Journal of Medical Entomology, 58(2), 2021, 730–738
doi: 10.1093/jme/tjaa195
Advance Access Publication Date: 12 October 2020
Vector Control, Pest Management, Resistance, Repellents Research

Phenotypic Insecticide Resistance in *Anopheles gambiae* (Diptera: Culicidae): Specific Characterization of Underlying Resistance Mechanisms Still Matters

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Subject Editor: Roberto Pereira

Received 8 July 2020; Editorial decision 14 August 2020

Abstract

An effective control of malaria vectors requires an extensive knowledge of mechanisms underlying the resistance-phenotypes developed by these vectors against insecticides. We investigated *Anopheles gambiae* mosquitoes from Benin and Togo for their intensity of insecticide resistance and we discussed the involvement of genotyped mechanisms in the resistance-phenotypes observed. Three- to five-day-old adult mosquitoes emerged from field and laboratory *An. gambiae* larvae were assayed using WHO tube intensity tests against various doses of deltamethrin: 1× (0.05%); 2× (0.1%); 5× (0.25%); 7.5× (0.375%) and those of pirimiphos-methyl: 0.5× (0.125%); 1× (0.25%). Members of *An. gambiae* complex were screened in field populations using polymerase chain reaction (PCR) assays. The presence of *kdr*^{R}(1014F/1014S) and *ace-1*^{R}(119S) mutations was also investigated using TaqMan and PCR-RFLP techniques, respectively. *Anopheles gambiae* from field were very resistant to deltamethrin, whereas KisKdr and AcerKdrKis strains displayed 100% mortality rates at 2× the diagnostic dose. In contrast, the field mosquitoes displayed a low resistance-intensity against 1× the diagnostic dose of pirimiphos-methyl, whereas AcerKis and AcerKdrKis strains showed susceptibility at 0.5× the diagnostic dose. *Anopheles gambiae s.s.*, *Anopheles coluzzii*, and *Anopheles arabiensis* were identified. Allelic frequencies of *kdr*^{R} (1014F) and *ace-1*^{R} (119S) mutations in the field populations varied from 0.65 to 1 and 0 to 0.84, respectively. The field *An. gambiae* displayed high-resistance levels against deltamethrin and pirimiphos-methyl when compared with those of the laboratory *An. gambiae*-resistant strains. These results exhibit the complexity of underlying insecticide resistance mechanisms in these field malaria vectors.

Graphical Abstract

Summary: The field *An. gambiae* displayed high resistance levels against deltamethrin and pirimiphos-methyl when compared to those of the laboratory *An. gambiae* resistant strains. These results exhibit the complexity of underlying insecticide resistance mechanisms in these field malaria vectors.

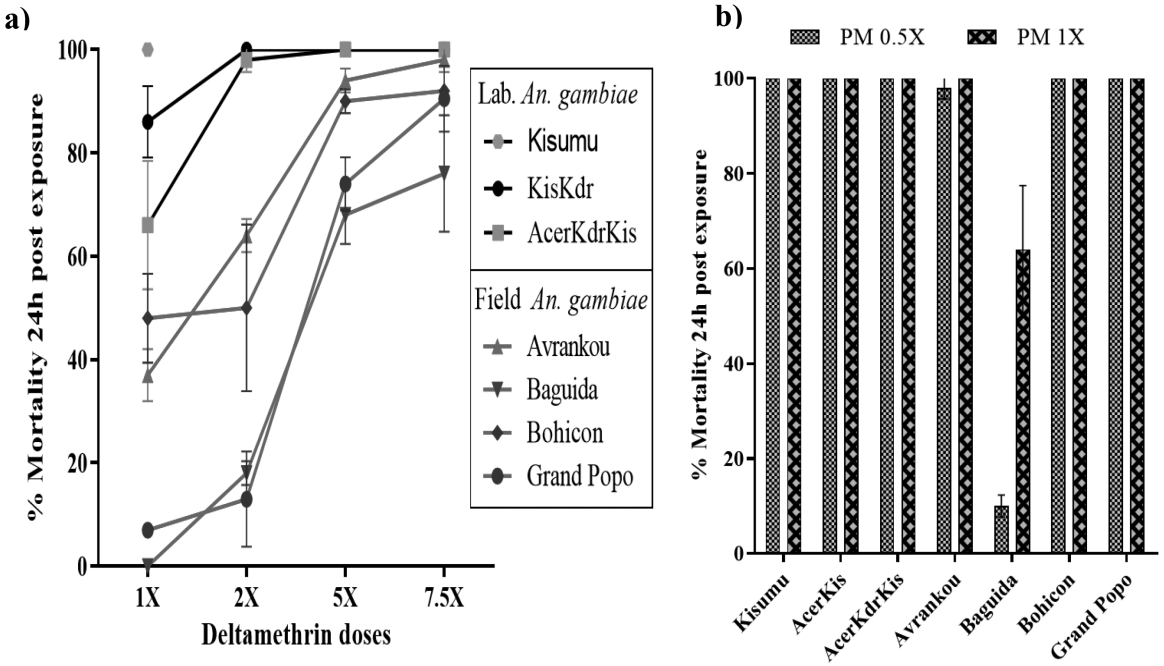

Figure. (a) Deltamethrin and (b) pirimiphos-methyl exposures.

