Countrywide insecticide resistance monitoring and first report of the presence of knock down resistance east in Niger, West Africa

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Abstract

Background

Mass distribution of insecticide treated nets (ITNs) is the principal malaria vector control strategy adopted by Niger. To better inform on the most appropriate ITNs to distribute, the Niger National Malaria Control Programme (NMCP), with the support of the U.S. President's Malaria Initiative and its partners, conducted insecticide resistance monitoring in sixteen sites selected across the country.

Methods

The susceptibility of *Anopheles gambiae* sensu lato (s.l.) to chlorfenapyr and pyrethroid insecticides was investigated in nine sites in 2019 and fifteen sites in 2020, including eightfrom the previous year. The susceptibility status, pyrethroid resistance intensity at 5 and 10 times the diagnostic concentrations, and piperonyl butoxide (PBO) synergism with diagnostic concentrations of deltamethrin, permethrin and alpha-cypermethrin were assessed using WHO bioassays. Two doses (100 and 200 µg/bottle) of chlorfenapyr were tested using the CDC bottle assay method. Species composition and allele frequencies for knock-down resistance (*kdr*-west and *kdr*-east) and acetylcholinesterase (*ace-1*) mutations were further characterized using polymerase chain reaction (PCR).

Results

High resistance to all pyrethroids tested was observed in all sixteen sites during both years. Pre-exposure to PBO substantially increased susceptibility with average increases in mortality from 30 to 70% for deltamethrin, 0 to 60% for permethrin, and 5 to 70% for alpha-cypermethrin. Susceptibility to chlorfenapyr (100 ug/bottle) was recorded in all sites except in Tessaoua and Magaria where susceptibility was recorded at the dose of 200 ug/bottle. The *kdr*-e allele, investigated for the first time, was detected in the country. Both *kdr*-w and *kdr*-e were present in all 15 sites surveyed in 2020, at frequencies between 0.46–0.81 and 0.41–0.87, respectively. *An. coluzzii* was the predominant malaria vector species in the 16 sites surveyed for insecticide susceptibility followed by *An. gambiae* s.s. and *An. arabiensis.*

Conclusion

The data collected showed high resistance to pyrethroids with the involvement of various resistance mechanisms, which will guide the NMCP in making evidence-based decisions to better adapt vector control strategies and insecticide resistance management in Niger, starting with the introduction of new generation ITNs such as interceptor G2 and PBO ITNs for mass distribution campaigns, by targeting sites where the vector population showed susceptibility to chlorfenapyr or increased mortality using PBO synergism.

Background

Malaria is one the leading health concerns in endemic countries and particularly in Sub-Saharan Africa [1, 2]. Several negative outcomes, including disability, death, and slow economic growth, are attributed to malaria. However, measures to prevent and/or treat the disease are limited and costly [3]. For decades, mass distribution of insecticide treated nets (ITNs) and indoor residual spraying (IRS) have represented the core malaria prevention strategies recommended by the World Health Organization (WHO) and have been implemented in many endemic countries [4]. In recent years, the development and spread of insecticide resistance in malaria vectors has become an increasingly large threat to vector control and disease prevention efforts [5, 6, 7, 8, 9, 10, 11, 12].

In Niger, malaria is endemic and is the primary cause of illness, death and disability combined, disproportionately affecting children under 5 years of age. It accounts for 28% of all illness in the country and 50% of all recorded deaths [2]. The National Malaria Control Programme (NMCP) National Epidemiological Report indicates that there were 4,490,328 cases and 4,170 malaria deaths in 2021, putting the country among those with the highest per capita rates of malaria fatalities globally [13]. In 2019, 29 countries accounted for 95% of malaria cases worldwide, among which five countries including Niger accounted for about 51% of all cases globally [2].

