Insecticide Resistance Status of Anopheles Arabiensis in Irrigated and Non-irrigated Areas in Western Kenya

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Abstract

Background: Malaria control in Kenya is based on case management and vector control using long lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Irrigation practices maintain vector population and thus transmission during dry season. Development of insecticide resistance further compromises the effectiveness of insecticide-based vector control programs. The aim of this study was to assess the status and mechanism of insecticide resistance in malaria vectors in irrigated and non-irrigated areas in western Kenya and the contribution of public health interventions and agriculture to insecticide resistance.

Methodology: The study was carried out in 2018–2019 in Homa Bay County, western Kenya. Anopheline larvae were collected in irrigated and non-irrigated fields, reared to F1 adults and 2-5 day-old female vector mosquitoes were subjected to standard WHO insecticide susceptibility tests. The test specimens were then screened for knock-down resistance, *kdr* alleles, and analyzed for presence of acetyl-cholinesterase inhibiting enzyme; angiotensin-converting enzyme (*Ace-1*) genes. All field-collected samples were preserved for species identification by polymerase chain reaction. To ascertain the probable cause of vector resistance to insecticides, a questionnaire was administered to farmers, households and veterinary officers in the study area to assess the use of public health and agricultural insecticides/pesticides.

Results: *Anopheles arabiensis* was the only species tested in irrigated (100%, n=154) area and predominant species in the non-irrigated areas (97.5%, n= 162) and the rest were *An. gambiae* sensu stricto. In 2018, susceptibility was observed in the vector species in the irrigated area and phenotypic resistance in the non-irrigated area while in 2019, phenotypic resistance was observed from all areas However, susceptibility to malathion (mortality 100%), DDT (98.98%-100%) and PBO- deltamethrin (100%) was observed. Molecular analysis of the vectors from the irrigated and non-irrigated areas revealed low levels of leucine- serine/ phenylalanine substitution at position 1014 (L1014S/ L1014F) with a mutation frequencies of 1%-16%, and almost zero mutation in *Ace-1*R gene (0.7%). In addition to very high coverage of LLINs impregnated with pyrethroids and IRS with organophosphate insecticides, pyrethroids were the predominant chemical class in pesticides used for crop and animal protection.

Conclusion: Extensive use of pyrethroids in agriculture and public health could have resulted in the initial development of insecticide resistance. The susceptibility of these malaria vectors to organophosphates and PBO synergist in pyrethroids offers a promising future for IRS and ITN based vector control interventions.

Introduction

Frequent and prolonged droughts have resulted in the need to increase food production. This has led to altered ecosystems, with adverse consequences on human health [1, 2]. The ongoing deforestation, reclamation of marsh lands and establishment of irrigation systems for food production has resulted in

increased vector populations and hence malaria transmission [3–8]. Agricultural activities have been intensified in many regions in Africa with increasing interest on household- owned irrigation[9, 10]. As a result, creation of extensive malaria vector breeding habitats in irrigation systems has led to increased malaria and schistosomiasis transmission [11–17] over-use of pesticides has led to the development of insecticide resistance [18–20].

In Kenya, 80% of the land surface area is classified as arid and semi-arid [21, 22], thus necessitating irrigation for food production to sustain the ever growing population. This has led to introduction of several irrigation systems across the country such as Ahero area in western Kenya [23–25] and Mwea area in Central Kenya [14–17]. *Anopheles arabiensis* has been observed as a predominant malaria vector in arid and semi-arid areas in Kenya [26, 27], and in the irrigated areas in high densities [14, 28, 29]. This species is an important vector in rice irrigation schemes [30, 31], and exhibits exophily and significant exophagy behavior [32] increasing its transmission potential especially outdoors [29, 33, 34]. Although this species showed strong zoophilic behavior, it is partly anthropophilic depending on the availability of other animal blood meal source [29, 35, 36]. This vector usually has low sporozoite rate [29, 36], nevertheless, its high density in irrigated areas makes it an important and probably the major malaria vector in these irrigated areas [14, 29] in the absence of *Anopheles funestus.*

Use of pesticides/insecticides in agriculture and in public health has been incriminated in the development of insecticide resistance in major malaria vectors [19]. The scale-up of LLINs and IRS has been considered the major causes of malaria vector insecticide resistance in many malaria endemic African countries [37–40]. Extensive use of pyrethroid based pesticides in agriculture for crop and livestock protection may further enhance/ induce the resistance [41–43].

