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Insecticide Resistance in Malaria Vectors in Dielmo and Ndiop: Comparative Analysis of Knock Down Resistance Patterns using Two Sampling Methods in a Malaria Pre-elimination Context

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Abstract: Introduction: Vector control is still confronted with the issue of insecticide resistance. This study investigates the impact of this phenomenon on entomological parameters and its distribution according to the mosquito sampling method in Dielmo and Ndiop, two malaria pre-elimination communities. **Methods:** Mosquitoes were sampled using human landing catch (HLC) and larval collection (LC) methods. Susceptibility tests were performed on LC samples. Larvae were collected using the dipping method from georeferenced breeding sites in the immediate vicinity of the study site. Five pyrethroids (alphacypermethrin, deltamethrin, lambdacyhalothrin, permethrin and etofenprox), one carbamate (bendiocarb) and one organophosphate (fenitrothion) were tested, using 2–5-day-old females. PCR techniques were used to identify *Anopheles* species and detect the *Kdr* mutation. *Kdr* frequencies were calculated under Hardy–Weinberg equilibrium and comparatively analyzed in terms of sampling method, biting behavior, longevity, vector species and study site, using a linear regression model. **Results:** Mosquitoes were fully susceptible to carbamates and organophosphates. For pyrethroids, mortality rates ranged from 80.3 to 100%. PCR revealed three species, *An. coluzzii, An. gambiae* and *An. arabiensis*. The latter predominated at both sites. *Kdr* frequencies ranged from 2.4 to 14% in Dielmo and from 17.3 to 40% in Ndiop. In Dielmo, *An. arabiensis* showed a low endophagic rate (36.6%). Biting behavior was independent of *Kdr* mutation, night period, species and mosquito lifespan but was dependent on the study site (p = 0.008). Comparing the sampling methods, there was a 43% lower chance of finding the *kdr* mutation in LC females (OR = 0.57; p = 0.03) in Dielmo, against a 3 times higher probability of detecting a *Kdr* allele in Ndiop (OR = 2.64; p = 0.001). **Conclusion**: This study underlines the importance of investigating insecticide resistance in host-seeking females, particularly in malaria pre-elimination settings.

Keywords: malaria; *An. gambiae* s.l.; insecticide resistance; Dielmo; Ndiop; pre-elimination

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Introduction

The burden of malaria is ever-present in sub-Saharan Africa, which continues to pay the heaviest price, despite undertakings to progress towards the elimination of malaria in many countries [1]. Malaria prevention is largely based on effective vector control tools such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). In 2020, 65% of people at risk were protected by LLINs and 2.6% by IRS [1]. Because of their low toxicity to humans and mammals and their high lethal effect on mosquitoes, pyrethroids are the main chemical class recommended by the World Health Organization (WHO) for treating bed nets [2]. However, the spread of pyrethroid resistance in malaria vector populations is threatening control strategies [1,3,4]. Intensive research has provided a description of a wide variety of insecticide resistance mechanisms in the main malaria vectors worldwide [5–10], among which is the knock down resistance (*kdr*) mutation, which undoubtedly confers resistance to pyrethroids and DDT [11]. On the other hand, malaria control could also be hindered by mosquito behavioral changes, which emerge as adaptive responses to implemented insecticide-treated tools [12–14]. To mitigate insecticide resistance, vector control research is generating new and promising approaches to control pyrethroid-resistant mosquitoes by manufacturing new generations of LLINs containing pyrethroids mixed with other insecticides that have different modes of action [15–18], or with synergist compounds [19–22], or even with insect growth regulators [23–26]. Faced with the challenges of the current context, assessing both insecticide resistance and vector behavioral adaptations should be the key components of control strategies in malaria endemic settings [27]. Measuring insecticide resistance in malaria vectors relies on bioassays, using either WHO insecticide susceptibility test kits or US Centers for Disease Control and Prevention (CDC) glass bottles, for mortality determination of adult mosquitoes against discriminating doses [28–31]. Most often, a random sub-sample of tested specimens, i.e., those from larval collections (LCs), is used for species identification and detection of resistance mechanisms such as the *Kdr* mutation [32–40]. However, as demonstrated in *Anopheles sinensis*, when using different sampling methods to assess insecticide resistance, a certain disparity often arises in resistance measurements [41]. The current malaria epidemiology, especially in pre-elimination areas, requires a careful analysis of the insecticide resistance issue; hence, the detection of the *Kdr* mutation should not only be restricted to a fraction of specimens used for bioassays but should also include indoor-resting [42,43] and host-seeking females.