The estimated number of cases decreased by 7.9% between 2015 and 2019 (from 204 cases per 1000 population to 131 per 1000 of the population at risk) and the number of deaths decreased by 25.9% in the same period (from 0.919 deaths per 1000 population to 0.730 deaths per 1000 of the population at risk) [14]. ITN distribution represented the main vector control strategy that was implemented in the country, coupled with diagnosis and treatment of malaria cases. Niger conducted mass ITN distributions in 2005, 2009, 2014 and 2017 targeting different regions across the country and guided by 'high burden high impact' approaches during each campaign, in addition to the routine distribution for pregnant women and children under the age of 5 years. To protect children under the age of 5 during the peak

transmission season, the NMCP has also been conducting annual seasonal malaria chemoprevention (SMC) since 2013 and has reached yearly more than 4 million children across58 of the 61 eligible districts. Additional measures to combat malaria in Niger included intermittent preventive treatment during pregnancy (IPTp), and case management.

In 2020, the Niger NMCP reviewed the country's malaria risk map to allow the selection of priority interventions according to the endemicity level and to align interventions with the 'high burden high impact' approaches. Therefore, the country was divided into four new endemicity strata including a very low transmission strata, also referred to as sporadic malaria cases and characterized by an incidence of less than 100 cases per 1000 population, low transmission representing areas of > 100 and < 250 cases per 1000 population, moderate transmission with > 250 and < 450 cases per 1000 population, and a high transmission strata recording more than 450 cases per 1000 population where the highest incidences were recorded, considering both malaria cases among children under five years of age and transmission data collected over the last five years. The vast majority of the population (94%) resides in the two southernmost (moderate and high transmission) zones where malaria is most prevalent.

Previous entomological activities conducted in the country have already highlighted malaria control challenges including insecticide resistance in the main malaria vectors in the country [15, 16, 17]. Since 2018, the U.S. President's Malaria Initiative (PMI) has supported Niger's NMCP and its partners to conduct insecticide susceptibility testing in several sites across the country to help update the country's malaria vector control strategy and selection of control tools.

Methods

Study sites

Niger is a West African country covering a land area of almost 1,270,000 km^2, making it the largest country in West Africa with a population of about 22 million living mostly in clusters in the far south and west of the country. Over 80% of its land area lies in the Sahara Desert. The climate is mainly very dry and very hot with a peak temperature of about 45°C between January and February. In the extreme South there is a tropical climate on the edges of the Niger River basin. The rainy season in Niger lasts three to four months, from June through September, with peak malaria transmission during the second half (August–September).

The Niger NMCP selected nine sites in 2019 and this was increased to fifteen sites in 2020 across the different endemicity zones (Fig. 1) to conduct insecticide susceptibility tests on local malaria vectors to generate data for appropriate ITN decision making at country level. Most of the sites are in areas of intensive agriculture such as rice cultivation (particularly in Gaya, Niamey V and Tillabery), cotton growing, sugar cane, onion, and market gardening with intensive pesticide use.

Insecticide susceptibility and resistance intensity tests

From August through December 2019 and September through December 2020, insecticide susceptibility tests were conducted once a year per site. Larvae and pupae of *An. gambiae* s.l. were collected in each site from several larval habitats, pooled, and reared to adulthood in the field laboratory. Insecticide susceptibility tests were conducted on two- to five-day-old adult females using WHO tube tests [18]. For each tube test, 80–100 female *An. gambiae* s.l. (in four tubes with 20–25 per tube) were exposed to the insecticide for one hour and mortality assessed after 24 hours (Table 1). An additional 40–50 mosquitoes in two tubes were tested in parallel as controls.

Insecticide impregnated papers were supplied by Universiti Sains Malaysia (USM). The diagnostic concentrations of permethrin (0.75%), deltamethrin (0.05%), alpha-cypermethrin (0.05%), were tested in all sites. When resistance to alpha-cypermethrin, deltamethrin and permethrin was confirmed (mortality below 90%), resistance intensity assays were carried out using 5 and 10 times the diagnostic concentration. Mosquitoes were exposed to the insecticides for one hour, and susceptibility was assessed according to WHO tube test procedures [18].

Piperonyl butoxide (PBO) synergist assays

Synergist assays with PBO were conducted for deltamethrin, permethrin, and alpha-cypermethrin according to the WHO tube test protocol [18] to determine the involvement of cytochrome P450s in pyrethroid resistance. The synergist assays were conducted by pre-exposing mosquitoes to a 4% PBO paper for one hour. Mosquitoes were then transferred to tubes with one of the three pyrethroids for one additional hour of exposure. For all tests, resistance status, synergist effect, and resistance intensity were defined following the WHO criteria [18].