The purpose of irrigation is to increase agricultural productivity, but as a by-product, irrigation also increases malaria vector breeding habitats and thus increase vector population density and malaria transmission [11–17]. It is not known if the increase in agricultural productivity in turn leads to the increase in pesticides usage in these irrigated areas. This is important because previous studies show that higher resistance in mosquito populations was associated with agricultural insecticide use [18, 41–45]. There is therefore a need to monitor vector behavior and insecticide resistance against all classes of insecticides in order to understand the mechanisms responsible for widespread resistance and to assess if irrigation contributes to the development of resistance. This is essential for vector insecticide resistance management.

The current study was undertaken in an irrigated and non-irrigated area of Homa Bay County, western Kenya where malaria vector control with high very coverage of LLINs and indoor residual spraying (IRS) using pirimiphos methyl (Actellic 300®SC), an organophosphate insecticide is ongoing [46]. The aim of this study was therefore to determine how different eco-systems have impacted the susceptibility of malaria vectors to insecticides and to investigate the insecticide usage in public health and in agriculture practice.

Materials And Methods

Study site and design. This study was conducted in Homa Bay County, western Kenya, a semi-arid malaria endemic area situated along the southern shores of Lake Victoria's Winam Gulf at an altitude of 1,040 – 1,330 m above sea level (Fig. 1). This region experiences average annual temperatures of 22.5^{o}C and rainfall of 1,100 mm, with two rainy seasons. The long rains occur between March – May and the short rains between September – November. A concrete channel irrigation system was constructed in the study area by the Ministry of Environment, Natural Resources and Regional Development Authorities of Kenya in 2007 and named Kimira- Oluch Small-holder Farm Improvement Project (KOSFIP). This project was undertaken to support subsistence and cash crop production like cotton and fruits. The local community practice crop and animal farming in addition to fishing. The main malaria vectors are *An. arabiensis* and *An. funestus* sensu latu. Over time, malaria control in this area relied on pyrethroid-based insecticide treated nets. However, in 2018 and 2019, IRS using an organophosphate, pirimiphos methyl (Actellic® 300SC) was implemented resulting in dramatic reduction of malaria vector populations [46]. Significant decrease in *An. funestus* s.l. population occurred tending to near extinction levels [46].

Mosquito larval samples were collected from different village clusters in the irrigated and non-irrigated areas of the study area (Fig. 1). The non-irrigated clusters are at least 2 km away from the irrigation channels. Larval samples were collected between February and July 2018 and 2019 from all the indicated clusters in the irrigated and non-irrigated (10 clusters each) areas (Fig. 1). Various habitat types were sampled during each sampling season. After the long rains (May- July), more habitats types were encountered compared to the dry season (February- March). The habitat types included man-made ponds, swamps, irrigation lining, drainage ditches, natural ponds, river edges, and hoof/ foot prints.

Malaria vector larval sampling. Larval sampling was carried out using standard larval 350 ml dippers. Anopheline larvae were collected and transported to the International Center of Excellence for Malaria Research (ICEMR) insectary in Tom Mboya University College, for rearing to adults pending phenotypic insecticide resistance tests and molecular analyses.

Larval and adult mosquito rearing in the insectary. Field collected mosquito larvae were placed in larval trays in the larval rearing room and fed daily on Whiskas® cat food (Trademarks© Mars, Incorporated, McLean, Virginia, U.S.A.). Temperature and humidity in the larval room were maintained between 27^{o}C – 32^{o}C and 40% – 60%, respectively. Pupae were collected daily and placed in holding cages covered with mosquito mesh netting where they emerged into adults. Emerged *An. gambiae* s.l. mosquitoes were maintained in the insectary adult rearing room with regulated temperatures (25^{o}C-28^{o}C) and humidity (60%-75%) and fed on 6% glucose solution soaked in cotton wool. The females that emerged in the insectary were used for insecticide resistance bioassays, knock-down resistance (*kdr*) and *Ace-1* enzyme molecular analyses.

