Status and Genes Involved in Insecticide Resistance in *Anopheles gambiae* Sibling Species in Lomé, (Togo), West Africa

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**Abstract**
Malaria vector control relies on mosquito susceptibility to insecticides. Nowadays, the phenomenon of mosquitoes’ resistance to insecticides is growing wider and wider, including all chemical families of insecticides. In order to update data on the insecticides susceptibility, the species’ distribution and genes involved in insecticide resistance in *Anopheles* in the capital of Togo, we tested local strains of *An. gambiae* s.l. from three study sites in Lomé, with five insecticides namely DDT, Permethrin, Deltamethrin, Bendiocarb, and Fenitrothion. The tests had been performed with the WHO kits from 2013 to 2015. The results of the tests showed mortality rates of 16.0% with 4% DDT, 28.0% with 0.75% Permethrin, 33.0% with 0.05% Deltamethrin, 44.0% with 0.1% Bendiocarb and 98.8% with 1% Fenitrothion. The major malaria vectors were shown, across all sites, to be resistant to all of the classes of insecticides used in the experiments except Fenitrothion. PCR analyses for the species’ identification showed, proportions of 81% of *An. gambiae* s.s. and 19% of *An. coluzzii* in the city. For the Kdr gene, PCR analyses showed proportions of 57.94% RR, 33.33% RS and 8.73% of SS, revealing a high prevalence of kdr resistance in the *Anopheles* population in Lomé. However, analyses showed mosquitos without Ace1R gene. The multiple resistance to various insecticides is a major concern for the control of malaria and other vector-borne diseases in Lomé, as well as in Togo.

**Keywords**
Resistance; Insecticides; *Anopheles gambiae* sibling species; Kdr and Ace1R genes distribution; Lomé

**Background**
The face of malaria control has deeply changed over the last few years; in the 2016 report of WHO, by 2015, the number of cases of malaria was estimated at 212 million and the related deaths at 429,000 worldwide. The WHO African region remains the most affected, with about 90% of cases of malaria and 92% of associated deaths in 2015. Globally, three-quarters of malaria cases and associated deaths are concentrated in fewer than 15 countries and one-third in Nigeria and the Democratic Republic of Congo (WHO, 2016). Several factors have contributed to this sharp decrease; predominately vector control through the use of pyrethroid as major insecticides for the Long-Lasting Impregnated Nets (LLINs) and household Indoor Spraying (IRS), during years, had played a fundamental role (Chandre et al., 1999a; 1999b; Diabate et al., 2015). However, the hopes aroused by this advance in the disease control and the substantial decrease in cases face the challenge of increasing insecticides resistance among major malaria vectors in African countries (Coluzzi et al., 1985; Weill et al., 2000; Favia et al., 2001; della Torre et al., 2001; Diabate et al., 2002b; Diabate et al., 2003; Ranson and Lissenden, 2016).

Resistance to insecticides is increasingly widespread and is now reported to affect nearly two thirds of malaria cases in Africa. Furthermore, other chemical families of insecticides such as organochlorines and organophosphates had fallen into the ties of this phenomenon which now extends to all major vector species and all classes of insecticides. In 2011, the World Health Assembly and the Board of the Roll Back Malaria Partnership requested that action be taken to develop a global strategy on insecticide resistance management to serve as the foundation of a coordinated multi-stakeholder response. It becomes then necessary to set up a
permanent watch of the evolution of the disease and especially the vectors’ bioecology. For this purpose, insecticide susceptibility tests were conducted to determine the level of vector resistance in the city of Lomé. The tests were carried out on wild-type *Anopheles gambiae* mosquitoes, using five insecticides. These include Permethrin, Deltamethrin, Bendiocarb, Fenitrothion and DDT. Moreover, a batch of mosquitoes was analyzed by PCR to identify vector species and detect mutations of the Kdr and Ace1R genes.

The results will enable malaria control programs to update data on the vector's resistance to insecticides in order to guide strategies for resistance management leading to a more efficient vector control.

