Interaction Between Insecticide Resistance-Associated Genes and Malaria Transmission in Anopheles Gambiae S. L. During a Cluster-Randomized Controlled Trial of A “lethal House Lure” in Central Côte D’ivoire

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Research

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Abstract

Background

There is evidence that the Kdr L1014F and Ace-1R G119S mutations involved in pyrethroid and carbamate resistance in Anopheles gambiae influence malaria transmission in sub Saharan Africa. This is likely due to changes in behavior, life history, vectorial competence and capacity. In the present study, performed as part of a two-armed cluster randomized controlled trial (CRT) evaluating the impact of household screening plus a novel insecticide delivery system (In2Care EaveTubes), we investigated the distribution of insecticide target site mutations and their association with the infection status in wild An. gambiae s.l populations.

Methods

Mosquitoes were captured in 40 villages around Bouaké by human landing catches (HLC), from May 2017 to April 2019. Randomly selected sample of infected and uninfected An.gambiae s.l. with Plasmodium sp. were identified to species and then genotyped for Kdr L1014F and Ace-1R G119S mutations using quantitative polymerase chain reaction (qPCR) assays. The frequencies of the two alleles were compared between An. coluzzii and An. gambiae and then between infected and uninfected groups for each species.

Results

The presence of An. gambiae (49 %) and An. coluzzii (51%) was confirmed in Bouaké. Both species seemed to transmit equally Plasmodium parasites. Over the study period, the average frequency of the Kdr L1014F and Ace-1R G119S mutations did not vary significantly between study arms. However, the frequency of the Kdr L1014F and Ace-1R G119S resistance alleles were significantly higher in An. gambiae than in An. coluzzii (OR [95%CI]: 59.64 [30.81-131.63] for Kdr and OR [95%CI]: 2.79 [2.17–3.60], for Ace-1R). For both species, there were no significant differences in Kdr L1014F or Ace-1R G119S genotypic and allelic frequency distribution between infected and uninfected specimens (p > 0.05).

Conclusions

Either alone or in combination, Kdr L1014F and Ace-1R G119S showed no significant association with Plasmodium infection in wild An.gambiae and An. coluzzii, demonstrating similar competence for Plasmodium transmission in Bouaké. Additional factors influencing competence in natural population and those outside allele measurements contributing to resistance should be consider when establishing link between insecticide resistance and vector competence.

Introduction

Anopheles gambiae complex mosquitoes are the main malaria vectors in sub-Saharan Africa[1]. Its remarkable vectorial capacity[2] is largely due to its propensity to blood feed on human and rest indoors[3]. This great ability to adapt to human behaviour led to the development of insecticide-based vector control measures targeting indoor biting. These measures are primarily long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) and are used to limit the human-vector contacts and reduce mosquito survival[4]. These insecticide-based vector control tools have been highly effective against malaria vectors by considerable reductions in disease burden[5] However, long-term effectiveness of both strategies is threaten by the emergence of insecticide resistance in malaria vector populations[6, 7].

There are several mechanisms responsible for insecticide resistance of which metabolic and target site resistance are the most recurrent[8–10]. Metabolic resistance increases enzymes responsible for the insecticide degradation, while modification of the insecticide target site prevents the molecule from binding the site. The molecular basis of resistance mediated by target site mutations has been characterized in several mosquito populations[11–13]. For example, the G119S mutation in the Ace-1R gene (a single amino acid substitution, from a glycine to a serine at the locus 119 in the acetylcholinesterase catalytic site) is responsible for organophosphate and carbamate resistance among malaria vectors in West Africa[14]. Likewise, the L1014F gene also called the Kdr-west mutation (an amino acid substitution, from leucine to phenylalanine in the voltage gated sodium channel gene, at the 1014
locus typically causing knock down resistance (kdr) is responsible for pyrethroid and dichlorodiphenyltrichloroethane (DDT) resistance in mosquito populations[12].

Despite the rise of insecticide resistance, its operational significance has never been elucidated clearly. In many instances, insecticide-based tools seem to continue to protect against malaria[15–18] whereas a community trial of LLINs clearly demonstrated that resistance is having an impact [19]. Resistance is dynamic and therefore cannot be randomized to assess its epidemiological impact. Several studies have evaluated the association between single insecticide resistance genes mutation (Kdr or Ace-1R) and vectorial competence in An. gambiae[20–22]. Nevertheless, this were laboratory assays utilizing mosquito colonies or wild strains infected with malaria parasites in laboratories. The coexistence of both Kdr and Ace-1R in wild population of An gambiae s.l. is common in west Africa, including Côte d'Ivoire[23, 24]. The impact of such association on vectorial competence has never been studied.

We took advantage of a two-armed cluster randomized controlled trial evaluating the impact of household screening plus a novel insecticide delivery system (In2Care EaveTubes) to capture mosquitoes in study villages around Bouaké by human landing catches, between May 2017 and April 2019. Mosquitoes were identified to species and then genotyped for KdrL1014F and Ace-1RG119S mutations using quantitative polymerase chain reaction assays and the frequencies of the two alleles were compared between An. coluzzii and An. gambiae and then between infected and uninfected groups for each species.

**Methods**

**Study area**

The trial was conducted from May 2017 to April 2019 in central Côte d'Ivoire. The methodology used in this study has been well described by Sternberg et al. [25]. Briefly, forty (40) villages were identified within a 60 km radius within the district of Bouaké. All households in the study villages received insecticide treated nets while half of the study villages (20) received screening (S) plus In2Care eave tubes (ET).

**Mosquito collections and processing**

Mosquito collection process was initially described by Sterenberg et al. [25]. Each month, mosquitoes were sampled by human landing catches (HLC) both indoors and outdoors at four randomly selected houses in each of the 40 study villages. HLC were done per month during the trial, from 6 p.m to 8 a.m the following day. Mosquitoes collected were sorted and morphologically identified to species using key described by Gillies and Meillon [26] and counted. All malaria vectors stored for further analysis, but for the interaction study, only An. gambiae s.l., the main malaria vector in Côte d'Ivoire was considered.