An. gambiae **s.l. resistance bioassay**. Two-five day old female adult *An. gambiae* s.l. mosquitoes were used to determine susceptibility to diagnostic concentrations of pyrethroid (0.05% deltamethrin),

organophosphate (5% malathion) and organochlorine (4% DDT) insecticides. Standard WHO tube bioassay tests were conducted as per the WHO guidelines [47]. Mosquitoes used as control samples were simultaneously placed in WHO tubes lined with untreated papers. Mosquitoes were exposed to insecticide impregnated test papers for 60 minutes and the number of knocked down females recorded every 10 minutes. 15–25 female mosquitoes were used in each test. At the end of the exposure period, mosquitoes were transferred into holding tubes, fed on 6% glucose and observed after 24 hours for mortality. The positive controls used were insectary colony of the susceptible *An. gambiae* s.s. Kisumu strain. The final mortality was recorded 24 h post exposure. Additionally, a synergist, piperonylbutoxide (PBO)–deltamethrin bioassay was conducted and exposures done for 120 minutes, 60 min PBO exposure followed by 60 min deltamethrin exposure, with mortalities recorded 24 h post exposure. Knock- down was recorded every 10 minutes during exposure and mortalities recorded after 24 h exposure to PBO alone and to PBO- deltamethrin combined. Both live and dead mosquitoes were preserved individually at -20^{0}C for molecular species identification and for the detection of *kdr* mutations and multiple copies of the acetyl-cholinesterase (AChE) enzyme encoded by the *Ace-1* gene.

An. gambiae **s.l. DNA extraction and species identification**. All field collected specimens were morphologically identified as *An. gambiae* s.l. using Gillies and De Meillon taxonomic keys [48]. DNA from the whole female body was extracted from a proportion of the bioassayed adult females following Musapa *et al*., protocol [49]. The molecular species identification was carried out as described by Scott *et al*., [50] and Paskewitz and Collins protocols [51].

Assessment of frequency of kdr **and** Ace-1 **alleles.** DNA samples of *An. gambiae* s.l. mosquitoes were assayed to detect the voltage-gated sodium channel (*vgsc*) L1014S (*kdr*-east) and L1014F (*kdr*-west) mutations and mutations in the *Ace-1* gene. Assays were done on both live and dead mosquitoes post bioassay tests using the published protocols [52–54].

Investigation of households using chemicals in public health, agriculture (farms) and veterinary (livestock). Different questionnaires were prepared and surveys were conducted with randomly selected farmers, households and veterinary officers/ agricultural extension workers to identify the chemicals used in public health, crops and animals pest control. Other methods for personal protection against malaria vector control tools employed by the selected households were also surveyed.

Data analysis. During field work, all larval data was entered into Open Data Kit (ODK) in tablets and then uploaded to online database. In the insectary and laboratory, data was recorded in respective laboratory processing forms and later entered in Microsoft Excel spreadsheets followed by error checks and corrections.

The WHO bioassay knock down recorded after every 10 minutes for one hour and final mortality at 24 hours was recorded for all test runs with corresponding negative and positive controls. Abbots formula was used to correct percentage mortality in cases where the negative control mortality was between 5% and 20%; experiments where negative control mortality was above 20% were discarded. Mortalities of

98%-100% in the sample population indicated susceptibility to the tested insecticide. A mortality of between 90%-98% suggested possible resistance and less than 90% mortality indicated resistance in the tested species [47]. Probit analysis was done using Poloplus Version 1 software to determine the knockdown time 50 (KDT_{50}).

The allele frequencies of *kdr* L1014 mutations and *Ace-1* G119 mutations were determined. Genepop Hardy Weinberg exact tests were used to determine the differences between the *kdr* alleles in the irrigated and non-irrigated areas.

