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# Resistance profiles and mechanisms in pyrethroid-resistant Aedes aegypti populations: first detection of the V1016G kdr mutation in Abidjan, Côte d'Ivoire

**Claver N. Adjobi**
Laboratoire de Biologie et Santé, UFR Biosciences, Université Félix Houphouët-Boigny
**Maurice A. Adja**
Laboratoire de Biologie et Santé, UFR Biosciences, Université Félix Houphouët-Boigny
**Aboubacar Sombié**
Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo
**Diakaridia Fofana**
Institut National d'Hygiène Publique
**Behi K. Fodjo**
Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
**Mahamadi Yaro**
Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo
**Nicolas Zanré**
Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo
**Benjamin G. Koudou**
Unité de Formation et de Recherche Sciences de la Nature, Université Nangui-Abrogoua
**Pie Müller**
Swiss Tropical and Public Health Institute
**David Weetman**
Department of Vector Biology, Liverpool School of Tropical Medicine
**Audrey E. Lenhart**
Entomology Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention
**Athanase Badolo**
Laboratoire d'Entomologie Fondamentale et Appliquée, Université Joseph Ki-Zerbo
**Julien Z. B. Zahouli**
julien.zahouli@csrs.ci

Centre Suisse de Recherches Scientifiques en Côte d'Ivoire

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## Abstract

# Background

Côte d'Ivoire is facing an acceleration of outbreaks of *Aedes* -borne arboviral diseases (e.g., dengue and yellow fever), with the highest incidence rate reported in the city of Abidjan where control responses are mostly based on insecticide space spraying (ISS). We investigated the insecticide resistance profiles and underlying mechanisms, including target site *knock-down resistance* (*kdr*) mutations and metabolic resistance, in wild *Aedes aegypti* populations across Abidjan.

# Methods

*Aedes aegypti* larvae were collected from their natural breeding habitats in four study sites (Anono, Ayakro, Entente and Gbagba) and reared to adults. Adult female *Ae. aegypti* aged 2–5 days were tested against multiple pyrethroids (deltamethrin, alphacypermethrin and permethrin) with and without pre-exposure to the synergist piperonyl butoxide (PBO), the carbamate bendiocarb, and the organophosphate pirimiphos-methyl, using standard World Health Organization (WHO) insecticide susceptibility bioassays. Additionally, mosquito samples were screened for five *kdr* mutations (V410L, S989P, V1016I, V1016G, and F1534C) using an allele-specific polymerase chain reaction (AS-PCR).

# Results

Overall, *Ae. aegypti* populations showed low to moderate mortality and evidence of multiple insecticide resistance mechanisms, including V1016G, a new *kdr* mutation. Mortality was low with all three pyrethroids (8%-33%), and moderate with bendiocarb (58%-85% mortality), and pirimiphos-methyl (60%-79% mortality) in all four study sites. Increased susceptibility to all three pyrethroids following PBO pre-exposure suggests the potential involvement of cytochrome P450 oxidase-mediated metabolic resistance. The 1534C mutation was nearing fixation at 93%, while the respective allelic frequencies for 1016I and 410L were approximately 55% and 45%, respectively. Overall, F1534C, V1016I and V410L *kdr* mutations were associated with phenotypic resistance, with the triple homozygous mutant CIL/CIL genotype being mostly found in alive individuals (89.2%) compared with dead individuals (10.8%). The V1016G *kdr* mutation, typically found in Asia, was detected in a single individual, representing the first report in *Ae. aegypti* in Côte d'Ivoire.

# Conclusions

*Aedes aegypti* populations from Abidjan are resistant to all five insecticides across the four study sites. Pyrethroid resistance was associated with multiple *kdr* mutations and is also likely due to P450 oxidase-mediated metabolic resistance. In addition, the present study reports the first detection of the V1016G *kdr* mutation in *Ae. aegypti* in Côte d'Ivoire. The presence of these multiple resistance mechanisms may be compromising the effectiveness of ISS interventions. Therefore, neither insecticide is an option and alternatives ought to be looked for. Our findings are important for developing effective and sustainable control of local *Aedes* vectors of arboviral diseases.

## Introduction

*Aedes aegypti* is a main vector of several arboviruses, including dengue (DENV), yellow fever (YFV), chikungunya (CHIKV) and Zika (ZIKV) [1]. Arboviral diseases are increasingly threatening public and global health worldwide [1], and their control is further complicated by widespread resistance to insecticides in *Aedes* vectors around the world [2]. As a result, more than half the world's population is at risk of arboviral infection, with both disease cases and deaths increasing rapidly. In Africa, arboviral disease outbreaks occur predominantly in densely populated cities, where transmission is fueled by fast and unplanned urbanization that promotes increased *Ae. aegypti* densities [3]. Specific treatments and licensed vaccines are

lacking for most arboviruses in Africa [3]. Therefore, *Aedes* vector control remains the key strategy for arboviral disease prevention and outbreak responses [4]. Current outbreak responses target primarily adult *Aedes* vectors through insecticide space spraying (ISS) with either organophosphates or pyrethroids [5]. However, the effectiveness of ISS is increasingly threatened by insecticide resistance in local *Aedes* vector populations to these insecticides [6]

Insecticide resistance in *Ae. aegypti* has been attributed to multiple mechanisms, including target site mutations and metabolic resistance. In *Aedes* vectors, the most widely reported mechanisms associated with insecticide resistance are mutations in the voltage-gated sodium channel (VGSC) at the target site for pyrethroids and dichlorodiphenyltrichloroethane (DDT), causing knockdown resistance (*kdr*), and metabolic resistance involving the overexpression of detoxification enzymes [7]. Among *kdr* mutations, the most common one is F1534C, which occurs worldwide [7] and is most frequently associated with V1016I and V410L mutations in Latin America, as well as S989P and V1016G mutations found in Asia [8]. Whereas V1016I and S989P alone do not confer resistance, they may amplify resistance in combination with other mutations [9]. The *kdr* mutations V1016I and V410L are also increasingly detected in *Ae. aegypti* in West and Central Africa [10, 11]. In contrast, the Asian variants, S989P and V1016G, have been detected only occasionally in Africa [12]. Worryingly, the simultaneous presence of the S989P, V1016G and F1534C mutations in the same individuals has been detected in *Ae. aegypti* populations from Benin [13]. The mutations show a synergistic effect, and resistance ratios of around 1,000 against a susceptible colony have been reported in pyrethroid-resistant *Ae. aegypti* mosquitoes [14].

Metabolic resistance involves major detoxification enzyme families, including cytochrome P450 monooxygenases (P450), carboxyl-cholinesterases (CCEs), and glutathione S-transferases (GSTs) [7], as well as other genes, such as ABC transporters, UDP-glycosyltransferases (UDPGTs), and sulfotransferases [15]. Despite limited knowledge of genes conferring metabolic resistance in African *Ae. aegypti* populations, studies in Burkina Faso [16], Cameroon [17], and Senegal [18] have reported overexpression of specific detoxification genes, including P450s.

