First identification of \(kdr\) allele F1534S in VGSC gene and its association with resistance to pyrethroid insecticides in \(Aedes albopictus\) populations from Haikou City, Hainan Island, China

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Abstract

**Background:** \(Aedes albopictus\) is distributed widely in China, as a primary vector of Dengue fever and Chikungunya fever in south of China. Chemical insecticide control is one of the integrated programmes to prevent mosquito-borne diseases. Long-term applications of pyrethroids have resulted in the development of resistance in \(Ae. albopictus\) populations in China. However, the susceptibility of \(Ae. albopictus\) to pyrethroids in Hainan Island was unclear. Knockdown resistance (\(kdr\)), caused by point mutations in the VGSC gene, is one of the mechanisms that confer resistance to DDT and pyrethroids. This study was to investigate the resistance level of \(Ae. albopictus\) populations in Haikou City to three pyrethroid insecticides, and elucidate the relationship between the resistant phenotype and \(kdr\) mutations.

**Methods:** The \(Aedes albopictus\) samples were collected in Xinbu Island (XI), Longtang Town (LT), Shishan Town (ST), Baishamen Park (BP), and Flower Market (FM) from Haikou City, Hainan Island, China. The larval susceptibility to deltamethrin, permethrin and beta-cypermethrin was tested by larval bioassays, and adult susceptibility to deltamethrin and DDT was determined by adult bioassays. The degree of resistance was determined by resistance ratio value (\(RR_{50} > 3\)) for larvae and by mortality for adult. The \(kdr\) alleles at codon 1534 of the VGSC gene were genotyped. The relationship between \(kdr\) genotypes and resistant phenotypes was analyzed by Chi-square test.

**Results:** Out of five populations, assessed by larval bioassays, XI was susceptible to deltamethrin and permethrin; LT was susceptible to permethrin and beta-cypermethrin; and ST was susceptible to permethrin. FM and BP both were resistant to all of the three pyrethroids, and FM showed the highest degree of resistance, with \(RR_{50}\) values from 65.17 to 436.36. A total of 493 individuals from the larval bioassays were genotyped for \(kdr\) alleles. Five alleles were detected, including two wildtype alleles, TTC(F) (67.04 %) and TTT(F) (0.41 %), and three mutant alleles, TGC(C) (0.30 %), TCC(S) (31.54 %) and TTG(L) (0.71 %). There was a clear correlation between mutant alleles (or F1534S) and resistant phenotypes (\(P < 0.01\)).

**Conclusion:** Two novel \(kdr\) mutant alleles F1534S and F1534L were detected in the pyrethroid resistant populations of \(Ae. albopictus\) in Haikou Hainan, China. For the first time, the mutant F1534S was associated with pyrethroid resistance in \(Ae. albopictus\).

**Keywords:** \(Aedes albopictus\), Pyrethroids, Resistance, \(kdr\) mutation, China
Multilingual abstracts
Please see Additional file 1 for translation of the abstract into the six official working languages of the United Nations.

Background
Aedes albopictus Skuse is a primary vector of Dengue fever and Chikungunya fever in China [1, 2]. Mosquito control is one of the integrated programmes to prevent transmission of mosquito-borne diseases. Chemical insecticides have been extensively used for vector management since the 1940s. There were four major categories of insecticides: organochlorines, organophosphates, carbamates and pyrethroids [3]. The pyrethroids have been used to indoor/outdoor residual sprays since 1980s for mosquito control in China. The long-term utilization has resulted in the development of resistance in many populations of Ae. albopictus in China [2, 4–10]. The pyrethroids function as neurotoxins that target voltage-gated sodium channels (VGSC) and interfere electronic signaling in the nervous system, which results in paralysis and death, an effect known as knockdown [11]. One of the mechanisms that mosquitoes have developed for the resistance to pyrethroids is the target insensitivity, which is caused by mutations in the VGSC gene and generated knockdown resistance (kdr) [12–15]. In Anopheles mosquitoes, substitution of leucine at residue position 1014 was correlated to the resistance to pyrethroids and DDT [14–17]. In Aedes aegypti Linn, mutants have been detected in several codons of the VGSC gene from different countries, including three mutants, V1016G/I and F1534C, all were correlated with kdr [18–27]. In Ae. albopictus, the relationship between kdr and pyrethroid resistance was unclear. In a DDT and pyrethroid resistant population of Ae. albopictus in India, no kdr mutations were detected [28]. Similarly, no kdr mutations were found in Ae. albopictus populations in Malaysia where F1534C and V1016G/I were detected in the populations of Ae. aegypti [29]. So far, only one study has identified the F1534C mutant allele in a population of Ae. albopictus in Singapore with frequency of 73.1% [13].