Pesticide use questionnaire data was entered and analyzed in Excel. Chi-square test and t-test were used to determine the difference in pesticide use in the irrigated and non- irrigated areas.

Results

An. gambiae **s.l. resistance bioassay and species identification.** A total of 1,657 female mosquitoes were tested for susceptibility against deltamethrin, malathion and DDT in 2018 and 2019 (Table 1). Nine hundred and fifty nine (959) females were tested from irrigated and 698 from non-irrigated areas, they were all *An. arabiensis*. Mortality in the positive control Kisumu strain was 100% in all tests while the mortality in the negative control ranged between 4.7%-15.4% in all the tests. Resistance to deltamethrin was observed in the non-irrigated areas in 2018 with possible resistance in the irrigated areas (Table 1). Significantly higher mortality in non-irrigated than irrigated area in 2018 against deltamethrin (Z test; z-stat = 5.4, $p < 0.00001$) was observed. However, in 2019, resistance to deltamethrin was observed in both the irrigated and the non-irrigated areas, and the mortalities were comparable in the two areas (78% in irrigated and 83% in non-irrigated area) (Table 1). Susceptibility to malathion (100%) and DDT (98.98%-100%) was recorded in both zones in the study site. All the mosquitoes tested on PBO-deltamethrin were susceptible (100%) in irrigated and non-irrigated areas (Table 1). The KDT_{50} of all the chemicals tested were less than 30 minutes.

Table 1
The status of phenotypic resistance of *Anopheles arabiensis* and the estimated time to 50% mortality (KDT_{50}) when exposed to insecticides for 60 min in the irrigated and non-irrigated areas in 2018 and 2019.

Insecticide	Year	Zone	Sample size	KDT_{50},min	Adjusted mortality rate (%)	95% (CI Lower)	95% CI (Upper)
Deltamethrin	2018	Irrigated	324	13.0	97.8***	96.2	99.4
		Non-irrigated	114	20.9	84.0	77.3	90.7
	2019	Irrigated	180	23.2	78.2	72.2	84.2
		Non-irrigated	117	21.5	83.3	76.5	91.1
PBO-Deltamethrin	2019	Irrigated	86	8.2	100	100	100
		Non-irrigated	76	7.6	100	100	100
DDT	2019	Irrigated	107	26.1	99.0	97.1	100
		Non-irrigated	123	24.2	100	100	100
Malathion	2018	Irrigated	104	27.0	100	100	100
		Non-irrigated	158	23.2	100	100	100
	2019	Irrigated	158	18.0	100	100	100
		Non-irrigated	111	21.1	100	100	100

*** Significantly different in mortality between irrigated and non irrigated areas.

Frequency of kdr **and** Ace-1 **alleles.** A total of 553 mosquitoes were tested for the presence of mutation in the *vgsc* gene. Generally, both *kdr*-east and *kdr*-west mutation were observed in both the irrigated and the non-irrigated areas (Table 2). The mutation frequency was low in all tests, ranging from 1–16%. However, regardless of study area, no mutation in the *Ace-1* gene was detected in 2018 and very low mutation frequency (0.007%) in the non-irrigated area in 2019. The *kdr* allele and genotype frequencies were significantly different between irrigated and non-irrigated zones (Pearson Chi- square = 17.804, df = 2, P = 0.0001 and Pearson Chi- square = 14.848, df = 4, P = 0.012 respectively). The *kdr* genotypes results show significant deviation from Hardy-Weinberg equilibrium in non-irrigated zones, due to heterozygote deficit ($P < 0.01$), while marginally significant of heterozygote deficit was observed in irrigated zone (P = 0.0528). Overall, *kdr* genotype frequencies were not consistent with HWE (Chi- square > 36.7, df = 4, P < 2.08e-07), indicating that *kdr* allele has been under strong selective pressure.

Table 2
Allele frequency of *vgsc* and *Ace*-1 mutations in *Anopheles arabiensis* in irrigated and non- irrigated areas in western Kenya in 2018 and 2019.