**1 Materials and Methods**

**1.1 Study area and study sites**

The study was conducted in three sites in Lomé (Latitude: 6.1375° N / Longitude: 1.2122° E, Figure 1), southern Togo, in West Africa. The capital city, as the south part of the country, has a Guinean type tropical climate, with two wet seasons, a longer in March to July and a shorter in October to November, and two dry seasons. The mean annual temperature is 26°C and the mean annual rainfall is approximately 810 mm.

![Figure 1 Map of Lomé showing the study sites](image-url)
The population of the city, according to 2010 Population and Housing census data, exceeds the one million population mark (RGPH; 2010). The relief of the city presents two geographical entities consisting of a sandy plain and a clay plateau separated by a lagoon. The three test sites identified, representative of the main ecological features of the city (Figure 1) are as follows: the area of Adakpamé (1.282275° E, 6.167656° N), Doumasese-Dogbeavu neighbourhoods (1.212151° E, 6.157137° N) and the Nyékonakpoè district (1.205440° E, 6.134076° N).

The main criterion for selecting these sites is the presence of surface water all the year, potential mosquitoes breeding sites, for larval collections, in relation with urbanization level.

1.2 Larval collections
Visits to the study sites were conducted monthly from 2013 to 2015. The collection of larvae were performed using very fine mesh larvae net. In small puddles or shallow water, ladles are used to collect the water containing the larvae. Plant debris and other wastes are removed from the water and these are brought back to the laboratory for breeding. The larvae were fed with enriched flour (BLEDINA®) for children. The larvae were reared in a room where the temperature is 28±3°C and an averaged relative humidity around 75±5% until they emerge into adults.

1.3 Insecticide bioassays
3 to 5 days old female An. gambiae s.l. were selected for the susceptibility tests according to the standard procedure for testing adult anopheline susceptibility to insecticides (WHO, 1998). Between 15 and 25 mosquitoes were used for the tests in each WHO tube. These tests were carried out with papers impregnated with Deltamethrin 0.05%, Permethrin 0.75%, DDT 4%, Bendiocarb 0.1% and Fenitrothion 1%. The choice of diagnostic doses of impregnated papers is based on WHO recommendations for these tests (WHO, 1998). The sensitivity of mosquitoes to Delthametrin was compared to the other insecticides and a control paper, according to standard WHO procedures. DDT was tested for cross-resistance between pyrethroid and organochlorines while the test with Fenitrothion was to show cross-resistance between pyrethroid and organophosphates. The mosquitoes were exposed to the impregnated papers for 60 minutes, and observed for 24 hours in order to assess the mortality rates.

As soon as the mosquitoes were exposed to the impregnated papers, the number of mosquitoes "knocked down" (Kd) at the bottom of the WHO tubes was registered after 10, 20, 30, 45 and 60 minutes. After the tests, the dead and live specimens were stored separately on silica gel in 1.5 ml Eppendorf tubes and stored in a freezer (at -20°C) for later biochemical and molecular analyses. In the present study, a population was considered as susceptible when the mortality is greater than or equal to 96%. When mortality is less than 90% the population is considered as resistant. Between the two values, the situation was considered as a suspicion of resistance (decrease in sensitivity). A control batch had been prepared with the sensitive Kisumu (SLAB) which is a reference strain reared in laboratory.

1.4 Extraction of DNA from the female mosquitoes
Each mosquito from the insecticide susceptibility assay was cut into two parts: one part constituted of the head-thorax and the other, with the abdomen, wings and legs. The two parts were put into separate tubes. These were ground in 200 μl of 2% CTAB buffer containing 100 mM Tris HCL, pH8.0, 1.4 M NaCl, 10 mM EDTA and 2 % Cetyl Trimethyl Ammonium Bromide, and introduced into a water bath at 65°C for 5 minutes. 200 μl of chloroform was added and then centrifuged at 14,000 rpm for 5 min. The supernatant was carefully transferred in another sterile tube with 200 μl of isopropanol and then centrifuged at 12,000 rpm for 15 minutes. The supernatant was discarded and the pellet was washed with 200 μl 70% ethanol and centrifuged at 14,000 rpm for 5 min. The contents of the tube were delicately inverted to preserve the pellet which was then dried for 3 hours on the pallet. At the end, 20 μl of double distilled water was added to the pellet and kept on the pallet all night. The DNA was used for the identification of species and molecular forms of the Anopheles gambiae complex.
1.5 Identification of *Anopheles gambiae* sibling species

