT), organophosphate (0.8% malathion), and carbamate (0.1%

propoxur). The susceptible Foshan strain was used as a control. For each population of the field mosquitoes and each insecticide, eight replicates (four each in urban and suburban) of 20 non-blood-fed 3–5 day-old female mosquitoes for each replicate were subjected to WHO tube bioassays. Data analysis used pooled values of the eight replicates at each site. For control populations, two replicates were used for each insecticide. WHO test and control papers were supplied by the School of Biological Sciences, Universiti Sains Malaysia (Penang, Malaysia). Mosquitoes were exposed to test papers for 1 h. The knockdown number of females was recorded every 10 min during the 60 min exposure period. Mortality was scored after the 24 h recovery period. After the bioassay, both survivors and dead mosquitoes were stored individually in 95% alcohol at −80 °C for subsequent DNA analysis.

2.3.3 Synergist assay with piperonyl butoxide

To investigate the role of oxidase-specific metabolic resistance mechanisms, synergist assays with piperonyl butoxide (PBO) were performed together with the six insecticides tested above: deltamethrin, permethrin, cyfluthrin, malathion, propoxur, and DDT. Nonblood-fed adult mosquitoes which were 3–5-days old were pre-exposed to 4% PBO test paper for 1 h, followed by exposure to insecticide-treated test papers for 1 h. There were three replicates for each insecticide, with 25 mosquitos per replicate. Mortality was recorded after the 24 h recovery period. Two replicates of 1 h PBO exposure were performed without subsequent exposure to an insecticide for the controls.

2.3.4 Molecular assays

Genomic DNA was extracted using the method published by Chang *et al*.[33] One adult mosquito leg for each mosquito was used for DNA extraction. Extracted DNA was stored at −20 °C or used immediately for PCR. The primers and PCR conditions used for amplification and sequencing of the voltage-gated sodium channel (VGSC) gene and the acetylcholinesterase (*ace-1*) gene of *Ae. albopictus* were according to the protocol from Su *et al* and Kasai *et al*.[13,34] PCR products were purified and directly sequenced in both directions with the same primers. PCR products were purified with ExoSAP-IT (USB, Cleveland, OH, USA) according to the manufacturer's protocol and directly sequenced by Retrogen, Inc. (San Diego, CA, USA). The sequences were analyzed using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and Codon Code (http://www.codoncode.com/).

2.3.5 Community survey of insecticide usage

Questionnaire surveys were conducted in the study communities to determine insecticide usage for public health and agricultural pest control. Questionnaire surveys were conducted from September to October of 2019 in all study sites. Each survey site

included urban, suburban, and rural areas with a total of 30 questionnaires. Oral consent was obtained from all participants after explanation of the study purpose to them.

2.3.6 Statistical analysis

Resistance status of adult mosquito was classified according to WHO criteria: resistant for <90% mortality, probable resistance for 90–98% mortality, and susceptible for >98% mortality.[31] In the larval bioassay, the median lethal concentration (LC_{50}), the 90% lethal concentration (LC_{90}), and the corresponding 95% confidence intervals were calculated based on the recorded data using Schoofs and Willhite's probit analysis program.[35] The degree of resistance intensity was determined by the resistance ratio (RR_{50}), calculated as the ratio of LC_{50} value for the field population over the LC_{50} value for the susceptible laboratory colony. By WHO definition, $RR_{50} < 5$ is considered susceptible, $5 < RR_{50} < 10$ is moderately resistant, and $RR > 10$ is highly resistant.[31] For the adult bioassays, χ^2-tests were used to examine the association between *kdr* mutations and the resistance phenotype. Here, mosquitoes dead after the 24 h recovery period were classified as susceptible and surviving mosquitoes as resistant. For each population and each insecticide, the mortalities with mosquitoes exposed to PBO + insecticide were compared with those exposed with insecticide alone using *t*-tests.