Year	Zone	Sample size	*vgsc*			Sample size	*ACE-1*
			Locus 1014				**Locus 119**
			L1014	**L1014S**	**L1014F**		**G119S**
2018	Irrigated	81	0.92	0.01	0.07	73	0
	Non-irrigated	72	0.80	0.16	0.04	60	0
2019	Irrigated	76	0.94	0.03	0.03	55	0
	Non-irrigated	88	0.9	0.05	0.05	75	0.007

Public health, agricultural and veterinary chemical use. Among the 200 households surveyed (98 were in the irrigated area and 102 in the non-irrigated area), the proportion of the households that used LLINs, IRS and other commercial insecticides was 91.8%, 84.4% and 51% in the irrigated area respectively and 91.2%, 91.2% and 39.2% in the non- irrigated area respectively (Table 3). There were a higher proportion of households in non-irrigated area (84.3%) that used pesticides in agriculture and veterinary pest cont control as compared to those in the irrigated area (80.6%). There was however no differences in the use of public health and agricultural/ veterinary chemicals between the two zones (T test; df = 6, t- stat = 0.1, p = 0.9).

Table 3
Frequency of responses to chemical use in Public health, agriculture (farms) and veterinary (animals) use in households in irrigated and non irrigated areas.

Category	**Use**	**Irrigated (n = 98)**	**Non - irrigated (n = 102)**	**P-value**
Public Health	LLINs	91.8 (89.0–94.6%)	91.2 (88.4– 94%)	0.88
	IRS	84.4% (77.1–91.6%)	91.2% (85.7–96.7%)	0.14
	Commercial insecticides	51.0 (46.0–56.0%)	39.2 (34.4– 44.0%)	0.09
Agricultural/ Veterinary	Vet and Agric pesticides	80.6 (76.6–84.6%)	84.3 (80.7– 87.9%)	0.49

No difference in the combined chemical use both in public health and agriculture/ veterinary in the irrigated (75.5%) and the non-irrigated (83.3%) area (T test; df = 10, t- stat = 0.2, p = 0.9) was detected, however a significantly higher use of pyrethroids in the irrigated than the non-irrigated area (Z test; Z- stat = 2.7, p = 0.007) was detected. Households that confirmed the use of chemicals but did not know the chemicals used (unknown classes) were also significantly higher in the non- irrigated area (Z test; Z- stat

= -3.2, p = 0.001) (Table 4). No difference in the duration of chemical use for crop protection and livestock pest control (Table 5) was reported.

Table 4
Proportion of household that use chemicals and the chemical classes used.

Chemical Class	Percentage (95% CI)		
	Irrigated (98)	Non- irrigated (102)	P- value
Use Chemicals	75.5% (67–84%)	83.3% (76.1–90.5%)	0.17
· Pyrethroids	62.2% (52.6–71.8%)	28.2% (19.5–36.9%)	< 0.00001
· Organophosphates	6.8% (1.8–11.8%)	2.4% (0- 5.4%)	0.14
· Carbamates	2.7% (0- 5.9%)	0% (0%)	0.09
· Other classes	21.6% (13.5–29.7%)	4.7% (0.6–8.8%)	0.00038
· Unknown	16.2% (8.9–23.5%)	67.1% (58- 76.2%)	< 0.00001
Don't use chemicals	24.5% (16–33%)	16.7% (9.5–23.9%)	0.17

Table 5
Proportion of farmers using pesticides and duration since the first use of pesticides in agriculture (crops) and livestock (veterinary) in the irrigated and non- irrigated areas.

Class	n	< 6 m	6– 12 m	13– 36 m	37– 60 m	61 – 12 < 60 yrs	> 10 yrs	Don't know
Irrigated								
Pyrethroids	46	-	2.2% (0- 4.4%)	15.2% (9.9– 20.5%)	4.3% (1.3– 7.3%	26.1% (19.6– 32.6%)	45.7% (38.4– 53%)	6.5% (2.9– 10.1%)
Organophoshates	5	-	-	60% (38.1– 81.9%)	-	20% (2.1– 37.9%)	20% (2.1– 37.9%)	-
Carbamates	2	-	-	-	-	-	100% (100%)	-
Non- irrigated								
Pyrethroids	24	12.5% (5.7– 19.3%)	-	8.3% (2.7– 13.9%)	4.2% (0.1– 8.3%)	16.7% (9.1– 24.3%)	45.8% (35.6– 56%)	12.5% (5.7– 19.3%)
Organophoshates	2	-	-	50% (14.6– 85.4%)	-	-	50% (14.6– 85.4%)	-