Haikou city is a provincial capital of Hainan Island, in south of China, located at marginal zone of tropic. In the past, dengue fever outbreaks have occurred twice in 1979–1982 and 1985–1988 in Hainan Island and surrounding areas; the mortality rate was 0.785% [30–35]. In recent years, dengue fever epidemic situations remain in Guangdong, Fujian and Yunnan Provinces in China [30, 36–38]. Especially in 2014, a large-scale outbreak of dengue fever with more than 45,000 cases occurred in Guangdong Province [2, 37, 39, 40]. Hainan Island is near to but separated by a strait from Guangdong Province, and there were also reported local cases during the dengue outbreak in 2014 [2]. Upon the pressure of dengue epidemics, residual and aerial spraying of pyrethroids have become a major routine method for the control of Aedes mosquitoes in the endemic areas in China. The most commonly used pyrethroid was deltamethrin [2, 41]. Pyrethroid resistance has been detected in the populations of Ae. aegypti and Ae. albopictus in Hainan [42, 43]. In this study, we investigated the susceptibility to pyrethroid resistance and examined the kdr mutations in Ae. albopictus in five locations in Haikou City, Hainan Island. The bioassays revealed that resistance to deltamethrin, permethrin and beta-cypermethrin was developed in certain populations. In addition to the known kdr mutant, F1534C, two novel mutant alleles, F1534S and F1534L, were detected.

Methods

Ethics statement
No permits were required for the described field studies. Mosquito collections in breeding sites were consent by the owners at each location.

Mosquito samples
Mosquito larvae were collected from breeding sites in Xinbu Island (XI, 110°37’E, 20°06’N), Longtang Town (LT, 110°42’E, 19°89’N), Shishan Town (ST, 110°22’E, 19°94’N), Baishamen Park (BP, 110°34’E, 20°08’N) and Flower Marker (FM, 110°29’E, 20°02’N) in Haikou city, Hainan Province during April and May 2015 (Fig. 1). The collected larvae were brought back to the insectary and reared to adults at 26 ± 1 °C and 70 ± 5% (RH), under a 14:10 h (light: dark) photoperiod. The larvae of F2 generation were used for larval bioassays. The species of Ae. albopictus was identified by adult morphology [1]. The susceptible laboratory colony of Ae. albopictus was provided by Department of Tropical Infectious Diseases, Second Military Medical University, which was established from a population originally collected from Hangzhou, China. The colony has been maintained in insectary for 15 years without exposure to any insecticides.

Larval bioassay
The susceptibility of larvae to three pyrethroid insecticides, deltamethrin (>98%, Sigma, USA), permethrin (>98%, Sigma, USA) and beta-cypermethrin (>99%, Sigma, USA), was determined using a procedure recommended by WHO [44]. In the assay, 20–25 late 3rd and early 4th instar larvae were placed in a glass container that held 199 mL H2O and 1 mL of insecticide solution. Analytical grade insecticides were diluted five to seven concentrations with acetone. The solution with no insecticide was used as control. Larval mortality was recorded 24 h after treatment. The larvae that were motionless or convulsive upon a sharp stimulation were counted as dead [44]. Larval mortality was determined by dividing the number of dead larvae by the total number tested. Dead and survival larvae were collected and preserved in 95% alcohol for subsequent DNA analysis. No
food was provided to larvae during the procedure. If a test with pupation rate greater than 10 %, or mortality rate in control greater than 20 %, the test was invalid and was removed. All bioassays were repeated three times. In the larval bioassay, the median lethal concentration (LC50), the 90 % lethal concentration (LC90) and 95 % confidence interval of different pyrethroids were calculated based on the recorded data using Schoofs and Willhite’s probit analysis program [45]. The degree of resistance was determined by the resistance ratio (RR50), obtained by the LC50 value for a population compared with the LC50 value for the insecticide for susceptible laboratory colony. The RR50 ≤ 3 was considered as susceptible, and 3 < RR50 ≤ 10 as low degree of resistance, 10 < RR50 ≤ 20 as median degree of resistance, and RR50 > 20 as high degree of resistance [44].

