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-1^R* 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-1^R* 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-1^R* G119S mutations did not vary significantly between study arms. However, the frequency of the *Kdr* L1014F and *Ace-1^R* 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-1^R*). For both species, there were no significant differences in *Kdr* L1014F or *Ace-1^R* G119S genotypic and allelic frequency distribution between infected and uninfected specimens ($p > 0.05$).

Conclusions

Either alone or in combination, *Kdr* L1014F and *Ace-1^R* 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-1^R* 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-1^R*) and vectorial competence in *An. gambiae* [20–22]. Nerveless, this were laboratory assays utilizing mosquito colonies or wild strains infected with malaria parasites in laboratories. The coexistence of both *Kdr* and *Ace-1^R* 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 *Kdr* L1014F and *Ace-1^R* G119S 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 Sternberg *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-1^R G119S* 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 TCC AG-3'), Mopint (5'-GCC CCT TCC TCG ATG GCA T-3') and B/Sint (5'-ACC AAG ATG GTT CGT TGC-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'-CATTTTTCTTGGCCACTGTAGTGAT-3'), and *Kdr*-Reverse (5'-CGATCTTGGTCCATGTTAATTTGCA-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 fluorescences 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 fluorescences 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:

$$R\ allelic\ frequency = \frac{RS + 2(RR)}{2(RS + RR + SS)}$$

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-1^{R}* between the control and Eave tube areas for each species (p 0.05) (Table 1).

Table 1

Kdr L1014F and *Ace-1^{R} G119S* allelic frequencies between study arms

		Kdr L1014F					χ^2 (P-value)	*Ace-1^{R}* G119S					χ^2 (P-value)
		N	SS	RS	RR	R (%)		N	SS	RS	RR	R (%)	
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-1^{R} G119S mutations

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

Table 2

Genotypic and allelic frequencies of *Kdr* L1014F and *Ace*-1^R *G119S* genes in *An. gambiae and An. coluzzii*

SNP/species	N	Genotypic frequencies (%)			Allelic frequencies (%)		OR [95%CI]	HWE χ^2 (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	0.105(0.744)
An. gambiae	624	616(98.72)	7(1.12)	1(0.16)	1232(99.28)	9 (0.72)	59.64[30.81-131.63]	6.96(0.008)
Ace-1^R G119S		RR	RS	SS	R	S		
An. coluzzii	630	14 (2.22)	76 (12.06)	540(85.72)	104 (8.25)	1156(91.75)	1	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]	51.48(< 0.001)

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-1^R* 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-1^R* 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-1^R* 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 ($p \geq 0.05$) (Table 3).

Table 3

Genotypic and allelic frequencies of *Kdr* L1014F and *Ace-1*^{R} *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		RR	RS	SS	R	S	
An. coluzzii	Control	Infected	213	102(47.89)	96(45.07)	15(7.04)	300(70.42)	126(29.58)	1
		Uninfected	208	102(49.04)	86(41.35)	20(9.62)	290 (69.71)	126 (30.29)	1.03[0.76–1.38]
	SET	Infected	92	40(43.48)	46(50.00)	6(6.52)	126 (68.48)	58 (31.52)	1
		Uninfected	118	60(50.85)	43(36.44)	15(12.71)	163(69.07)	73 (30.93)	0.97 [0.62–1.5]
An. gambiae	Control	Infected	187	183 (97.86)	3(1.60)	1(0.53)	369 (98.66)	5(1.35)	1
		Uninfected	208	207(99.52)	1 (0.47)	0(0)	415(99.76)	1(0.24)	0.17[0.003-1.6]
	SET	Infected	119	117(98.32)	2(1.68)	0(0)	236 (99.16)	2(0.84)	1
		Uninfected	110	109(99.1)	1(0.9)	0(0)	219 (99.55)	1(0.45)	0.53[0.009–10.4]
		Ace-1^{R}*G119S*		RR	RS	SS	R	S	
An. coluzzii	Control	Infected	213	4(1.88)	23(10.80)	186 (87.32)	31 (7.28)	395 (92.72)	1
		Uninfected	207	8(3.86)	29(14.01)	170(82.13)	45(10.87)	369(89.13)	0.64[0.38–1.06]
	SET	Infected	92	0(0)	9(9.78)	83(90.22)	9(4.89)	175(95.11)	1
		Uninfected	118	2(1.69)	15(12.71)	15(85.60)	19(8.05)	217(91.95)	0.58[0.22–1.40]
An. gambiae	Control	Infected	186	15(8.06)	42(22.58)	129 (69.35)	72(19.32)	300(80.64)	1
		Uninfected	208	21(10.10)	52(25.00)	135(64.90)	94(22.60)	322(77.40)	0.82[0.57–1.17]
	SET	Infected	119	7(5.88)	25(21.01)	87(73.11)	39(16.39)	199(83.61)	1
		Uninfected	109	9(8.26)	19(17.43)	81(74.31)	37(16.97)	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^{R} genotypes and infection status