**DNA extraction**

Polymerase chain reaction (PCR) assays were used to assess sporozoite prevalence in a monthly random sub-sample of up to 30 females per village. Mosquitoes were identified to sibling species and kdr L1014F and Ace-1RG119S mutations detected. Genomic DNA was extracted from the head and thorax of individual females using cetyl trimethyl ammonium bromide (CTAB) 2 % as described by Yahouedo et al.[27].

**Detection of Plasmodium infection**

Plasmodium spp. (P. malariae, P. falciparum, P. ovale, and P. vivax) infection was detected by quantitative real-time PCR according to Mangold et al.[28]. The sequences of the primers were synthesized and supplied by Eurofins Genomics (Ebersberg, Germany) and were as follows: forward PL1473F18 (5’-TAA CGA AGA ACG TCT TAA-3’) and reverse PL1679R18 (5’-GTT CCT CTA AGA AGC TTT-3’). The reactions were prepared in a total reaction volume of 10 µl, which contained 2 µl of 5x HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis Biodyne, Tartu, Estonia), 0.3 µl of each primer, 6.4 µl of sterile water, and 1 µl of DNA template. The real-time PCR mixture were preincubated at 95°C for 12 min followed by amplification for 50 cycles of 10 sec at 95°C, 5 sec at 50°C and 20 sec at 72°C with fluorescence acquisition at the end of each cycle. Characterization of the PCR product was performed with melt curve analysis of the amplicons (95°C for 60 sec, 60°C for 60 sec, then 60°C to 90°C for 1 sec, with fluorescence acquisition at each temperature transition. Plasmodium species were identified by melting curve generated at different temperatures (i.e., P. malariae: 73.5–75.5°C; P. falciparum: 75.5–77.5°C; P. ovale: 77.5 to 79.5°C and P. vivax: 79.5 to 81.5°C).
Species identification

A subsample of 1,392 An. gambiae s.l. (686 infected with Plasmodium sp. and 706 uninfected randomly selected) was analysed for molecular sibling species identification. The molecular identification was performed using the classic PCR assay according to Favia et al.[29] The primers were R3 (5'-GCC AAT CCG AGC TGA TAG CGC-3'), R5 (5'-CGA ATT CTA GGG AGC AGC AGC-3'), Mopint (5'-GCC CCT TCC TCG ATG AGC T-3') and B/Sint (5'-ACC AAG ATG GTT CGT C-3'). The reaction mixture consisted of 14 µl of sterile water, 0.75 µl of each primer R3 and R5, 1.5µl of each primer Mopint and B/Sint, and 5 µl of the master mix. The reaction mixture of 23.5µl was distributed into 0.5ml PCR tubes along with 1µl of each DNA sample. Amplifications were performed on the MJ Research PTC-100 Thermal Cycler PCR machine (Marshall Scientific, Watertown, Massachusetts, USA) with cycling conditions of 95°C for 3 min, followed by 30 cycles at 95°C for 30 sec, 72°C for 45 sec and 72°C for 60 sec. Amplified fragments were analysed on a 2% agarose gel with 4µl of Sybr Green. The results were analysed as described in Favia et al.[29] to determine An. coluzzii (1300 bp band (R3/R5) + 727 bp band (Mop-int)) or An. gambiae (1300 bp band (R3/R5) + 475 Pb band (B/S-int)).

Detection of Kdr L1014F mutation in An. gambiae s.l.

Detection of the Kdr L1014F mutation was performed using the TaqMan real time PCR assay as described by Bass et al.[30]. The reactions were carried out in a total reaction volume of 10 µl, which contained 2 µl of the 5x HOT FIREPol® Probe Universal qPCR Mix (Solis Biodyne, Tartu, Estonia), 0.125µl primer/probe mix, 6.875 µl of sterile water, and 1 µl of DNA template.

Primers Kdr-Forward (5'-CATTTTTCTTGGCACCAGTGATGAT-3'), and Kdr-Reverse (5'-CGATCTTGGTCATGTTAATTTGCA-3') were standard oligonucleotides with no modification. The probes were labelled with two distinct fluorophores: VIC to detect the susceptible allele and FAM to detect the resistant allele. Amplifications were performed on the LightCycler® 96 Systems real-time qPCR machine (Roche LifeScience, Meylan, France) with cycling conditions of 95°C for 10 min, followed by 45 cycles at 95°C for 10 sec, 60°C for 45 sec and 72°C for 1 sec. FAM and VIC fluoroscences were captured at the end of each cycle and genotypes were called from endpoint fluorescence using the LightCycler® 96 software (Roche LifeScience, Meylan, France) for results analysis.

Detection of Ace-1 R G119S mutation in An. gambiae s.l.

Allelic and genotypic frequencies for insensitive acetylcholinesterase phenotypes characterized by the G119S mutation were determined in An. gambiae s.l., using the TaqMan assay, according to Bass et al.[31]. The reactions were carried out in a total reaction volume of 10 µl, which contained 2 µl of the 5x HOT FIREPol® Probe Universal qPCR Mix (Solis Biodyne, Tartu, Estonia), 0.125µl primer/probe mix, 6.875 µl of sterile water, and 1 µl of DNA template. Primers Ace-1-Forward (5'-GGC CGT CAT GCT GTG GAT-3'), and Ace-1-Reverse (5'-GCG GTG CCG GAG TAG A-3') were standard oligonucleotides with no modification. The probes were labelled with two distinct fluorophores: VIC to detect the susceptible allele and FAM to detect the resistant allele. Amplifications were performed on the LightCycler® 96 Systems real-time qPCR machine (Roche LifeScience, Meylan, France) with cycling conditions of 95°C for 10 min, followed by 55 cycles at 92°C for 15 sec, 60°C for 60 sec and 72°C for 1sec. FAM and VIC fluoroscences were captured at the end of each cycle and genotypes were called from endpoint fluorescence using the LightCycler® 96 software (Roche LifeScience, Meylan, France) for results analysis.