Adult bioassay
Field-collected larvae were reared to adults in the insectary. Female unfed adults at day 2 or 3 post emergence were tested for the susceptibility to deltamethrin and DDT, using the standard WHO tube bioassay [46]. So far, there has been no sufficient data for a standard diagnostic concentration for resistance monitoring for *Ae. albopictus* in China. The test papers with deltamethrin (0.1 %) and DDT (4 %) were used for the assay, which were provided by National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention. For each insecticide, approximately 100 female mosquitoes were tested. Paraffin oil-treated papers without insecticide were used as control. The knockdown time of individual mosquitoes was recorded at 10 min, 30 min, 50 min and 60 min. Post 1 h exposure, mosquitoes were transferred to a recovery tube and maintained on 6 % of sucrose solution for 24 h. Dead and survival mosquitoes were collected and preserved in 95 % ethanol for subsequent DNA analysis, respectively. Mosquitoes were considered dead if they were motionless, when they were mechanically stimulated, following the method of Gonzalez Audino [47].

Detection kdr alleles and correlation with the larval bioassay
The individual mosquito larvae or adult was used for DNA extraction with the DNAzol Reagent (Invitrogen, USA). To identify kdr alleles, a partial sequence of S6 segment of domain III of the VGSC gene was amplified from 20 to 50 ng genomic DNA using primers aeSCF7 (5’-AGG TAT CCG AAC GTT GCT GT-3’) and aeSCR8 (5’-TAG CTT TCA GCG GCT TCT TC-3’) [13]. The PCR kit was from Aidlab, China. PCR reaction was carried out in Verity 96 well 157 Thermal Cycler (Applied Biosystems, USA). The cycling parameter included an initial step of denaturation at 94 °C for 2 min, followed by 35 cycles of amplification at 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, with a final extension step at 72 °C for 8 min. After electrophoresis, PCR products were purified and directly sequenced in both directions with the same primers. There were 4 specimens, of which the PCR products were cloned into plasmids (pGEMX-T Easy Vector, Aidlab, China), and then
sequenced, due to the double peaks at two positions of the codon 1534.

The codon 1534 was examined by sequence analysis, and genotypes were determined. In each sample, for a particular allele, the allele frequency was calculated as: number of alleles/(sample size × 2). The mutation frequency was defined as frequency of sum of wildtype/mutant heterozygotes and mutant/mutant homozygotes, which was calculated as: (sum of wildtype/mutant + mutant/mutant individuals)/sample size.

Chi-square tests were used to examine the association between kdr mutation and the resistance phenotype. In the present study, the dependent variables were the mosquito status (alive or dead) at 24 h post larval bioassay.

Results

Insecticide susceptibility bioassays

The larval susceptibility to three pyrethroids was tested for five populations of Ae. albopictus, which revealed a heterogeneous pattern (Table 1). Among five tested populations, XI (RR<sub>50</sub> = 2.38), LT (RR<sub>50</sub> = 1.17) and ST (RR<sub>50</sub> = 1.67) were susceptible to permethrin; BP was resistant with a median level (RR<sub>50</sub> = 8.83) and FM was resistant with a high level of resistance (RR<sub>50</sub> = 182.00). Besides, four of the five populations had developed resistance to deltamethrin and beta-cypermethrin, only XI was susceptible to deltamethrin and LT was susceptible to beta-cypermethrin. FM appeared to be the population having high level of resistance, with RR<sub>50</sub> = 436.36 to deltamethrin and RR<sub>50</sub> = 65.17 to beta-cypermethrin (Table 1).

The adult bioassay was conducted to determine the susceptibility to DDT and deltamethrin. In the present study, the dependent variables were the mosquito status (alive or dead) at 24 h post larval bioassay.

Detection of mutant kdr gene and correlation with the bioassay

The VGSC gene was genotyped for kdr alleles. A total of 493 specimens from larval bioassay samples were typed. At codon 1534, in addition to the wildtype codon TTC encoding phenylalanine (F), four other alleles were detected. Codon TTT codes for phenylalanine (F), codon TCC for serine (S), TGC for cysteine (C) and TTG for leucine (L). The allele frequency was TTC (F) (67.04 %), TTT (F) (0.41 %) TGC (C) (0.30 %), TCC (S) (31.54 %), and TTG (L) (0.71 %). The most frequent mutant allele was TCC (S) (Table 3). A total of eight genotypes were determined, due to the double peaks at two positions of the codon 1534.

