Re-Visiting Insecticide Resistance Status in Anopheles gambiae from Côte d’Ivoire: A Nation-Wide Informative Survey

Alphonsine Amanan Koffi, Ludovic Ahoua Alou Phamien, Jean-Paul K K Kabran, Raphaël N’Guessan, Cédric Pennetier

To cite this version:

Alphonsine Amanan Koffi, Ludovic Ahoua Alou Phamien, Jean-Paul K K Kabran, Raphaël N’Guessan, Cédric Pennetier. Re-Visiting Insecticide Resistance Status in Anopheles gambiae from Côte d’Ivoire: A Nation-Wide Informative Survey. PLoS ONE, Public Library of Science, 2013, 10.1371/journal.pone.0082387. hal-03249028

HAL Id: hal-03249028
https://hal.archives-ouvertes.fr/hal-03249028
Submitted on 3 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Re-Visiting Insecticide Resistance Status in *Anopheles gambiae* from Côte d’Ivoire: A Nation-Wide Informative Survey

Alphonsine A. Koffi¹, Ludovic P. Ahoua Alou¹-², Jean-Paul K. Kabran¹, Raphael N’Guessan³⁵, Cédric Pennetier⁴⁵*

¹ Institut Pierre Richet (IPR), Bouaké, Côte d’Ivoire, ² Laboratoire de Zoologie et Biologie Animale, Université Félix Houphouët Boigny, Abidjan, Côte d’Ivoire, ³ London School of Hygiene and Tropical Medicine, Keppel Street, London, United Kingdom, ⁴ Institut de Recherche pour le Développement (IRD), Maladies Infectieuses et Vecteurs, Ecologie, Génétique, Evolution et Contrôle (MIVEGEC), UM1-UM2-CNRS 5290 IRD 224, Montpellier, France, ⁵ Centre de Recherche Entomologique de Cotonou (CREC), Cotonou 04, Bénin

Abstract

Insecticide resistance constitutes a major threat that may undermine current gain in malaria control in most endemic countries. National Malaria Control Programmes (NMCPs) need as much information as possible on the resistance status of malaria vectors and underlying mechanisms in order to implement the most relevant and efficient control strategy. Bioassays, biochemical and molecular analysis were performed on *An. gambiae* collected in six sentinel sites in Côte d’Ivoire. The sites were selected on the basis of their bioclimatic status and agricultural practices. *An. gambiae* populations across sites showed high levels of resistance to organochloride, pyrethroid and carbamate insecticides. The *kdr* and *ace-1* mutations were detected in almost all sentinel sites with mosquitoes on the coastal and cotton growing areas mostly affected by these mutations. At almost all sites, the levels of detoxifying enzymes (mixed-function oxidases (MFOs), non-specific esterases (NSE) and glutathione-S-transferases (GSTs)) in *An. gambiae* populations were significantly higher than the levels found in the susceptible strain Kisumu. Pre-exposure of mosquitoes to PBO, an inhibitor of MFOs and NSEs, significantly increased mortality rates to pyrethroids and carbamates in mosquitoes but resistance in most cases was not fully synergised by PBO, inferring a residual role of additional mechanisms, including *kdr* and *ace-1* site insensitivity. The large distribution of resistance in Côte d’Ivoire raises an important question of whether to continue to deploy pyrethroid-based long-lasting insecticidal nets (LLINs) and insecticide residual spraying (IRS) towards which resistance continues to rise with no guarantee that the level of resistance would not compromise their efficacy. Innovative strategies that combine insecticide and synergists in LLINs or spatially LLIN and an effective non-pyrethroid insecticide for IRS could be in the short term the best practice for the NMCP to manage insecticide resistance in malaria vectors in Côte d’Ivoire and other endemic countries facing resistance.

Background

Malaria vector control programmes rely largely on long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) in sub-Saharan Africa [1]. For LLINs thirteen brands impregnated with only three pyrethroid insecticides (permethrin, deltamethrin and α-cypermethrin) are recommended by the World Health Organization Pesticide Scheme (WHOPECS) [2]. WHOPECS also recommends 12 insecticide formulations for IRS, involving four insecticide classes: organochloride, organophosphates, carbamates and pyrethroids [3].

Unfortunately insecticide resistance mechanisms are spreading faster than thought across Africa [4]. This threatens to undermine the efficacy of IRS and LLINs that contain the four insecticide classes cited above. Four types of resistance mechanisms against public health insecticides have been identified: metabolic resistance, target site resistance, penetration resistance and behavioural resistance [4–6]. Nevertheless only metabolic and target site resistance mechanisms have been extensively investigated at both the phenotypic and genetic levels [7].

So far, all insecticides used in malaria vector control are neurotoxic. The acetylcholinesterase which hydrolyses the neurotransmitter, acetylcholine in the synaptic gap is the target of organophosphate and carbamate insecticides. The voltage-gated sodium channel that triggers impulse along the neuron membrane via discharge of Na⁺ is the target site of pyrethroid and organochlorine insecticides. The c-aminobutyric acid (GABA) is a neurotransmitter of the inhibitory synapses in the insect nervous system [8]. Non-silent point mutations that render these targets less sensitive to insecticides are respectively: the *ace-1R* mutation [9–11]. Three main groups of enzymes are involved in metabolic resistance mainly through an overproduction of detoxifying enzymes (mixed-function oxidases (MFOs), non-specific esterases (NSE) and glutathione-S-transferases (GSTs)) in *An. gambiae* populations which are significantly higher than the levels found in the susceptible strain Kisumu. Pre-exposure of mosquitoes to PBO, an inhibitor of MFOs and NSEs, significantly increased mortality rates to pyrethroids and carbamates in mosquitoes but resistance in most cases was not fully synergised by PBO, inferring a residual role of additional mechanisms, including *kdr* and *ace-1* site insensitivity. The large distribution of resistance in Côte d’Ivoire raises an important question of whether to continue to deploy pyrethroid-based long-lasting insecticidal nets (LLINs) and insecticide residual spraying (IRS) towards which resistance continues to rise with no guarantee that the level of resistance would not compromise their efficacy. Innovative strategies that combine insecticide and synergists in LLINs or spatially LLIN and an effective non-pyrethroid insecticide for IRS could be in the short term the best practice for the NMCP to manage insecticide resistance in malaria vectors in Côte d’Ivoire and other endemic countries facing resistance.