Statistical analysis

To analyse the distribution of Kdr L1014F and Ace-1 R G119S genotypic and allelic frequencies, data collected in the same study arm between May 2017 and April 2019 were compared between species. The association between genotypic and allelic frequencies for these mutations and infection status were determined using Pearson Chi-square test in R software (version 4.0.3). Both Kdr L1014F and Ace-1 R G119S combined genotypic frequencies distribution within infection status in each species were also included. The Fisher test was used when individual number available for test was less than 30. The significance threshold was set at 5%. Odds ratios were computed to assess the strength of difference or association between resistance alleles and infection status. The allelic frequencies were tested to Hardy-Weinberg equilibrium (HWE) conformity using the Exact HW test and calculated as follows:

\[
R \text{ allelic frequency} = \frac{RS + 2(RR)}{2(RS + RR + SS)}
\]
NB: Kdr L1014F and Ace-1 R G119S mutations comprise three genotypes expressing different allelic variants on the targeted loci. RR indicates the resistant homozygous genotype; RS, the heterozygous genotype and SS, the susceptible homozygous. The resistant (R) and susceptible (S) alleles are possible versions of these genes.

Ethical clearance

Ethical approval was obtained from the Côte d’Ivoire Ministry of Health ethics committee (ref: 039/MSLS/CNER-dkn), the Pennsylvania State University Human Research Protection Program under the Office for Research Protections (ref.:STUDY00003899 and STUDY00004815), and the London School of Hygiene and Tropical Medicine ethical review board (No. 11223). Verbal and written informed consents, using local language, was obtained from all participants (mosquito collectors and household heads) prior to their enrolment in the study. Mosquito collectors were vaccinated against yellow fever and the project provided treatment of confirmed malaria cases free of charge for any study participant according to national policies.

Results

Genotypic and allelic frequency distribution in Anopheles gambiae s.l. species

Out of 1,392 mosquitoes analysed in PCR, 1255 were successfully identified to species (<10% failure rate). Both An. gambiae (n = 624; 49.7%) and An. coluzzii (n = 631; 50.3%) were found. For each species, the proportions of infected vs uninfected were similar (Fig. 1). There were no significant differences in the allelic frequency of Kdr or Ace-1R between the control and Eave tube areas for each species (p>0.05) (Table 1).

Table 1
Kdr L1014F and Ace-1R G119S allelic frequencies between study arms

|        | Kdr L1014F | Ace-1R G119S |        |
|--------|------------|-------------|--------|
|        | N          | SS          | RS     | RR     | R (%) | N          | SS          | RS     | RR     | R (%) | χ² (P-value) |        | χ² (P-value) |
| An. coluzzii |           |             |        |        |       |             |             |        |        |       |             |        |             |
| Control | 421        | 35          | 182    | 204    | 70.10 | 0.15       | 420          | 356    | 52     | 12     | 9.05    | 1.79  |
| SET    | 210        | 21          | 89     | 100    | 68.81 (0.69) | 210          | 184    | 24     | 2      | 6.67    | (0.195) |
| An. gambiae |          |             |        |        |       |             |             |        |        |       |             |        |             |
| Control | 395        | 1           | 4      | 390    | 99.24 | 3.87.10⁻²⁸ | 394          | 264    | 94     | 36     | 21.07   | 3.29  |
| SET    | 229        | 0           | 3      | 226    | 99.34 (1) | 228          | 168    | 44     | 16     | 16.67   | (0.069) |

N = number of mosquitoes. SET: Screening plus In2Care Eave Tubes

Distribution of Kdr L1014F and Ace-1R G119S mutations

Genotypic and allelic frequencies of Kdr L1014F and Ace-1R G119S genes for An. coluzzii and An. gambiae are shown in Table 2.
Table 2
Genotypic and allelic frequencies of Kdr L1014F and Ace-1R G119S genes in An. gambiae and An. coluzzii

| SNP/species       | N  | Genotypic frequencies (%) | Allelic frequencies (%) | OR [95%CI] | HWE χ² (P value) |
|-------------------|----|---------------------------|-------------------------|------------|-----------------|
| Kdr L1014F        |    | RR                        | RS                      | SS         | R               | S             |
| An. coluzzii      | 631| 304(48.18)                | 271(42.95)              | 56(8.87)   | 879 (69.65)     | 383 (30.35)  |
|                   |    | 1 (0.16)                  | 1232(99.28)             | 9 (0.72)   | 59.64[30.81-131.63] | 6.96(0.008) |
| An. gambiae       | 624| 616(98.72)                |                         |            |                 |               |
|                   |    | 7(1.12)                   | 1232(99.28)             | 9 (0.72)   | 59.64[30.81-131.63] | 6.96(0.008) |
| Ace-1R G119S      |    | RR                        | RS                      | SS         | R               | S             |
| An. coluzzii      | 630| 14 (2.22)                 | 76 (12.06)              | 540(85.72) | 104 (8.25)      | 1156(91.75)  |
|                   |    | 23.66(< 0.001)            |                        |            |                 |               |
| An. gambiae       | 622| 52 (8.36)                 | 138(22.19)              | 432(69.45) | 250 (20.10)     | 994(79.90)   |
|                   |    | 2.79 [2.17–3.60]          |                        |            |                 |               |

For the genotypic frequency distribution, values between An. coluzzii and An. gambiae species were significantly different (p < 0.001). Degree of freedom for Chi square (df) = 2, OR: odds ratio, HWE: Hardy-Weinberg Equilibrium, CI: confidence interval. N: number of mosquitoes, SNP: Single Nucleotide Polymorphism.

Kdr allelic frequency was significantly greater in An. gambiae than in An. coluzzii (OR [95%CI]: 59.64 [30.81-131.63]) (Table 2). By contrast, the frequency of heterozygous individuals was significantly higher in An. coluzzii (42.95%) than in An. gambiae (1.12%), indicating deviation from Hardy-Weinberg expectations in An. gambiae populations with excess of resistant homozygous genotypes (Table 2) (p < 0.001).