In previous surveys carried out in Dielmo, western Senegal, the effect of malaria control tools on vector dynamics has been assessed [44], and a snapshot of the insecticide resistance profile has been taken, following nearly a decade of intense use of LLINs in this setting [32]. Dielmo and Ndiop are both in a malaria pre-elimination phase, and entomological surveillance activities, as well as insecticide resistance monitoring, are being performed in these villages.

This study aimed to update the insecticide susceptibility profile and make a comparative analysis of *Kdr* patterns in *An. gambiae* s.l., a major malaria vector in Dielmo and Ndiop, using two sampling methods, larval collection (LC) and human landing catch (HLC).

Methods

Study sites. This study was conducted during the rainy season, in September 2016, in Dielmo and Ndiop (Figure 1), two sites in western Senegal, in the Fatick region, 280 km from Dakar. Dielmo and Ndiop are 5 km apart and were included in a research project on malaria epidemiology that lasted over 25 years [45,46]. A description of Dielmo village was detailed at the beginning of the project, in 1990 [45], as was that of Ndiop, which joined the project three years later [46]. Since then, the sociodemographic status of Dielmo and Ndiop has been updated on a fairly regular basis [46–49]. Serere and Wolof are still the dominant ethnic groups in Dielmo and Ndiop, respectively. The main activities of the villagers remain agriculture (millet, groundnuts, maize). However, market gardening is relatively important in Dielmo thanks to the presence of a small permanent river, the *Nema*. In Ndiop, a large part of the population is engaged in rice growing in flood-prone areas a few miles from the village.

Socio-economically, one of the most significant events in these villages was their electrification between 2012 and 2015, enabling the regular use of television and the installation of a millet mill for women. However, access to electricity has also been associated with malaria epidemic rebounds, especially among people spending much of the night outdoors watching television [50]. Vector control was implemented in 2008 and since then, only LLIN universal coverage has been strategically used both in Dielmo and Ndiop. LLIN universal coverage was renewed in 2011, 2014, 2016, 2019 and 2022. During the period of the present study, in 2016, Ndiop was fully covered with permethrin-impregnated mosquito nets (*Olyset® Net*) and Dielmo with deltamethrin-impregnated ones (*PermaNet 2.0®*).

Field mosquito sampling and bioassays. Host-seeking mosquito females were captured both indoors and outdoors, from 7 p.m. to 7 a.m., using the human landing catch (HLC) method. Monthly captures were carried out over two consecutive nights in three rooms, using two volunteers per catching point, one inside and the other outside the room. The method consisted of catching females landing on the exposed body parts of the trapper by hypnotizing them with a torchlight before enclosing them in a hemolysis tube. Mosquito immature stages were collected in different breeding sites, as described in a previous study carried out in Dielmo [32]. Larvae were fed with a fish feed (TetraMin Baby®) and were maintained in a local insectary, where the temperature and the relative humidity conditions were controlled (25 $\pm$ 2 °C and 75 $\pm$ 10%). Pupae were collected daily and were transferred into crystallizers placed in cages covered with untreated mosquito nets. On emergence, adults were fed using a cotton swab soaked in a 10% sugar solution placed on the upper surface of the hatching cage. Larval collections (LCs) in the immediate vicinity of the study sites were coupled with HLC activities on the same monthly capture run, so that comparisons could be made between host-seeking females (HLC) and those collected from LCs. Captured host-seeking females were morphologically identified using dichotomous keys [51,52] and their ovaries were dissected for the determination of lifespan (longevity) through the calculation of the parity rates (ratio of the number of parous females to the total number dissected), as described by Detinova [53]. Susceptibility tests were carried out by exposing 3–5-day-old females from LCs to different discriminating doses of insecticides, using the Centers for Disease Control and Prevention (CDC, Atlanta GA) bottle bioassays, as described in a previous survey in Dielmo [32]. Insecticides were supplied by the CDC in hyperconcentrated formulations. In the laboratory, they were diluted in Falcon tubes by adding a volume of pure acetone (solvent) in order to obtain working solutions (50 mL) so that 1 mL of each corresponded to the diagnostic dose used to impregnate one bottle test. Control bottles were coated with 1 mL of solvent (pure acetone). Female mosquitoes were exposed for 30 minutes, which was the diagnostic time for all the insecticides used, including five pyrethroids (alphacypermethrin 12.5 µg/bottle, deltamethrin 12.5 µg/bottle, etofenprox 12.5 µg/bottle, lambdacyhalothrin 12.5 µg/bottle