Key words: *Anopheles gambiae*, phenotype, resistance mechanism, deltamethrin, pirimiphos-methyl

Currently, the most effective way to prevent malaria transmission episodes remains the use of malaria vectors control trials alongside with chemical insecticides contained in long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) formulations (Katureebe et al. 2016). Due to their properties, pyrethroid chemistries remain the only class of insecticides authorized for the treatment of LLINs (World Health Organization 2004, 2006). Pirimiphos-methyl insecticide (Actellic capsule suspension) has been recently used as an alternative molecule to control the pyrethroid-resistant *Anopheles gambiae* in the field (Fuseini et al. 2011, Rowland et al. 2013, Tchicaya et al. 2014).

Most often, the resistance-phenotypes reported in natural populations of mosquitoes relies on four relevant resistance mechanisms such as target-site insensitivity (Chandre et al. 1999), metabolic (Li et al. 2007), behavioral (Reddy et al. 2011, Russell et al. 2011, Moiroux et al. 2012), and cuticular (Vannini et al. 2014, Huang et al. 2018). Target-site resistance is induced by punctual mutation in specific gene encoding for specific protein that interact with target insecticide through its mechanisms of action. The known common target-site resistance mechanisms that occur in malaria vectors are the voltage-gated sodium channel (*Vgsc*) mutations encoded by L1014F or L1014S, N1575Y and the insensitivity acetylcholinesterase *ace-1*(G119S) mutations that cause resistance to pyrethroid/DDT and carbamate/organophosphate insecticides, respectively (Martinez-Torres et al. 1998, Ranson et al. 2000, Djogbénou et al. 2007, Jones et al. 2012). However, genetic technologies developed in the last 20 yr cannot yet allow researchers to specifically link the characterized mechanisms to the resistance-phenotypes observed in field-collected malaria vectors after susceptibility assays.

The resistance of malaria vectors to insecticides used in public health relies on a genetic phenomenon which once selected is transmitted from generation to generation (Corbel and N'Guessan 2013). Its evolution over time is mainly favored by the selection pressure exerted by the pesticides and other xenobiotics residues present in malaria vector environments (Diabate et al. 2002, Djouaka et al. 2008, Djogbénou et al. 2011, Nkya et al. 2014). Unfortunately, the widespread of insecticide resistance in natural populations of *An. gambiae* mosquitoes represent a threat for implementation of malaria prevention programs based on the use of insecticide compounds (Ranson et al. 2011, Aïkpon et al. 2013, Corbel and N'Guessan 2013, Mnzava et al. 2015). To slow the threats of emergence and spread of resistance on vector control measures, the Global plan for insecticide resistance management in malaria vectors (GPIRM) was launched in May 2012 and one of its objectives was to fill gaps in knowledge on mechanisms of insecticide resistance and the impact of current insecticide resistance management approaches (World Health Organization 2012).

Recently, several studies have attempted to reveal the association between genotype at the kdr^R locus and occurrence of the pyrethroids/DDT resistance-phenotypes in wild populations of *An. gambiae s.s.* (Dabiré et al. 2009, Ibrahim et al. 2014, Djegbe et al. 2017). Meanwhile, other research works demonstrated a lack of such a correlation which means that kdr^R genotyping is not the only predictor of the pyrethroids/DDT resistance-phenotypes (Matambo et al. 2007, N'Guessan et al. 2007, Abdalla et al. 2008) often observed in

field-collected *Anopheles* mosquitoes. Overall, the role of kdr^R mutation in the expression of pyrethroids/DDT resistance-phenotypes remains a matter of debate (Donnelly et al. 2009).

In order to enlighten the ongoing debate on the involvement of resistance mechanisms in phenotypic insecticide resistance occurring in *An. gambiae*, we performed herein the resistance-intensity assays against four colonies of well-known genotypes and four field-collected mosquitoes using two insecticides currently applied in malaria vector control interventions. The more likely reasons explaining why the specific characterization of resistance mechanisms which confer the resistance-phenotypes still matters are discussed.