The allelic frequency of Ace-1R G119S mutation was detected at lower rate in both An. coluzzii and An. gambiae although it was significantly more prevalent in An. gambiae than in An. coluzzii (OR [95%CI]: 2.79 [2.17–3.60]). Deviation from Hardy-Weinberg expectations for Ace-1R G119S was observed within both An. gambiae and An. coluzzii populations.

**Insecticides resistance genes and infection status**

Genotypic and allelic frequencies of Kdr L1014F and Ace-1R G119S genes among infected and uninfected individuals are shown in Table 3. Regardless of the species and study arms, there were no significant differences in genotypic or allelic frequencies between infected and uninfected individuals (p ≥ 0.05) (Table 3).
Table 3
Genotypic and allelic frequencies of *Kdr* L1014F and *Ace-1*<sup>R</sup> G119S genes between infected and uninfected *An. gambiae s.l.* species

| Species       | Study arm | SNP/status | N   | Genotypic frequencies (%) | Allelic frequencies (%) | OR [95%CI] |
|---------------|-----------|------------|-----|---------------------------|------------------------|------------|
|               |           |            |     | *Kdr* L1014F              |                        |            |
| *An. coluzzii*| Control   | Infected   | 213 | RR 102(47.89) RS 96(45.07) SS 15(7.04) | R 300(70.42) S 126(29.58) | 1          |
|               |           | Uninfected | 208 | RR 102(49.04) RS 86(41.35) SS 20(9.62) | R 290(69.71) S 126(30.29) | 1.03 [0.76–1.38] |
|               | SET       | Infected   | 92  | RR 40(43.48) RS 46(50.00) SS 6(6.52) | R 126(68.48) S 58(31.52) | 1          |
|               |           | Uninfected | 118 | RR 60(50.85) RS 43(36.44) SS 15(12.71) | R 163(69.07) S 73(30.93) | 0.97 [0.62–1.5] |
| *An. gambiae* | Control   | Infected   | 187 | RR 183(97.86) RS 3(1.60) SS 0(0.53) | R 369(98.66) S 5(1.35) | 1          |
|               |           | Uninfected | 208 | RR 207(99.52) RS 1(0.47) SS 0(0.00) | R 415(99.76) S 1(0.24) | 0.17 [0.003–1.6] |
|               | SET       | Infected   | 119 | RR 117(98.32) RS 2(1.68) SS 0(0.00) | R 236(99.16) S 2(0.84) | 1          |
|               |           | Uninfected | 110 | RR 109(99.1) RS 1(0.9) SS 0(0.00) | R 219(99.55) S 1(0.45) | 0.53 [0.009–10.4] |
|               |            |            |     | *Ace-1*<sup>R</sup> G119S       |                        |            |
| *An. coluzzii*| Control   | Infected   | 213 | RR 4(1.88) RS 23(10.80) SS 186(87.32) | R 31(7.28) S 395(92.72) | 1          |
|               |           | Uninfected | 207 | RR 8(3.86) RS 29(14.01) SS 170(82.13) | R 45(10.87) S 369(89.13) | 0.64 [0.38–1.06] |
|               | SET       | Infected   | 92  | RR 0(0.00) RS 9(9.78) SS 83(90.22) | R 9(4.89) S 175(95.11) | 1          |
|               |           | Uninfected | 118 | RR 2(1.69) RS 15(12.71) SS 15(85.60) | R 19(9.05) S 217(91.95) | 0.58 [0.22–1.40] |
| *An. gambiae* | Control   | Infected   | 186 | RR 15(8.06) RS 42(22.58) SS 129(69.35) | R 72(19.32) S 300(80.64) | 1          |
|               |           | Uninfected | 208 | RR 21(10.10) RS 52(25.00) SS 135(64.90) | R 94(22.60) S 322(77.40) | 0.82 [0.57–1.17] |
|               | SET       | Infected   | 119 | RR 7(5.88) RS 25(21.01) SS 87(73.11) | R 39(16.39) S 199(83.61) | 1          |
|               |           | Uninfected | 109 | RR 9(8.26) RS 19(17.43) SS 81(74.31) | R 37(16.97) S 181(83.03) | 0.95 [0.56–1.62] |

For the genotypic frequency distribution, values between infected and uninfected groups did not differ significantly (p > 0.05).

Degree of freedom for the Chi square (df) = 2, OR: odds ratio, SET: Screening plus In2Care Eave Tubes, CI: confidence interval. N: number of mosquitoes, SNP: Single Nucleotide Polymorphism.

**Frequencies of combined Kdr and Ace-1<sup>R</sup> genotypes and infection status**

There are nine possible combinations for the *Kdr* L1014F and *Ace-1*<sup>R</sup> G119S mutations that were analysed in this study (Fig. 2). For all combined genotypes, the two first alleles refer to *Kdr* genotypes whereas the two last alleles refer to *Ace-1*<sup>R</sup> genotypes: (1) Kdr-Ace-1<sup>R</sup>(RRRR), (2) Kdr-Ace-1<sup>R</sup>(RRRS), (3) Kdr-Ace-1<sup>R</sup>(RRSS), (4) Kdr-Ace-1<sup>R</sup>(RSRR), (5) Kdr-Ace-1<sup>R</sup>(RSRS), (6) Kdr-Ace-1<sup>R</sup>(RSSS), (7) Kdr-Ace-1<sup>R</sup>(SSRR), (8) Kdr-Ace-1<sup>R</sup>(SSRS), (9) Kdr-Ace-1<sup>R</sup>(SSSS). Figure 2 showed that in areas where *Kdr* and *Ace-1*<sup>R</sup> coexist in *An. gambiae s.l.*, the frequency of individuals bearing the Kdr RR genotype was significantly higher in *An. gambiae* than *An. coluzzii* and
this was observed in both control and SET areas. By contrast, the frequencies of those bearing the \textit{Kdr} heterozygous genotype were significantly higher in \textit{An. coluzzii} than in \textit{An. gambiae}, confirming the trend in isolation of this genotype (Fig. 2). Overall, there were no significant differences between infected and uninfected for each combined genotypes in \textit{An. coluzzii} or \textit{An. gambiae}.