3 RESULTS

3.1 Larval *Ae. albopictus* developed resistance to pyrethroid insecticides

Larval bioassays revealed that *Ae. albopictus* have developed different levels of resistance ($RR_{50} \geq 5$) to all pyrethroid insecticides at nearly all study sites, but they were all susceptible ($RR_{50} < 5$) to all nonpyrethroid insecticides (Table 2 and Fig. 2). All larval populations were highly resistant to deltamethrin ($LC_{50} = 0.17–0.47$ mg/L; RR_{50} range 17–47), and most of them were moderately or highly resistant to permethrin ($LC_{50} = 0.36–1.86$ mg/L, RR_{50} range 7–37). The highest RR_{50} was 53 for beta-cypermethrin ($LC_{50} = 1.06$ mg/L) in the Haikou population, and the lowest RR_{50} was 3 for beta-cypermethrin ($LC_{50} = 0.06$ mg/L) in the Danzhou population (Fig. 2 and Table 2). In addition, all of the populations tested were susceptible to Propoxur ($LC_{50} = 0.89–4.78$ mg/L, RR_{50} range 0.8 to 4.2), malathion ($LC_{50} = 2.40–5.97$ mg/L, RR_{50} range 0.7–2.4), temephos ($LC_{50} = 0.07–0.16$ mg/L, RR_{50} range 1.8–4.0), DDT ($LC_{50} = 1.92–4.96$ mg/L, RR_{50} range

0.8–2.1), and *Bti* ($LC_{50} = 0.55–0.99$ mg/L, RR_{50} range 0.7–1.2). (Table 2 and Fig. 2).

3.2 Adult *Ae. albopictus* developed resistance to all the insecticides used

WHO tube bioassays revealed that nearly all *Ae. albopictus* populations were resistant (<90% mortality) to the six synthetic insecticides tested, as indicated by the low mortality rate after the 24 h recovery period (Fig. 3(a)). Although mortality rates varied, mosquitoes were highly resistant to DDT (mortality range 28–46%), permethrin (mortality range 47–71%), and malathion (mortality range 61–82%) at all study sites (Fig. 3(a)). Mosquito resistance levels ranged from highly resistant to suspected resistant to cyfluthrin (mortality range 69–91%), propoxur (mortality range 56–96%), and deltamethrin (mortality range 70–96%) (Fig. 3(a)).

3.3 Synergist PBO moderately increased mosquito mortality

Pre-exposure of *Ae. albopictus* mosquitoes to the PBO synergist before exposure to insecticides led to the full recovery of *Ae. albopictus* susceptibility to deltamethrin, propoxur, and cyfluthrin (except Wanning population, mortality 89%) and partial recovery of susceptibility to malathion (mortality range 72–100%) and permethrin (mortality range 82–96%) (Fig. 3(b)). However, *Ae. albopictus* mortality was still low (31–83%) against DDT after PBO treatment (Fig. 3(b)). The effect of pre-exposure to PBO on mosquito mortality varied substantially for a given insecticide among different sites and at a given site among different insecticides (Fig. 3, bottom panel), i.e. increase in mosquito mortality was observed overall, but showed strong heterogeneity (Fig. 3, bottom panel).

3.4 *kdr* mutations were associated with resistance phenotypes in adult mosquitoes

A total of 479 *Ae. albopictus* mosquitoes (234 resistant and 245 sensitive) were examined for *kdr* mutations. We detected F1534S and F1534C mutations in the VGSC gene. Overall, the total *kdr* mutation frequency was significantly higher in the resistant mosquitoes (mean 78.6%, range 69.8–89.3%) than that in the susceptible mosquitoes (mean 46.5%, range 38.1–50.9%) in four of the five populations (χ^2-test, $P < 0.05$; Table 3). The odds ratio for the F1534S mutation ranged from 2.4 to 10.8 (but was 0.8 in Haikou) in resistant mosquitoes compared to susceptible mosquitoes, indicating the association between pyrethroid phenotypic