Materials and Methods

Mosquito Strains

Larvae and pupae were collected from four selected localities in Benin and Togo (based on the levels of insecticide resistance recorded by these populations in previous studies) in 2018 using the techniques previously described (Service 1977). Prospected areas were Avrankou (6°33′42″ N–2°38′55″ E), Bohicon (7°11N–2°49E), Grand Popo (6°14′28″ N–1°37′60″ E) in Republic of Benin and Baguida (06°09′47″ N–01°19′05″ E) in Republic of Togo (Fig. 1). Collected larvae and pupae were transported in labeled plastic bottles to the insectary of Laboratory of Infectious Vector-Borne Diseases based at Regional Institute of Public Health/University of Abomey-Calavi (Benin) and reared to adults as the *An. gambiae s.s.* of well-known genotypes used. All mosquito strains were maintained under standard insectary conditions of 70 ± 8% relative humidity and 27 ± 2°C ambient temperature. The field samples used for resistance-intensity assays were females F0 adults that emerged from the collected larvae and pupae. The *An. gambiae s.s.* colonies of well-known genotypes and the field strains used for resistance-intensity assessments, susceptibility status, insecticide resistance mechanisms, and references are presented in Table 1.

WHO Insecticide Resistance Tests for Determining Insecticide Resistance-Intensity

Intensity assays were performed on 3- to 5-d-old non-blood-fed females from both field and laboratory mosquitoes using the classical WHO susceptibility test kits (WHO 2016) with slight modifications. Filter papers impregnated with 0.5–7.5 times the diagnostic dose of the both deltamethrin (pyrethroid) and pirimiphos-methyl (organophosphate) were supplied by Liverpool School of Tropical Medicine (LSTM) and stored at 4°C before, during and after each test. These insecticides were chosen because they are currently used in West Africa for malaria vector control (deltamethrin in bed nets and pirimiphos-methyl in indoor residual spraying).

The doses of deltamethrin used were 0.05, 0.1, 0.25, and 0.375% (respectively termed 1×, 2×, 5×, and 7.5×) and those of pirimiphos-methyl were 0.125 and 0.25% (respectively termed 0.5× and 1×). Note that the strains used in the resistance-intensity experiments were not all exposed to the same insecticide (Table 2). The tests were