and permethrin 21.5 µg/bottle), one carbamate (bendiocarb 12.5 µg/bottle) and one organophosphate (fenitrothion 50 µg/bottle).

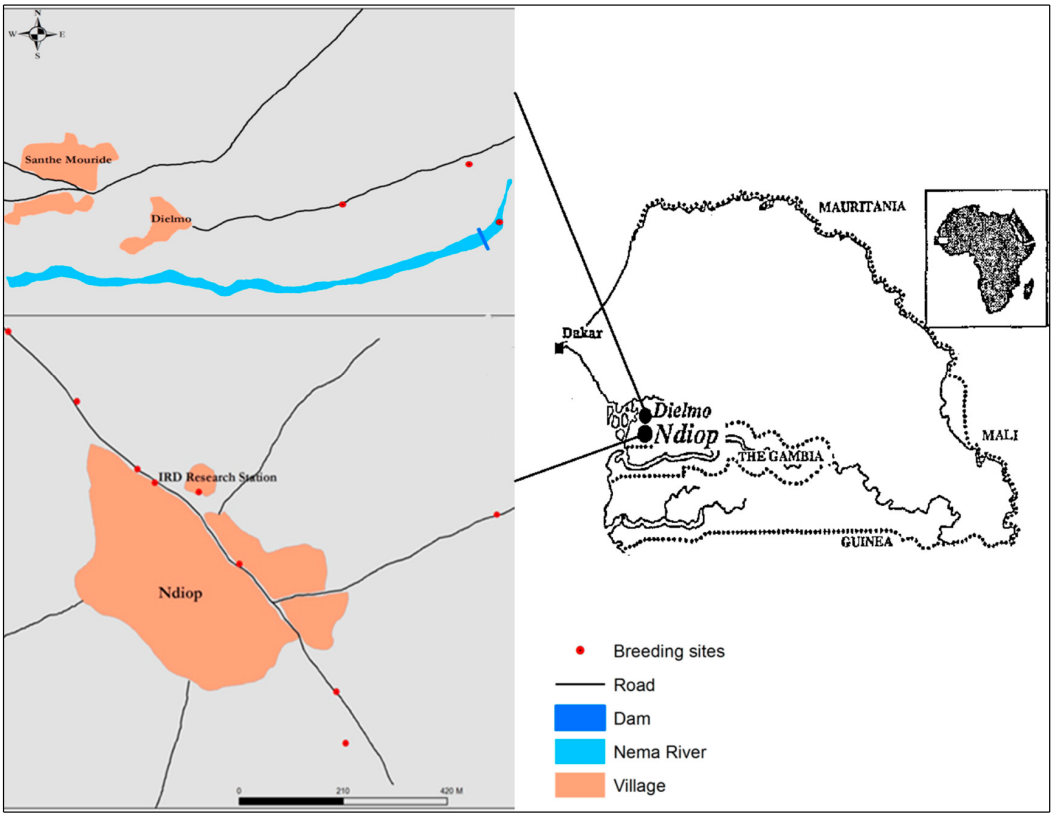

Figure 1: Map showing the location of the study sites (Dielmo and Ndiop) with breeding sites and the distinctive *Nema* river in Dielmo.