The DNA extracted permitted species identification which was based on the protocol of Scott et al. (1993). Four primer sets: UN (GTGTGCCGTTCCTCGATGT), AG (CTGGTTTGGTCGGCACGTTT), AA (AAGTGTCTCTCCCCATCTCA) and AM (GTGACCAACCCCACCTCCTTGA), derived from ribosomal gene sequences (IGS of the rDNA located in the heterochromatin of the X chromosome) specific for the *Anopheles* species (*gambiae*, *arabiensis* and *melas*) were used. The molecular forms were determined using four primers: R5 (GCAATCCGAGCTGA TAGCGC), R3 (CGAATTCTAGGGAGCTCCAG), Mop int (GCCCCTTCTCGATGGAAT) and B/S int (ACCAAGATGTTCTGTTGC) corresponding to ribosomal gene sequences (rDNA IGS), according to the method of Favia et al. (2001).

1.6 Detection of the kdr mutation in mosquitoes

100 adult mosquitoes tested with DDT and Permethrin were used to detect the Knock down resistance gene following the PCR method of Martinez et al. (1998). Four primers were used; the first two: D1 5’ATAGATTCCCACCCATG3’ D2 5’AGACAAGGATGA TGAACC3’ in amplifying the Na-canal gene containing the site of Kdr mutation and the last two: D3 5’AAATTCATTCTACTACGACA3’ and D4 5’CTGTAGTGTAGAAATTTTA3’ for genotyping the resistant allele mutation Kdr and the susceptible allele Kdr respectively.

1.7 Detection of the Ace-1 mutation in mosquitoes

The characterization of the Ace-1 polymorphism was done using two primers: Ex3AGdir GATCGTGGACACCGTGTGC and Ex3AGrev AGGA TGGCCCGCTGGAACAG, and following the methods of Weill, et al. (2003).

1.8 Data analyses

The kdr PCR results for the genotypes obtained were analyzed using the Genepop software (version 3.2a) of Raymond and Rousset (1995) to calculate the allele frequencies, to compare the populations with each other, and to differentiate the genotypic frequencies between populations. Tests regarding kdr and Ace-1 mutations was tested using Fisher’s exact test. For each enzyme system, the mean activity per population is calculated and compared with that of the "Kisumu" reference susceptible strain with the nonparametric Kruskall-Wallis and Mann-Whitney U tests at the CI of 5%.

2 Results

2.1 Insecticide susceptibility testing

The results of *Anopheles* susceptibility to the five insecticides used during the tests are presented in Figure 2 (1-5). Throughout the three years, approximately 7,500 mosquitoes were tested with the five insecticides. The results revealed resistance to Bendiocarb with mortality rates of 38.2%, 47.2% and 46.8% respectively in Adakpame, Doumasese and Nyé konakpoè (Figure 2-1). High resistance to organochlorine DDT was also detected, from the study sites, with mortality rates of 17% for the peripheral zone, 24% for Adakpame, Doumasese and Nyé konakpoè (Figure 2-2).

There was resistance to type I (Permethrin) and type II (Deltamethrin) pyrethroid in the *An. gambiae s.l.* tested from the various study sites, with mortality rates being 38.6% (Permethrin) and 33.5% (Deltamethrin) in Adakpame, 49% and 45% (Permethrin and Deltamethrin respectively)) in Doumasese areas, while in Nyékaponakô, the mortality rates were 38.6% and 30% respectively for Permethrin and Deltamethrin (Figure 2-3; Figure 2-4).

The organophosphate Fenitrothion revealed a high susceptibility with mortality rates of 98.6% in Adakpame, 98.4% in Doumasese zone and 98.5% and Nyékonaâkô (Figure 2-5).