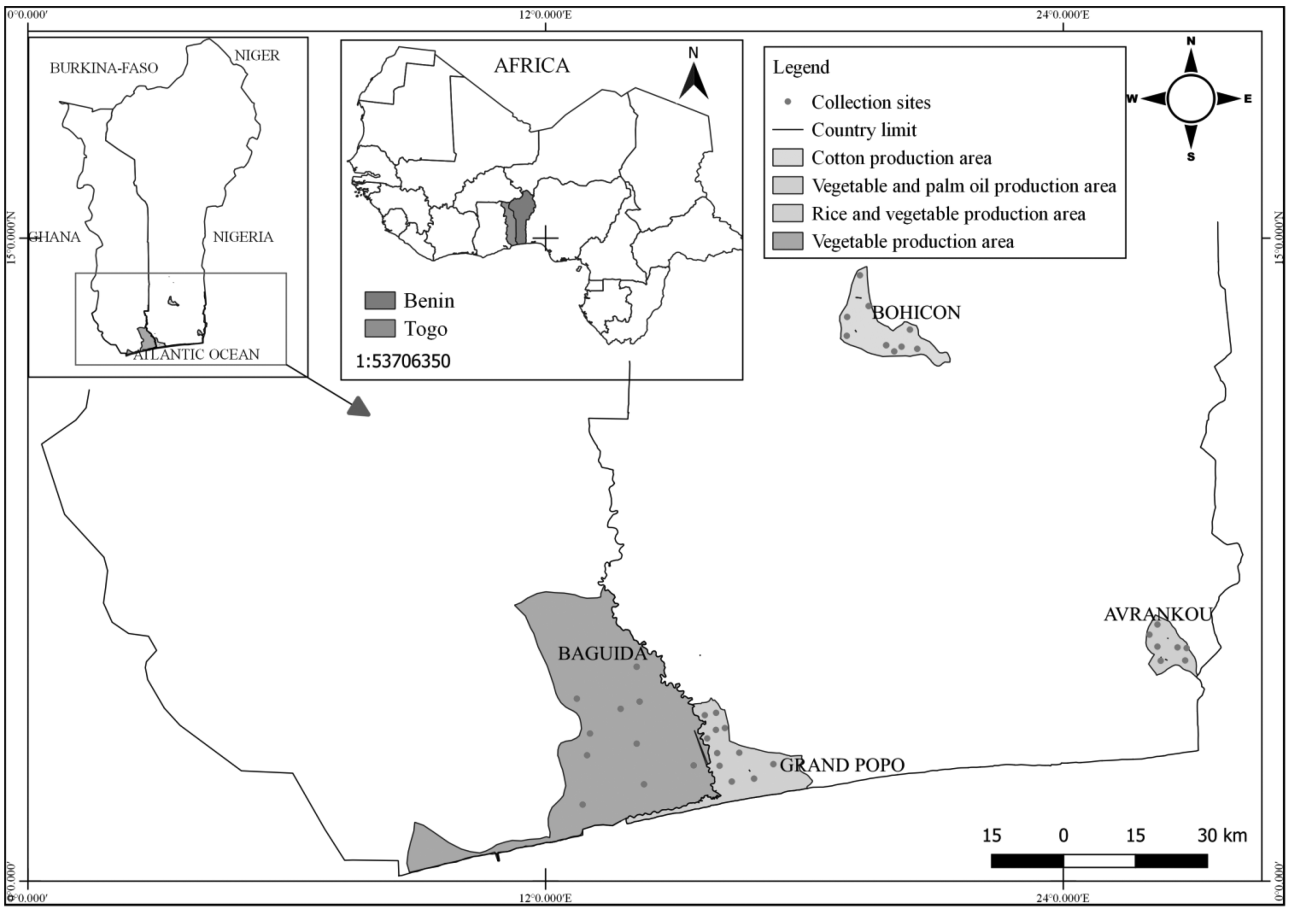

Fig. 1. Map of Benin and Togo showing the prospected breeding sites in Avrankou (vegetable and palm oil production area), Baguida (vegetable production area), Bohicon (cotton production area) and Grand Popo (rice and vegetable production area).

Table 1. Resistance status of the different mosquito strains (field and laboratory *Anopheles gambiae* strains) used in this study

Names of the strains	Type of the strains	Resistance profiles	Known resistance mechanisms	References
Kisumu	Laboratory strains sharing the same genetic background	Susceptible for all insecticides	None	Shute (1956)
KisKdr		Pyrethroids and DDT resistant	Homozygous for kdr^R(1014F) allele	Alout et al. (2013)
AcerKis		Carbamates and organophosphates resistant	Homozygous for $ace\text{-}1^R$(119S) allele	Djogbénou et al. (2007)
AcerKdrKis		Pyrethroids and DDT, carbamates and organophosphates resistant	Homozygous for both kdr^R(1014F) and $ace\text{-}1^R$(119S) mutations	Assogba et al. (2014)
Avrankou	Field strain	Permethrin resistant	Unknown	Assogba et al. (2020)
Baguida	Field strain	Pyrethroids and DDT, carbamates and organophosphates resistant	Unknown	Amoudji et al. (2019)
Bohicon	Field strain	Pyrethroids and DDT resistant	Unknown	Djègbè et al. (2011)
Grand Popo	Field strain	Permethrin resistant	Unknown	Assogba et al. (2020)

Table 2. *Anopheles gambiae* mosquitoes used in each intensity bioassays for deltamethrin and pirimiphos-methyl exposures

Insecticides	Deltamethrin				Pirimiphos-methyl	
Intensity doses	1×	2×	5×	7.5×	0.5×	1×
Mosquito strains	Kisumu KisKdr[a] AcerKdrKis[a] Avrankou Baguida Bohicon Grand Popo				Kisumu AcerKis[b] AcerKdrKis[b] Avrankou Baguida Bohicon Grand Popo	

[a]Homozygous for kdr^R(1014F) mutation.
[b]Homozygous for $ace\text{-}1^R$(119S) mutation.

implemented using batches of 20–30 females for 1 h at 70 ± 8% relative humidity and 27 ± 2°C ambient temperature. For a single insecticide dose, four batches were exposed against impregnated filter papers, whereas four other were exposed to nonimpregnated filter papers serving as controls. For deltamethrin insecticide, knocked-down mosquitoes were recorded at 10-min intervals along the hour of exposure. After insecticide exposures, final mortality rates were recorded 24-h holding period later during which a 10% honey solution was made available to survivors mosquitoes (WHO 2016).

Identification of *An. gambiae* Species and Detection of kdr^R(1014F/1014S) and $ace\text{-}1^R$(119S) Mutations in Field-Collected Mosquitoes

In order to assess the real resistant allele frequencies as occurred in natural populations, *Anopheles* specimens were randomly selected from the batches of each field unexposed populations (control tubes) and genomic DNA from these mosquito samples was individually extracted using the DNA extraction protocol previously developed (Collins et al. 1987). The members of *An. gambiae* species present were identified using the SINE-PCR method (Santolamazza et al. 2008). The presence of both West and East African kdr^R mutations was characterized in the same specimens applying a TaqMan real-time PCR assays (Bass et al. 2010). Specimens were also tested for $ace\text{-}1^R$(119S) mutation using Weill et al.'s protocol (Weill et al. 2004). Genotyping results' analysis was performed using a Mx3005Pro Software (Agilent Technologies, Stratagene, San Diego, CA).