Mosquito laboratory processing. Mosquito genomic deoxyribonucleic acid (DNA) was extracted using CTAB method [32,54]. A random sub-sample of specimens from HLC and a batch of insecticide post-exposed females (consisting of randomly selected dead individuals and all survivors) was used for species identification and the detection of the *Kdr* mutation, as described by Wilkins et al. [55], and Hunyh et al. [56], respectively. Both *Kdr-west* (L1014F) and *Kdr-East* (L1014S) mutations were investigated. The steps in PCR procedures were detailed in a recent study [32].

Data analysis. The mortality rates of *An. gambiae* s.l. females exposed to insecticides were interpreted according to the WHO criteria, in which a 90% resistance threshold was defined. The allelic frequencies of the *Kdr* mutation (*L1014F* and *L1014S*) were calculated using the Hardy–Weinberg model. Wright's fixation index (F_{is}) was determined and departures from the Hardy–Weinberg equilibrium were analyzed using Pearson's Chi-2 test or Fisher's test through the '*HWExact*' function from the '*GWASExactHW*' package in R software [57]. Means were compared using the Student test or the Kruskall–Wallis test, and 95% confidence intervals were calculated using the binomial exact test. Endophagic rates were determined as the ratio of the number of mosquitoes collected indoors (endophagous females) over the total captured using the HLC technique. A generalized linear model (GLM) analysis of indoor biting behavior was performed, where the odds ratio and 95% confidence intervals were calculated by negative binomial distribution, using the following explanatory variables: *Kdr* mutation, nocturnal biting behavior, *Anopheles* species, lifespan and study site. The mosquito nocturnal collection time was divided into two nighttime periods, which, for the purposes of analysis, will be referred to as "early" (7 p.m.–1 a.m.) and "later" (1–7 a.m.).

GLM analysis of the *Kdr* mutation was also performed, using the sampling method (HLC or LC) and *Anopheles* species as explanatory variables.

Logistic regression analyses were performed using the '*GLM*' function of R software. The significance level was set at 5%.

Results

Susceptibility of *An. gambiae* s.l. to insecticides. Bioassays were performed on a sample of 1070 specimens from Dielmo (n = 647) and Ndiop (n = 430). The results showed full susceptibility (100% mortality) to carbamates (bendiocarb) and organophosphates (fenitrothion) where these insecticides were tested (Table 1). Susceptibility to pyrethroids was relatively heterogeneous. *Anopheles* mosquitoes were fully susceptible (100% mortality) to lambdacyhalothrin and alphacypermethrin, which were tested only in Dielmo. However, a suspected resistance to etofenprox was noted, with respective mortality rates of 95.7% and 95.3% in Dielmo and Ndiop. Regarding deltamethrin and permethrin (Table 1, Figure 2), resistance was reported in Ndiop (87.5% and 80.3% mortality rates, respectively). However, a suspected resistance of *An. gambiae* s.l. to these latter pyrethroids was observed in Dielmo (95.2% and 92.5%, respectively). Molecular species identification indicated that the *An. gambiae* complex was represented by *An. arabiensis*, *An. gambiae and An. coluzzii*, with respective proportions of 74.66% ($CI_{95\%}$: 69.85–79,05), 12.36% ($CI_{95\%}$: 12.36–20.16) and 8% ($CI_{95\%}$: 6.57–12.84) in Dielmo and 76.28% ($CI_{95\%}$: 70.55–81.39), 9.49% ($CI_{95\%}$: 6.17–13.78) and 14.23% ($CI_{95\%}$: 10.17–19.15) in Ndiop (Figure 2).