Table 1 Susceptibility of Aedes albopictus larva to three pyrethroid insecticides in Haikou City, Hainan Island, China

| Insecticides | Sites | LC<sub>50</sub> (mg/L) | LC<sub>50</sub> (95%CI) | LC<sub>90</sub> (mg/L) | LC<sub>90</sub> (95% CI) | RR<sub>50</sub> |
|--------------|------|------------------------|------------------------|------------------------|------------------------|---------------|
| Deltamethrin | XI   | 0.0001                 | 0.0001–0.0002          | 0.0003                 | 0.0003–0.0004          | 1.27          |
|              | LT   | 0.0012                 | 0.0011–0.0014          | 0.0032                 | 0.0027–0.0040          | 9.09          |
|              | ST   | 0.0020                 | 0.0010–0.0020          | 0.0070                 | 0.0050–0.0100          | 18.18         |
|              | BP   | 0.0080                 | 0.0070–0.0090          | 0.0210                 | 0.0180–0.0270          | 72.73         |
|              | FM   | 0.0480                 | 0.0420–0.0550          | 0.1650                 | 0.1300–0.2320          | 436.36        |
|              | S    | 0.0001                 | 0.0001–0.0001          | 0.0003                 | 0.0003–0.0005          |               |
| Permethrin   | XI   | 0.0143                 | 0.0134–0.0159          | 0.0259                 | 0.0232–0.0300          | 2.38          |
|              | LT   | 0.0070                 | 0.0060–0.0070          | 0.0120                 | 0.0110–0.0130          | 1.17          |
|              | ST   | 0.0100                 | 0.0100–0.0110          | 0.0220                 | 0.0190–0.0270          | 1.67          |
|              | BP   | 0.0530                 | 0.0490–0.0580          | 0.1130                 | 0.0990–0.1320          | 8.83          |
|              | FM   | 1.0920                 | 0.9540–1.2530          | 4.6740                 | 3.5090–7.1620          | 182.00        |
| Beta-cypermethrin | XI   | 0.0047                 | 0.0043–0.0052          | 0.0132                 | 0.0114–0.0158          | 5.31          |
|              | LT   | 0.0020                 | 0.0020–0.0020          | 0.0040                 | 0.0030–0.0040          | 2.25          |
|              | ST   | 0.0040                 | 0.0030–0.0040          | 0.0100                 | 0.0080–0.0120          | 4.49          |
|              | BP   | 0.0130                 | 0.0120–0.0140          | 0.0310                 | 0.0260–0.0400          | 14.61         |
|              | FM   | 0.0580                 | 0.0530–0.0640          | 0.1740                 | 0.1500–0.2120          | 65.17         |
|              | S    | 0.0009                 | 0.0008–0.0010          | 0.0020                 | 0.0020–0.0031          |               |

The data of deltamethrin and permethrin was from the literature [52]

XI Xinbu Island, LT Longtang Town, ST Shishan Town, BP Baishamen Park, FM Flower Market, S: susceptible colony
### Table 2

kdrl alleles in relation to mosquito survival phenotype determined by the deltamethrin and DDT susceptibility adult bioassay in *Aedes albopictus* populations in Haikou City, Hainan Island, China

| Insecticide | Bioassay | Individuals (N) | Dead (N) after 24 h recovery period | Mortality rate (%) | Individuals (N) | Wildtype Mutant Frequency (%) |
|-------------|----------|-----------------|------------------------------------|-------------------|-----------------|-----------------------------|
| Deltamethrin | Alive | 104 | 98.40 | 102 | Alive | 0.00 |
| | Dead | 17 | 34 | 0 | 0 | 0.00 |
| DDT | Alive | 198 | 87.50 | 173 | Alive | 60.53 |
| | Dead | 17 | 32 | 2 | 0 | 5.89 |

### Table 3

kdrl alleles in relation to mosquito survival phenotype determined by three pyrethroids larval bioassay groups in Haikou City, Hainan Island, China