There are nine possible combinations for the *Kdr* L1014F and *Ace-1*^{R} 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*^{R} genotypes: (1) Kdr-Ace-1^{R}(RRRR), (2) Kdr-Ace-1^{R}(RRRS), (3) Kdr-Ace-1^{R}(RRSS), (4) Kdr-Ace-1^{R}(RSRR), (5) Kdr-Ace-1^{R}(RSRS)), (6) Kdr-Ace-1^{R}(RSSS), (7) Kdr-Ace-1^{R}(SSRR), (8) Kdr-Ace-1^{R}(SSRS), (9) Kdr-Ace-1^{R}(SSSS). Figure 2 showed that in areas where *Kdr* and Ace-1^{R} 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 *Kdr* heterozygous genotype were significantly higher in *An. coluzzii* than in *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 *An. coluzzii* or *An. gambiae*.

Discussion

This study evaluated the effects of the *Kdr* L1014F and *Ace-1^R* G119S genes on *Plasmodium sp*. infection status in natural *An. gambiae s.l.* populations. The presence of both *An. coluzzii.* and *An. gambiae* in similar proportions in this longitudinal study was consistent with previous studies in the area of Bouaké[24, 32] but it contrasts with another study conducted in adjacent areas within Bouaké which found *An. coluzzii* to be predominant [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[33]. We observed no difference in infection rate between *An. gambiae* and *An. coluzzii*. This aligns with previous studies conducted in Burkina Faso and Senegal [21, 34], which reported equivalent *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 *Kdr* or *Ace-1^R* between the control and Eave tube areas regardless of the species. This is because *Kdr* was already close to fixation in *An. gambiae s.l.* species prior to the eave tubes intervention (> 80%)[24] leaving tiny window for further selection. Also, the insecticide deployed in the eave tube trial was a pyrethroid (beta-cyfluthrin) [35] which could not induce a selection pressure on the *Ace-1^R* since this gene is associated with organophosphate and carbamate resistance[14, 24].

We found significantly higher *Kdr* L1014F and *Ace1^R* G119S genotypic and allelic frequencies in *An. gambiae* than in *An. coluzzii*, which was in agreement with observations of Koukpo *et al*.[36] in Benin and by Zogo *et al*.[37] in Côte d'Ivoire. There were 59 times greater probability of encountering *Kdr* L1014F resistance allele of *An. gambiae* relating to *An. coluzzii*, whereas the frequency of *Kdr* L1014F heterozygous individuals was reversely higher in *An. coluzzii* (42.95%) than in *An. gambiae* (1.12 %). This clearly highlighted a deviation from Hardy-Weinberg expectations within both malaria vector species for the *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 [38].

Furthermore, *Ace-1^R* G119S allelic frequency in *An. gambiae* was significantly higher than in *An. coluzzii*, although the amplitude was moderate. The low proportion (< 10%) of homozygous resistant (RR) genotypes observed in *An. gambiae* and *An. coluzzii* population could indicate the high fitness cost associated with A*ce-1^R* G119S gene[39, 40]. Conversely, this fitness cost associated with A*ce-1^R* seems to be resorbed by the duplication of this gene which induced various heterozygous genotypes by increasing their proportions[41]. Further studies focusing on *Ace-1^R* genotype distribution, including the duplication in *An. gambiae s.l.* is needed. Our study showed that in areas where *Kdr* L1014F and *Ace-1^R* G119S coexist in *An. gambiae s.l.*, the frequency of individuals bearing the *Kdr* L1014F RR genotype appeared significantly higher in *An. gambiae* than in *An. coluzzii*. By contrast, the frequencies of those bearing the *Kdr* L1014F heterozygous genotype were reversely significantly higher in *An. coluzzii* than *An. gambiae*, confirming the trend when this genotype is in isolation. This is the first study evaluating the distribution of individual *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 *Plasmodium* infection status and *Kdr* L1014F or *Ace-1^R* G119S genes. These results were similar to those found in Guinea where these target site mutations (*Kdr* L1014F or *Ace-1^R* G119S) were not associated with *Plasmodium* infection in wild *An. gambiae* [42], but that the phenotypic resistance was rather associated with infection. By contrast, a study in Tanzania found a link between *Kdr*-east and *Plasmodium* infection in wild *An. gambiae*[43].