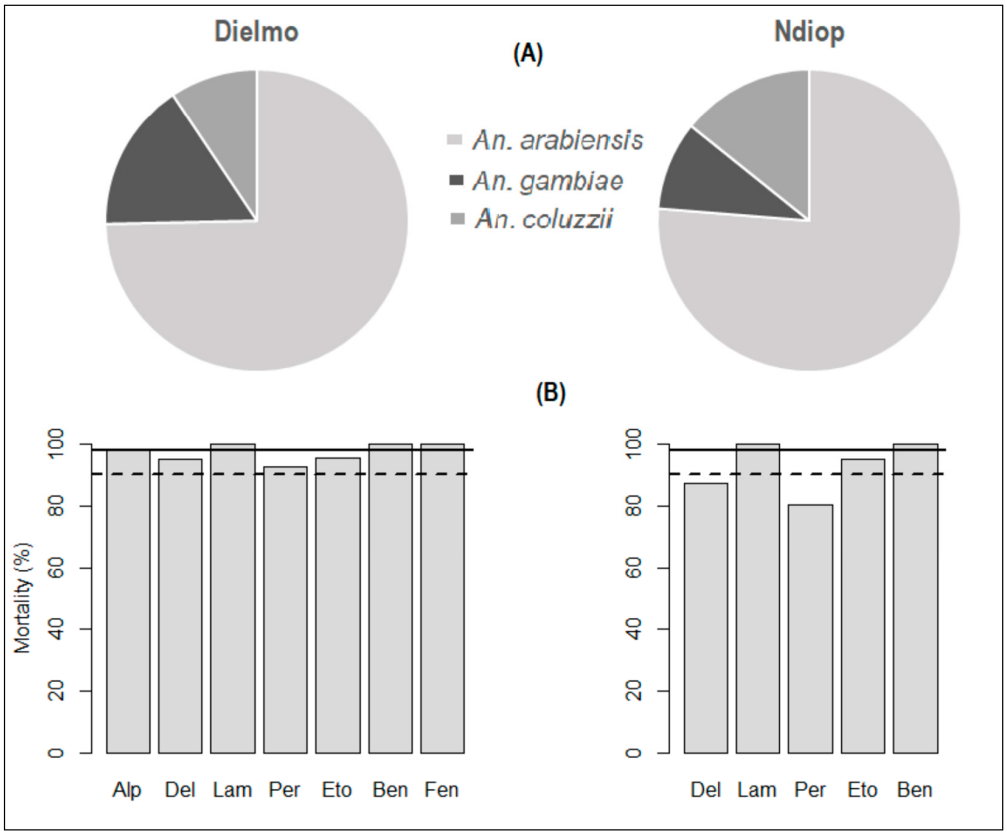

Figure 2: Species composition (**A**) and insecticide susceptibility status (**B**) in Dielmo and Ndiop. Solid and dotted horizontal bars indicate, respectively, susceptibility (98%) and resistance (90%) thresholds according to WHO criteria (WHO, 2016).

Table 1: Mortality rates of *Anopheles gambiae* s.l. from Dielmo and Ndiop following exposure to diagnostic doses of pyrethroid, carbamate and organophosphate insecticides.

Insecticides	Mortality Rates (%)		Mosquito Phenotype	
	Dielmo [$CI_{95\%}$]	Ndiop $CI_{95\%}$]	Dielmo	Ndiop
α-cypermethrin (97, 0)	98 [92.7–99.7]	-	S	-
Deltamethrin (83, 80)	95.2 [88.1–98.7]	87.5 [78.2–93.8]	R*	R
λ-cyhalothrin (106, 93)	100 [96.6–100]	100 [96.1–100]	S	S
Permethrin (94, 71)	92.5 [82.3–97]	80.3 [69.1–88.7]	R*	R
Etofenprox (93, 86)	95.7 [89.4–98.8]	95.3 [88.5–98.7]	R*	R*
Bendiocarb (83, 100)	100 [95.7–100]	100 [96.4–100]	S	S
Fenitrothion (91, 0)	100 [96.0–100]	-	S	-

Mortality rates were interpreted using WHO criteria. Figures between brackets represent the total number of specimens tested for each insecticide in Dielmo and Ndiop. Figures between square brackets represent 95% confidence intervals for mortality rates. S, susceptible; R, resistant; R*, suspected of resistance.