* Citation: Koffi AA, Ahoua Alou LP, Kabran J-PK, N’Guessan R, Pennetier C (2013) Re-Visiting Insecticide Resistance Status in *Anopheles gambiae* from Côte d’Ivoire: A Nation-Wide Informative Survey. PLoS ONE 8(12): e82387. doi:10.1371/journal.pone.0082387

* Editor: Georges Sounouou, Université Pierre et Marie Curie, France

* Received August 11, 2013; Accepted October 28, 2013; Published December 16, 2013

* Copyright: © 2013 Koffi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

* Funding: The study was supported by the National Malaria Control Programme of Côte d’Ivoire as a part of a work package of the Global Fund initiative in the country. The NMCP of Côte d’Ivoire participated to the study design and approved the decision to publish. This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.

* Competing Interests: The authors have declared that no competing interests exist.

* E-mail: koffi_alphonsine@yahoo.fr (AAK); cedric.pennetier@ird.fr (CP)
Ethics statement

Methods

Ethics statement

No specific permissions were required for the larvae sampling in urban areas because mosquito breeding sites were located in public areas. In contrast in rural areas, owners of the sampled cultivated areas gave their permission before the larvae collections. Field collections did not involve endangered or protected species.

Study sites

The study was conducted in six sentinel sites currently involved in malaria surveillance by the NMCP in Côte d’Ivoire. These sites were selected on the basis of bioclimatic parameters and agricultural practices in order to capture a broader range of ecological patterns found in Côte d’Ivoire (Figure 1 and Table 1). Where possible, mosquito larvae were sampled in breeding sites from both the urban and rural within sentinel sites:

- San Pedro and Abidjan, belonging to the Guinean bioclimatic zone were characterized by hyper-ombrophilous rainy forest. The average annual rainfall is between 1400 mm and 2000 mm, with two rainy seasons in a year (April to July and September to November) with an average temperature of 26±2 °C according to records from the local weather stations. The main agricultural practices include coffee, cocoa, rubber, palm and coconuts. Breeding sites investigated at San Pedro and three localities selected within the economic capital city, Abidjan, (i.e. Yopougon, Port-Bouët and Bingerville) were situated in urban or peri-urban areas.
- Man, is a city located in the Western zone of the country consisting of tropical rainy forest with hills and mountains. It is characterized by a single rainy season starting in February up to November. The average annual rainfall ranged from 1600 mm to 2500 mm and the annual temperature averaged 24.5 °C. Coffee and cocoa are grown but at a small scale. There are valleys suitable for the production of rice. A nearby rural area, Zele, with intense farming was also considered for investigation.
- Abengourou, situated in the Eastern part of Côte d’Ivoire has a Guinean bioclimatic profile too. It has one rainy season with an average annual rainfall between 1200 mm and 1700 mm and an annual average temperature of 25.8 °C. Coffee and cocoa are the main cash crops produced in the area. The breeding sites surveyed in Abengourou are all from urban areas.
- Yamoussoukro, located in the centre of the country is a transitional zone characterized by a wet savannah. This region has one rainy season and an average annual rainfall of 1200 mm and an annual average temperature of 25.8 °C.
- Korhogo, in the North is a city located in a sub-Sudanian bioclimatic zone. The rainy season generally starts between May-June, peaks in August and ends in late October. The mean annual rainfall generally plateaus at 1200 mm with temperature varying over the seasons and the bioclimatic zone matching the savannah type. Cotton is the major commercial cash crop grown in this area. Some vegetables, rice and mangoes are also grown but at a small scale. Kaforo a nearby rural area where cotton is also intensively produced was included.

Mosquito collection

The sampling of *An. gambiae* larvae was done between June and July 2012 at the different sentinel sites (urban and rural). Larvae were collected from natural breeding sites such as ponds, puddles, footprints maintained by rainfall, and in rice and vegetable farms. They were then brought to the insectary at Institut Pierre Richet (IPR) and reared to adults.

Insecticide susceptibility tests

Bioassays on adult mosquitoes were conducted using WHO test kits [21]. Filter papers were impregnated with diagnostic concentrations of eight insecticides from 4 distinct classes as follows:

- Pyrethroids: permethrin (0.75%), deltamethrin (0.05%) and α-cypermethrin (0.05%);
- Pseudo-pyrethroid: etofenprox (0.05%);
- Organochlorides: Dichlorodiphenyldichloroethane (DDT) (4%) and dieldrin (4%);
- Carbamates: carbosulfan (0.4%);
- Organophosphate: pyrimiphos-methyl (1%).

Bioassays were performed with batches of 25 unfed females of *An. gambiae*, 2–3 days old (four replicates per concentration). Mosquitoes were exposed to insecticide-impregnated papers for 60 min at 25±2 °C and 80% relative humidity (RH). At the end of the exposure period, mosquitoes were transferred to observation
Insecticide Resistance in Ivorian Malaria Vectors

tubes, supplied with 10% honey solution and held for 24 h before scoring delayed mortality. Batches of mosquitoes from each site exposed to untreated papers were used as negative controls.

In order to assess the involvement of detoxifying enzymes in the resistance phenotypes, complementary tests were performed with a 1 hour pre-exposure to piperonyl butoxide (PBO), an inhibitor of multiple function oxidases (MFO) and non-specific esterases (NSE). The wild mosquito populations were compared to the susceptible reference strain of An. gambiae s.s. Kisumu. All control survivor specimens (including the susceptible reference mosquito) were stored at −80°C for biochemical analysis. All samples exposed to the different insecticides were kept individually with silicagel at −20°C for molecular analysis.