The control strain KISLAB exhibited very high sensitivity to the insecticides tested; 99% mortality to carbamate, 100% mortality to the organochlorine and the type I pyrethroid, 99.7% mortality to the organophosphate and 99.3% mortality to the type II pyrethroid.
2.2 Resistance mechanisms in the *Anopheles gambiae* strains from the study sites:

2.2.1 Detection of the Kdr and Ace-1r genes

Mosquitoes from each study site were used for the detection of the Kdr gene.

In the peripheral zone, in Adakpame, the Kdr gene was strongly expressed with a frequency of 0.79 as well as in suburban zone Doumasese with a frequency of 0.78; In the urban area of Nyékonakpoè, the frequency of expression of the Kdr gene was 0.89 (Table 1). Over all, the frequency of the Kdr gene in Lomé was 0.82 (Table 1).

| Study sites | Localities     | Number tested | Kdr Mutation | Ace-1Mutation |
|-------------|----------------|---------------|--------------|---------------|
|             |                | RR | RS | SS | F(R) | RR | RS | SS | F(R) |
| Peripheral  | Adakpame       | 50 | 31 | 16 | 3   | 0.79* | 0  | 0  | 50 | 0.0 |
| Suburban    | Doumasese      | 50 | 32 | 14 | 4   | 0.78* | 0  | 0  | 50 | 0.0 |
| Urban       | Nyékonakpoè    | 50 | 39 | 11 | 0   | 0.89* | 0  | 0  | 50 | 0.0 |
| Average     |                | 50 | 34 | 14 | 2   | 0.82* | 0  | 0  | 50 | 0.0 |

Note: F= frequency; ns = no significant difference with the Fisher’s test (p> 0.05)

Analyses for the Ace 1 mutation releaved all mosquitoes tested to be sensitive SS homozygotes (Frequency Ace-1r =0).

2.2.2 Identification of *Anopheles gambiae* sibling species

Concerning the species of the *Anopheles* collected in the study sites, the results from the PCR analyses showed an exclusivity of *Anopheles gambiae s.l.* with *An. gambiae s.s.* and *An. coluzzii* with respective frequencies of 92% and 8% in Adakpame, 84 and 16% in Doumasese and 66 and 34% in Nyékonakpoè. The results showed an average in forms in Lomé of 81% *An. gambiae* and 19% *An. coluzzii* (Table 2).

Table 1: Sorting of Kdr and Ace-1 Mutations in Wild type *Anopheles gambiae s.l.* in the study sites of Lomé

| Study sites | Localities     | Number tested | Kdr Mutation | Ace-1Mutation |
|-------------|----------------|---------------|--------------|---------------|
|             |                | RR | RS | SS | F(R) | RR | RS | SS | F(R) |
| Peripheral  | Adakpame       | 50 | 31 | 16 | 3   | 0.79* | 0  | 0  | 50 | 0.0 |
| Suburban    | Doumasese      | 50 | 32 | 14 | 4   | 0.78* | 0  | 0  | 50 | 0.0 |
| Urban       | Nyékonakpoè    | 50 | 39 | 11 | 0   | 0.89* | 0  | 0  | 50 | 0.0 |
| Average     |                | 50 | 34 | 14 | 2   | 0.82* | 0  | 0  | 50 | 0.0 |
Table 2  *Anopheles sibling species* in the three study sites in Lomé

| Localities  | Species      | An. gambiae | An. coluzzii |
|------------|--------------|-------------|--------------|
| Adakpame   | 92%          | 8%          |              |
| Doumasese  | 84%          | 16%         |              |
| Nyékonakpoè| 65%          | 35%         |              |
| Average    | 80%          | 20%         |              |

3 Discussions

The present study aimed to update the entomological data of malaria vector, *Anopheles gambiae s.l.*, in three selected representative bio ecologic areas: an urban, a suburban and a peripheral areas of the city of Lomé. These data took in account the actual susceptibility to insecticides, the various species subservient to the town, the status of the Knock down gene as well as the Ace1 and the metabolic resistance mechanisms.