Discussion

This study evaluated the effects of the \textit{Kdr} L1014F and \textit{Ace-1$^R$} G119S genes on \textit{Plasmodium sp.} infection status in natural \textit{An. gambiae s.l.} populations. The presence of both \textit{An. coluzzii} and \textit{An. gambiae} in similar proportions in this longitudinal study was consistent with previous studies in the area of Bouaké\cite{24, 32} but it contrasts with another study conducted in adjacent areas within Bouaké which found \textit{An. coluzzii} to be predominant \cite{33}. The difference observed is likely due to the study sampling period covering both rainy and drying seasons in our study compared to rainy season only\cite{33}. We observed no difference in infection rate between \textit{An. gambiae} and \textit{An. coluzzii}. This aligns with previous studies conducted in Burkina Faso and Senegal \cite{21, 34}, which reported equivalent \textit{Plasmodium} susceptibility to these species. Our current results demonstrate that both sibling species are equally dangerous vectors of human malaria in the central region of Côte d'Ivoire.

With regard to resistance genes, there were no significant differences in the allelic frequency of \textit{Kdr} or \textit{Ace-1$^R$} between the control and Eave tube areas regardless of the species. This is because \textit{Kdr} was already close to fixation in \textit{An. gambiae s.l.} species prior to the eave tubes intervention (>80%)\cite{24} leaving tiny window for further selection. Also, the insecticide deployed in the eave tube trial was a pyrethroid (beta-cyfluthrin) \cite{35} which could not induce a selection pressure on the \textit{Ace-1$^R$} since this gene is associated with organophosphate and carbamate resistance\cite{14, 24}.

We found significantly higher \textit{Kdr} L1014F and \textit{Ace-1$^R$} G119S genotypic and allelic frequencies in \textit{An. gambiae} than in \textit{An. coluzzii}, which was in agreement with observations of Koukpo \textit{et al}\cite{36} in Benin and by Zogo \textit{et al}\cite{37} in Côte d’Ivoire. There were 59 times greater probability of encountering \textit{Kdr} L1014F resistance allele of \textit{An. gambiae} relating to \textit{An. coluzzii}, whereas the frequency of \textit{Kdr} L1014F heterozygous individuals was reversely higher in \textit{An. coluzzii} (42.95%) than in \textit{An. gambiae} (1.12%). This clearly highlighted a deviation from Hardy-Weinberg expectations within both malaria vector species for the \textit{Kdr} L1014F mutations. It is possible that evolutionary factors affect mosquito population structure through the excess use of insecticides. These factors induce the selection of rare and existing mutations in natural population of both species which become later variably widespread \cite{38}.

Furthermore, \textit{Ace-1$^R$} G119S allelic frequency in \textit{An. gambiae} was significantly higher than in \textit{An. coluzzii}, although the amplitude was moderate. The low proportion (<10%) of homozygous resistant (RR) genotypes observed in \textit{An. gambiae} and \textit{An. coluzzii} population could indicate the high fitness cost associated with \textit{Ace-1$^R$} G119S gene\cite{39, 40}. Conversely, this fitness cost associated with \textit{Ace-1$^R$} seems to be sorbed by the duplication of this gene which induced various heterozygous genotypes by increasing their proportions\cite{41}. Further studies focusing on \textit{Ace-1$^R$} genotype distribution, including the duplication in \textit{An. gambiae s.l.} is needed. Our study showed that in areas where \textit{Kdr} L1014F and \textit{Ace-1$^R$} G119S coexist in \textit{An. gambiae s.l.}, the frequency of individuals bearing the \textit{Kdr} L1014F RR genotype appeared significantly higher in \textit{An. gambiae} than in \textit{An. coluzzii}. By contrast, the frequencies of those bearing the \textit{Kdr} L1014F heterozygous genotype were reversely significantly higher in \textit{An. coluzzii} than \textit{An. gambiae}, confirming the trend when this genotype is in isolation. This is the first study evaluating the distribution of individual \textit{An. gambiae s.l.} bearing both mutations inside them. It calls for further studies to better understand the genotypic structure of their combinations.

The vectorial competence in association with resistance genes was investigated. We found no evidence of association between \textit{Plasmodium} infection status and \textit{Kdr} L1014F or \textit{Ace-1$^R$} G119S genes. These results were similar to those found in Guinea where these target site mutations (\textit{Kdr} L1014F or \textit{Ace-1$^R$} G119S ) were not associated with \textit{Plasmodium} infection in wild \textit{An. gambiae} \cite{42}, but that the phenotypic resistance was rather associated with infection. By contrast, a study in Tanzania found a link between \textit{Kdr}-east and \textit{Plasmodium} infection in wild \textit{An. gambiae}\cite{43}.

However, the non-association between \textit{Plasmodium} infection status and resistance genes under natural condition contrasts with several other studies reporting that resistance associated genes affect vector competence to transmit \textit{Plasmodium} parasites\cite{20, 21, 44}. Reasons for the difference could be three-fold: (i) These contrasting results could derived from studies that used colonies maintained in laboratory over years, which can decrease resistance, including loss of genetic diversity\cite{45, 46}. (ii) Some genetic susceptibility studies do not take account of additional factors influencing competence in natural vector population; e.g. mosquito
blood feeding rate, age at infection, longevity, exposure to insecticide and other pathogens that could influence mosquito immune status\cite{47, 48, 49, 50, 51}. Natural infection study also implies the effects of ecology and behavior on vectorial competence\cite{52, 53}. (iii) Resistance is a package encompassing mutations plus metabolic components with different functions; therefore isolating one from the other, may not be representative of the phenotypic resistance. The absence of association between genotypes in combination (\textit{Kdr} L1014F-Ace-1\textsuperscript{R}G119S) with infection status in \textit{An. coluzzii} or \textit{An. gambiae} requires further attention by control programmes, given that this is now common observation in many parts of west Africa \cite{13, 24}.