Identification of sibling species and M and S molecular of An. gambiae s.s.
Ribosomal DNA was extracted from individual mosquitoes following Collins et al [22] and used for polymerase chain reaction (PCR) analysis to determine the species from the An. gambiae complex following Scott et al [23] and M or S molecular form of An. gambiae s.s. according to Favia et al [24].

PCR detection of the L1014F and L1014S kdr and G119S ace-1 mutations
The presence of L1014F and L1014S kdr alleles was assessed using hot oligonucleotide ligation assay (HOLA) technique according to the protocol of Lynd et al [25]. The PCR-RFLP diagnostic test was used to detect the presence of G119S mutation (ace-1 gene) as described by Weill et al [26].

Biochemical analysis
Biochemical assays were performed to compare the amount of MFO and the activity levels of NSE and GST) of Kisumu and the wild mosquito populations. When less than 80% mortality was observed the population was considered ‘susceptible’, between 80% and 97% mortality was considered ‘resistant’ (or ‘suspected of resistance’) and when the mortality was above 98% the population was considered ‘susceptible’.

Data analysis
WHO criteria [28] were adopted to define the resistance status of the mosquito populations. When less than 80% mortality was observed the population was considered ‘susceptible’, between 80% and 97% mortality was considered ‘resistant’ (or ‘suspected of resistance’) and when the mortality was above 98% the population was considered ‘susceptible’.

Results
Species and molecular forms of An. gambiae in Côte d’Ivoire
Table 1 illustrates the distribution of M and S An. gambiae s.s. molecular forms at the different sentinel sites. In 318 mosquito samples analysed for sibling species identification across the ecological sentinel sites, only An. gambiae s.s. were found. The S molecular form was predominant in the sub-Saharan area in the north both in urban and rural areas (71.9% in Korhogo and 65.6% in Kaforo) and in the rural area of Zele (78.1%) in the Western part of Côte d’Ivoire. The distribution in the neighbouring urban area of Man was different, the S form representing 43.3% only of the An. gambiae population. By contrast, in the pre-forested (Yamoussoukro) and the rain forest areas (San Pedro, Bingerville, Yopougon, Port-Bouet), the M form was predominant (65.6% in Bingerville and 100% in San-Pedro, Yopougon and Port-Bouet).

Table 1. Description of sampling sites, agricultural practices, rural/urban status, bioclimatic zones and the distribution of the molecular forms of An. gambiae s.s.

| Localities       | Bio-climate status | Longitude | Latitude | Status | Main agricultural practices | Molecular forms | N |
|------------------|--------------------|-----------|----------|--------|-----------------------------|-----------------|---|
| Korhogo          | sub-sudannian      | −5.626144 | 9.450654 | urban  | Rice, cotton, vegetables    | S (71.9) | 32|
| Kaforo           | sub-sudannian      | −5.685582 | 9.277895 | rural  | Rice, cotton, vegetables    | M (28.1) | 32|
| Yamoussoukro     | pre-forest         | −5.662728 | 6.820336 | urban  | Rice, vegetables            | S (16.1) | 32|
| Man              | rain forest, mountains | −7.562108 | 7.412094 | urban  | Cocoa, coffee, rice         | S (43.8) | 32|
| Zele             | rain forest, mountains | −7.58967  | 7.36381  | rural  | Cocoa, coffee, rice         | M (56.2) | 32|
| Abengourou       | rain forest         | −3.496321 | 6.729096 | urban  | Cocoa, coffee, rice         | S (9.4)  | 32|
| San-Pedro        | rain forest         | −6.616667 | 4.733333 | urban  | Rubber, palm trees, coconut palms, vegetable | M (100) | 32|
| Bingerville      | rain forest         | −3.90043  | 5.35929  | urban  | Vegetables                  | S (34.4) | 32|
| Port-Bouet       | rain forest         | −3.919482 | 5.24823  | urban  | Vegetable, horticultural    | M (65.6) | 32|
| Yopougon         | rain forest         | −4.021887 | 5.324857 | urban  | Vegetables                  | S (100) | 32|
Resistance status

Figure 2 shows the insecticide resistance status of *An. gambiae* populations from the sentinel sites compared with the susceptible reference strain Kisumu. All insecticides tested killed 99–100% of susceptible mosquitoes indicating the accuracy of the active ingredient deposits on the filter papers used for the bioassays. *An. gambiae* populations from all sites showed strong resistance to permethrin and DDT with some levels of tolerance to permethrin observed in the urban areas of Man, San-Pedro and Yopougon.

![Insecticidal effects of diagnostic concentrations of insecticides against Anopheles gambiae mosquitoes from different sentinel sites (60 min contact in WHO tube tests).](http://doi:10.1371/journal.pone.0082387.g002)
Dieldrin resistance was reported in the few representative sites tested (Man, Yopougon, Yamoussoukro and Kaforo) (Fig. 2). Populations from all 10 sites were more vulnerable to deltamethrin than any other insecticides tested; mortality ranged from 74–100% between locations, with Korhogo and Kaforo (cotton areas) showing susceptibility (90–100% mortality) despite they displayed strong resistance to permethrin and DDT.

There was resistance to alpha-cypermethrin at all sites, except populations from Yopougon. A high frequency of resistance to etofenprox was recorded in all populations of An. gambiae s.s. (Fig. 2).

An. gambiae s.s. was strongly resistant to carbofuran (14–52% mortality) at all site but showed reduced susceptibility to pyrimiphos-methyl in Yamoussoukro, Korhogo, Yopougon and Port-Bouet (66–87%) and full susceptibility in Abengourou and Man (99–100%).

Detection of resistance genes by PCR

The frequency of the kdr mutations was investigated in every sample from all sentinel sites. The distribution of the L1014F kdr mutation in the molecular forms of An. gambiae s.s. populations is shown in Table 2. The L1014F kdr was detected in both M and S molecular forms. Its average frequency ranged from 0.47 to 0.86 with the highest values (>0.70) reported in the coastal localities (Bingerville, Port-Bouet, San-Pedro) and the areas with cotton in the North (Korhogo, Kaforo). No L1014F kdr type was detected in all samples analysed [N = 317].