Data Analysis

For different doses of deltamethrin bioassays, times for 50% knock-down of mosquitoes (KdT_{50}) and their CIs were generated by probit analysis through a log-time probit model with Polo-plus 1.0 software (Russell et al. 1977).

The percentage mortality (24-h postinsecticide exposure) of the mosquitoes exposed against each dose of each insecticide was determined as the proportion of mosquitoes that died at the times the diagnostic doses of each insecticide.

To assign resistance-intensity levels, the mortalities obtained from the WHO intensity bioassays as described above were interpreted using the following criteria as a guide:
For deltamethrin

- Mortality between 98 and 100% at 2× the diagnostic dose indicates low resistance-intensity.
- Mortality between 98 and 100% at 5× the diagnostic dose indicates moderate resistance-intensity.
- Mortality between 98 and 100% at 7.5× the diagnostic dose confirms moderate resistance-intensity.
- Mortality <98% at 7.5× the diagnostic dose indicates high resistance-intensity.

For pirimiphos-methyl

- Mortality <98% at 1× the diagnostic dose indicates low resistance-intensity.

The main objective of the data analysis was to compare the levels of insecticide resistance-phenotype for the field populations to those of the laboratory strains of well-known genotypes used in present study.

Results

Knock-Down Time Effects of Deltamethrin

The knock-down time (KdT_{50}) values induced by deltamethrin exposure at 1× the diagnostic dose were relatively higher for the field mosquitoes when compared with those of laboratory

Table 3. Knock-down times (KdT_{50} and KdT_{95}) with deltamethrin of the field-collected and laboratory *Anopheles gambiae* mosquitoes

Deltamethrin doses	Mosquito strains	*N*	Knock-down times with CI			
			$KdT_{50,}$ min	95% CI	$KdT_{95,}$ min	95% CI
1×	KisKdr	100	53.537	(47.923–62.290)	140.164	(107.007–218.236)
	Kisumu	100	14.659	(12.751–16.556)	36.798	(30.963–47.047)
	AcerKdrKis	100	49.259	(45.615–54.097)	97.401	(81.739–129.235)
	Avrankou	100	67.390	(57.222–84.425)	398.278	(258.241–758.440)
	Baguida	100	No kd	No kd	No kd	No kd
	Bohicon	100	74.607	(61.543–98.200)	536.946	(321.811–1173.022)
	Grand Popo	108	186.085	(111.776–986.760)	715.898	(270.373–18436.883)
2×	KisKdr	100	26.015	(23.847–28.283)	59.661	(52.189–71.187)
	Kisumu	100	11.297	(8.175–14.377)	33.583	(24.498–61.482)
	AcerKdrKis	100	30.128	(26.993–33.429)	58.461	(50.206–73.483)
	Avrankou	100	40.886	(34.630–50.729)	182.232	(120.517–369.668)
	Baguida	100	No kd	No kd	No kd	No kd
	Bohicon	100	47.722	(40.691–59.010)	265.192	(172.598–521.039)
	Grand Popo	92	168.252	(108.897–472.839)	802.533	(331.040–6935.958)
5×	KisKdr	100	17.906	(15.465–20.411)	40.980	(33.878–55.066)
	Kisumu	100	8.936	(6.054–11.565)	27.654	(20.045–52.464)
	AcerKdrKis	100	17.315	(15.213–19.432)	40.673	(34.542–51.236)
	Avrankou	100	23.726	(18.786–29.644)	98.042	(66.309–203.916)
	Baguida	100	94.128	(72.470–164.946)	318.972	(177.122–1224.283)
	Bohicon	100	24.867	(21.756–28.393)	104.032	(79.746 –152.937)
	Grand Popo	100	27.571	(25.467–29.866)	98.798	(83.633–122.038)
7.5×	KisKdr	100	17.906	(15.465–20.411)	40.980	(33.878–55.066)
	Kisumu	100	8.936	(6.054–11.565)	27.654	(20.045–52.464)
	AcerKdrKis	100	17.138	(15.610–18.678)	37.243	(32.838–43.991)
	Avrankou	100	21.208	(16.829–26.207)	94.858	(64.944–186.126)
	Baguida	100	80.850	(66.039–120.226)	236.477	(147.908–641.252)
	Bohicon	100	21.946	(18.418–25.928)	117.689	(83.490–201.220)
	Grand Popo	100	80.850	(65.969–120.722)	236.477	(147.562–648.107)

KdT_{50}, knock-down time for 50% of mosquitoes; KdT_{95}, knock-down time for 95% of mosquitoes; *N*, sample sizes; No kd, complete loss of knock-down effect (<10% knock-down after 60 min exposure).

strains (Table 3). From 2×, the diagnostic dose and above, knock-down time (KdT_{50}) values of the field populations showed two to four times increase in the mean KdT_{50} compared with the well-known resistant laboratory strains of *An. gambiae* (Table 3).