In Côte d'Ivoire, arboviral disease outbreaks have predominantly occurred in Abidjan, the country's economic capital with a population of 7 million, where *Ae. aegypti* is highly abundant and displays differing patterns of resistance to insecticides [19]. Since 2017, the frequency and scale of dengue outbreaks in Abidjan has risen, often coupled with outbreaks of yellow fever [20, 21]. Alone in 2024, 4,450 confirmed dengue cases and two fatalities have been reported [21]. In response to the outbreaks, the National Institute of Public Hygiene deployed ISS based on either deltamethrin a combination formulation with transfluthrin and flupyradifurone [20, 22]. However, the *Ae. aegypti* populations recovered quickly following the campaigns, leading to new outbreaks demonstrating a time-limited impact of the ISS applications as reported by local population.

To what extent this resurgence is driven by insecticide resistance remains unknown, and very little information is available regarding the insecticide resistance status of *Ae. aegypti* populations in Abidjan. Only one study specifically addressed insecticide resistance patterns and underlying mechanisms in the local *Ae. aegypti* populations [19]. The study documented the presence of three *kdr* mutations, V410L, V1016I, and F1534C

This study aimed at assessing insecticide resistance and its underlying mechanisms in the *Ae. aegypti* populations across four additional sites within emerging arboviral disease foci across Abidjan. The results are important for understanding and improving the management of insecticide resistance and control of *Aedes* vectors to prevent dengue, yellow fever and other *Aedes*-borne viruses in Abidjan, Côte d'Ivoire.

## Methods

# Study areas

*Aedes* larvae were collected in in the city of Abidjan across four sites (Fig. 1). They included the neighborhoods of Anono (5° 20' 32" N; 3° 58' 25" W), Ayakro (5° 22' 05" N; 4° 06' 04" W), Entente (05° 18' 05" N; 04° 00' 09" W), and Gbagba (5° 21' 1" N;

3°53’ 19”W), which are located in the municipalities of Cocody, Yopougon, Treichville, and Bingerville, respectively. These municipalities are heavily infested with *Ae. aegypti* [23] and have regularly experienced dengue and yellow fewer epidemics. In response to those outbreaks, the authorities have deployed ISS with either deltamethrin (Aqua-K-Othrine, Bayer West-Central Africa SA, Environmental Science, Abidjan, Côte d’Ivoire) or a combination transfluthrin and flupyradifurone (Fludora Co-Max, Bayer) [24].

## << Fig. 1 near here >>

**Figure 1. Study sites in the city of Abidjan, southeastern Côte d’Ivoire**. The study was conducted across four sites, including Anono, Gbagba, Entente, and Ayakro (inlets) in the municipalities of Cocody, Bingerville, Treichville and Yopougon, respectively.

## Mosquito collections and rearing

*Aedes* larvae were collected from various types of breeding sites (e.g., tires, water storage containers, discarded containers, cans, and flowerpots) during the long rainy seasons (May to June) in 2021 and 2022. *Aedes* immatures were transported to the insectary of the Centre Suisse de Recherches Scientifiques (CSRS) in Abidjan and reared to adults. Emerging adult identified as *Ae. aegypti* were kept in cages and provided with a 10% sugar solution ad libitum and maintained at a temperature of 27 ± 2°C, a relative humidity of 70 ± 5%, and a photoperiod of 12:12 h dark:light until they were 2 to 5 days old. In addition to the colony from an insecticide susceptible *Ae. aegypti* Rockefeller colony was obtained directly from hatched eggs sourced from the Swiss Tropical and Public Health Institute (Swiss TPH) in Allschwil, Switzerland, in 2017.

## Insecticide bioassays

Five insecticides belonging to three classes were tested on custom-made impregnated filter papers, including the pyrethroids alpha-cypermethrin (0.05%), deltamethrin (0.05%), and permethrin (0.75%), the carbamate bendiocarb (0.1%), and the organophosphate pirimiphos-methyl (0.25%). Following the WHO protocol [25], the diagnostic dose for bendiocarb was suitable for *Ae. aegypti*. The diagnostic doses for deltamethrin, alpha-cypermethrin and pirimiphos-methyl were in line with those recommended by WHO for *Ae. aegypti* (deltamethrin: 0.03%, alphacypermethrin: 0.05%, and pirimiphos-methyl: 40 mg/$m^2$). However, the diagnostic dose of permethrin used exceeds the recommended diagnostic dose (0.40%) for *Ae. aegypti*, providing a less robust assessment of resistance.

Insecticide susceptibility bioassays were conducted following WHO guidelines [25]. For each insecticide, six batches of 20–25 non-blood-fed 2- to 5-day-old female mosquitoes were tested. Four batches were (n = 100) were exposed to insecticide-impregnated papers, and two batches (n = 50) were exposed to controls tubes with untreated filter papers. Bioassays were performed at 25–26°C, and 70–80% relative humidity. Post-exposure, surviving mosquitoes were maintained over night with a 10% sugar solution ad libitum, and delayed mortality was assessed at 24 h. The mosquitoes tested in the first set of assays were collected in 2021. As a control, bioassays were repeated with the insecticide-susceptible *Ae. aegypti* Rockefeller colony.

Additional synergist bioassays were conducted to assess the presence of metabolic insecticide resistance mechanisms involving P450s [26]. These assays were carried out with four batches of 25 mosquitoes exposed to each of four groups. In group 1, mosquitoes were pre-exposed for 1 h to filter papers impregnated with 4% piperonyl butoxide (PBO) before being exposed to one of the three pyrethroids. In group 2, mosquitoes were directly exposed to the pyrethroids. In group 3, mosquitoes were only exposed to PBO, and in group 4 they were exposed to control papers [26]. The mosquitoes tested in this second set of assays had been collected in 2022. Bioassays with PBO combined with pyrethroids were conducted only on fields strains from each study site

## Molecular analysis

Molecular analyses were done in the Laboratoire d'Entomologie Fondamentale et Appliquée of Université Joseph Ki-Zerbo in Ouagadougou, Burkina Faso. *Aedes aegypti* samples were screened for the *kdr* loci F1534C, V1016I, V410L, S989P, and V1016G. DNA was individually extracted from field-collected adult *Ae. aegypti* from two batches exposed per pyrethroid (n = 492), following the protocol of Livak [27]. The resulting DNA pellets were re-suspended in 30 µl Tris-EDTA buffer and stored at -20°C.

One microliter of template DNA per individual was used in 12.5 µl reaction volumes for simultaneous detection of the F1534C and V1016I *kdr* mutations, using the multiplex method developed by Sombié et al. [28]. Resulting amplicons were visualized on 3% agarose gels stained with ethidium bromide. For the F1534C mutation, amplicons were 232 bp (wild-type) and 180 bp (mutant); for V1016I, they were 98 bp (wild-type) and 78 bp (mutant).

Detection of the V419L *kdr* mutation was performed following the PCR protocol of Granada et al. [29], using 12.5 µl reaction volumes with 1 µl of template DNA. The assay comprises two allele-specific PCRs (AS-PCR), each employing a primer pair targeting either the wild-type or mutant allele, both producing 350 bp amplicons. Resulting amplicons were visualized on 1.5% agarose gels stained with ethidium bromide.

Because frequencies of S989P and V1016G *kdr* mutants were expected to be low or zero, DNA from 10 mosquitoes was pooled, yielding 42 pools. Detection of S989P and V1016G was performed using the AS-PCR assay of Li et al. [30], with 1 µl pooled DNA in 20 µl reactions. PCR products were separated by 1.5% agarose gels stained with ethidium bromide. Amplicons were 240 bp for S989 and 348 bp for V1016 (wild-type and the mutant), with non-specific bands at 594 bp (S989) and 592 bp (V1016). When the pool was positive, AS-PCRs were repeated on individual DNAs to confirm *kdr* mutations.