The G119S mutation conferring resistance to organophosphates and carbamates was detected in 4 localities out of 6 in both molecular forms (Table 3). The M and S specimens carrying the mutation were heterozygotes, except for 5 specimens out of 26 (19.2%) found homozygous in Korhogo in the North and 1 out of 26 (3.8%) in Man in Western Côte d’Ivoire. Based on the Hardy-Weinberg equilibrium, this excess of heterozygous supports the presence of a duplicated allele (ace-1D) in An. gambiae s.s. from the sentinel sites. Table 3 shows the predicted distribution of the ace-1D mutation in the S and M forms of An. gambiae s.s. The presence of the ace-1D mutation in both M and S forms of An. gambiae s.s. from all sites except Abengourou and San-Pedro (Table 3). It is only significantly supported (after Bonferroni correction) in M samples from Port-Bouet and S from Bingerville, the model suggesting a frequency around 0.47 for those from Port-Bouet (p<0.01) and 0.57 from Bingerville (p = 0.01).

Synergist and biochemical analysis

Synergist. Figure 3 shows the toxicity of permethrin and carbofuran with and without PBO against An. gambiae s.s. from the sentinel sites. Pre-exposure of mosquitoes to PBO significantly increased the mortality rates to permethrin from all locations (p<0.05) except Yopougon (p = 0.539) (Figure 3a). Interestingly, PBO increased mortality to permethrin to full susceptibility level in populations from Korhogo (from 39% to 100%) and Abengourou (62% to 97%), suggesting that the resistance phenotype in these two areas is almost entirely mediated by the metabolic activities of MFOs and NSE.

Resistance to carbofuran was largely pronounced across sites, mortality ranging from 14% to 63%. Pre-exposure of mosquitoes to PBO significantly increased mortality rates to carbofuran at all sites but resistance was not fully synergised by this MFO and NSE inhibitor, inferring a residual role of additional mechanisms, including the ace-1 gene insensitivity.

Biochemical assays. Table 4 compares the mean amount of MFO and the mean activities of the NSE and GST found in An. gambiae s.s. from the sentinel sites relative to the levels in the susceptible An. gambiae Kisumu.

All wild samples analyzed displayed significantly higher levels of esterase activity (using β-naphtyl acetate as a substrate) than that measured for Kisumu (p<0.05), except samples from Abengourou and Zele. The trend in activity for assays using β-naphtyl acetate as a substrate was similar to the α-naphtyl assays. The highest α- and β-esterase activities (>2.5-fold) were recorded on the coast (Yopougon, Port-Bouet) and the cotton growing area of Korhogo.

The production of MFO (cytochrome P450s) amount in An. gambiae from all sites but not Zele was significantly higher than the content measured in Kisumu (p<0.05), with broadly similar mean levels of activity expressed between locations.

The GST levels of activity in Yopougon, Port-Bouet, Korhogo, Yamoussoukro and Man populations were significantly higher than in Kisumu and the other field populations (p<0.05).

---

Table 2. Distribution of genotypes and allelic frequency of the L1014F kdr mutation in An. gambiae s.s. populations from sentinel sites.

| Molecular form   | M     | S     | n1 | n2 | n1+n2 | f(R) |
|------------------|-------|-------|----|----|-------|------|
|                  | SS    | RS    | RR | SS | RS    | RR   | f(R) |
| Bingerville      | 21    | 1     | 3  | 17 | 0.881 |      |      |
| Port-Bouet       | 31    | 3     | 3  | 25 | 0.855 |      |      |
| Yopougon         | 31    | 2     | 29 | 0  | 0.468 |      |      |
| Yamoussoukro     | 26    | 4     | 17 | 3  | 0.479 | 5    | 0    | 5    | 0.500 | 11   | 2    | 3    | 6    | 0.682 | 32   | 0.813 |
| Korhogo          | 9     | 0     | 5  | 4  | 0.722 |      |      |
| Kaforo           | 10    | 0     | 4  | 6  | 0.800 |      |      |
| Man              | 15    | 2     | 13 | 0  | 0.433 |      |      |
| Zele             | 7     | 0     | 7  | 0  | 0.500 |      |      |
| San-Pedro        | 32    | 4     | 10 | 18 | 0.719 |      |      |
| Abengourou       | 29    | 3     | 22 | 4  | 0.517 |      |      |

n: number analysed; f(R): frequency of the mutation.
Overall a patchy distribution of overproduced quantities of NSE, MFO and GST was found in most *An. gambiae* s.s. populations analysed relative to the normal strain Kisumu; the most affected areas being on the coastal urban part of the country (Yopougon and Port-Bouët with vegetables and horticulture) and Korhogo in the North where cotton is produced.

### Discussion

A nation-wide survey of insecticide resistance in *An. gambiae* s.s. in Côte d’Ivoire was conducted in order to have an overview of the resistance status and establish a baseline dataset that would guide the National Malaria Control Programme.

The bioassays data showed high resistance levels of *An. gambiae* s.s. to organochlorides, carbamates and pyrethroids but at a lesser extent toward deltamethrin. The resistance levels to the organophosphate (pyrimiphos-methyl) varied greatly from susceptibility to resistance across sites. The *kdr* and ace-1R mutations were highly expressed in all *An. gambiae* s.s. populations with the exception for the ace-1R in samples from Abengourou and San-Pedro. The ace-1D duplication was present in Bingerville and Port-Bouët respectively in S and M molecular forms confirming previous finding highlighting its presence in Côte d’Ivoire [31,32]. Its spread seemed to be wider as it was suspected in the urban areas of Yopougon, Yamoussoukro and Man although the low sample size did not allow to confirm it.

The MFO quantities and, NSE and GST activities in almost all *An. gambiae* populations were significantly higher than in the susceptible strain Kisumu and the bioassays data with PBO indicated that at least the MFO and NSE were involved in the phenotypic expression of the resistance.