Insecticide Resistance-Phenotypes

The levels of resistance-phenotype for deltamethrin and pirimiphos-methyl in the field populations (Avrankou, Baguida, Bohicon, and Grand Popo) were assessed and compared to those of the laboratory strains of well-known genotypes (see supplementary data: Phenotypic Insecticide Resistance).

For Deltamethrin

Using the criteria explained above in present study, Kisumu was as expected, susceptible at 1× the diagnostic dose, whereas KisKdr, AcerKdrKis, Avrankou, Baguida, Bohicon, and Grand Popo mosquitoes displayed mortalities of less than 98%. At 2×, the diagnostic dose, both KisKdr and AcerKdrKis strains showed susceptibility (100% mortality), whereas Grand Popo, Bohicon, Baguida, and Avrankou samples were still resistant and confirmed moderate-resistant, respectively, against 7.5× the diagnostic dose (Fig. 2). In addition, even if they were purely homozygote for kdr^R(1014F) allele, KisKdr and AcerKdrKis displayed low intensity of resistance and the field mosquitoes showed relatively high resistance-intensity against deltamethrin insecticide.

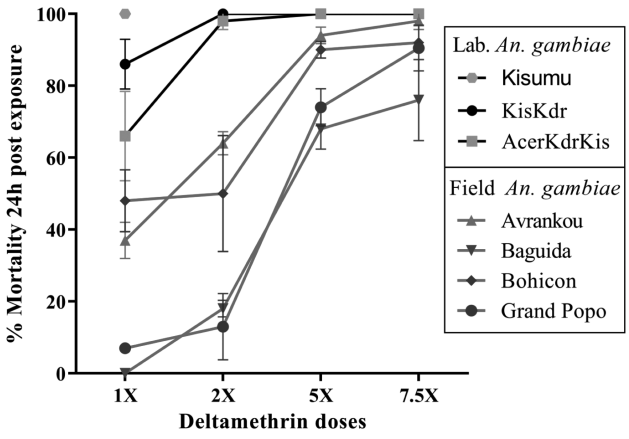

Fig. 2. Levels and evolutions of resistance-phenotype for the field-collected *Anopheles gambiae* in deltamethrin exposure compared to those of laboratory strains of well-known genotypes. Error bars indicate 95% CIs.

For Pirimiphos-Methyl

Based on the criteria explained above, all laboratory strains (Kisumu, AcerKis, and AcerKdrKis) exposed to 0.5× the diagnostic dose showed susceptibility while Baguida mosquitoes were still resistant against 1× the diagnostic dose with mortality of less than 98% (Fig. 3). Even if they were purely homozygote for *ace-1*^{R}(119S) allele, AcerKis and AcerKdrKis displayed susceptibility phenotypes and Baguida *An.*

gambiae recorded low resistance-intensity (64% mortality) against 1× the diagnostic dose of pirimiphos-methyl insecticide.

Detection of *kdr*^{R}(1014F/1014S) and *ace-1*^{R}(119S) Mutations in Natural Populations

Species identification was performed on a total of 320 individuals of *Anopheles* mosquitoes (80 from each locality). *Anopheles gambiae s.s.*, *An. coluzzii*, and *An. arabiensis* species were identified. Overall, 13 *An. gambiae s.l.* were *An. gambiae s.s.* (16.25%) and 67 (83.75%) belonged to *An. coluzzii* among Avrankou specimens. All of Baguida individuals investigated were *An. gambiae s.s.* From Bohicon *Anopheles* mosquitoes, 39 were *An. gambiae s.s.* (48.75%), 36 were *An. coluzzii* (45%) and 5 (6.25%) were *An. arabiensis*. All of Grand Popo samples were *An. coluzzii*. These specimens were then genotyped for *Vgsc-kdr*^{R}(1014F/1014S) and *ace-1*^{R}(119S) mutations to evaluate their frequencies in natural populations of *An. gambiae s.l.* tested. The *Vgsc-kdr*^{R}(1014F) West resistance allele frequencies were relatively high (ranging 0.65–1) among all the field *An. gambiae s.l.* populations (Table 4). From Avrankou, Baguida, Bohicon, and Grand Popo *kdr* resistant specimens, 67.74, 100, 96.1, and 76% of individuals were detected homozygotes [RR], respectively, and the remaining are heterozygotes [RS]. No individual was detected bearing *kdr*^{R}(1014S) East resistance allele.