**Conclusion**

We saw no significant association of the \textit{Kdr} L1014F and Ace-1\textsuperscript{R}G119S mutations alone or in association with infection status in wild \textit{An. gambiae} and \textit{An. coluzzii} demonstrating similar competence for \textit{Plasmodium} transmission within Bouaké areas. Nevertheless, the frequencies for the \textit{Kdr} and Ace-1\textsuperscript{R} genotypes and alleles were significantly higher in \textit{An. gambiae} than in \textit{An. coluzzii}. Additional factors influencing competence in natural vector population and those outside alleles or genotypes measurements contributing to resistance should be consider when establishing link between insecticide resistance and vector competence.

**Abbreviations**

WHO

World Health Organization; LLINs:Long Lasting Insecticidal Nets; IRS:Indoor Residual Spraying; SET:Screening plus In2Care Eave Tubes; SNP:Single Nucleotide Polymorphism; L1014F \textit{Kdr}:West knockdown resistance; Ace-1\textsuperscript{R}:Acetylcholinesterase-1 resistance; VCPEC:Vector Control Evaluation Centre; IPR:Institut Pierre Richet; Ace-1\textsuperscript{R}G119S:G119S mutation in Ace-1\textsuperscript{R}; DDT:Dichlorodiphenyltrichloroethane; OR:odds ratio, HWE:Hardy-Weinberg Equilibrium, CI:confidence interval; SNP:Single Nucleotide Polymorphism; R:Resistant; S:Susceptible

**Declarations**

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**Authors’ contributions**

RZW, AAK and RN designed the study. RZW, FHAY, AAPL, EDS, IZT, WAO and SC conducted the field, laboratory and data management work. RZW, AD, and MHK analysed the data. RZW wrote the manuscript. KAA, ONA, EDS, AAPL, SPAN, MBT and NR supervised the study and revised the manuscript. All authors reviewed and approved the final manuscript.

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**Availability of data and materials**

The data supporting the conclusions of this manuscript are included within the manuscript and are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Ethical clearance and consent information are included within the manuscript.
Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

1. Sinka ME, Bangs MJ, Manguin S, Rubio-Palis Y, Chareonviriyaphap T, Coetzee M, et al. A global map of dominant malaria vectors. Parasit Vectors. 2012;5:69. https://doi.org/10.1186/1756-3305-5-69.
2. Garrett-Jones C, Shidrawi GR. Malaria Vectorial Capacity of a Population of Anopheles gambiae. Bull World Health Organ. 1969;40:531–45. https://apps.who.int/iris/handle/10665/267721.
3. Coluzzi M, Sabatini A, Petrarca V, Di Deco MA. Chromosomal differentiation and adaptation to human environments in the Anopheles gambiae complex. Trans R Soc Trop Med Hyg. 1979;73:483–97. https://doi.org/10.1016/0035-9203(79)90036-1.
4. Zaim M, Aitio A, Nakashima N. Safety of pyrethroid-treated mosquito nets. Med Vet Entomol. 2000;14:1–5. https://doi.org/10.1046/j.1365-2915.2000.00211.x.
5. Bhatt S, Weiss DJ, Cameron E, Bianszio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207–11. https://doi.org/10.1038/nature15535.
6. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011;27:91–8. https://doi.org/10.1016/j.pt.2010.08.004.
7. Strode C, Donegan S, Garner P, Enayati AA, Hemingway J. The Impact of Pyrethroid Resistance on the Efficacy of Insecticide-Treated Bed Nets against African Anopheline Mosquitoes: Systematic Review and Meta-Analysis. PLoS Med. 2014;11:e1001619. https://doi.org/10.1371/journal.pmed.1001619.
8. Mitchell SN, Rigden DJ, Dowd AJ, Lu F, Wilding CS, Weetman D, et al. Metabolic and Target-Site Mechanisms Combine to Confer Strong DDT Resistance in Anopheles gambiae. PLoS ONE. 2014;9:e92662. https://doi.org/10.1371/journal.pone.0092662.
9. Chouaibou M, Zivanovic GB, Knox TB, Jamet HP, Bonfoh B. Synergist bioassays: A simple method for initial metabolic resistance investigation of field Anopheles gambiae s.l. populations. Acta Trop. 2014;130:108–11. https://doi.org/10.1016/j.actatropica.2013.10.020.
10. Stevenson BJ, Bibby J, Pignatelli P, Muangnoicharoen S, O'Neill PM, Lian L-Y, et al. Cytochrome P450 6M2 from the malaria vector Anopheles gambiae metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. Insect Biochem Mol Biol. 2011;41:492–502. https://doi.org/10.1016/j.ibmb.2011.02.003.
11. Chouaibou M, Kouadio FB, Tia E, Djogbenou L. First report of the East African kdr mutation in an Anopheles gambiae mosquito in Côte d’Ivoire. Wellcome Open Res. 2017;2:8. https://doi.org/10.12688/wellcomeopenres.10662.1.
12. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, Devonshire AL, et al. Molecular characterization of pyrethroid knockdown resistance (Kdr) in the major malaria vector Anopheles gambiae s.s. Insect Mol Biol. 1998;7:179–84. https://doi.org/10.1046/j.1365-2583.1998.72062.x.
13. Dabiré RK, Namountougou M, Diabaté A, Soma DD, Bado J, Toé HK, et al. Correction: Distribution and Frequency of kdr Mutations within Anopheles gambiae s.l. Populations and First Report of the Ace-1 G119S Mutation in Anopheles arabiensis from Burkina Faso (West Africa). PLOS ONE. 2015;10:e0141645. https://doi.org/10.1371/journal.pone.0101484.
14. Essandoh J, Yawson AE, Weetman D. Acetylcholinesterase (Ace-1) target site mutation 119S is strongly diagnostic of carbamate and organophosphate resistance in Anopheles gambiae s.s. and Anopheles coluzzii across southern Ghana. Malaria J. 2013;12:404. https://doi.org/10.1186/1475-2875-12-404.

15. Cook J, Hergott D, Phiri W, Rivas MR, Bradley J, Segura L, et al. Trends in parasite prevalence following 13 years of malaria interventions on Bioko island, Equatorial Guinea: 2004–2016. Malaria J. 2018; 17:62. https://doi.org/10.1186/s12936-018-2213-9.