Discussion

Whereas the relationship between vector phenotypic resistance and failure of a control programme is currently contentious [55], insecticide susceptibility tests are usually used as the baseline indicator of a functional vector control strategy [56]. However, conflicting records of susceptibility of malaria vectors against different chemical compounds [55] have been reported due to different factors including the choice of assay used. Notwithstanding, this has remained the most ideal data to determine susceptibility within a population against different classes of insecticides. This study was carried out to determine the levels of susceptibility of *An. arabiensis* to pyrethroid, organophosphate and organochloride insecticides in an area where public health vector control was undertaken using pyrethroids in insecticidal nets and organophosphates in IRS. The observations herein highlight the importance of multi- disciplinary co-ordination between relevant ministries including agriculture, public health and environment (IRM plan Kenya, 2020–2024; unpublished).

In Homa Bay, previous studies reported *kdr* mutation [57]. The current study reports higher frequencies of *kdr* compared to previous studies. Initial possible resistance was observed in the irrigated area in 2018 against a pyrethroid -deltamethrin, a chemical compound used in long lasting insecticide treated nets (LLINs). With the scaled-up mass distribution of bed nets in 2006, 2012, 2014 and 2015, increase in insecticide resistance in malaria vectors across sub- Saharan Africa against different classes of insecticides in use (http://www.irmapper.com) has been reported. In 2019, resistance was confirmed in the irrigated areas of Homa Bay against deltamethrin. In the non-irrigated areas, resistance was earlier observed in 2018 and again in 2019. This is an indication that there is resistance against 0.05% deltamethrin in *An. arabiensis* in Homa Bay. This might be due to contribution from both public health interventions (LLINs) and agricultural activity.

IRS was conducted in Homa Bay in 2018 and 2019 using pirimiphos methyl- Actellic® 300SC, an organophosphate. *An arabiensis* from Homa Bay were susceptible to malathion, an organophosphate. This is an indication that the organophosphates are a better alternative for vector control in Homa Bay. This study didn't detect resistance against DDT in 2019 an indication that the *An. arabiensis* population in this area, currently has not cross- resistance between pyrethroids and organochlorides. This is concurrent with other studies conducted in Africa where no cross-resistance was observed in *An. gambiae* s.l. [58] and *An. funestus* [59] species.

The study confirmed that PBO can synergize deltamethrin and decrease the effect of mono-oxygenase enzyme detoxification [60] against *An. arabiensis* in the study area. These results indicate that mono-oxygenase enzyme activity is responsible for the observed phenotypic resistance. With the observed susceptibility of the malaria vectors in Homa Bay against PBO– deltamethrin, introduction of PBO-impregnated nets in the study area will be effective in malaria vector control. However, previous studies have shown that use of PBO-deltamethrin nets has no effect on mosquito mortality in comparison to standard LLINs in areas with little or no insecticide resistance [61].

With the development of reduced nervous sensitivity at the para-type sodium channel, reduced susceptibility to pyrethroid insecticides have been observed resulting in knockdown resistance. *Kdr* has

evolved in *An. gambiae* s.s and *An. arabiensis* separately [62–64]. *Kdr* mutation; a substitution at position 1014 resulting in L1014F (*kdr*-west) predominantly occurs in West Africa [64, 65] while L1014S (*kdr*–east) is commonly encountered in Kenya [66]. Two different allele-specific polymerase chain reactions (AS-PCR) are required to detect these known *kdr* mutations. The frequencies of *kdr*- east was observed to be less than the frequencies of *kdr*-west in the irrigated region in the study area. This might be as a result of agricultural practices as it has been observed that the mutation might have originally arisen as a result of use of agricultural pesticides [67, 68].