# Data analysis

All statistical analyses were performed using R version 4.4.2 [32]. Significance level was set at 0.05.

Phenotypic insecticide resistance was classified according to WHO guidelines [26], with populations defined as resistant if mortality was < 90%, potentially resistant if mortality ranged from 90% to 97%, and susceptible if mortality ≥ 98%.

For the synergist assays with PBO, the hypothesis was that PBO increases mortality. To test the hypothesis, a generalized linear model (GLM) with a binomial family and logit link function was fitted to compare the mortality induced by the insecticides tested alone vs. with PBO pre-exposure.

Another hypothesis was that mortality rates were associated with *kdr* genotypes. To test this hypothesis, $\chi^2$-square tests were utilized to compare *kdr* genotypes between dead and alive *Ae. aegypti* exposed to insecticides. The relationship between genotypes or alleles and phenotype (dead/alive) of mosquitoes was determine using prop.test.

To investigate for potential signatures of selection for resistance alleles, deviation of *kdr* alleles from Hardy-Weinberg equilibrium and the linkage disequilibrium between the two *kdr* mutations V410L and V1016I was determined using R *Genetics* package version 1.3.8.1.3. To control for multiple testing, p-values were adjusted using the Bonferroni correction.

## Results

# Susceptibility to insecticides

Whereas the Rockefeller strain showed 100% mortality, the field-collected *Ae. aegypti* exhibited resistance (mortality < 90%) to all five insecticides (Fig. 2A).

Pre-exposure of wild *Ae. aegypti* populations to PBO resulted in partial restoration of susceptibility to all three pyrethroids across study areas (Table 1 and Fig. 2B). Mortality rates without PBO pre-exposure ranged from 12.5% to 28.2% for permethrin, 11.0% to 25.3% for alphacypermethrin, and 34.5% to 50.0% for deltamethrin. This pyrethroids-induced mortality remained consistent between 2021 and 2022 (all $P > 0.05$) (Additional file 3: Table S3). With PBO pre-exposure, mortality rates ranged from 38.1% to 66.3% for permethrin, 42.1% to 76.2% for alphacypermethrin and 66.1% to 77.8% for deltamethrin. A significant difference was observed between mortality rates with the insecticides alone and pre-exposure to PBO ($P < 0.001$). Similarly, the GLM showed a significant difference in mortality induced by the combination of PBO-deltamethrin in Anono compared to that observed with PBO-permethrin in Ayakro (OR = 0.19, 95% CI = 0.07–0.46, $p < 0.001$) and in Entente for the PBO-alphacypermethrin (OR = 3.61, 95% CI = 1.45–9.00, $P = 0.005$) (Table 1 and Fig. 2B).

Table 1
Generalized linear model showing the Ae. aegypti mortality against pyrethroids insecticides with WHO tube bioassays in Abidjan, southeastern Côte d'Ivoire

| Predictor | Odds Ratio | 95% CI | *P*-value |
|---|---|---|---|
| **Intercept** | **0.61** | **0.39–0.94** | **0.025** |
| **Insecticide [Deltamethrin]** | | | |
| **Alphacypermethrin** | **0.20** | **0.08–0.45** | **< 0.001** |
| PBO + Alphacypermethrin | 1.67 | 0.90–3.10 | 0.098 |
| **PBO + Deltamethrin** | **4.53** | **2.36–8.70** | **< 0.001** |
| **PBO + Permethrin** | **3.21** | **1.71–6.02** | **< 0.001** |
| **Permethrin** | **0.50** | **0.26–0.99** | **0.049** |
| **Locality [Anono] X Insecticide [Deltamethrin]** | | | |
| Ayakro x Alphacypermethrin | 1.23 | 0.42–3.64 | 0.697 |
| **Entente x Alphacypermethrin** | **2.99** | **1.04–8.58** | **0.042** |
| **Gbagba x Alphacypermethrin** | **2.96** | **1.04–8.41** | **0.042** |
| Ayakro x PBO + Alphacypermethrin | 0.44 | 0.18–1.06 | 0.068 |
| **Entente x PBO + Alphacypermethrin** | **3.61** | **1.45–9.00** | **0.005** |
| Gbagba x PBO + Alphacypermethrin | 1.14 | 0.48–2.70 | 0.758 |
| Ayakro x PBO + Deltamethrin | 0.44 | 0.17–1.09 | 0.079 |
| Entente x PBO + Deltamethrin | 1.46 | 0.56–3.78 | 0.432 |
| Gbagba x PBO + Deltamethrin | 0.84 | 0.34–2.10 | 0.723 |
| **Ayakro x PBO + Permethrin** | **0.19** | **0.07–0.46** | **< 0.001** |
| Entente x PBO + Permethrin | 1.03 | 0.42–2.50 | 0.944 |
| Gbagba x PBO + Permethrin | 0.56 | 0.23–1.34 | 0.195 |
| **Ayakro x Permethrin** | **0.26** | **0.09–0.76** | **0.013** |
| Entente x Permethrin | 1.46 | 0.57–3.73 | 0.425 |
| Gbagba x Permethrin | 1.32 | 0.51–3.40 | 0.554 |

Reference factor levels of predictors are shown in brackets with the Odds Ratio, 95% confidence intervals (CI), and associated probabilities for predictors included in the model. Significant predictor terms are shown in bold.

# << Fig. 2 >>

**Figure 2. Mortality of Aedes aegypti females tested against insecticides using WHO insecticide susceptibility bioassays.** A: Mortality rate to insecticides. B: Mortality rate to insecticides without or with piperonyl butoxide (PBO) pre-exposure. The insecticide susceptibility bioassays were performed according to current WHO procedures [26]. The red dashed line indicates the resistance threshold, while the black dashed line depicts the susceptibility threshold. Error bars represent 95% confidence interval of the mean.

# << Table 1 near here >>

### V410L, V1016I and F1534C kdr mutations

The genotypic frequencies of F1534C, V1016I, and V410L *kdr* mutations are shown in Table 2. Allele frequency of the 1534C was notably high across all study sites, ranging from 0.89 to 0.96. For the V1016I *kdr* mutation, the allele frequency varied between 0.48 and 0.57 across study sites, with no statistically significant differences ($\chi^2$ = 1.78, df = 3, $P$ = 0.62). Regarding the V410L *kdr* mutation, the highest allele frequency was observed in Ayakro (0.59) ($\chi^2$ = 14.21, df = 3, $P$ = 0.002).