16. Dossou-Yovo J, Guillet P, Rogier C, Chandra F, Carnevale P, Assi S-B, et al. Protective efficacy of lambda-cyhalothrin treated nets in Anopheles gambiae pyrethroid resistance areas of Côte d'Ivoire. The Am J Trop Med Hyg. 2005;73:859–64. https://doi.org/10.4269/ajtmh.2005.73.859.

17. Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, Mbogo C, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. Lancet Infect Dis. 2018;18:640–9. https://doi.org/10.1016/S1473-3099(18)30172-5.

18. Tokponnon FT, Sissinto Y, Ogouyémi AH, Adéothy AA, Adechoubou A, Houansou T, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: evidence from health facility data from Benin. Malaria J. 2019;11:550. https://doi.org/10.1186/s13071-018-3101-4.

19. Protopopoff N, Mosha JF, Lukole E, Charwood JD, Wright A, Mwalimu CD, et al. Effectiveness of a long-lasting permethrin butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. Lancet. 2018;391:1577–88. https://doi.org/10.1016/S0140-6736(18)30427-6.

20. Alou H, Ndam NT, Sandeu MM, Djégbe I, Chandra F, Dabiré RK, et al. Insecticide Resistance Alleles Affect Vector Competence of Anopheles gambiae s.s. for Plasmodium falciparum Field Isolates. PLoS ONE. 2013;8:e63849. https://doi.org/10.1371/journal.pone.0063849.

21. Ndiath M, Cailleau A, Diedhiou S, Gaye A, Boudin C, Richard V, et al. Effects of the kdr resistance mutation on the susceptibility of wild Anopheles gambiae populations to Plasmodium falciparum: a hindrance for vector control. Malaria J. 2014;13:340. https://doi.org/10.1186/1475-2875-13-340.

22. Mitri C, Markianos K, Guelbeogo WM, Bischoff E, Gneme A, Eiglmeier K, et al. The kdr-bearing haplotype and susceptibility to Plasmodium falciparum in Anopheles gambiae: genetic correlation and functional testing. Malaria J. 2015;14:391. https://doi.org/10.1186/s13071-014-0924-8.

23. Dabiré RK, Namountougou M, Diabaté A, Soma DD, Bado J, Toé HK, et al. Distribution and Frequency of kdr Mutations within Anopheles gambiae s.l. Populations and First Report of the Ace1G119S Mutation in Anopheles arabiensis from Burkina Faso (West Africa). PLoS ONE. 2014;9:e101484. https://doi.org/10.1371/journal.pone.0101484.

24. Camara S, Koffi AA, Ahoua Alou LP, Koffi K, Kabran J-PK, Koné A, et al. Mapping insecticide resistance in Anopheles gambiae (s.l.) from Côte d'Ivoire. Parasit Vectors. 2018;11:19. https://doi.org/10.1186/s13071-017-1706-1.

25. Sternberg ED, Cook J, Ahoua Alou LP, Aoura CJ, Assi SB, Doudou DT, et al. Evaluating the impact of screening plus eave tubes on malaria transmission compared to current best practice in central Côte d'Ivoire: a two armed cluster randomized controlled trial. BMC Public Health. 2018; 18:894 https://doi.org/10.1186/s12889-018-5746-5.

26. Gillies MT, Coetzee M, A supplement to the Anophelinae of Africa south of the Sahara (Afrotropical Region). Johannesburg; 1987; 143:15. ISBN: 0620103213.

27. Yahouédo GA, Chandre F, Rossignol M, Giniibre C, Balabanidou V, Mendez NGA, et al. Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector Anopheles gambiae. Sci Rep. 2017; 7:11091. https://doi.org/10.1038/s41598-017-11357-z.

28. Mangold KA, Manson RU, Koay ESC, Stephens L, Regner M, Thomson RB, et al. Real-Time PCR for Detection and Identification of Plasmodium spp. J Clin Microbiol. 2005;43:2435–40. https://doi.org/10.1128/JCM.43.5.2435-2440.2005.

29. Favia G, Lanfrancotti A, Spanos L, Sidén-Kiamos I, Louis C. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of Anopheles gambiae s.s.: An. gambiae s.s. rDNA polymorphisms. Insect Mol Biol. 2001;10:19–23. https://doi.org/10.1046/j.1365-2583.2001.00236.x.

30. Bass C, Nikou D, Donnelly MJ, Williamson MS, Ranson H, Ball A, et al. Detection of knockdown resistance (kdr) mutations in Anopheles gambiae: a comparison of two new high-throughput assays with existing methods. Malaria J. 2007;6:111.
31. Bass C, Nikou D, Vontas J, Williamson MS, Field LM. Development of high-throughput real-time PCR assays for the identification of insensitive acetylcholinesterase (ace-1R) in Anopheles gambiae. Pestic Biochem Physiol. 2010; 96:80–5. https://doi.org/10.1016/j.pestbp.2009.09.004.

32. Koffi AA, Ahoua Alou LP, Djenontin A, Kabran J-PK, Dosso Y, Kone A, et al. Efficacy of Olyset ® Duo, a permethrin and pyriproxyfen mixture net against wild pyrethroid-resistant Anopheles gambiae s.s. from Côte d'Ivoire: an experimental hut trial. Parasite. 2015;22:28. https://doi.org/10.1051/parasite/2015028.

33. Zoh DD, Ahoua Alou LP, Toure M, Pennetier C, Camara S, Traore DF, et al. The current insecticide resistance status of Anopheles gambiae (s.l.) (Culicidae) in rural and urban areas of Bouaké, Côte d'Ivoire. Parasit Vectors. 2018;11:118. https://doi.org/10.1186/s13071-018-2702-2.

34. Gnémé A, Guelbéogo WM, Riehle MM, Sanou A, Traoré A, Zongo S, et al. Equivalent susceptibility of Anopheles gambiae M and S molecular forms and Anopheles arabiensis to Plasmodium falciparum infection in Burkina Faso. Malar J. 2013;12:204. https://doi.org/10.1186/1475-2875-12-204.