#### Table 3. ace-1 allele frequency distribution in *An. gambiae* s.s. molecular forms from Côte d’Ivoire.

| Localities | M molecular form | S molecular form |
|------------|------------------|------------------|
|             | N   | S    | R   | D   | FHW | N   | S    | R   | D   | FHW |
| Bingerville| 20   | 0.74 | 0.00 | [0-0.301] | 0.26 | [0-0.420] | 0.10 | 11   | 0.43 | 0.00 | [0-0.400] | 0.57 | [0.182-0.819] | 0.01* |
| Port-Bouët | 32   | 0.53 | 0.00 | [0-0.241] | 0.47 | [0.251-0.617] | 0.00* | 0    |      |      |      |      |      |      |
| Yopougon   | 32   | 0.81 | 0.00 | [0-0.235] | 0.19 | [0-0.304] | 0.13 | 0    |      |      |      |      |      |      |
| Yamoussoukro| 26  | 0.85 | 0.00 | [0-0.225] | 0.15 | [0-0.262] | 0.30 | 5    | 0.89 | 0.00 | [0-0.367] | 0.11 | [0-0.390] | 0.74 |
| Korhogo    | 8    | 0.94 | 0.00 | [0-0.245] | 0.06 | [0-0.255] | 0.80 | 23   | 0.72 | 0.28 | [0.122-0.422] | 0.00 | [0-0.228] | 1.00 |
| Kaforo     | 9    | 0.61 | 0.39 | [0.180-0.617] | 0.00 | [0-0.299] | 1.00 | 21   | 0.76 | 0.22 | [0.053-0.379] | 0.03 | [0-0.264] | 0.82 |
| Man        | 17   | 0.87 | 0.00 | [0-0.241] | 0.13 | [0-0.269] | 0.46 | 14   | 0.85 | 0.00 | [0-0.284] | 0.15 | [0-0.325] | 0.41 |
| Zele       | 7    | 1.00 | 0.00 | [0-0.128] | 0.00 | [0-0.128] | 1.00 | 25   | 0.72 | 0.28 | [0.048-0.381] | 0.08 | [0-0.316] | 0.46 |
| San-Pedro  | 32   | 1.00 | 0.00 | [0-0.030] | 0.00 | [0-0.030] | 1.00 | 0    |      |      |      |      |      |      |
| Abengourou | 29   | 1.00 | 0.00 | [0-0.033] | 0.00 | [0-0.033] | 1.00 | 3    | 1.00 | 0.00 | [0-0.274] | 0.00 | [0-0.274] | 1.00 |

N: number analysed; Values in brackets represent the confidence interval at 95% S, R and D represent the estimated frequencies of the susceptible, resistant and duplicated alleles respectively. The probability of the departure from Hardy-Weinberg expectation ($F_{HW}$) is bold when significant and has a star when still significant after Bonferroni correction.

doi:10.1371/journal.pone.0082387.t003

![Figure 3. Insecticidal effects of diagnostic concentrations of one pyrethroid insecticide (permethrin) and one carbamate insecticide (carbosulfan) (60 min contact in WHO tube tests) with or without a 60 min pre-exposure to PBO.](https://doi.org/10.1371/journal.pone.0082387.g003)
This study provides a wider view of the spread of insecticide resistance in Côte d’Ivoire and adds a significant baseline knowledge to recent reports of high insecticide resistance levels in M’Bé [19], Yaokoffikro [20] near Bouaké in central Côte d’Ivoire, Tiasalé [18,33] and Adzopé [17] in southern Côte d’Ivoire. All these reports including the present highlight the level of spread of resistance to all class of insecticides deployed for vector control, either as LLIN already in place or IRS under consideration for implementation in Côte d’Ivoire; an increasing trend commonly shared by several countries in West Africa [34–39].

The association between agricultural practices and the build up of insecticide resistance has been intensively investigated. It is worth noticing that An. gambiae s.s. population from areas with massive cotton production (Korhogo, Kaforo) displayed the highest resistance levels against almost all insecticides classes (organochlorides, pyrethroids and carbamates) as previously reported [34,40–44]. Urban vegetable farming in Yopougon, Port-Bouet and Yamoussoukro areas was also associated with high insecticide resistance in such context are scarce [40,44] but where moderate to high resistance levels was found in An. gambiae [49].

There is a growing expansion of rubber production in Côte d’Ivoire and generally across part of Africa. So far reports on insecticide resistance in such context are scarce [50]. In-depth studies documenting the use of insecticides both socially and in the o agricultural sector is highly stressed in order to better appraise factors selecting and driving the evolution of insecticide resistance mechanisms [32].

The significance of the present study for the NMCP in Côte d’Ivoire raises an important question of whether to continue to deploy pyrethroid based LLINs and IRS towards which resistance continues to rise with no guarantee that the level of resistance seen in the country would not compromise their efficacy. No doubt it would be difficult to demonstrate the impact of resistance on the effectiveness of any of these interventions. There have been extensive randomized controlled trials (RCTs) (phase III) in part of Africa aiming at investigating the efficacy of ITNs for malaria prevention [14,51] but very few have assessed how pyrethroid resistance might affect the effectiveness of such intervention. RCTs entail a set of communities randomly divided into groups, one that receives the novel form of vector control intervention, and comparison arms that often receive the old form of vector control tools or nothing. The key difficulty is that it is impossible to address the question to whether vector control would produce a smaller reduction in malaria if the vector mosquitoes are resistant than it would have done if they were susceptible, using RCT methods. This is simply because resistance is not an easy factor that can be allocated randomly to some communities and not to others. The distribution of resistance is patchy and its severity seems to differ from one area (locality) to another as seen in the present study. Moreover there may be more resistance or survival trend of mosquitoes in some villages than others because of variations in the quality of vector control operations [52] or in mosquito behavior [53]. This means there is no straight forward solution to address resistance impact. Some researchers are currently striving to bring up to the market novel insecticides or combination of these with pyrethroids to circumvent insecticide resistance but this may take several years before they are approved and made available for use. In the short term, NMCPs could consider adopting intervention strategies that e.g. combine a synergist such as PBO with pyrethroids in LLINs. The technology is intended to alleviate the load of resistance by removing the metabolic component of resistance due to MFOs and NSE [54]. The well known commercialized prototypes PermaNet® 3.0 [55–58] and more recently Olyset Plus® [59] of this kind have proved to be highly active against pyrethroid resistant mosquitoes and could be the product of choice for NMCPs. Ultimately, where LLINs are already in place and vectors survive them because of pyrethroid resistance [12,13], NMCPs could consider spraying the homes with a non pyrethroid insecticide. In any event, insecticide combinations within homes for malaria control is an unavoidable reality. LLIN coverage is going universal and IRS with non-pyrethroid insecticides is being applied concurrently with LLINs as malaria control policy in many areas of high malaria transmission. Given the high resistance to carbamates detected across all sites but an appreciable level of susceptibility to the organophosphate, pyrimiphos methyl, it would be advisable to deploy this insecticide for IRS in combination with LLINs in Côte d’Ivoire. Recent