Table 2
Genotype and allelic frequency of the F1534C, V1016I and V410L mutations in Aedes aegypti populations from Abidjan, southeastern Côte d'Ivoire

| Study area | Genotype (F1534C) | | | | | Genotype (V1016I) | | | | | Genotype (V410L) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F/F | F/C | C/C | Total | Freq (C) | V/V | V/I | I/I | Total | Freq (I) | V/V | V/L | L/L | Total | Freq (L) |
| Anono | 0 | 15 | 103 | 118 | 0.94 | 30 | 48 | 40 | 118 | 0.54 | 42 | 63 | 20 | 125 | 0,41 |
| Ayakro | 0 | 12 | 106 | 118 | 0.95 | 29 | 64 | 25 | 118 | 0.48 | 20 | 57 | 40 | 117 | 0,59 |
| Entente | 0 | 10 | 109 | 119 | 0.96 | 22 | 71 | 26 | 119 | 0.52 | 51 | 51 | 18 | 120 | 0,36 |
| Gbagba | 2 | 24 | 97 | 123 | 0.89 | 15 | 77 | 31 | 123 | 0.57 | 45 | 59 | 20 | 124 | 0,40 |
| **Total** | 2 | 61 | 415 | 478 | 0.93 | 96 | 260 | 122 | 478 | 0.53 | 158 | 230 | 98 | 486 | 0,44 |

C, I and L are the mutated alleles of the F1534C, V1016I and V410L *kdr* mutation whilst F, V and V are the wilds alleles of the same mutations respectively, Freq represented allelic frequency

For the F1534C and V410L *kdr* mutations, the *Ae. aegypti* populations from all four study sites were in Hardy-Weinberg equilibrium (all $P$ > 0.05). Concerning the V1016I *kdr* mutation, the Hardy-Weinberg equilibrium test was not significant after multiple testing detection using the Bonferroni test ($P$ > 0.05) (Additional file 4: Table S4).

# << Table 2 near here >>

Overall, the V1016I and V410L mutations showed significant linkage disequilibrium (D' = 0.182, $r^2$ = -0.152, $P$ < 0.0001). The same observation was made in Gbagba (D' = 0.245, $r^2$ = -0,245, $P$ = 0.033) and Anono. (D' = 0.552, $r^2$ = -0.43, $P$ < 0.001). However, in Ayakro (D' = 0.092, $r^2$ = -0.075, $P$ = 0.25) and Entente (D' = 0.024, $r^2$ = 0.019, $P$ = 0.773), linkage disequilibrium was not observed between these two loci. When considering the three *kdr* mutations F1534C/V1016I/V410L, out of 27 possible genotypes, 18 were found, the most prevalent was CC/VI/VL at 23.7%. The triple-homozygote mutant CC/II/LL accounted for 7.8%, while the triple-homozygote wild-type represented 0.4%. The CC/VI/VL genotype was the most encountered in all the sites with 28% in Anono, 19.2% in Gbagba, 22.4% in Ayakro and 25.2% in Entente (Table 3). Comparison between the study sites showed no significant differences in the frequency of this genotype ($\chi^2$ = 2.81, df = 3, $P$ = 0.42). The *kdr* genotype with three mutated loci (CC/II/LL) was found in all localities. It was in highest frequency in Anono (14.4%, n = 17), followed by Ayakro (7.8%, n = 9), Gbagba (5.0%, n = 6) and Entente (4.2%, n = 5) ($\chi^2$ = 10.58, df = 3, $P$ = 0.01). However, the *kdr* genotype with the three wild loci (FF/VV/VV) was only observed in Gbagba (1.7%, n = 2) (Table 3). The

double mutant homozygotes CC/II (CC/II/VL and CC/II/VV) were the most common in all the study sites. Likewise, the double mutant genotypes CC/II and CC/LL were mostly observed in alive individuals of *Ae. aegypti* (> 70%). The double mutant genotype II/LL was recorded in only one alive individual in Anono (Additional file 5: Table 5). Only eight haplotypes were identified across all four study sites, with CIL (0.314) being the most prevalent, followed by CVV (0.282). The FVV haplotype (0.059) was found at a very low frequency. Except for Entente, where CVV was the most frequent, CIL was the most common haplotype in all the other sites (Anono, Ayakro, and Gbagba). The frequency of CIL was 0.347, 0.304, 0.353, and 0.252 in Anono, Ayakro, Entente, and Gbagba, respectively (Additional file 6: Table S6).

Table 3
Frequencies of the tri-loci genotypes crossing the F1534C, V1016I and V410L mutations in Aedes aegypti from Abidjan, southeastern Côte d'Ivoire

| Genotype | Study site | | | | Total |
|---|---|---|---|---|---|
| | Anono | Ayakro | Entente | Gbagba | |
| | N (%) | N (%) | N (%) | N (%) | N (%) |
| CC/II/LL | 17 (14,4) | 9 (7,8) | 5 (4,2) | 6 (5,0) | 37 (7,8) |
| CC/II/VL | 11 (9,3) | 12 (10,3) | 7 (5,9) | 18 (15,0) | 48 (10,1) |
| CC/II/VV | 10 (8,5) | 3 (2,6) | 14 (11,8) | 6 (5,0) | 33 (7,0) |
| CC/VI/LL | 2 (1,7) | 22 (19,0) | 11 (9,2) | 9 (7,5) | 44 (9,3) |
| CC/VI/VL | 33 (28,0) | 26 (22,4) | 30 (25,2) | 23 (19,2) | 112 (23,7) |
| CC/VI/VV | 11 (9,3) | 11 (9,5) | 24 (20,2) | 22 (18,3) | 68 (14,4) |
| CC/VV/LL | 0 (0) | 5 (4,3) | 2 (1,7) | 2 (1,7) | 9 (1,9) |
| CC/VV/VL | 9 (7,6) | 11 (9,5) | 7 (5,9) | 6 (5,0) | 33 (7,0) |
| CC/VV/VV | 10 (8,5) | 5 (4,3) | 9 (7,6) | 4 (3,3) | 28 (5,9) |
| FC/II/LL | 1 (0,8) | 0 (0) | 0 (0) | 0 (0) | 1 (0,2) |
| FC/II/VV | 1 (0,8) | 0 (0) | 0 (0) | 0 (0) | 1 (0,2) |
| FC/VI/LL | 0 (0) | 2 (1,7) | 0 (0) | 1 (0,8) | 3 (0,6) |
| FC/VI/VL | 1 (0,8) | 2 (1,7) | 2 (1,7) | 10 (8,3) | 15 (3,2) |
| FC/VI/VV | 1 (0,8) | 1 (0,8) | 4 (3,4) | 9 (7,5) | 15 (3,2) |
| FC/VV/LL | 0 (0) | 2 (1,7) | 0 (0) | 0 (0) | 2 (0,4) |
| FC/VV/VL | 5 (4,2) | 5 (4,3) | 4 (3,4) | 1 (0,8) | 15 (3,2) |
| FC/VV/VV | 6 (5,1) | 0 (0) | 0 (0) | 1 (0,8) | 7 (1,5) |
| FF/VV/VV | 0 (0) | 0 (0) | 0 (0) | 2 (1,7) | 2 (0,4) |
| **Total** | **118** | **116** | **119** | **120** | **473** |

C, I, and L represent the mutated alleles of the F1534C, V1016I, and V410L kdr mutations, while F, V, and V represent the wild alleles of the same mutations, respectively. N represents the number of mosquitoes, and % represents the percentage.