Results from the OMS susceptibility tests showed *Anopheles gambiae s.l.*, the major malaria vector in Africa, presenting strong resistance to four of the five insecticides at study sites: the peripheral zone of Adakpame, the suburban zone of Doumasese and the urban area of Nyékonakpoè in the city of Lomé. The data collected showed resistance to pyrethroid (types I & II), carbamates and organochlorines across all study sites. As stated by several studies (Mouchet et al., 1988; Yadouleton et al., 2011; Ranson et al., 2011; Mahande et al., 2012), the mass use of pesticides in vegetables’ protection confer the resistance in mosquitoes. The influence of crops and the use of insecticides for vegetable protection are not direct in the city, the vegetable producing sites in the town do not make a large proportion of areas in the city to influence deeply the emergence of resistance in the anopheline strains. The city of Lomé, according to its topography, with its clayey-sandy plateau, oriented East-West, dotted with Watersheds or Talwegs (Direction de l’Assainissement, unpublished data) which retain surface moving water during rains, presents no connection with any stream that could drain chemical residues to the city. The Zio River, passing from Northside by the close rice fields of Kovié and its surroundings, supplying the areas with water and collects the overflow from the fields which it drains downstream and towards North Lomé areas, could discharge the pesticide residue in the outskirts of Adakpame at the extreme East. However, given the amount of water drained and the current flowing to its outlet, which is Lake Togo, 35 km Eastside from the city, it is expected the amount of residues released at Adakpame should not be high enough to induce large-scale resistance. On the other hand, the migration of adults mosquitoes by the wind and the means of road communications towards Lomé, along a shorter distance than the river’s one, could suggest intersections between the strains with migration and dispersion of resistance genes (Weill et al., 2003; Migration and genetic drift, Poupardin, 2011).

Beyond the resistance expressed by the local strains collected in the study sites, the consequence of small-area crops and non-market gardening crops made up of beans, millet, sorghum, cassava, yam, sweet potatoes and vegetables that do not require insecticides or Fertilizer, it is very likely to suspect a "partial import" of resistance from the periphery to the city.

Concerning the household individual and collective protection against mosquitoes’ aggression and nuisance, the use of insecticide products, such as aerosols and mosquito coils must have rendered field selection pressure towards the family of insecticides in mosquitoes (Fonseca-Gonzalez et al., 2011; Koou et al., 2014); but the main idea is that in the name of the instinct of conservation and survival, only the trapped mosquitoes face the insecticide aggression; in free spaces, the mosquito escapes the influence of the insecticides by flying far from the dangerous zone. This leads to conclude that even though the household use of insecticides could lead to the emerging of resistance, it could be at a smaller part among the whole insecticide aggression towards the insects since the pre imaginal stages in shallows and breeding sites face longer the pesticide residues. The larvae of all instars face more selection pressure towards the families of insecticides in the aquatic stage of life; as reported by Yadouleton et al. (2016), pesticide treatments cause movement of chemical particles from pesticide residues to larval breeding habitats and are the major causes leading to selection of resistance in arthropods, particularly in *Cx. quinquefasciatus*. 

Indeed, it is necessary to imagine the migration of the resistant *Anopheles* strains from the intensive cultivation zones, in particular the close rice fields of Kovié and the whole Zio valley, along the winds and means of road transport towards the capital of Togo, and other agglomerations. This leads to dispersion of resistance genes achieved by mixing the local strains of the city with the field’s ones coming from areas with high pesticide use, as stated by and Raymond et al. (1991); Guillemaud et al. (1996); Ouedraogo et al. (2004); V. Corbel and R. N’Guessan (2013).

Two major types of resistance appeared in the results: the Knockdown resistance (kdr) showing the genetic aspect of resistance to DDT and pyrethroid and the "metabolic aspect of resistance", resulting from enhanced expression of detoxification enzymes (Hemingway et al., 2000). The second type of resistance will be discussed in another publication.