Table 2. Insecticide concentrations that caused 50% mortality (LC_{50}) of *Aedes albopictus* in different populations

Insecticide	Haikou	Wanning	Sanya	Qiongzhong	Danzhou	Susceptible strain
Deltamethrin	0.35 (0.25, 0.48)	0.47 (0.38, 0.56)	0.26 (0.20, 0.31)	0.27 (0.15, 0.45)	0.17 (0.09, 0.31)	0.01 (0.01, 0.01)
Beta-cypermethrin	1.06 (0.50, 2.48)	0.15 (0.09, 0.27)	0.17 (0.15, 0.19)	0.09 (0.06, 0.14)	0.06 (0.05, 0.06)	0.02 (0.02, 0.03)
Permethrin	1.86 (1.28, 2.57)	1.24 (0.37, 2.78)	0.51 (0.26, 0.94)	0.86 (0.48, 1.35)	0.36 (0.28, 0.44)	0.05 (0.03, 0.06)
Malathion	3.22 (2.90, 3.55)	2.81 (2.21, 3.49)	5.97 (4.45, 8.18)	2.83 (1.97, 3.98)	2.40 (1.88, 2.96)	3.37 (2.70, 4.18)
Temephos	0.12 (0.11, 0.13)	0.15 (0.14, 0.17)	0.13 (0.12, 0.14)	0.07 (0.06, 0.07)	0.16 (0.14, 0.17)	0.04 (0.04, 0.04)
Propoxur	4.78 (4.46, 5.12)	2.65 (2.43, 2.88)	3.83 (3.58, 4.09)	0.89 (0.71, 1.10)	1.48 (1.22, 1.78)	1.14 (1.07, 1.23)
DDT	3.72 (2.62, 5.44)	3.42 (2.94, 3.98)	1.92 (1.11, 3.41)	4.96 (4.19, 5.89)	3.46 (3.07, 3.89)	2.41 (1.82, 3.28)
Bti	0.55 (0.43, 0.68)	0.60 (0.53, 0.70)	0.82 (0.52, 1.92)	0.99 (0.86, 1.20)	0.95 (0.89, 1.02)	0.81 (0.66, 1.03)

LC_{50} lethal concentration that kills 50% of the population (mg/L), values are LC_{50} (95% CI).
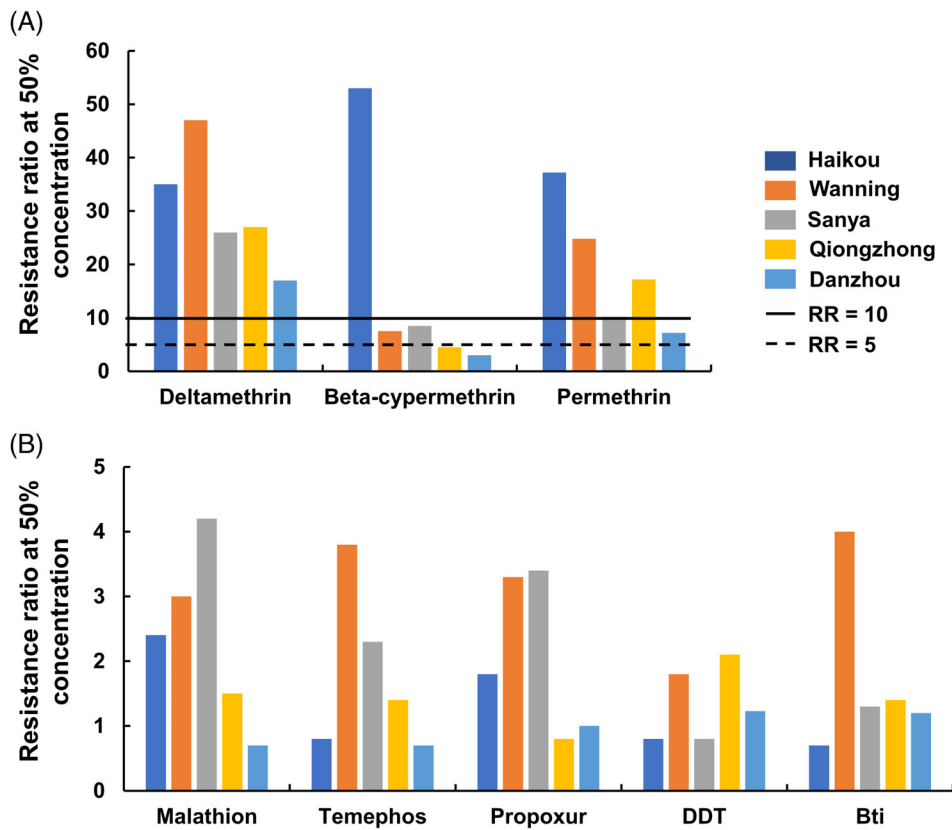*Bti*: 7000 ITU/mg.