Table 5
Generalized linear model showing the haplotypes of kdr mutations of Aedes aegypti to pyrethroids in Abidjan, southeastern Côte d'Ivoire

| Predictor | Odds ratio | 95% CI | *P*-value |
|---|---|---|---|
| **Intercept** | **1.19** | **0.466–2.12** | **0.563** |
| Insecticide [Deltamethrin] | | | |
| **Alphacypermetrin** | **0.35** | **0.24–0.50** | **< 0.001** |
| **Permethrin** | **0.36** | **0.25–0.53** | **< 0.001** |
| Haplotypes [FVV] | | | |
| **CIL** | **0.40** | **0.22–0.75** | **0.004** |
| CIV | 0.56 | 0.29–1.60 | 0.073 |
| **CVL** | **0.28** | **0.13–0.610** | **0.001** |

Reference factor levels of predictors are shown in brackets with the odds ratio, 95% confidence intervals (CI), and associated probabilities for predictors included in the model. Significant predictor terms are shown in bold. Given the very low presence of haplotypes FIL, FIV and FVL, they were removed from the predictive model.

# << Table 3 near here >>

### Association of F1534C, V1016I and V410L kdr mutations with phenotypic resistance

Overall, the triple homozygous mutant CIL/CIL genotype was commonly found in both alive (89.2%) and dead (10.8%) *Ae. aegypti* individuals (Table 4). This genotype accounted for 88.2% (n = 15) and 11.7% (n = 2) in dead and alive *Ae. aegypti* individuals respectively. Whilst in Entente and Gbagba, it was found only in alive individuals. With the 1534C allele almost fixed, GLM analysis of genotypes based on the 1016I and 410L mutations showed that the VV/LL (p = 0.98) genotype was weakly associated with pyrethroid resistance, whilst the genotypes II/LL, II/VL, II/VV, VI/LL, VI/VL, VI/VV, and VV/VL were found associated, with VV/VV considered as the reference genotype (Additional file 7: Table S7). The GLM model revealed that the FVV, CIV, and CVV haplotypes were not associated with pyrethroid resistance (all $P > 0.05$). However, the other two haplotypes (CIL and CVL) demonstrated an association with resistance to alpha-cypermethrin and permethrin (all $P < 0.01$) (Table 5).

Table 4
Triple-loci kdr genotypes (CIL/CIL) in dead and alive Aedes aegypti mosquitoes in association with pyrethroid resistance

| Insecticide | Phenotype | Anono | Ayakro | Entente | Gbagba | Total |
|---|---|---|---|---|---|---|
| | | **n (%)** | **n (%)** | **n (%)** | **n (%)** | n (%) |
| Deltamethrin | Alive | 3 (0,6) | 2 (50) | 3 (100) | 0 (0) | 8 (66,7) |
| | Dead | 2 (0,4) | 2 (50) | 0 (0) | 0 (0) | 4 (33,3) |
| Alphacypermethrin | Alive | 10 (100) | 3 (100) | 1 (100) | 5 (100) | 19 (100) |
| | Dead | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Permethrin | Alive | 2 (100) | 2 (100) | 1 (100) | 1 (100) | 6 (100) |
| | Dead | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Total** | **Alive** | **15 (88,2)** | **7 (77,8)** | **5 (100)** | **6 (100)** | **33 (89,2)** |
| | **Dead** | **2(11,7)** | **2 (22,2)** | **0 (0)** | **0 (0)** | **4 (10,8)** |
| | **Total** | **17 (100)** | **9 (100)** | **5 (100)** | **6 (100)** | **37 (100)** |

n : number of mosquito, % : percentage

# << Table 4 and Table 5 near here >>

**Detection of S989P and V1016G kdr mutations**

No S989P *kdr* allele was detected. However, one pool from Anono was positive for the 1016G allele. Hence, the individual DNA samples from that pool were individually tested, and one individuum was hererozygous for the 1016G *kdr* allele (Fig. 3). This individuum carried the V/V genotype for the *kdr* V410L gene. After three successive PCR attempts to detect the *kdr* F1534C and V1016I mutations, genotyping remained unsuccessful.

# << Fig. 3 near here >>

**Figure 3 Gel electrophoresis bands of AS-PCR products corresponding to the pooled (a) and individual (b, c) Aedes aegypti DNA samples for the V1016G kdr mutation**. M: marker, V: V1016G Wild-type allele (V1016), G: V1016G mutant allele (1016G). P8-P14: Pool of DNA. 101–110: Individual sample. NC: Negative Control. The red box indicates the positive pool and sample for V1016G *kdr* mutation.

## Discussion

The present study investigated the insecticide resistance profiles and underlying mechanisms in local *Ae. aegypti* populations from the large city of Abidjan, Côte d'Ivoire. Adult females were tested against the pyrethroids deltamethrin, alphacypermethrin and permethrin with and without pre-exposure to PBO, the carbamate bendiocarb, and the phosphorothiate pirimiphos-methyl and screened for insecticide resistance genes. The results showed that the wild *Ae. aegypti* Abidjan populations testes displayed to pyrethroids that was associated with multiple *kdr* mutations and also P450-based metabolic resistance.

*Aedes aegypti* populations across the four study sites in Abidjan showed resistance to all five insecticides tested (alpha-cypermethrin, deltamethrin, permethrin, bendiocarb, and pirimiphos-methyl).

Similar results have been obtained in studies conducted in different countries, where *Ae. aegypti* has developed resistance to pyrethroids [33–36]. In Côte d'Ivoire, Konan et al. [19] attributed insecticide resistance to uncontrolled use of pesticides, leading to increased selection pressure towards pyrethroids. Additionally, pyrethroid insecticides are predominantly

promoted and utilized during mosquito control campaigns in Côte d'Ivoire [35]. There is no consensus on the origin of the selection pressure responsible for resistance development in *Ae. aegypti* populations [37]. However, some authors suggest that domestic use of pyrethroids through spraying by private companies and household use of insecticides could contribute to insecticide resistance development in *Ae. aegypti* [13].

According to Konan et al. [19], various pyrethroids are present in the formulation of household aerosols available in Côte d'Ivoire. However, bendiocarb was only used in 2017 in preparation for the Eighth Francophonie Games in Abidjan. While PBO was not tested with bendiocarb, the bendiocarb resistance that was detected in *Ae. aegypti* in the present study may also involve P450-mediated mechanisms, similar to those reported in *Anopheles gambiae* [38]. Similarly, *Ae. aegypti* resistance to bendiocarb has been documented in Burkina Faso and Cameroon [39, 40]. Our findings also revealed that the *Ae. aegypti* populations in all four study areas exhibited resistance to 0.25% pirimiphos-methyl. This resistance is more difficult to explain since pirimiphos-methyl is not typically included in the insecticides used for ISS interventions in response to arboviral disease outbreaks in Abidjan [19]. However, this resistance may be linked to cross-resistance to other insecticides such as carbamates. This resistance could also be attributable to *Ace-1*-mediated mechanisms, which were not investigated in this study. According to Moyes et al. [7], although *Ace-1* mutations have not been reported in *Ae. aegypti*, resistance to carbamate and organophosphate insecticides has been observed worldwide including Africa. Furthermore, Spada et al. [41], showed that *Ace-1* gene which is know to confer resistance to organophosphates and carbamates remains insufficiently documented globally.

The resistance observed with pirimiphos-methyl in *Ae. aegypti* populations in Abidjan aligns with similar findings from Ouagadougou [42] as has been the case in Dakar [43].and Konongo in Ghana [44]. However, populations of *Ae. aegypti* from the seaport of Cotonou in Benin showed complete sensitivity to pirimiphos-methyl [45].

In our study, the concentrations used to assess insecticide sensitivity were mainly those recommended for WHO tube tests for *Anopheles*, which are higher than those recommended for *Aedes* mosquitoes, thus providing a likely underestimate of *Ae. aegypti* insecticide resistance.