Results of the study showed the mosquitoes resistant to DDT, and pyrethroid and were close to several authors ones evoking cross resistance. The cross-resistance between DDT and pyrethroid in *An. gambiae* is closely linked to the kdr mutation in this species as noted in reviews by Martinez-Torres et al. (1998); Chandre et al. (1999); Hemingway and Ranson (2000); Ranson et al. (2000). The fact of cross-resistance to DDT (organochlorine), to Deltamethrin and Permethrin (type I and II pyrethroid) (Chandre et al., 1999; Hemingway and Ranson, 2000) in strains originating from study sites is a consequence of intensive use of agricultural pesticides directed against crop pests in vegetable, rice and cotton fields, causing collateral damage to the non-target entomofauna namely the *Cuicidae* (Yadouleton et al., 2016).

The resistance of *An. gambiae s.l.* to carbamate Bendiocarb, showed by the results, could be explained by the use of this chemical product during intra-home spraying by the National Malaria Control Program since 2010 in Benin. Carbamate have as target the Ace1 gene in the mosquitoes. The activity of intra room spraying with carbamate was not carried out in Togo. This could explain the homozygote susceptible (100% SS) Ace-1 gene found in the study sites of Lomé. In this border country of Togo, the insecticide had been used as an alternative to pyrethroid (Padonou, 2012) and had reduced the transmission of malaria in the country, as pointed out by Akogbeto et al. (2010); Aikpon et al. (2013), but the selection of the acetylcholinesterase mutation within the populations of *An. gambiae s.l.* has been recognized in relation with its repeated frequency in the use of carbamate in domestic hygiene. The fact of meeting carbamate resistant strains however bearing Ace-1 SS homozygote mosquitoes in Lomé seemed to show no relationship between the two situations as stated by Donnelly et al. (2009) inviting to deeper analysis of the relationship presented by several previous work.

The work of Yadouleton et al. (2011), reported that in addition to the use of this carbamate in public health, intensive and especially uncontrolled use of the Tihan, insecticide of the carbamate family, was made in Natitingou and its surroundings to control cultures pests.

The results of this research work still revealed the selection of resistance within populations of *An. gambiae s.l.* to pyrethroid, organochlorines and carbamates. The work of Aheidji-Dabla et al. (2014), reported this situation without mentioning the case of carbamates. These authors claim that the strong resistance to DDT could be linked to the use of this organochlorine by the colonizer in 1952 in the malaria vectors control activities in Lomé at the creation of the Malaria Control Service which had become the National Malaria Control Program (NMCP). Moreover, the high expressions of the Kdr mutation in the *Anopheles gambiae s.l.* wild strains could suggest the NMCP to take appropriate measures for a judicious use of chemical pesticides in household hygiene as well as in the protection against crops pests.

Concerning the organochlorine DDT, the results of this work had showed mortality rates of 17% for the peripheral zone, 24% for the suburban and 29% for the urban area, showing thus a decrease in resistance to this product (DDT) from the periphery to the urban environment. Data of Coetzee et al., 2006, reported higher mortality rates with DDT than in Lomé. From assays against wild mosquitoes’ strains, they showed a level of resistance to DDT of 60.9% as final mortality rate but without cross resistance to pyrethroid; these authors had presented 100% as final mortality rate recorded with Deltamethrin and cyfluthrin. These authors linked the discrepancy in the results
with the age at which the strains were exposed to the insecticides suspecting an age dependent variability in the physiological status in the batches of the mosquitoes tested.

In the present work, experiments on the Ace-1 gene had showed the anopheline wild strains to be exclusively sensitive homozygotes (frequency Ace-1 = 0). This gene, as stated by Weill et al. (2003), is the target of the organochlorine and carbamate insecticides. The wild-type An. gambiae s.l. strains tested in the experiments during this work did not show an Ace-1R mutation. Okoye et al., in 2008 in Ghana, reported many Culicidae families presenting reduced acetylcholinesterase sensitivity to propoxur inhibition. Coetzee et al. (2006), by the standard WHO insecticide susceptibility method for wild mosquito strains, showed Obuasi An. funestus strains carrying resistance to the carbamate Bendiocarb thus the Ace1R gene with a final mortality rate of 71.4%, 24 hours after exposure.