| Insecticides | Collecting sites | Bioassay status | Individuals (N) | Wildtype Mutant Frequency (%) |
|-------------|-----------------|-----------------|-----------------|-----------------------------|
| Deltamethrin XI | Alive | 17 | 27 | 0 | 0 | 20.59 |
| | Dead | 15 | 28 | 0 | 0 | 6.67 |
| | Alive | 21 | 36 | 2 | 0 | 9.52 |
| | Dead | 13 | 26 | 0 | 0 | 0.00 |
| | Alive | 20 | 40 | 0 | 0 | 0.00 |
| | Dead | 17 | 34 | 0 | 0 | 0.00 |
| | Alive | 17 | 16 | 0 | 2 | 52.94 |
| | Dead | 13 | 20 | 0 | 6 | 23.08 |
| | Alive | 19 | 1 | 36 | 1 | 97.37 |
| | Dead | 16 | 7 | 0 | 23 | 78.13 |
| Permethrin XI | Alive | 16 | 28 | 0 | 0 | 12.50 |
| | Dead | 16 | 29 | 0 | 3 | 9.38 |
| | Alive | 15 | 28 | 2 | 0 | 0.00 |
| | Dead | 11 | 22 | 0 | 0 | 0.00 |
| | Alive | 20 | 40 | 0 | 0 | 0.00 |
| | Dead | 18 | 36 | 0 | 0 | 0.00 |
| | Alive | 15 | 9 | 0 | 20 | 1 | 70.00 |
| | Dead | 12 | 14 | 0 | 9 | 1 | 41.67 |
| | Alive | 19 | 2 | 36 | 0 | 0 | 94.74 |
| | Dead | 17 | 9 | 0 | 25 | 0 | 73.53 |
| Beta-cypermethrin XI | Alive | 13 | 12 | 0 | 14 | 0 | 53.85 |
| | Dead | 18 | 32 | 0 | 4 | 0 | 11.11 |
| | Alive | 19 | 38 | 0 | 0 | 0 | 0.00 |
| | Dead | 14 | 28 | 0 | 0 | 0 | 0.00 |
| | Alive | 14 | 28 | 0 | 0 | 0 | 0.00 |
| | Dead | 15 | 30 | 0 | 0 | 0 | 0.00 |
| | Alive | 20 | 12 | 0 | 27 | 1 | 70.00 |
| | Dead | 19 | 25 | 0 | 13 | 0 | 34.21 |
| | Alive | 20 | 1 | 39 | 0 | 0 | 97.50 |
| | Dead | 14 | 3 | 0 | 25 | 0 | 89.29 |

XI xinbu Island, LT Longtang Town, ST Shishan Town, BP Baishamen Park, FM Flower Market
detected, including wildtype genotype TTC/TTC (57.40 %) and TTC/TTT (0.81 %), wildtype/mutant heterozygotes TTC/TCC (17.85 %), TTC/TTG (0.20 %), TTC/TGC (0.41 %), and mutant genotypes TTC/TCC (21.91 %), TCC/TTG (1.22 %), TCC/TGC (0.20 %). Overall, the frequency of mutant genotypes (S/S, S/L and S/C) was 23.33 %, and the frequency of wildtype/mutant heterozygotes (F/S, F/C and F/L) was 18.46 % (in Additional file 2: Table S1). The mutant frequency was high in both BP and FM while low or none in LT and ST populations of *Ae. albopictus* (Table 3).

The distributions of wildtype and mutant genotypes in larval populations were shown in Fig. 2. In *Aedes albopictus* resistant population, the frequencies of mutant genotypes were 41.04 % in deltamethrin group, 56.47 % in permethrin group and 60.15 % in beta-cypermethrin group. The frequencies of mutant alleles were 35.11 % in alive individuals and 22.30 % in dead individuals in deltamethrin group, 35.88 % in alive and 25.68 % in dead in permethrin group, 47.09 % in alive and 26.25 % in dead in beta-cypermethrin group. In each case, the mutant alleles were associated with resistant alive mosquitoes (*P* < 0.05). There were all significant differences between the wildtype and mutant alleles in every pyrethroid insecticides bioassay groups (*P* <0.05). The difference was more significant if the individuals from all of the pyrethroid bioassays were pooled together (*P* <0.01).

In the samples from adult bioassay, three alleles were detected, namely TTC (F) (73.64 %), TCC (S) (24.55 %) and TGC (C) (1.82 %), which formed four genotypes: wildtype homozygote TTC/TTC, and wildtype/mutant genotypes, TTC/TGC and TTC/TCC and mutant homozygote TCC/TCC (Table 2). The genotypes of the two resistant mosquitoes that survived the exposure to 0.1 % deltamethrin were both mutant homozygotes of TCC(S). The frequency of mutant alleles was 60.53 % in 19 resistant mosquitoes that survived in the 4 % DDT treatment (Table 2). Significant correlation was detected between *kdr* mutations and deltamethrin or DDT resistant phenotypes by Chi-test (*P* < 0.05).