Understanding the mechanisms involved in insecticide resistance in vectors is crucial for optimizing the effectiveness of a control program. PCR analysis revealed the presence of F1534C, V1016I, and V410L *kdr* mutations in the *Ae. aegypti* Abidjan populations. The F1534C mutation was detected with very high frequencies in all the study sites (> 90%), suggesting a trend towards the disappearance of the wild type allele (F) and a strong expansion or even fixation of the mutated allele (C) in the mosquito populations, as reported in Konan et al. [19]. Consistent with our findings, the F1534C mutation has been frequently reported in various African countries: Burkina Faso [46], Ghana [44], Nigeria [47] Cameroon [48], and Cape Verde [10]. Indeed, the fixation of the F1534C mutation suggests that it was the initial mutation established in *Ae. aegypti* [49, 50]. Furthermore, the elevated frequency of the 1534C mutated allele may pave the way for the broader emergence of additional mutations like V1016I and V410L, as suggested in Vera-Maloof et al. [50].The high prevalence of the F1534C mutation combined with V1016I mutations detected in the present study sites could contribute to heightened resistance [39, 49].

The frequency of the 1016I allele was moderate, ranging between 0.48 and 0.57. These findings are consistent with observations reported in Burkina Faso by Toe et al. [42], which detected 1016I allele frequencies ranging from 0.29 to 0.32 in Ouagadougou. However, our results showed higher frequencies than those reported by Konan et al. [19] in 2021, which ranged between 0.25 and 0.38, indicating an increasing trend of this mutation in Abidjan. Another study conducted in Burkina Faso reported an increase of this mutations between 0.20 and 0.47 in 2016, and reaching 0.64 in 2017 [39]. According to Sombié et al. [39], the surge in *kdr* mutations might be attributed to the increased use of insecticide products, such as ISS against *Aedes*, and IRS against *Anopheles* malaria vectors.

The V410L mutation was detected in all four study sites at varying frequencies. The mutant allele was found with a high frequency (0.59) in Ayakro. This area contains chemical processing, food production, and other manufacturing industries. The untreated waste from these operations pollutes the environment (soil, air, and water) and could infiltrate mosquito

breeding sites, potentially contributing to the selection pressure for this resistance mechanism. Further investigations in our current study sites are needed to evaluate the impact of industrial environmental contamination with pesticides on resistance. Sombié et al. [28] identified the 410L mutated allele with a frequency of 0.60 in Ouagadougou. When our allele frequency [0.59 (95%, CI, 0.49–0.67)] was compared to that reported by Konan et al. [19] [0.33 (95%, CI, 0.17–0.53)], this mutation seems to be increasing over time and could enhance resistance in conjunction with the F1534C and V1016I mutations [42]. The coexistence of the three *kdr* mutations F1534C, V1016I, and V410L in *Ae. aegypti* populations in Abidjan corroborates findings reported in Konan et al. [19].

The present study reported the first detection of the V1016G *kdr* mutation in *Ae. aegypti* from Côte d'Ivoire, which was unexpected given that this mutation is almost exclusively reported in Asia. Since this *kdr* mutation was found in only one *Ae. aegypti* individual and at very low frequency (0.1%), no statistical analysis was conducted to correlate it with phenotypic resistance. This mutation was identified in an alive individual exposed to permethrin with a heterozygous genotype (V/G) from Anono. Nevertheless, surveillance of this mutation is important due to its physiological effects and its connection with the S989P mutation, which provides significant resistance to pyrethroids [51, 52]. Numerous studies have established that the co-occurring of *kdr* mutations S989P, V1016G, and F1534C confers high level of resistance to pyrethroids [13, 14]. Further investigations are needed to assess the geographical extent of the distribution of this mutation among *Ae. aegypti* populations in Côte d'Ivoire and to discern its contributions to local insecticide resistance.

The combination of the three *kdr* loci enabled us to identify 18 genotypes, including the homozygous triple-resistant genotype CC/II/LL (1534C/1016I/410L). A similar level of genotype diversity was observed in Mexico City, Mexico and Harris County, USA, where 15 and 23 genotypes were respectively reported [53, 54]. Our findings contrast with those of Sombié et al. [28], who identified fewer than five genotypes and haplotypes in Burkina Faso, indicating a lack of genotype diversity. Additionally, near-fixation of the F1534C mutation is likely to decrease the number of genotypes and haplotypes [11]. As demonstrated in our study, the triple-mutant genotype CC/II/LL was identified. This genotype was previously reported by Konan et al. [19] in Abidjan. Similar studies conducted by several authors have reported this genotype at significantly higher frequencies in some countries, such as Burkina Faso (42.3%) [28] and the United States (ranging from 35.9% to 96.42%) [53]. Furthermore, individuals carrying the CC/II/LL genotype exhibited increased resistance to pyrethroids such as deltamethrin and permethrin due to the resistance conferred by the 410L mutation in combination with one of the other two mutations [53]. The triple mutant genotype was mainly observed in alive *Ae. aegypti* individuals. This suggested it could be associated with phenotypic resistance in Abidjan district. Thus, this genotype is predictive of pyrethroids resistance. Although the triple mutant CC/II/LL was predominantly found in *Ae. aegypti* its occurrence in a small proportion of dead individuals exposed to deltamethrin show that in addition to *kdr* genes, other mechanisms may be involved in the vector's resistance. The partial restoration of *Ae. aegypti* susceptibility to pyrethroids after pre-exposure to the synergist PBO suggests that other resistance mechanisms, such as metabolic resistance involving cytochrome P450 oxidases, may explain at least partially the pyrethroid resistance. Similar results have been observed in several other West African countries [11, 19, 37, 55].

The presence of the CC/II/LL genotype in *Ae. aegypti* from the city of Abidjan may constitute a public health risk. Given the observed resistance to pyrethroids and the presence of homozygous resistant genotypes in local *Ae. aegypti* populations, improved resistance management is essential. This requires alternating insecticides or adopting new formulations, such as Fludora Co-Max (a combination of transfluthrin, a volatile pyrethroid, and flupyradifurone, a butenolide). In fact, phase II trials of Fludora Co-Max, conducted by Zahouli et al. [22] on *Ae. aegypti* strains from Abidjan, showed high efficacy, with similar findings reported by Ordonez-Gonzalez et al. [56]. However, one of the components of Fludora Co-Max, transfluthrin, is a pyretrhoid insecticide, so understanding the role of *kdr* genes in conferring transfluthrin resistance would be important. Indeed, multiple studies conducted by Andreazza et al. [57], Yang et al. [58], and Wagman et al. [59] stated that in *Ae. aegypti* carrying the V1016I *kdr* mutation, the repellent effect of transfluthrin can be reduced.