(A)

(B)

Figure 2. The resistance of *Aedes albopictus* larvae to currently used insecticides (resistance ratio at 50% concentration). (A) Insecticide pyrethroids (cyfluthrin, deltamethrin, and permethrin); (B) organophosphate (malathion, temephos), organochlorine (DDT), carbamate (propoxur), and *Bti*. RR$_{50}$, resistance ratio, LC$_{50}$ test population/LC$_{50}$ laboratory-susceptible strain.

(A) Without pre-exposure to PBO

(B) With pre-exposure to PBO

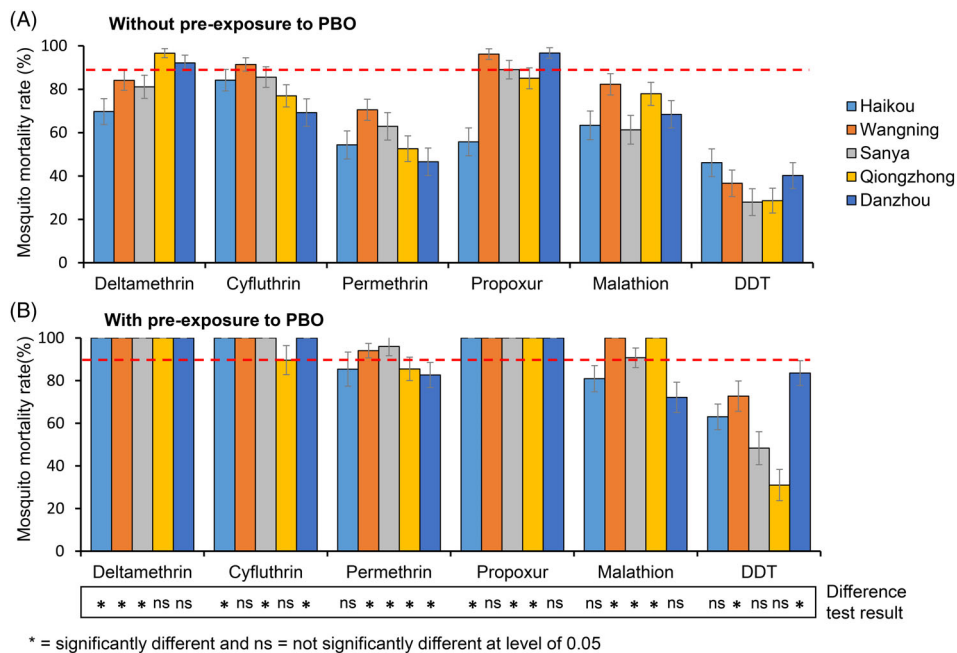* = significantly different and ns = not significantly different at level of 0.05

Figure 3. Mortality rates of adult *Aedes albopictus* after exposure to insecticides without (A) and with (B) pre-exposure to PBO. Error bars represent standard error of the mean. The bottom panel indicated significant level of difference in mortalities for the same insecticide between PBO and non-PBO pre-exposed populations from the same study site.