***Kdr* allelic frequencies under Hardy–Weinberg equilibrium.** *Kdr* allelic frequencies were calculated by separating *L1014F* and *L1014S* mutations, thus focusing on a bi-allelic system approach. For *An. arabiensis*, *An. coluzzii* and *An. gambiae*, respectively, *kdr-w* (*1014F*) allele frequencies were 11.34%, 2.4% and 14% in Dielmo and 26.2%, 40% and 17.3% in Ndiop, while *kdr-e* (*1014S*) allele frequencies were 36.08%, 16.67% and 15.12% in Dielmo and 43%, 27.5% and 30.77% in Ndiop (Table 2). All *An. arabiensis* and *An. gambiae* populations presented heterozygote deficiencies, with fixation indices ranging from 0.692 to 0.821 ($p < 0.05$) in Dielmo and from 0.352 to 0.866 in Ndiop ($p < 0.05$), regardless of *Kdr* mutation type (Table 2). On the other hand, *An. coluzzii* populations showed an excess of heterozygotes in Dielmo ($F_{is} = -0.024$; $p = 0.989$) and Ndiop ($F_{is} = -0.128$; $p = 0.003$), when considering *L10114F* and *L1014S* mutations, respectively (Table 2).

Table 2: Genotypes and *Kdr-west* (*L1014F*) and *Kdr*-east (*L1014S*) mutations in Dielmo and Ndiop under Hardy–Weinberg equilibrium.

		Genotypes				***1014F/S***	HWE	
L1014F mutation		SS	RS	RR	Total	Freq. (%)	F_{IS} (Wright)	*P-value*
An. arabiensis	Dielmo	166	12	16	194	11.34	+0.692	<0.0001
	Ndiop	140	36	38	214	26.20	+0.565	<0.0001
An. coluzzii	Dielmo	20	1	0	21	2.40	−0.024	0.989
	Ndiop	21	6	13	40	40.00	+0.686	<0.0001
An. gambiae	Dielmo	36	2	5	43	14.00	+0.806	<0.0001
	Ndiop	21	1	4	26	17.3	+0.866	<0.0001
L1014S mutation								
An. arabiensis	Dielmo	116	16	62	194	36.08	+0.821	<0.0001
	Ndiop	88	68	58	214	43.00	+0.352	<0.0001
An. coluzzii	Dielmo	17	1	3	21	16.67	+0.829	<0.0001
	Ndiop	20	18	2	40	27.50	−0.128	0.002
An. gambiae	Dielmo	35	3	5	43	15.12	+0.728	<0.0001
	Ndiop	16	4	6	26	30.77	+0.639	0.003

HWE: Hardy–Weinberg equilibrium.

Impact of *Kdr* mutation on mosquito biting behavior. A mean endophagy rate of 36.6% ($CI_{95\%}$: 26.2–47.9) was observed in Dielmo (Table 3), indicating an outdoor biting behavior that particularly characterized *An. arabiensis* (binomial exact test: $p = 0.02$). In Ndiop, the mean endophagic rate was estimated to be 50.6% ($CI_{95\%}$: 42.7–58.5), showing a certain plasticity in biting activity, with a similar likelihood of indoor and outdoor biting behavior (binomial exact test: $p = 0.94$).

Multiple regression analysis demonstrated that indoor biting behavior was independent of *Kdr* mutation, nocturnal biting pattern (early or later), vector species (*An. arabiensis* versus *An. gambiae* and *An. coluzzii*) and longevity of host-seeking females. However, biting behavior was more influenced by the study site, with marked outdoor biting activity in Ndiop compared to Dielmo ($p = 0.008$) (Table 4).

Table 3: Relative proportions (%) of three sibling species within *Anopheles gambiae* complex during indoor and outdoor human landing catches in Dielmo and Ndiop villages, Senegal.