## Conclusion

Through the present study, it was observed that *Ae. aegypti* populations from Abidjan displayed resistance to many of the insecticides commonly used in public health. The tests with PBO partially restored mortality, indicating a contribution of metabolic mechanisms to resistance. The study detected the presence of *kdr* mutations in *Ae. aegypti* across all study sites, with a high frequency of the F1534C mutation. The V1016I and V410L mutations were detected at moderate frequencies. The triple homozygous mutant (CC/II/LL) genotype was found in the *Ae. aegypti* and is thought to be associated with phenotypic resistance in the district of Abidjan. Given the additional detection of the V1016G *kdr* mutation, it would be important to expand monitoring across the Abidjan region, where outbreaks of DEN and YF are prevalent. This expansion should include screening for all mechanisms that could contribute to resistance in *Ae. aegypti*. Such an approach will be instrumental in refining *Aedes* vector control strategies and establishing a comprehensive database of the diverse mechanisms present. The findings from our study offer valuable insights into the resistance profiles and underlying mechanisms in *Ae. aegypti* resistance to insecticides in Abidjan, informing future vector control strategies.

# Abbreviations

*Ace*-1
Acetylcholinesterase-1
*Ae*.
*Aedes*
AS-PCR
Allele-specific polymerase chain reaction
CHIKV
Chikungunya virus
CI
Confidence interval
DDT
Dichlorodiphenyltriphosphate
DENV
Dengue virus
Freq
Frequence
GLM
Generalized linear model
ISS
Insecticide space spraying
*kdr*
Knockdown resistance
OR
Odds ratio
PBO
Piperonyl butoxide
YFV
Yellow fever virus
ZIK
Zika virus.

# Declarations

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## Authors' contributions

Conceptualization: CNA, MAA, AB and JZBZ:

Data curation: CNA, MY, AS and AB.

Formal analysis: CNA, DF, AS, AB and JZBZ.

Funding acquisition and resources: BGK and JZBZ.

Investigation: CNA, MY, BKF, AS and JZBZ.

Methodology: CNA, MAA, DF, AS, BGK, PM, DW, AEL, AB and JZBZ.

Project administration: BGK and JZBZ.

Data collection: CAN, BKF, MY and AS.

Supervision: MAA, BGK, AB and JZBZ.

Visualization: CNA, MAA, DF, AS, DW, AEL, AB and JZBZ.

Writing – original draft: CNA, AS, AB and JZBZ.

Writing – review & editing: CNA, MAA, DF, AS, BKF, BGK, PM, DW, AEL, AB and JZBZ.

The final version of the manuscript was read and approved by all the authors.

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Data are provided within the manuscript or supplementary files.

## Ethics approval and consent to participate

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## Consent for publication

All the authors consent the publication of the manuscript

## Competing interest

The authors declare no competing interests

## Disclaimer

The views expressed in this manuscript are those of the authors and do not necessarily reflect the official policy or position of the Centers for Disease Control and Prevention.

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# Figures

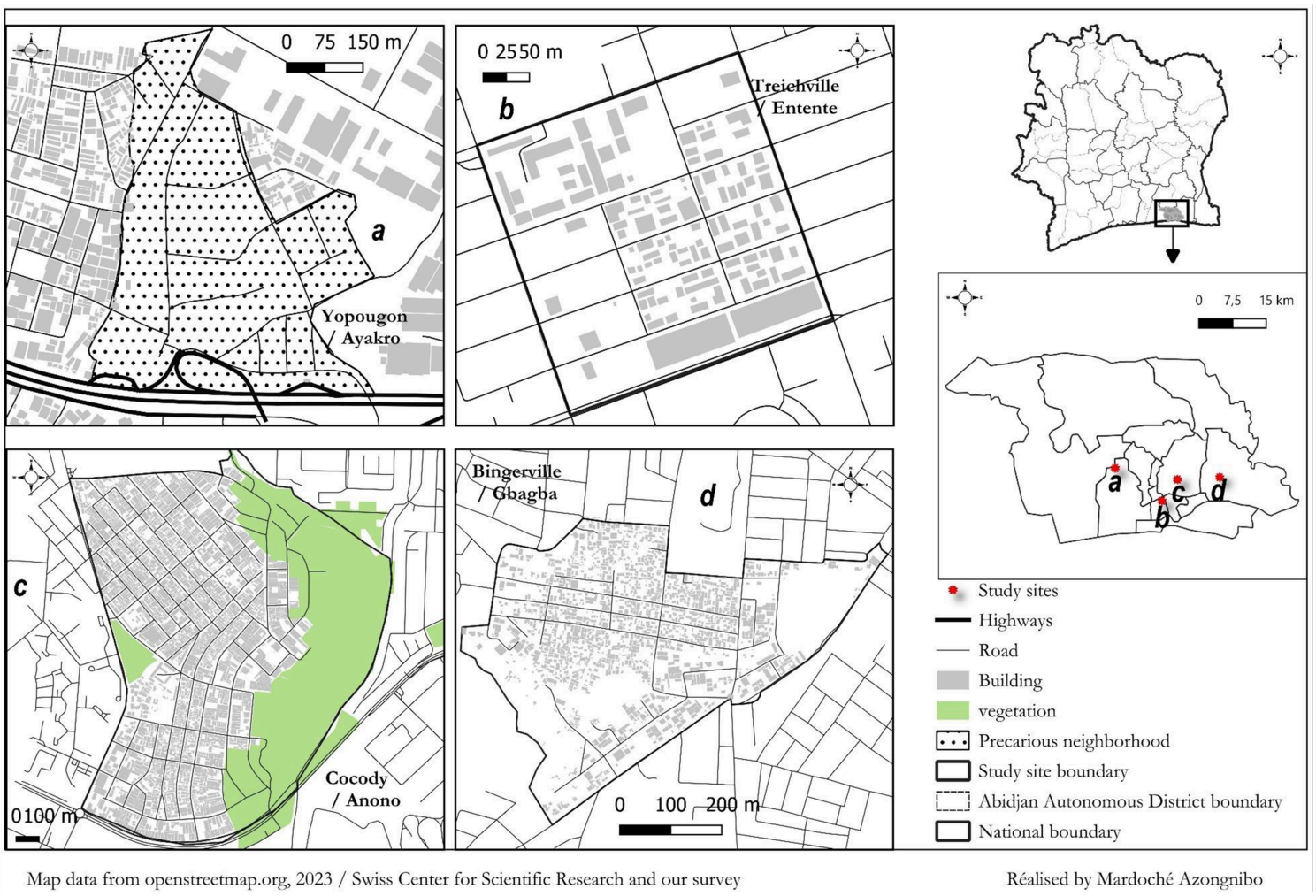



**Figure 1**

**Study sites in the city of Abidjan, southeastern Côte d'Ivoire**. The study was conducted across four sites, including Anono, Gbagba, Entente, and Ayakro (inlets) in the municipalities of Cocody, Bingerville, Treichville and Yopougon, respectively.