Chlorfenapyr CDC bottle assays

The CDC bottles were coated following the protocol described by Brogdon *et al*., [19] with 1 mL of chlorfenapyr diluted in acetone at the concentrations of 100 µg/bottle and 200 µg/bottle. Each dose active ingredient was pre-weighed at the U.S. Centers for Disease Control and Prevention (CDC, Atlanta, USA) to enable the coating of 50 bottles. The test was conducted following the CDC bottle assay standard testing procedures, with the exception that the tested mosquitoes were removed from the bottles after the exposure time and held in disposable cups with access to 10% sucrose solution, and mortality was scored up to 72 hours [19]. Both concentrations (100 µg/bottle and 200 µg /bottle) were simultaneously tested in all nine sites in 2019 as the molecule was tested for the first time in the country and to avoid any difference of testing conditions to enable interpretation and comparison of the results of both tests with less deviation. But in 2020, only the 200 µg/bottle concentration was tested in sites where 100 µg/bottles did not achieve 98% mortality, following the previous year's lesson learned.

Table 1: List of tested insecticides and concentrations

		Intensity Assay		Diagnostic Exposure Time	Delayed Mortality Post-exposure
Insecticides	**Diagnostic Concentration (DC)**	**5X DC**	**10X DC**		
Deltamethrin	0.05%	0.25%	0.50%	60 mins	24 hours
Permethrin	0.75%	3.75%	7.50%	60 mins	24 hours
Alpha-cypermethrin	0.05%	0.25%	0.50%	60 mins	24 hours
Piperonyl butoxide + pyrethroids	4.00%	Synergist assay		60 mins synergist and 60 mins pyrethroid	24 hours
Chlorfenapyr	100 & 200 µg/bottles	-	-	60 mins	72 hours

Species identification and characterization of insecticide resistance markers

A subsample of about one-hundred *An. gambiae* s.l. mosquitoes was randomly selected among the population tested per site for species identification and molecular markers of resistance detection using polymerase chain reaction (PCR) analysis. DNA was extracted following the protocol described by Rudbeck *et al.* [20]. PCR species identification of the *An. gambiae* complex was conducted following the SINE PCR protocol described by Santolamazza *et al*. [21]. The presence of the knock down resistance West *(kdr*-w*)* and knock down resistance East (*kdr*-e) alleles was characterized using the PCR restriction fragment length polymorphism (RFLP) method as described by Martinez-Torres *et al*. [22]. The protocol described by Weill *et al*. [23] was used to detect the acetylcholinesterase (*ace*-1*)* gene mutation.

Statistical analysis

Insecticide resistance status was defined following WHO criteria [18], with mortality after 24 hours < 90% as confirmed resistance, between 90% and < 98% as possible resistance, and ≥ 98% as susceptible. Mortality was corrected using Abbott's formula [24] when the mortality of the control tubes was above 5% and less than 20%.

For intensity assays, corrected mortality of:

- 98–100% at 5X the diagnostic dose indicated low resistance intensity
- Less than 98% at 5X diagnostic dose implied testing 10X the diagnostic dose
- 98–100% at 10X the diagnostic dose confirmed a moderate resistance intensity
- Less than 98% at 10X the diagnostic dose indicated high resistance intensity

For the synergist assays, an increase in the mortality after pre-exposure to PBO compared to the diagnostic dose of the insecticide alone indicated the involvement of oxidase enzymes such as P450s in the resistance in the population tested.

Results

Pyrethroid and synergist bioassays

Resistance to all three pyrethroids was observed in 2019 and 2020 in all sites. Mortality against the diagnostic dose of alpha-cypermethrin (0.05%) was between 0% in Agadez and 26.4% in Boboye in 2019 while the lowest mortality was observed in Madaoua (3.0%)

and the highest in Keita (75.0%) in 2020 (Fig. 2). Mortality against the diagnostic dose of deltamethrin (0.05%) was between 1.1% in Gaya and 35.8% in Guidimouni in 2019, and 6.6% in Guidimouni and 74.0% in Niamey V in 2020 (Fig. 3). For permethrin (0.75%), mosquito mortality was between 1.2% in Agadez and 51.9% in Boboye in 2019, while in 2020, the lowest mortality of (3.3%) was in Balleyara and highest (76.2%) in Tillabery (Fig. 4).