Species	Dielmo						Ndiop					
	Indoor		Outdoor		Total		Indoor		Outdoor		Total	
	n_i	(%)	n_o	(%)	n_t	(%)	n_i	(%)	n_o	(%)	n_t	(%)
An. arabiensis	23	(33.8)	45	(66.2)	68	(82.9)	66	(53.2)	58	(46.8)	124	(75.6)
An. coluzzii	3	(37.5)	5	(62.5)	8	(9.8)	13	(41.9)	18	(58.1)	31	(18.9)
An. gambiae	4	(66.7)	2	(33.3)	6	(7.3)	4	(44.4)	5	(55.6)	9	(5.5)
Overall	30	(36.6)	52	(63.4)	82	(100)	83	(50.6)	81	(49.4)	164	(100)

$n_{(i,\ o,\ t)}$, number of mosquitoes captured indoor (n_i) and outdoor (n_o) on total collections (n_t).

Table 4: Generalized linear model (GLM) analysis of indoor biting behavior. Odds ratio and 95% confidence intervals were calculated by negative binomial distribution using the following explanatory variables: *kdr* mutation, nocturnal biting activity (time of night), species, parity and study site.

Indoor Biting		Odds Ratio	[$CI_{95\%}$]	*p-Value*
Kdr mutation	RR *vs* SS	0.754	[0.413–1.364]	0.353
Time of night *	Later *vs* Early	1.270	[0.730–2.224]	0.402
Species	*An. coluzzii* vs *An. arabiensis*	1.614	[0.776–3.464]	0.207
	An. gambiae vs *An. arabiensis*	0.845	[0.271–2.624]	0.767
Parity	Parous *vs* Nulliparous	1.344	[0.283–7.106]	0.708
Village	Dielmo vs Ndiop	0.441	[0.236–0.803]	0.008

RR, homozygote resistant for *Kdr* mutation; SS, homozygote susceptible (K*dr*-free); (*) time of night was divided into two periods: early (7 p.m.–1 a.m.) and later (1–7 a.m.)

Interactions between sampling method, *Kdr* mutation and *Anopheles* species. Generalized linear model (GLM) analysis revealed that the probability of encountering the *Kdr* mutation (in its heterozygous or homozygous form) varied significantly according to the sampling method (HLC vs. LC) and *Anopheles* species (Table 5). In Dielmo, there was an approximately 43% lower chance of finding the *kdr* allele in LC females compared to HLC females (OR = 0.57; $p = 0.03$). In contrast, in Ndiop, the probability of detecting individuals carrying the resistant allele was 3-fold higher in LC females compared to HLC females (OR = 2.64; $p = 0.001$). Likewise, the probability of finding the *kdr* allele was increased in *An. coluzzii* from Dielmo (OR =3.86; $p = 0.007$) and in *An. gambiae* from Dielmo (OR = 2.33; $p = 0.012$) and Ndiop (OR = 2.91; $p = 0.019$), compared to *An. arabiensis*.

Table 5: Generalized linear model (GLM) analysis of *Kdr* mutation performed on 616 mosquitoes from Dielmo (*N* = 363) and Ndiop (*N* = 253) with odds ratio and 95% confidence intervals calculated by negative binomial distribution using sampling method (HLC or LC) and species as explanatory variables.

***Kdr* Mutation**		**Site**	**Odds Ratio**	**[$CI_{95\%}$]**	***p-Value***
Method	LC *vs* HLC	Dielmo	0.568	[0.336–0.941]	0.030
		Ndiop	2.636	[1.443–4.914]	0.001
Species	*An. coluzzii* vs *An. arabiensis*	Dielmo	3.860	[1.564–11.655]	0.007
		Ndiop	1.460	[0.594–3.370]	0.387
	An. gambiae vs *An. arabiensis*	Dielmo	2.326	[1.234–4.627]	0.012
		Ndiop	2.910	[1.182–7.165]	0.019

Kdr, knock down resistance; HLC, human landing catch; LC, larval collection.