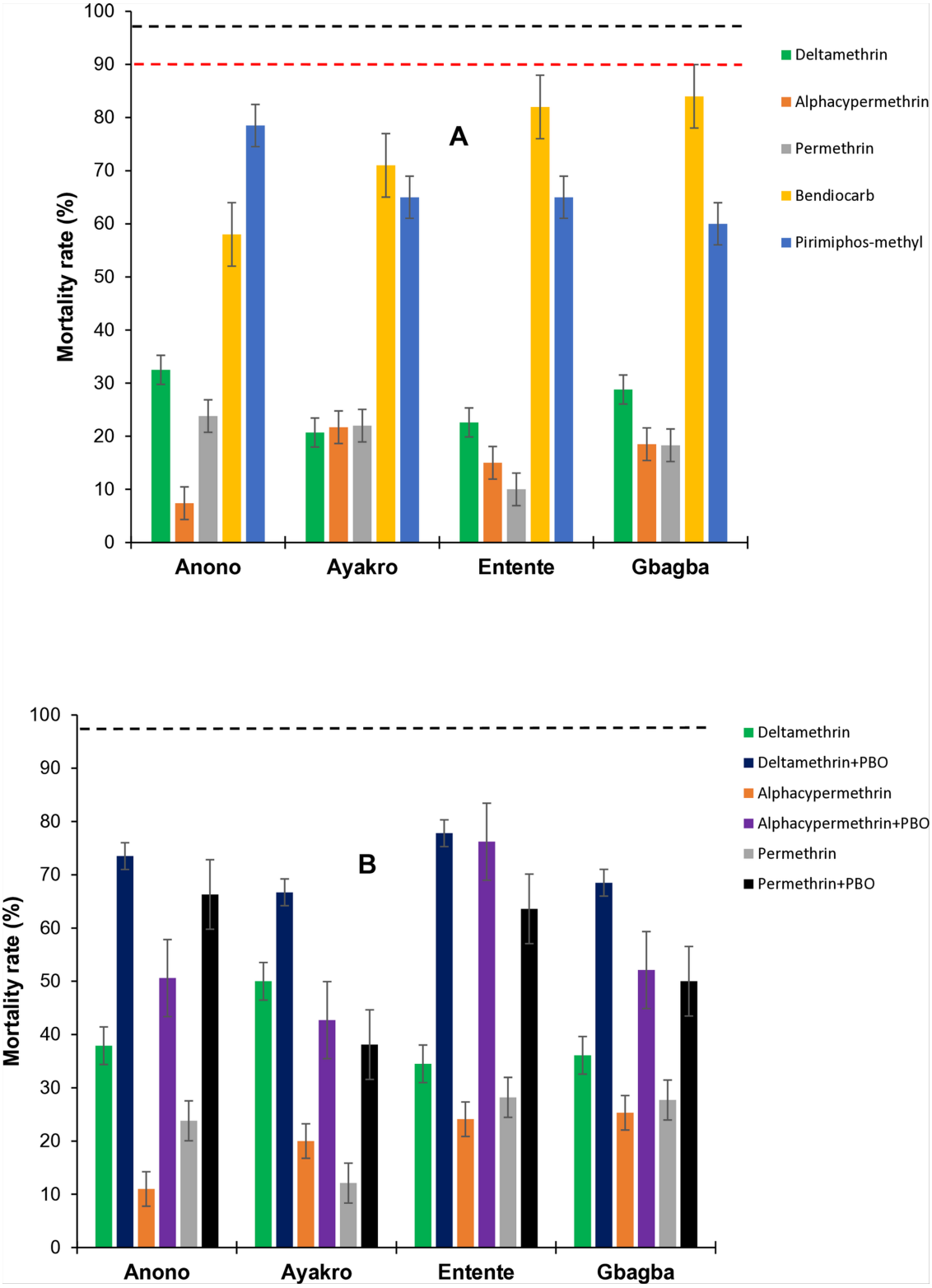



Figure 2

**Mortality of *Aedes aegypti* females tested against insecticides using WHO insecticide susceptibility bioassays.** A: Mortality rate to insecticides. B: Mortality rate to insecticides without or with piperonyl butoxide (PBO) pre-exposure. The insecticide susceptibility bioassays were performed according to current WHO procedures [26]. The red dashed line indicates the resistance threshold, while the black dashed line depicts the susceptibility threshold. Error bars represent 95% confidence interval of the mean.

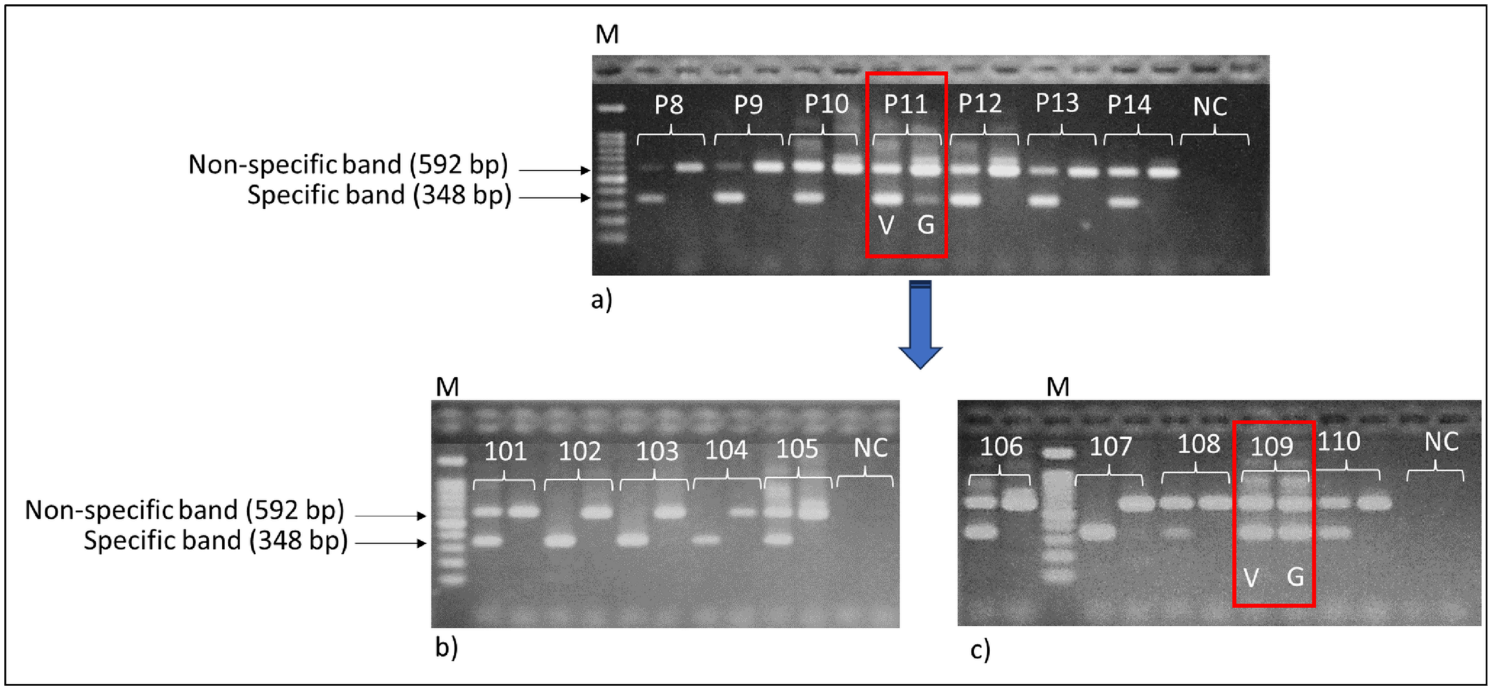



**Figure 3**

**Gel electrophoresis bands of AS-PCR products corresponding to the pooled (a) and individual (b, c) *Aedes aegypti* DNA samples for the V1016G *kdr* mutation**. M: marker, V: V1016G Wild-type allele (V1016), G: V1016G mutant allele (1016G). P8-P14: Pool of DNA. 101-110: Individual sample. NC: Negative Control. The red box indicates the positive pool and sample for V1016G *kdr* mutation.

## Supplementary Files

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