In Baguida mosquito specimens, the *ace-1*^{R}(119S) point mutation was detected at high frequency (0.84). From these *ace-1* resistant mosquitoes, 83.56% of individuals were homozygotes [RR] and the remaining were heterozygotes [RS]. However, relatively low *ace-1*^{R}(119S) frequencies were detected among the other field mosquitoes (Table 4) (see supplementary data: Phenotypic Insecticide Resistance).

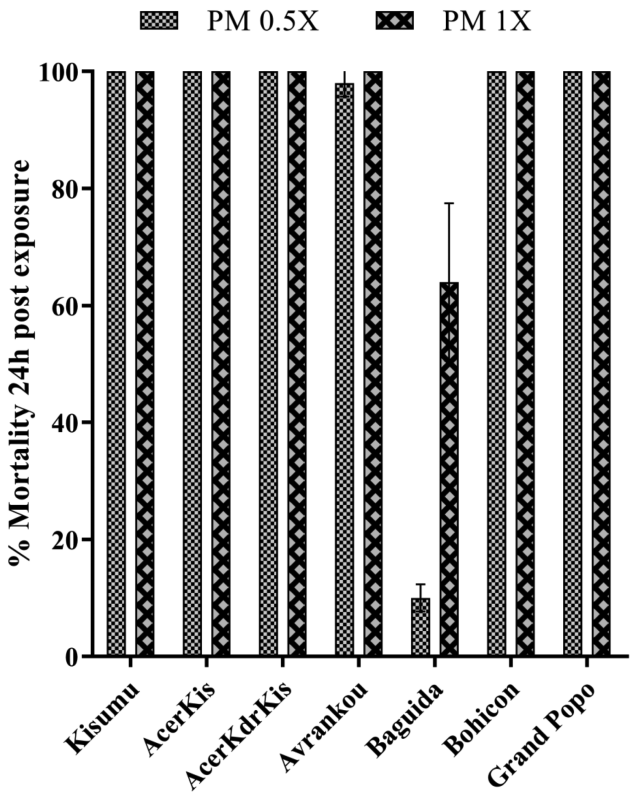

Fig. 3. Levels of resistance-phenotype for the field-collected *Anopheles gambiae* in pirimiphos-methyl (PM) exposure compared to those of laboratory strains of well-known genotypes. Error bars indicate 95% CIs.

Discussion

Specific mechanisms involved in the insecticide resistance-phenotypes occurring in natural populations of malaria vectors remain unclear. With regard to the *kdr*^{R}(1014F or 1014S) mutations, the current question is whether the levels of resistance-phenotype observed toward pyrethroids in the field *Anopheles* vectors is associated or not with the frequency of these resistance alleles (Donnelly et al. 2009). The present study was conducted in order to enhance the debate about the question above. Here, we have compared knock-down times and mortality values of *An. gambiae* strains bearing the well-known target-site insensitivity mechanisms at homozygous state (KisKdr, AcerKis, and AcerKdrKis) with those of field-collected populations (Avrankou, Baguida, Bohicon, and Grand Popo).

Results from intensity bioassays showed that all of the well-known homozygous resistant individuals for *kdr*^{R}(1014F) mutation (KisKdr and AcerKdrKis strains) died at 2× the diagnostic dose of deltamethrin, whereas significant survival percentages: 2, 24, 8, and 9% of adult female mosquitoes from Avrankou, Baguida, Bohicon, and Grand Popo localities (field strains) were recorded at 7.5× the diagnostic dose, respectively. Moreover, the molecular biology analysis among all the field strains revealed globally, the presence of *An. gambiae* species (*An. coluzzii*, *An. gambiae s.s.*, and *An. arabiensis*) with very high frequency of *kdr*^{R}(1014F) mutation (Table 4). Furthermore, by using the average knock-down time (KdT_{50}) data obtained with the range of deltamethrin doses and the WHO bioassay method, it was shown that the knock-down time expressed in the field *An. gambiae* is approximately two to four times higher than the one displayed by the insecticide-resistant laboratory strains (resistance ratio calculated for the field strains using KisKdr KdT_{50}

Table 4. Frequency of the *kdr*^{R}(1014F) and *ace-1*^{R}(119S) mutations in the field-collected *Anopheles gambiae s.l.*

Locality	*Anopheles coluzzii*				*Anopheles gambiae s.s.*				*Anopheles arabiensis*				Frequency mutation in field *An. gambiae s.l.*		
	kdr^R (1014F)		*ace-1*^{R} (119S)		*kdr*^{R} (1014F)		*ace-1*^{R} (119S)		*kdr*^{R} (1014F)		*ace-1*^{R} (119S)				
	n^s	*F*	n^s	*F*	n^s	*F*	n^s	*F*	n^s	*F*	n^s	*F*	n^l	*F* (*kdr*^{R})	*F* (*ace-1*^{R})
Avrankou	67	0.73	67	0	13	0.23	13	0	–	–	–	–	80	0.65	0
Baguida	–	–	–	–	80	1	80	0.84	–	–	–	–	80	1	0.84
Bohicon	36	0.90	36	0	39	1	39	0.01	5	0.8	5	0	80	0.94	0.006
Grand Popo	80	0.83	80	0.05	–	–	–	–	–	–	–	–	80	0.83	0.05

n^s, number of mosquitoes tested by species; n^l, total number of mosquitoes tested; *F*, allele frequency; –, no data.