Table 3. *Kdr* mutation frequency relationship to pyrethroid resistance in different *Aedes albopictus* populations

Study site	Phenotype	Sample size	Allele frequency (%)			Odds ratio (95% CI)	
			Wildtype	F1534C	F1534S	F1534C	F1534S
Haikou	Resistant	61	13.1	26.2	60.7	1.1 (0.6–2.1) [NS]	0.9 (0.5–1.6)[NS]
	Susceptible	50	13.0	24.0	63.0		
Qiongzhong	Resistant	14	10.7	3.6	85.7	1.5 (0.1–25.3) [NS]	10.8 (3.2–37.0)***
	Susceptible	21	61.9	2.4	35.7		
Sanya	Resistant	36	16.7	26.4	56.9	1.5 (0.7–3.2) [NS]	3.3 (1.7–6.4)**
	Susceptible	42	52.4	19.0	28.6		
Wanning	Resistant	48	30.2	4.2	65.6	0.6 (0.2–2.3) [NS]	2.4 (1.4–4.2)**
	Susceptible	56	49.1	6.4	44.5		
Danzhou	Resistant	75	28.0	2.0	70.0	NA	2.4 (1.5–3.8)***
	Susceptible	76	50.7	0.0	49.3		

** *P* < 0.01.
*** *P* < 0.001.
NS, no significance.

Table 4. Summary of public health and agricultural use of pesticides based on questionnaire survey

Site	Purpose	Environment	Insecticide	Percentage of households (%)			
				Never	Weekly	Biweekly	Monthly
Sanya	Public health	Indoor*	Pyrethroids	3.3	96.7	0.0	0.0
	Agricultural	Outdoor	Pyrethroids	80.0	13.3	0.0	6.7
			Organophosphate	66.7	6.7	13.3	13.3
			Microbial insecticide	90.0	3.3	3.3	3.3
Wanning	Public health	Indoor*	Pyrethroids	10.0	36.7	33.3	20.0
	Agricultural	Outdoor	Pyrethroids	93.3	0.0	6.7	0.0
			Organophosphate	56.7	0.0	33.3	10.0
			Nitromethyl	86.7	0.0	6.7	6.7
Haikou	Public health	Indoor*	Pyrethroids	26.7	43.3	13.3	16.7
			Carbamate	93.3	0.0	3.3	3.3
	Agricultural	Outdoor	Pyrethroids	86.7	0.0	6.7	6.7
			Organophosphate	73.3	0.0	16.7	10.0
Danzhou	Public health	Indoor*	Pyrethroids	26.7	40.0	13.3	20.0
	Agricultural	Outdoor	Organophosphate	63.3	0.0	20.0	16.7
			Nicotine insecticide	86.7	0.0	10.0	3.3
Qiongzhong	Public health	Indoor*	Pyrethroids	0.0	100.0	0.0	0.0
	Agricultural	Outdoor	Pyrethroids	56.7	0.0	3.3	40.0
			Organophosphate	66.7	0.0	0.0	33.3

Total number of households surveyed was 30 per site.
*Pyrethroid-based mosquito repellents.

resistance and the *kdr* mutation (Table 3). The F1534C mutation was not associated with pyrethroid resistance in any of the five populations (Table 2). The F1534L mutation was not detected at any study site. Genotyping of the *ace-1* gene in Haikou and Danzhou populations ($n = 120$), the two populations that exhibited the highest resistance to malathion, did not reveal any mutations at codon 119.