### Table 4. Mean level of NSE, MFO and GST activity in An. gambiae s.s. populations from the sentinel sites relative to the susceptible reference strain Kisumu.

| Localities | N | μmol o-naphthol/min/mg protein | AR | μmol β-naphthol/min/mg protein | AR | nmol P450/mg protein | QR | nmol GSH conj/min/mg protein | AR |
|------------|---|-------------------------------|----|-------------------------------|----|----------------------|----|--------------------------|----|
| Kisumu     | 40 | 0.086±0.007                   | 40 | 0.084±0.007                   | 38 | 0.095±0.008          | 40 | 0.295±0.032              |
| Yopougon   | 32 | 0.352±0.054                   | 32 | 0.313±0.058                   | 37 | 0.255±0.048          | 27 | 2.358±0.679              | 8  |
| Port-Bouet | 17 | 0.300±0.070                   | 35 | 0.365±0.171                   | 4.2| 0.236±0.044          | 25 | 1.065±0.308              | 3.6|
| Korhogo    | 32 | 0.340±0.052                   | 4.0| 0.206±0.031                   | 2.5| 0.209±0.059          | 22 | 1.236±0.354              | 4.2|
| Kaforo     | 15 | 0.155±0.036                   | 1.8| 0.105±0.027                   | 1.2| 0.330±0.052          | 15 | -                        |
| Yamoussoukro | 33 | 0.192±0.036                   | 2.2| 0.138±0.028                   | 1.6| 0.198±0.050          | 33 | 1.008±0.198              | 3.4|
| Abengourou | 32 | 0.036±0.011                   | 4.0| 0.043±0.009                   | 0.5| 0.251±0.075          | 2.6| 0.501±0.268              | 1.7|
| Mar        | 32 | 0.150±0.024                   | 1.7| 0.175±0.050                   | 2.1| 0.420±0.067          | 4.4| 0.664±0.142              | 2.3|
| Zele       | 32 | 0.107±0.016                   | 1.2| 0.089±0.014                   | 1.1| 0.163±0.037          | 1.7| 0.638±0.192              | 2.2|
| San-Pedro  | 40 | 0.152±0.011                   | 1.8| 0.178±0.024                   | 2.1| 0.216±0.028          | 3.6| 0.471±0.084              | 1.6|

N: total tested. Number in bold indicated samples where enzyme level or activity was significantly higher compared with Kisumu. (P<0.05) at the 5% level. AR: Activity Ratios. QR: Quantity ratios. GSH: Reduced form of glutathione.

doi:10.1371/journal.pone.0082387.t004
evaluation of this insecticide applied as IRS in experimental huts showed extremely high level of control of pyrethroid resistant mosquitoes [60].

Conclusion

The insecticide resistance data presented in the sentinel survey are of great interest for the NMCP of Côte d’Ivoire and beyond, to countries also facing similar rise in the level of pyrethroid resistance in their local vectors. Innovative strategies that combine insecticide and synergists in LLINs or spatially LLIN and effective insecticide for IRS within homes could be in the short term the best practice for NMCPs to manage insecticide resistance in malaria vectors in endemic countries.

Acknowledgments

Sincere thanks go to Dr Koffi San, former Head of the National Malaria Control Programme of Côte d’Ivoire and the research unit of NMCP (Dr Serge B Asso., Dr Florence-Judith Kadoji. We are very grateful to all the staff at the Institut Pierre Richet, Bouaké, Côte d’Ivoire, particularly Aboubacar Koné, for his hard work during the field and laboratory experiments. We also thank to Aboubacar Sidick from the Institut de Recherche pour le Développement (IRD)/Centre de Recherche Entomologique de Cotonou (CREC), Benin, for his technical assistance in biochemical and molecular experiments. We are very grateful to Dr Pierrick Labbé for his crucial help on the ace-1 frequency calculations. Special thanks also go to Bamomo Coulibaly for making the study site.

Author Contributions

Conceived and designed the experiments: AAK LPAA. Performed the experiments: LPAA JPJKK. Analyzed the data: AAK LPAA CP. Contributed reagents/materials/analysis tools: AAK CP. Wrote the paper: AAK LPAA RN CP.