In this study, the *kdr* allele and genotype frequencies were higher in the non-irrigated area than the irrigated areas. The *kdr* genotypes in non-irrigated areas had significant deviation from Hardy-Weinberg equilibrium due to heterozygote deficit, while minimal heterozygote deficit was observed in irrigated zone. This is an indication that *kdr* allele has been under strong selective pressure in this area.

The occurrence of a higher frequency of insecticide resistance in the non-irrigated area may be associated with the more frequent use of pesticides for animal pest control. This is because *An. arabiensis* are known to be zoophagic vectors and this might be resulting in frequent contact with livestock pest control chemicals enhancing resistance in these vectors. It was also observed that most livestock were treated with amidine compounds (Tritix®) which is effective in areas experiencing pyrethroid and organophosphate resistance. Several households in the non-irrigated area also reported not knowing the chemicals used for livestock pest control during treatment by the veterinary extension workers. These chemicals might be the driving force for the high resistance levels in the non-irrigated area.

The *Ace-1* gene is known to code for acetyl-cholinesterase (AChE) enzyme in the nerve synapses [69]. AChE neutralizes the neurotransmitter, acetylcholine (ACh) bound to receptors on post-synaptic membranes of neural synapses. Presence of insecticide usually binds irreversibly to AChE causing accumulation of ACh in the synaptic cleft resulting in death due to paralysis. *Ace-1* mutation (*Ace-1R*) is associated with carbamate and organophosphate resistance [70]. This mutation, a point mutation, is as a result of gene duplication providing a higher chance of survival to individuals with extra copies of the resistant *Ace-1* G119S alleles [71] preventing the blockage of synaptic neurotransmission by inhibiting AChE enzyme which is encoded by the *Ace-1* gene. This mutation has been observed in several insects [71–75]. The presence of this mutation in low frequencies in the non-irrigated area is a matter that needs to be investigated further.

These results indicate that *An. arabiensis* populations in Homa Bay County are still susceptible to organophosphates, a class of chemical compound used in IRS. Although this is promising, resistance was observed against pyrethroid, a class of chemical compound used in LLINs. The susceptibility of these malaria vectors to PBO synergist in pyrethroids offers a promising future for ITN based vector control interventions. Resistance against DDT is a useful marker of cross- resistance with pyrethroids. Absence of DDT resistance is promising as it suggests lack of cross- resistance between pyrethroids and organochlorides. These results show that there is need for continued monitoring of insecticide resistance

status as insecticide resistance is posing a major challenge to malaria vector control programmes. Additionally, collaboration between the agriculture, public health and environment sectors will be key to insecticide use and resistance management.

Abbreviations

Ace– angiotensin converting enzyme

ACh – acetylcholine

AChE – acetyl-cholinesterase

AS-PCR - allele-specific polymerase chain reactions

DDT - dichlorodiphenyltrichloroethane

DNA - deoxyribonucleic acid

F- phenylalanine

G – glycine

ICEMR - International Center of Excellence for Malaria Research

IRS- indoor residual spraying

kdr – knock down resistance

KOSPIF - Kimira- Oluch Small-holder Farm Improvement Project

L – leucine

LLINs - long lasting insecticide treated nets

ODK - Open Data Kit

PBO – piperonylbutoxide

S- serine

s.l. – sensu latu

s.s. – sensu stricto

vgsc – Voltage gated sodium channel

WHO – World Health Organization

Declarations

Conflict of interest:

All authors declare that they have no conflict of interest.

Authors' contributions:

GY, JWK and AKG conceived and designed the research. PWO, HA, BMO, KO and CJO participated in the field work and data collection. PWO and DZ participated in laboratory analysis. PWO, AKG, DZ and GZ did the data analysis. MCL determined the study site demarcations. PWO and AKG drafted the manuscript, JG edited the draft manuscript. All authors read and approved the final version of the manuscript. The final manuscript was edited by GY and AKG.