3.5 Local residents used all types of insecticides
Questionnaire surveys of local residents in the study area revealed that six classes of insecticides, i.e. pyrethroids, organophosphates, carbamates, microbial insecticide, nitromethyl, and nicotine insecticide, were used in Hainan Province (Table 4). Pyrethroids

were the most commonly used insecticide for public health and agricultural pest control, with 73.3–100.0% of the surveyed households reporting using different types of pyrethroid insecticides for mosquito control (Table 4). Organophosphates were primarily used for agricultural pest control, and carbamates were used only in the Haikou site for mosquito control among a small proportion of surveyed households (Table 4).

4 DISCUSSION
This study presents the first resistance profile of *Ae. albopictus* across Hainan Province. The results indicate that the majority of adult *Ae. albopictus* populations in Hainan are resistant or highly

resistant to the four classes of synthetic insecticides, especially the most commonly used pyrethroids. The occurrence of widespread insecticide resistance in *Ae. albopictus* adults potentially compromises the efficacy of current intervention measures in controlling *Ae. albopictus* populations on the island. While adult *Ae. albopictus* resistance to different insecticides has been reported in other places,[15] *Ae. albopictus* adults in Hainan showed high resistance to all insecticides at all study sites. In Hainan province, all types of insecticides have been frequently used for mosquito control, especially for epidemic controls. These insecticides have also been widely used for agricultural pest controls, which can lead to resistance. Resistance management strategies are urgently needed to help mitigate failures in future vector control interventions.

The reemergence of dengue fever has been reported in many countries and often has been caused by invading *Ae. albopictus*.[36] Dengue epidemics were reported in Hainan island in the 1980s and 1990s after approximately 30 years of no reported local transmission. In 2019, Dengue fever outbreak occurred in Hainan. The high insecticide resistance in the primary vector *Ae. albopictus* adds another dimension of risk in dengue outbreak control for the near future. Currently, larval control is a key measure to control the mosquito population in Hainan. In Hainan, pyrethoids are widely used for agricultural pests and mosquito adult control but not larval control. However, we found that *Ae. albopictus* larvae are resistant to pyrethroid insecticides, which is consistent with other studies in China.[19,37] This finding is likely caused by the residues left in the habitats from adult mosquito spraying or agricultural pest controls. However, we cannot rule out inherited resistance from adult mosquitoes, which needs further investigation. *Ae. albopictus* larvae remained sensitive to some other insecticides, including organophosphorus (malathion, temephos), propoxur, and the microbial larvicide *Bti*. However, it has been reported that *Aedes* larvae have developed high resistance to temephos in South-East Asia and South America.[13,38–40] Therefore, *Aedes* larval resistance to insecticides must be closely monitored and integrated strategies should be developed to combat resistance.

Due to widespread multiple insecticide resistance in the dengue vector *Ae. albopictus* in Hainan Province, new strategies should be developed to enhance the efficacy of currently available insecticides and to slow down the development of insecticide resistance. The main strategies for insecticide resistance management, as recommended by WHO, include rotations, mosaics, and mixtures of insecticides among which there is no cross-resistance.[41] For larval control, community-involved combinations of ecological, biological, and social strategies have been demonstrated to reduce *Aedes* mosquito abundance,[42,43] i.e. combining larval source reduction with larvicides, especially microbial larvicides, may prevent further development of insecticide resistance in *Ae. albopictus*. Using PBO-insecticides for adult mosquito control, which combines PBO with insecticides and is widely used in the USA, is another potential option to slow down insecticide resistance.[44] However, to our knowledge PBO insecticide has not been tested in China. Our study indicated that pre-exposure to PBO could potentially fully recover the efficacy of some insecticides. In addition, field studies have indicated that insecticide-treated mosquito nets impregnated with PBO can significantly enhance the efficacy of standard net and reduces malaria infections.[45,46] Therefore, adding PBO to insecticides may be an effective formulation for resistance management, and this strategy warrants further examination for *Ae. albopictus* control.