References

1. WHO (2011) World Malaria Report. Geneva. 259 p.
2. WHO (2012) WHO recommended long-lasting insecticidal mosquito nets. Geneva. Available: http://www.who.int/whopes/Long_lasting_insecticidal_nets_Jul_2012.pdf p.
3. WHO (2009) WHO recommended insecticides for indoor residual spraying against malaria vectors. Geneva. Available: http://www.who.int/whopes/Insecticides_IRS_Malaria_09.pdf p.
4. Hansson H, N’Guessan R, Lines J, Moureux N, Nkuni Z, et al. (2010) Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol.
5. Lapié Y, Penuetier C, Apaire-Marchais V, Lézec P, Corbel V (2009) Innovative applications for insect viruses: towards insecticide sensitization. Trends Biotechnol 27: 190–198.
6. Rivero A, Veale JC, Weil M, Read AF, Gondos S (2010) Insecticide control of vector-borne diseases when is insecticide resistance a problem? PLoS Pathog 6.
7. Hemingway J, Ranson H (2000) Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 45: 371–391.
8. Raymond-Delpech V, Matsuda K, Sattelle DB, Raoult D, Sattelle DB (2003) Ion channels: molecular targets of neuroactive insecticides. Invert Neurosci 5: 119–133.
9. Hemingway J, Hawkes NJ, McCarroll L, Ranson H (2004) The molecular basis of insecticide resistance in mosquitoes. Insect Biochem Mol Biol 34: 633–663.
10. Corbel V, N’Guessan R, Beaugros C, Chandre F, Djogbenou L, et al. (2007) Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Trop 101: 207–216.
11. VectorBase (2012) AnoBase: the Anopheles database. Insecticide resistance functions in the mosquito. Available: http://anobase.vectorbase.org/ir/. Accessed 2012 Oct 23.
12. N’Guessan R, Corbel V, Akogbeto M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in a pyrethroid resistance area, Benin. Emerging Infectious Diseases 13: 199–206.
13. Assidi A, N’Guessan R, Akogbeto M, Curtis C, Rowland M (2012) Loss of insecticide resistance data presented in the sentinel survey of Anopheles gambiae populations from Côte-d’Ivoire, West Africa. Malar J 9: 167.
14. Badolo A, Traore A, Jones CM, Sanou A, Flood L, et al. (2012) Three years of Long Lasting Insecticidal Nets (LLINs) in Adzope, Eastern Côte-d’Ivoire. Emerg Infect Dis 18: 1508–1511.
15. PNLP_Côte_d’Ivoire (2012) Plan straté gique 2012–2015 du programme de lutte contre le paludisme de la Côte d’Ivoire. Abidjan. 110 p.
16. Edi CV, Koudou BG, Jones CM, Weetman D, Ranson H (2012) Multiple insecticide resistance in Anopheles gambiae s.s. populations from M’Be´, a WHOPES CDS/CPC/MAL/1998.12.
17. Le Normand T, Guillemaud T, Bourguet D, Raymond M (1998) Appearance of resistance to Long-Lasting Insecticidal Nets (LLINs) in Adzope, Eastern Côte-d’Ivoire. Parasit Vectors 5: 289.
18. Dabire RK, N’Guessan R, Sawadogo SP, Yaro LB, Toe HK, et al. (2012) Malaria control? Trends Parasitol.
19. Koffi AA, Alou LP, Chandre F, Diakite J, Bass C, Sawadogo SP, et al. (2012) First report of the L1014S kdr mutation in wild populations of Anopheles gambiae M and S molecular forms in Burkina Faso. Acta Trop.
20. Djogbenou L, Labbé P, Chandre F, Pasteur N, Weil M (2009) Ace-1 duplication in Anopheles gambiae: a challenge for malaria control. Malar J 8: 70.
21. WHO editor (2006) Guidelines for testing mosquito adulticides intended for Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs). WHO/CDS/CPC/MAL/1998.12.
22. Collins FH, Finneyt V, Petracca V (1988) Ribosomal DNA-genes differentiate five cryptic species in the Anopheles gambiae complex. Parasitologia 30: 231–249.
23. Scott JA, Brodgon WG, Collins FH (1995) Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. Am J Trop Med Hyg 49: 520–529.
24. Favia G, Lanfranociti A, Spars L, Siden Kianno I, Louis C (2001) Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s. Insect Molecular Biology 10: 19–23.
25. Lynd A, Ranson H, McCall PJ, Rambale NP, Black WC, et al. (2005) A simplified high-throughput method for pyrethroid knock-down resistance (kdr) detection in Anopheles gambiae. Malar J 4: 16.
26. Weil M, Malcolm C, Chandre F, Mogensen K, Berthoumieu A, et al. (2004) The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol Biol 13: 1–7.
27. WHO (1998) Techniques to detect resistance mechanisms (Field and laboratory manual). Geneva. 42 p.
28. WHO (1996) Tests for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces. WHO/CDS/CPC/MAL/1998.12.
29. Raymond M, Rousett F (1995) GENEPOP Version 1.2 A population genetics software for exact tests and ecumenicism. Journal of Heredity: 249–249.
30. N’Gouamba M, Simard F, Baldet T, Diabate A, Ouedraogo JB, et al. (2012) Prevention and control of malaria in Benin: Emerging Infectious Diseases 13: 199–206.
31. Dabire RK, N’Guessan R, Sawadogo SP, Yaro LB, Toe HK, et al. (2012) Multiple insecticide resistance in Anopheles gambiae s.s. from Côte-d’Ivoire, West Africa. BMC Infect Dis submitted.
32. Edi CV, Koudou BG, Jones CM, Weetman D, Ranson H (2012) Multiple insecticide resistance in Anopheles gambiae mosquitoes, Southern Côte d’Ivoire. Emerg Infect Dis 18: 1500–1511.
33. Dabire RK, N’Guessan R, Sawadogo SP, Yaro LB, Toe HK, et al. (2012) Population dynamics of Anopheles gambiae s.l. in Bobo-Dioulasso city: bionomics, infection rate and susceptibility to insecticides. Parasit Vectors 5: 127.
34. Djogbenou L, Labbé P, Chandre F, Pasteur N, Weil M (2009) Ace-1 duplication in Anopheles gambiae: a challenge for malaria control. Malar J 8: 70.