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Figures

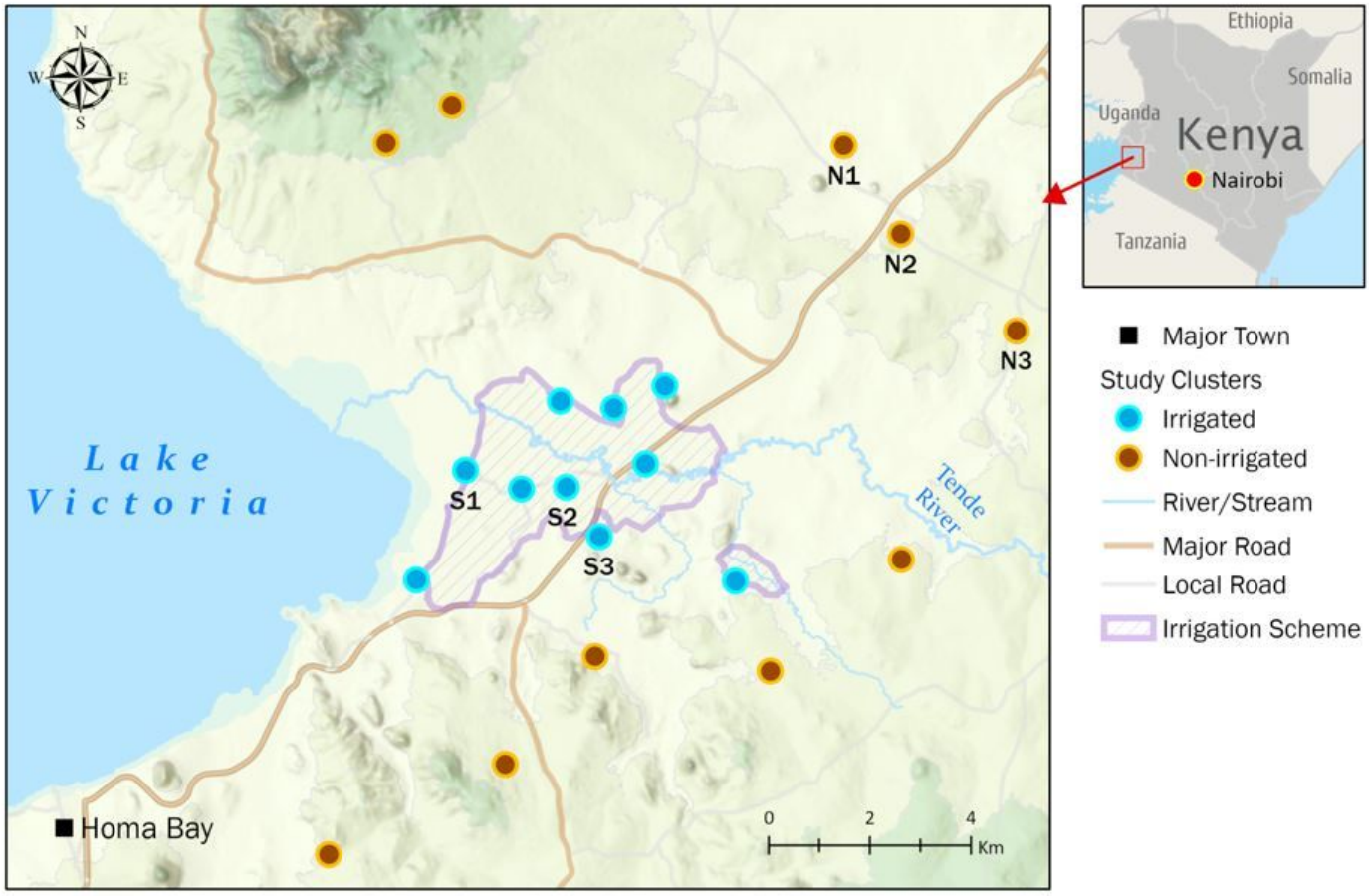

Figure 1

Map of Homa Bay study site. Samples were collected from all the above indicated irrigated and non-irrigated areas. S1-S3 and N1-N3 indicate where questionnaire survey was conducted in the irrigated and non- irrigated areas respectively. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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