As suggested above, a method of judicious chemical control would lead to the control of resistance in mosquitoes by a combination of insecticides (Pennetier et al., 2005; 2007) or by alternating insecticides (Yadouleton et al., 2016) such a method would result in long lasting in the impregnation activities of the bed-nets and their use against the Culicidae nuisance.

The work of Corbel et al. (2007) highlights this as a major consequence of the high Kdr gene frequencies in An. gambiae s.l. in the collected batches (Table 1). The Kdr mutation had been noted in Togo by Ketoh et al. (2009) and had also been reported by works from many other West African countries where high kdr frequencies had been showed in An. gambiae s.l. namely in Côte d’Ivoire, Burkina Faso, Benin, Ghana and Nigeria (Yawson et al., 2004; Awolola et al., 2005; Corbel et al., 2007; Djogbénoun et al., 2007; Oduola et al., 2010; Yadouleton et al., 2011; Dabire et al., 2012; Koffi et al., 2012), as well as in Central Africa particularly in countries such as Equatorial Guinea and Cameroon by Nwane et al. (2009), Etang et al. (2006) and Reimer et al. (2005).

As proposed by Yadouleton et al. (2016), to quantify both Kdr and Ace-1R genes in Benin where they had showed very high frequencies, in Togo it is necessary to quantify over time the Kdr gene by the qPCR technique.

The results of detection of Ace1 showed Malaria vectors in Lomé to be exclusively homozygote susceptible (SS). This could mean that the use of insecticides which targets are acetylcholinesterase, were did not yet lead to the mutation of the Ace1 gene. To monitor the Ace-1 gene which does not yet have presented a mutation becomes necessary with a view to better resistance management in An. gambiae s.l. for optimum control of malaria in the town.

In Mali, in the 1960s, the selective role of organochlorine (OC) treatments leading to developing resistance in Culicidae, particularly vectors, was observed in areas free from public health treatments but rather in Agriculture (Yadouleton et al., 2016). In other countries, notably Côte d'Ivoire and Burkina Faso, the late 1990s saw an increase in the level of resistance of vectors to pyrethroid insecticides resulting from the cotton season with pesticide treatments (Chandre et al., 1999).

4 Conclusions

The multi insecticide resistance levels in Anopheles gambiae s.l. reported in the present work, left to a continuous selection pressure in these vectors, will lead inexorably to a situation of mosquito and malaria management, by use of insecticide treated bed-nets and indoor residual spraying, beyond efforts of the Malaria Control Program authorities if unchecked. This resistance could spread rapidly and weaken or even undermine the hope generated by the control of the disease through vector control in efforts in reducing malaria across Africa.

Therefore, the results presented in the present work are aiming directing the NMCP authorities to plan an effective malaria control strategy in the Lomé area by a judicious resistance management in the city. An integrated vector management system including alternative and/or combination use of insecticides, for indoor residual spraying, for impregnation of bed-nets, in house screening and, finally, larval control by environmental and larval habitats control and management, can be taken into account by the malaria control authorities.
List of abbreviations
- CREC: Centre de Recherches Entomologiques de Cotonou
- EGESE: Ecole de Gestion et d’Exploitation de Systèmes d’Élevage
- ESTBA: Ecole Supérieure des Techniques Biologiques et Alimentaires
- FDS: Faculté des Sciences
- NMCP: National Malaria Control Program
- UAK: Université d’Agriculture de Ketou

Authors’ contributions
AK designed the study and carried out the experiments. OR participated in sampling the mosquitoes and controlled the laboratory results. SA conducted biochemical and biomolecular analyzes. OLM has been involved in the treatment of mosquitoes for biochemical analyzes. AK and MA drafted the manuscript. DAM and OLM read the first manuscript and provided pieces of advice for correction. GA and MA critically revised the manuscript. AK and DAM translated the manuscript. All the authors read and approved the final manuscript.

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