35. Badolo A, Traore A, Jones CM, Sanou A, Flood L, et al. (2012) Years of insecticide resistance monitoring in Anopheles gambiae in Burkina Faso: resistance on the rise? Malar J 11: 232.
36. Djebri RK, Namounoumou G, Savadogo SP, Yaro LB, Toe HK, et al. (2012) Population dynamics of Anopheles gambiae s.l. in Bobo-Dioulasso city: bionomics, infection rate and susceptibility to insecticides. Parasit Vectors 5: 127.
37. Namounoumou M, Simard F, Bala D, Diabate A, Ouedraogeb J, et al. (2012) Multiple Insecticide Resistance in Anopheles gambiae s.s. Populations from Burkina Faso, West Africa. PLoS ONE 7: e49412.
38. Alou LP, Koffi AA, Jones CM, Weetman D, Ranson H (2012) Update on resistance status of Anopheles gambiae s.s. to conventional insecticides at a previous WHO/PIE field site, “YaoSkollo” , 6 years after the political crisis in Côte d’Ivoire. Parasit Vectors 5: 68.
41. Yadouleton A, Martin T, Padonou G, Chandre F, Asidi A, et al. (2011) Cotton pest management practices and the selection of pyrethroid resistance in Anopheles gambiae population in northern Benin. Parasit Vectors 4: 60.
42. Chouaibou M, Etang J, Brevault T, Nwane P, Hinzoambwe CK, et al. (2008) Dynamics of insecticide resistance in the malaria vector Anopheles gambiae s.l. from an area of extensive cotton cultivation in Northern Cameroon. Trop Med Int Health 13: 476–486.
43. Muller P, Chouaibou M, Pignatelli P, Etang J, Walker ED, et al. (2008) Pyrethroid tolerance is associated with elevated expression of antioxidative and agricultural practice in Anopheles arabiensis sampled from an area of cotton fields in Northern Cameroon. Mol Ecol 17: 1145–1153.
44. Diabate A (2002) The Role of Agricultural Uses of Insecticides in Resistance to Pyrethroids in Anopheles gambiae s.l. in Burkina Faso. Am J Trop Med Hyg 67: 617–622.
45. Yadouleton A, N’Guessan R, Allagbe H, Asidi A, Boko M, et al. (2010) The impact of the expansion of urban vegetable farming on malaria transmission in major cities of Benin. Parasit Vectors 3: 118.
46. Tia E, Akogbeto M, Koffi A, Toure M, Adja AM, et al. (2006) [Pyrethroid and DDT resistance of Anopheles gambiae s.s. (Diptera: Culicidae) in five agricultural ecosystems from Cote d’Ivoire]. Bull Soc Pathol Exot 99: 270–282.
47. Antonio-Nkondjio C, Foussog BT, Ndo C, Djantio BM, Togouet SZ, et al. (2011) Anopheles gambiae distribution and insecticide resistance in the cities of Douala and Yaounde (Cameroon): influence of urban agriculture and pollution. Malar J 10: 154.
48. Jones CM, Toe HK, Sanou A, Namountougou M, Hughes A, et al. (2012) Additional selection for insecticide resistance in urban malaria vectors: DDT resistance in Anopheles arabiensis from Bobo-Dioulasso, Burkina Faso. PLoS ONE 7: e45995.
49. Bigoga JD, Ndangoh DN, Awono-Ambene PH, Patchoke S, Fondjo E, et al. (2012) Pyrethroid resistance in Anopheles gambiae from the rubber cultivated area of Niete, South Region of Cameroon. Acta Trop 124: 210–214.
50. Bigoga JD, Nfack FM, Awono-Ambene PH, Patchoke S, Atangana J, et al. (2012) Seasonal prevalence of malaria vectors and entomological inoculation rates in the rubber cultivated area of Niete, South Region of Cameroon. Parasit Vectors 5: 197.
51. Henry MC, Assi SB, Rojger C, Dossou-Yovo J, Chandre F, et al. (2005) Protective efficacy of lambda-cyhalothrin treated nets in Anopheles gambiae pyrethroid resistance areas of Cote d’Ivoire. Am J Trop Med Hyg 73: 839–864.
52. malERA, Aultman K, Burkom TR, Chandre F, Cortese M, et al. (2011) A research agenda for malaria eradication: vector control. PLoS Med 8: e1000401.
53. Moiroux X, Gomez M, Pennetier C, Elanga E, Djou midoua A, et al. (2012) Changes in Anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in Benin. J Infect Dis 206: 1622–1629.
54. Hemingway J (2000) The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. Insect Biochem Mol Biol 30: 1009–1015.
55. Koudou BG, Koffi AA, Malone D, Hemingway J (2011) Efficacy of PermaNet(R) 2.0 and PermaNet(R) 3.0 against insecticide-resistant Anopheles gambiae in experimental huts in Cote d’Ivoire. Malar J 10: 172.
56. Corbel V, Chabi J, Dahire RD, Etang J, Nwane P, et al. (2010) Field efficacy of a new mosaic long-lasting mosquito net (PermaNet 3.0) against pyrethroid-resistant malaria vectors: a multi centre study in Western and Central Africa. Malar J 9: 113.
57. N’Guessan R, Asidi A, Boko P, Odjo A, Akogbeto M, et al. (2010) An experimental hut evaluation of PermaNet(R) 3.0, a deltamethrin-piperonyl butoxide combination net, against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus mosquitoes in southern Benin. Trans R Soc Trop Med Hyg 104: 758–763.
58. Van Bortel W, Chinh VD, Berkvens D, Speybroeck N, Trung HD, et al. (2009) Impact of insecticide-treated nets on wild pyrethroid resistant Anopheles epiroctes population from southern Vietnam tested in experimental huts. Malar J 8: 248.
59. Pennetier C, Bouraima A, Chandre F, Paimai M, Etang J, et al. (2013) Efficacy of Olyset® Plus, a new long-lasting insecticidal net incorporating permethrin and piperonil-butoxide against multi-resistant malaria vectors. PLoS ONE submitted.
60. Rowland M, Boko P, Odjo A, Asidi A, Akogbeto M, et al. (2013) A new long-lasting indoor residual spray formulation of the organophosphate pirimiphos methyl for prolonged malaria vector control: an experimental hut trial against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus in Southern Benin. PLoS ONE 8: e69516.