value as denominator; Table 3). The present results show clearly that the observed phenotypes (in terms of resistance level) in the field populations is not associated only with the presence of the resistance allele *kdr*^{R}(1014F). Comments above on the *kdr*^{R} mutation can also be applied to the *ace-1*^{R}(119S) mutation. The findings have shown that, at 1× the diagnostic dose of pirimiphos-methyl, all resistant homozygous specimens for the *ace-1*^{R}(119S) mutation of laboratory strains used were killed, whereas, except the 100% mortalities displayed by Avrankou, Bohicon, and Grand Popo mosquitoes, only 64% of death was recorded especially in Baguida *An. gambiae*. Therefore, it can also be deducted here that the levels of resistance-phenotype observed against pirimiphos-methyl in this field *An. gambiae* population was not only associated with the presence of the resistance allele *ace-1*^{R}(119S) at the *ace-1* locus.

In most insecticide resistance studies using wild populations, the target-site insensitivity-mediated resistance such as *kdr*^{R}(L1014F, L1014S & N1575Y), *Rdl* and *ace-1*^{R}(G119S), is one of the most common resistance characterized in malaria vectors (Djogbénou et al. 2011, Alemayehu et al. 2017, Nardini et al. 2017, Camara et al. 2018). However, the role of metabolic resistance mechanisms through activities of the P450 and GST genes is increasingly being detected in *An. gambiae* vectors across different sites (Ochomo et al. 2013, Mitchell et al. 2014, Awolola et al. 2018, Stica et al. 2019). Thus, in this study, the wider discrepancies in the levels of resistance-phenotype observed between the field and laboratory resistant *An. gambiae* exposed against various doses of deltamethrin (Fig. 2) indicate that metabolic insecticide resistance mechanisms like P450-monooxygenase could contribute to the pyrethroid resistance-phenotypes observed.

Malaria control highly depends on an effective programmatic-scale vector control with wide distribution of insecticide-treated nets and the large-scale indoor residual spraying campaigns which have contributed to the recent decline in morbidity and mortality in endemic countries (Katureebe et al. 2016). Unfortunately, insecticide resistance is a cause of a great concern for vector control and it threatens to reverse these gains. To monitor the insecticide resistance mechanisms, relatively affordable diagnostic methods for the detection of target-site insecticide resistance mutations have been carried out and can be used in the endemic countries and also used to monitor their evolution in natural populations of malaria vectors (Martinez-Torres et al. 1998, Weill et al. 2004, Bass et al. 2010, Badolo et al. 2012). As for metabolic resistance mechanisms, there are still no tools to monitor their evolution in time and space due to their complex molecular basis despite their probable greater operational impact on malaria control (Corbel and N'Guessan 2013, David Jean-Philippe et al. 2013, Liu 2015). This is then posing a serious additional threat for malaria vector control measures. Furthermore, it is also possible that unknown insecticide resistance mechanisms may be occurring in wild populations of the dominant Afro-tropical malaria vectors *An. gambiae*. In this case, we cannot argue that the metabolic resistance plus the target-site insensitivity mechanisms may be the cause of the higher levels of insecticide resistance-phenotype observed. Recent studies have revealed the presence of previously undetectable insecticide resistance mechanisms in African malaria vectors *An. gambiae* (Balabanidou et al. 2018, 2019; Ingham et al. 2018). That illustrates thereby the complexity of mechanisms involved in the resistance-phenotypes observed in malaria vectors *An. gambiae*.

Our findings provide evidence that target-site insensitivity mutations alone cannot induce the resistance-phenotypes that occur in natural populations of *An. gambiae* mosquitoes. Therefore, the main concern of entomologists and other actors of the related disciplines would be to work for a better characterization of the specific resistance mechanisms that contribute to this insecticide resistance phenomenon in order to help appropriate decision-making process for an effective management of resistant *Anopheles* vectors.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

We express our sincere thanks to David Weetman and Martin Donnelly (LSTM) for providing WHO insecticide-impregnated papers. We would like to acknowledge Emilienne Fiogbe for her helpful English writing assistance. This study and AAM received financial support by grant to LSD from Wellcome Trust (grant 109917/Z/15/Z).

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