The *kdr* mutation in the VGSC genes is one indicator of mosquito resistance to pyrethroid insecticides.[47,48] In malaria vector *Anopheles* mosquitoes, previous studies have found strong associations between *kdr* mutation frequency and phenotypic resistance status.[49,50] However, this association does not necessarily hold true for all mosquito species. For example, the *kdr* mutation was not found in *An. funestus*[51] nor in some populations of *An. sinensis*,[52] even when WHO tube test mortality was very low (i.e. very high observed insecticide resistance). The *kdr* mutation, specifically the F1534 mutation, has been found in *Ae. albopictus* in China and elsewhere.[21,22,53] Some studies found F1534 mutations to be associated with phenotypic resistance,[54–56] but a separate study did not find this relationship.[57] Our results indicate that the F1534S mutation is associated with pyrethroid phenotypic resistance in *Ae. albopictus* in Hainan Province. However, *kdr* mutation frequency was high in both resistant and susceptible individuals, indicating that the F1534S mutation is not completely predictive of resistance status.[54] Similarly, although with very low mortality, resistant *Ae. albopictus* did not show higher F1534C mutations in comparison with susceptible mosquitoes, indicating that F1534C is also not likely to be a good indicator of resistance, which is consistent with other studies reported from China.[54] It is possible that there are regional differences in *kdr* mutations as shown in *An. sinensis*,[54,56] but this hypothesis requires further investigation. In this study, no F1534L mutations were detected, which is different from what has been reported from nearby Guangdong province.[13] This finding may be due to the geographic variation influenced by either environmental conditions or insecticide usage.

Ace-1 mutations have been found to confer insensitivity to organophosphates and carbamates in mosquito populations.[49] Our study did not detect any G119 mutations in *ace-1* in *Ae. albopictus* populations, which suggests either that *Ae. albopictus* in Hainan lacks mutations at the target sites for these insecticides or that the mutation frequency was extremely low. Other studies also found that resistance to *ace-1* agonists is common in *Aedes*, but that *ace-1* mutations do not occur widely.[13,21,58–60] Interestingly, exposure to the PBO synergist increased *Ae. albopictus* sensitivity to pyrethroids, DDT, and malathion, which suggests that metabolic resistance may play an important role in resistance. However, in high permethrin-, DDT-, and malathion-resistant *Ae. albopictus* populations, PBO exposure only partially recovered insecticide efficacy and mosquito mortality rate, which suggests that other mechanisms may also contribute to insecticide resistance in these populations.[61,62]

Although, in general, *Ae. albopictus* populations were resistant to insecticides, it is worth noting that resistance levels varied between study sites and between different insecticides. For example, *Ae. albopictus* populations were highly resistant to permethrin and DDT in all study sites although with variations in mortality, but resistance to deltamethrin in Qiongzhong was very low. These differences may be due to the differences in the usages of specific insecticide in local residents, as some insecticides may be more frequently used in certain areas but not in others. For example, in Danzhou, farmers used organophosphate twice a month. However, farmers in Qiongzhong used the same type of insecticide monthly. In Qiongzhong, all the surveyed households used pyrethroid for household pest controls, while only about half of the residents in Haikou did the same. These differences in insecticide usage in different sites may have led to variation in resistance levels in different *Ae. albopictus* populations. Consequently, the mechanisms of the resistance need to be further investigated.

5 CONCLUSION

Aedes mosquito resistance to insecticides is influenced by a complex interplay of factors, including environmental factors and intervention strategies. The development of insecticide resistance to multiple insecticides which has occurred in Hainan will complicate the current vector control strategies. Nonetheless, the findings from this study can help in guiding the selection of insecticides for *Aedes* mosquito control. First, high pyrethroid resistance calls for the use of alternative insecticides. Second, PBO insecticides may be an option to mitigate the negative impact of pyrethroid resistance. Finally, to achieve effective vector management, a public health response beyond routine larviciding or focal spraying is essential. Targeted vector management can make a difference in terms of reducing vector abundance and controlling mosquito-borne infectious disease outbreaks.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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