However, the non-association between *Plasmodium* infection status and resistance genes under natural condition contrasts with several other studies reporting that resistance associated genes affect vector competence to transmit *Plasmodium* parasites[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[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[47–51]. Natural infection study also implies the effects of ecology and behavior on vectorial competence[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 (*Kdr* L1014F-*Ace-1^R* G119S) with infection status in *An. coluzzii* or *An. gambiae* requires further attention by control programmes, given that this is now common observation in many parts of west Africa [13, 24].

Conclusion

We saw no significant association of the *Kdr* L1014F and *Ace-1^R* G119S mutations alone or in association with infection status in wild *An. gambiae* and *An. coluzzii* demonstrating similar competence for *Plasmodium* transmission within Bouaké areas. Nevertheless, the frequencies for the *Kdr* and *Ace-1^R* genotypes and alleles were significantly higher in *An. gambiae* than in *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 *Kdr*:West knockdown resistance; *Ace-1^R*:Acetylcholinesterase-1 resistance; VCPEC:Vector Control Evaluation Centre; IPR:Institut Pierre Richet; *Ace-1^R* G119S:G119S mutation in *Ace-1^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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Figures

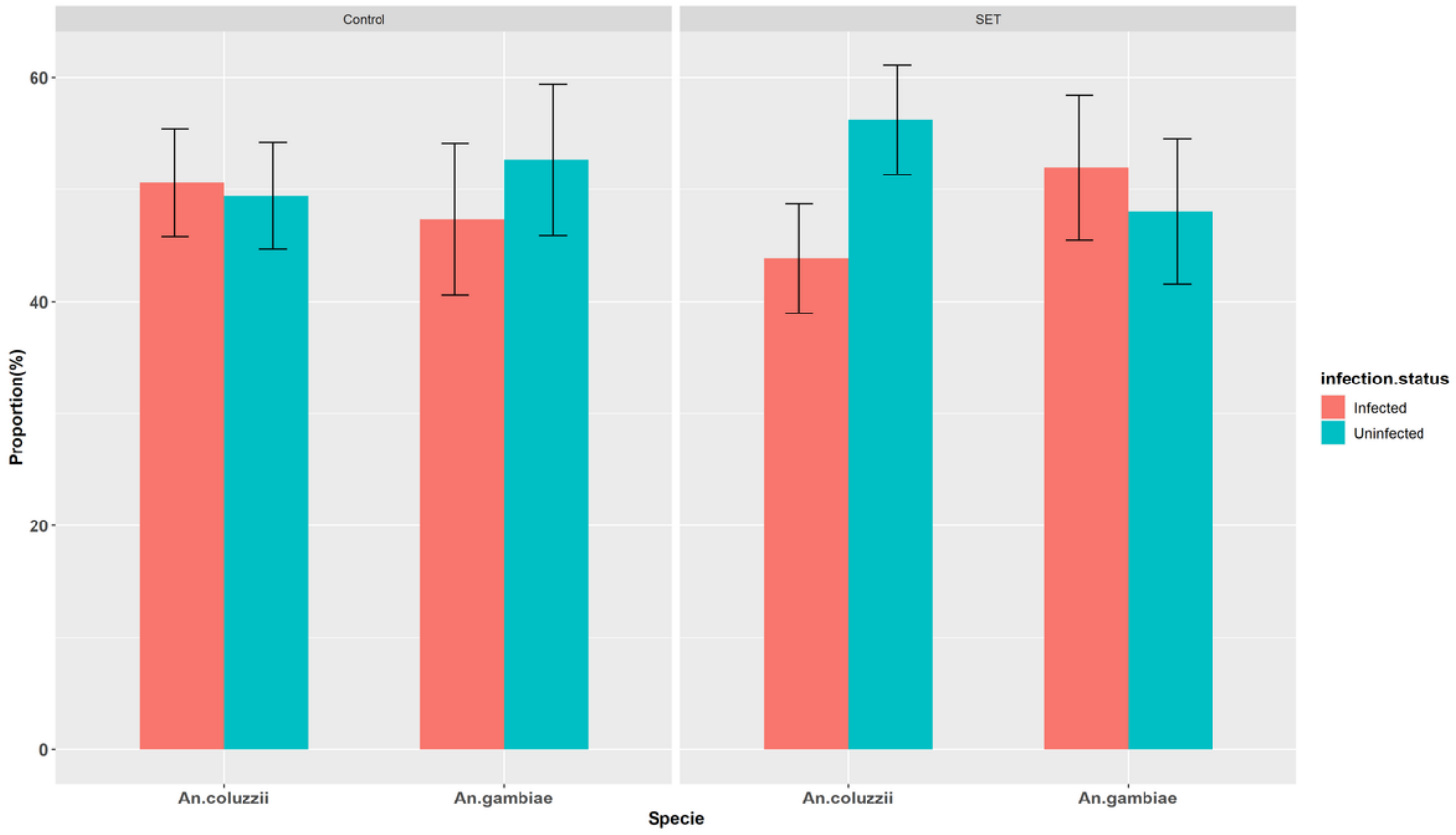

Figure 1

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

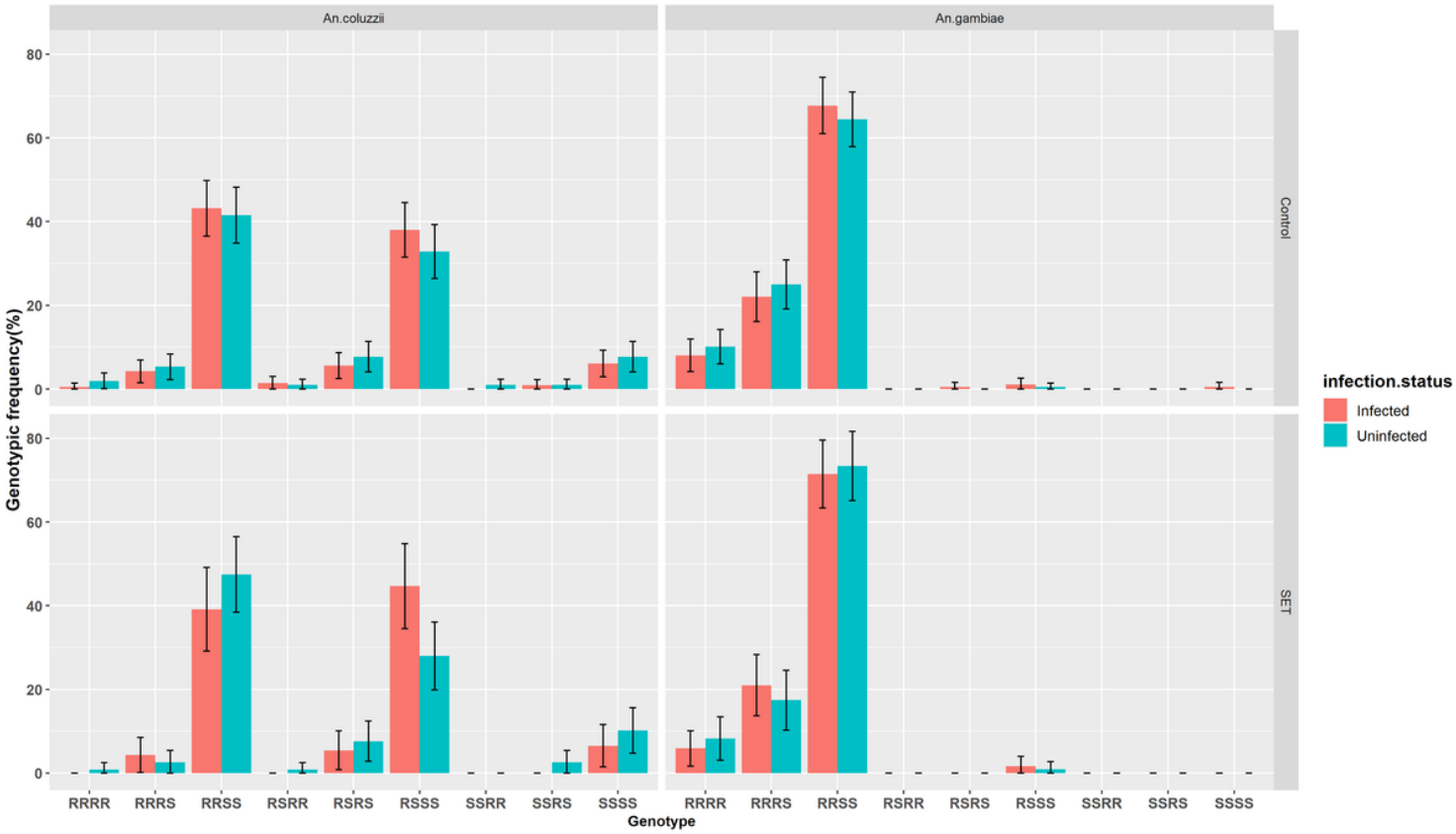

Figure 2

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.

Supplementary Files

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