Discussion

This study updates the insecticide resistance pattern of *An. gambiae* s.l. in Dielmo and Ndiop, two malaria pre-elimination settings. Compared to the first surveys, the overall trend has not changed for Dielmo, with persistently low *Kdr* frequencies (varying between 2% and 14% depending on the species). However, in Ndiop, where the insecticide resistance situation was not investigated during the first surveys, the *Kdr* frequencies were relatively high (17–40%). An outdoor biting behavior was observed in host-seeking mosquitoes, particularly at Dielmo.

In this study, three sibling species of the *An. gambiae* complex were encountered at the two study sites. Their respective populations generally showed a deviation from panmixia under the Hardy–Weinberg assumptions, with a generally closed reproductive pattern. The introduction of insecticide-treated mosquito nets was shown to have strongly influenced the genetic structure among *Anopheles* populations, with a degree of selection in favor of *An. Arabiensis* [1], which was the predominant species in our study. The phenotypic status of *An. arabiensis* towards insecticides did not change significantly compared to previous surveys [2], especially in Dielmo, where the *Kdr* mutation was still occurring at relatively low frequencies and with a suspected resistance to pyrethroids. In contrast, the situation was different in Ndiop, where pyrethroid resistance was confirmed, with higher *kdr* allelic frequencies compared to Dielmo. These disparities between Dielmo and Ndiop could be explained by some socio-economic and socio-anthropological factors. Indeed, agricultural activities were more developed in Ndiop, where the use of pesticides could induce a selection pressure and contribute to the increase in insecticide resistance. It is also worth noting that a study in Dielmo highlighted a higher malaria incidence among people who usually spent a good part of the night outside watching television [3]. This human behavior probably promoted some opportunism in host-seeking females, as expressed by the high exophagic rates recorded in this study. This outdoor biting behavior of host-seeking females was also consistent with the low *kdr* allelic frequencies observed in Dielmo, suggesting a low level of contact between *An. arabiensis* and insecticide-treated bed nets. Conversely, the mosquito indoor biting behavior observed in Ndiop was in line with the permethrin and deltamethrin resistance recorded in that site.

In this study, the *Kdr* mutation was estimated using two different sampling methods, i.e., larval collections (LCs), which targeted females immediately emerging from breeding sites (neonate females), and human landing catches (HLCs), focusing on host-seeking females taking action in households where insecticide-treated nets are used. The latter targeted population (HLC females) is of greater epidemiological significance and could provide *Kdr* frequencies that are probably more closely linked with the epidemiological picture, thus allowing for a better assessment of the impact of long-lasting insecticidal treated nets (LLINs) on the dynamics of vector populations and changes in their insecticide resistance patterns. This approach, which could be considered an entity of

epidemiological entomology, as described by Killen et al. [4], allows for a better evaluation of the insecticide-based interventions put in place to fight malaria [5]. Comparisons between the LC and HLC "subpopulations" highlighted the existence of a certain selection of resistant genes under the effect of LLINs, with a higher probability of finding the *kdr* allele in host-seeking mosquitoes, presumably older females, compared to neonate ones, which would not yet have come into contact with LLINs. However, in our study, we did not find any causal link between the presence of the *Kdr* mutation and the nocturnal biting behavior of *An. arabiensis*, in the idea of establishing a correlation between the *Kdr* mutation and convenient vector biting time, particularly during the time when LLNs are used (later in the night), contrary to what was observed in an experimental study on *An. gambiae* s.s [6].

Conclusion

In the context of malaria pre-elimination, classical entomological investigation methods must be refined so as not to underestimate existing threats. In this study, the *Kdr* mutation was examined in both neonate and host-seeking females in Dielmo and Ndiop, two villages where impregnated mosquito nets have been the only vector control strategy since 2008. Our study highlights a particular interest in characterizing the resistance mechanisms of vector populations that present a direct link with malaria transmission in the context of pre-elimination and allows for a better understanding of data from standard methods used in the detection of insecticide resistance.

Author Contributions: O.T., S.D. and C.S. designed this study. S.D., N.D. and C.S. supervised this study. O.T., C.B. and S.D. carried out the field collections. O.T. performed the experiments, analyzed the data and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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