35. Sternberg ED, Cook J, Alou LPA, Assi SB, Ko AA, Doudou DT, et al. Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: a two-arm, cluster-randomised controlled trial. Lancet. 2021;397(10276):805–15. https://doi.org/10.1016/S0140-6736(21)00250-6.

36. Koukpo CZ, Fassinou AJYH, Ossè RA, Agossa FR, Sovi A, Sewadé WT, et al. The current distribution and characterization of the L1014F resistance allele of the kdr gene in three malaria vectors (Anopheles gambiae, Anopheles coluzzii, Anopheles arabiensis) in Benin (West Africa). Malaria J. 2019; 18:175. https://doi.org/10.1186/s12936-019-2808-9.

37. Zogo B, Soma DD, Tchiekoi BN, Somé A, Ahoua Alou LP, Ko AA, et al. Anopheles bionomics, insecticide resistance mechanisms, and malaria transmission in the Korhogo area, northern Côte d'Ivoire: a pre-intervention study. Parasite. 2019;26:40. https://doi.org/10.1051/parasite/2019040.

38. Nkya TE, Poupardin R, Laporte F, Akhouayri I, Mosha F, Magesa S, et al. Impact of agriculture on the selection of insecticide resistance in the malaria vector Anopheles gambiae: a multigenerational study in controlled conditions. Parasit Vectors. 2014;7:480. https://doi.org/10.1186/s13071-014-0480-z.

39. Alout H, Dabiré RK, Djogbénoù LS, Abate L, Corbel V, Chandre F, et al. Interactive cost of Plasmodium infection and insecticide resistance in the malaria vector Anopheles gambiae. Sci Rep. 2016;6:29755. https://doi.org/10.1038/srep29755.

40. Djogbénoù L, Noel V, Agnew P. Costs of insensitive acetylcholinesterase insecticide resistance for the malaria vector Anopheles gambiae homozygous for the G119S mutation. Malar J. 2010;9:12. https://doi.org/10.1186/1475-2875-10-12.

41. Djogbénoù LS, Assogba B, Essandoh J, Constant EAV, Makoutodé M, Akogbéto M, et al. Estimation of allele-specific Ace-1 duplication in insecticide-resistant Anopheles mosquitoes from West Africa. Malar J. 2015;14:507. https://doi.org/10.1186/s12936-015-1026-3.

42. Collins E, Vaselli NM, Sylla M, Beavogui AH, Orsborne J, Lawrence G, et al. The relationship between insecticide resistance, mosquito age and malaria prevalence in Anopheles gambiae s.l. from Guinea. Sci Rep. 2019;9:8846. https://doi.org/10.1038/s41598-019-45261-5.

43. Kabula B, Tungu P, Rippon EJ, Steen K, Kisinza W, Magesa S, et al. A significant association between deltamethrin resistance, Plasmodium falciparum infection and the Vgsc-1014S resistance mutation in Anopheles gambiae highlights the epidemiological importance of resistance markers. Malaria J. 2016;15:289. https://doi.org/10.1186/s12936-016-1331-5.

44. Ndiath MO, Cohuet A, Gaye A, Konate L, Mazenot C, Faye O, et al. Comparative susceptibility to Plasmodium falciparum of the molecular forms M and S of Anopheles gambiae and Anopheles arabiensis. Malar J. 2011;10:269. https://doi.org/10.1186/1475-2875-10-269.

45. Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, et al. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim Methods Softw. 2012;27:233–49. https://doi.org/10.1080/10556788.2011.597854.

46. Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, et al. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol Evol. 2009;24:127–35. https://doi.org/10.1016/j.tree.2008.10.008.
47. Cohuet A, Harris C, Robert V, Fontenille D. Evolutionary forces on *Anopheles*: what makes a malaria vector? Trends Parasitol. 2010;26:130–6. https://doi.org/10.1016/j.pt.2009.12.001.

48. Glunt KD, Thomas MB, Read AF. The Effects of Age, Exposure History and Malaria Infection on the Susceptibility of *Anopheles* Mosquitoes to Low Concentrations of Pyrethroid. PLoS ONE. 2011;6:e24968. https://doi.org/10.1371/journal.pone.0024968.

49. Manguin S. Biodiversity of malaria in the world. Engl. version completely updated. Paris: John Libbey Eurotext; 2008; 133:427. http://hdl.handle.net/10390/2213.

50. Churcher TS, Lissenden N, Griffin JT, Worrall E, Ranson H. The impact of pyrethroid resistance on the efficacy and effectiveness of bednets for malaria control in Africa. eLife. 2016;2:5:e16090. https://doi.org/10.7554/eLife.16090.

51. Mbepera S, Nkwengulila G, Peter R, Mausa EA, Mahande AM, Coetzee M, et al. The influence of age on insecticide susceptibility of *Anopheles arabiensis* during dry and rainy seasons in rice irrigation schemes of Northern Tanzania. Malaria J. 2017; 16:364. https://doi.org/10.1186/s12936-017-2022-6.

52. Simard F, Ayala D, Kamdem G, Pombi M, Etouna J, Ose K, et al. Ecological niche partitioning between *Anopheles gambiae* molecular forms in Cameroon: the ecological side of speciation. BMC Ecol. 2009;9:17. https://doi.org/10.1186/1472-6785-9-17.

53. Gimonneau G, Bouyer J, Morand S, Besansky NJ, Diabate A, Simard F. A behavioral mechanism underlying ecological divergence in the malaria mosquito *Anopheles gambiae*. Behav Ecol. 2010;21:1087–92. https://doi.org/10.1093/beheco/arq114.

**Figures**

**Figure 1**

An. gambiae s.l. species distribution by infection status. Error bars represent 95% confidence intervals. SET: Screening plus In2Care Eave Tubes.
Figure 2

Combination of Kdr L1014F and Ace-1R G119S genotypic frequencies between infected and uninfected groups, in each study arm, Error bars represent 95% confidence intervals. SET: Screening plus In2Care Eave Tubes. For all combined genotypes, the two first alleles refer to Kdr genotypes and the two last refer to Ace-1R genotypes.

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