**Discussion**

In Hainan Island, *Aedes* mosquitoes are responsible for the Dengue fever transmissions. The application of ultra low-volume (ULV) spray of pyrethroids has been a major measure to control *Aedes* adults since the 1990s. The susceptibility to pyrethroids has been monitored, and pyrethroid resistance has been reported in wild populations of *Ae. albopictus* in Hainan in 2005 and 2010, respectively [42, 43]. In this study, the larval bioassays showed that the populations in rural areas (XI, ST, LT) were largely susceptible to the pyrethoids tested; while BP and FM, two urban populations, were resistant to all of three pyrethroids. BP represented a population in a city park, where ULV spraying was applied on a regular basis. FM was collected from a garden/nursery market, where containers with aquatic plants, flower pots and planters with sufficient water constitute a large quantity of habitats for *Aedes* larvae. Owners used spray insecticides frequently to reduce mosquito density in the market. In those habitats, mosquitoes expose persistently to high dose of pyrethroids at both larval and adult stages. In rural
area, no regular spray was applied, unless dengue patients were present in a village. This may explain why BP and FM mosquitoes were resistant to pyrethroids while the other three rural populations were susceptible.

In the adult assay, adults showed resistance to DDT. When exposed 0.1 % deltamethrin test paper, 98. 40 % of adults were dead. Since the concentration was 4 fold higher than the diagnostic concentration 0.025 % for Ae. aegypti [48], we rather not to make any conclusion upon the data. It is an urgent need to develop standard diagnostic concentration for adults of Ae. albopictus in China.

A number of mutations in the VGSC gene have been reported in pyrethroid resistant strains of Ae. aegypti [18–25, 49], a few of these mutations (I1011M/V, V1016G/ I, F1534C) have been clearly associated with the resistance phenotype [12, 20–23, 25]. However, very little is known about the molecular or biochemical basis of resistance in Ae. albopictus. No kdr mutations were found in Ae. albopictus resistant populations from India, Malaysia and Sri Lanka [28, 29, 49, 50]. Recently, F1534C was found in 24 of 26 individuals of Ae. albopictus in Singapore [13]. In this study, five alleles were identified in the codon 1534, including two wildtype codons, and three mutant codons TCC(S), TGC(C) and TTT(L). The allele TCC(S) was clearly correlated to the resistance to permethrin and beta-cypermethrin, both belong to Type I pyrethroids, similar to the situation in Ae. aegypti [51].

This was the first report that kdr mutants, particularly F1534S, is behind pyrethroid resistance in Ae. albopictus. Apparently, long term applications of DDT and pyrethroids have posed selection pressure on VGSC gene in Ae. albopictus. It is required to examine more loci of VGSC gene in more populations in different geographic areas worldwide. In addition, understanding of the resistance mechanisms and development of simple and accurate diagnostic tools to monitor the presence of resistance gene mutations is critical for effective management of pyrethroid resistance and sustainable use of pyrethroid insecticides in the future.

Conclusions

Some Ae. albopictus populations in Haikou City, Hainan Island of China have developed resistance to deltamethrin, permethrin and beta-cypermethrin. The results suggested that Ae. albopictus control should adjust the usage of insecticides timely based on the resistant status investigation, and slow down the production and development of resistance. Two novel kdr mutant alleles F1534S and F1534L were detected in the pyrethroid resistant populations of Ae. albopictus in Haikou City, Hainan Island of China. For the first time, the mutant F1534S was associated with pyrethroid resistance in Ae. albopictus.

Additional files

Additional file 1: Multilingual abstract in the six official working languages of the United Nations. (PDF 370 kb)

Additional file 2: Table S1. kdr genotypes of Aedes albopictus populations from pyrethroid larval bioassay groups in Haikou City, Hainan Island, China. Table S2. Frequencies of kdr genotypes in relation to mosquito survival phenotype determined by the deltamethrin and DDT susceptibility adult bioassay in Aedes albopictus populations in Haikou City, Hainan Island, China (ZIP 28 kb)

Competing interests
The authors declared that they have no competing interests.

Authors’ contributions
All authors read and approved the final version of the manuscript. YM designed the study, HC and KL did adult bioassay, XW, XY, YL, FC, WZ, CL and ZL collected mosquitoes in the field and did larval bioassay. YM, HC, KL and XY did data analysis. CH and YM wrote the manuscript. The authors would like to thank Prof. Xu Jiannong to participate in the discussion and to assist in the writing the manuscript.

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