Exposing mosquitoes to PBO before the pyrethroids did not completely restore susceptibility of the *An. gambiae* s.l. populations in any of the sites surveyed in either year, except in Tillabery where susceptibility to permethrin was recovered to 99.1% mortality. Nonetheless, in both 2019 and 2020 a substantial increase in mortality following PBO exposure was observed for pyrethroids overall and particularly for deltamethrin (Fig. 2-4). Resistant mosquitoes in five sites showed no significant mortality when pre-exposed to PBO prior to exposure to pyrethroid. Magaria for alpha-cypermethrin, Madaoua and Say for deltamethrin, Madarounfa, Magaria, Say and Tchintabaraden for permethrin yielded similar mortality rates with pyrethroid alone and combined with PBO.

Resistance Intensity of *Anopheles gambiae* s.l.

In 2019, resistance intensity was tested in eight of the nine sites for alpha-cypermethrin due to limited number of mosquitoes in Fararrat, and in all nine sites for deltamethrin and permethrin. High intensity resistance was observed for all three pyrethroids except for alpha-cypermethrin and permethrin in Gaya and for alpha-cypermethrin in Tessaoua where moderate resistance was recorded.

In 2020, resistance intensity was tested in fourteen sites for alpha-cypermethrin, twelve for deltamethrin, and ten for permethrin due to limited availability of impregnated papers received from the supplier. The resistance intensity was also high at all sites surveyed and for all three pyrethroids except in Balleyara, Keita and Tillabery with moderate resistance for alpha-cypermethrin and deltamethrin. Only Niamey V yielded low resistance intensity against deltamethrin and permethrin with 100% mortality recorded at 5X dose for both insecticides (Fig. 5-7).

Chlorfenapyr bioassays

In 2019, mortality recorded after a 72-hour holding period showed susceptibility at the dose of 100 µg/bottle only in two sites (Agadez and Niamey V) while all sites showed susceptibility at 200 µg/bottle. In 2020, susceptibility was recorded at 100 µg/bottle in all sites except in Magaria and Tessaoua with susceptibility at 200 µg/bottle (Fig. 8)

Anopheles species identification and insecticide resistance markers

An average of 110 mosquitoes tested for susceptibility per site per year were analyzed for species identification and resistance allele characterization. In 2019, 1,056 *An. gambiae* s.l. mosquitoes tested for insecticide susceptibility across all nine sites were analyzed for molecular species identification, while 1,708 specimens were analyzed across the 15 sites in 2020. The resistance markers were determined for 1,708, 1,747 and 1,692 mosquitoes for *kdr*-w, *kdr*-e and *ace*-1, respectively, in 2020.

Anopheles coluzzii represented the main vector at most of the sites during both years with an average of 79.9% of the total mosquitoes analyzed in 2019 and 60.4% in 2020. Other members of the *An. gambiae* complex identified included *An. arabiensis* (18%) in 2019 and *An. gambiae* s.s. (37.7%) in 2020. Fewer *An. arabiensis* were recorded in 2020 compared to 2019. Guidimouni recorded the highest proportion of *An. arabiensis* in 2019 with 75.2% of the total samples tested from this site while 95.0%, 89.8% and 78.8% of the tested samples of Balleyara, Matamey and Tessaoua, respectively, were *An. gambiae* s.s. in 2020 (Fig. 9).

Only *kdr*-w and *ace*-1 mutations were characterized in 2019, and *kdr*-w allele frequencies varied between 0.22 and 0.38 with a mean of 0.31 across all nine sites monitored. The highest *kdr*-w frequency was recorded in Guidimouni (0.38) followed by Balleyara (0.36), while the highest *ace*-1 frequency was found in Balleyara and Niamey V (0.09) (Table 2).

In 2020, the *kdr*-w frequencies varied from 0.45 to 0.80 with a mean of 0.63 across all sites. The highest *kdr*-w frequency was recorded in Gaya (0.81), followed by Tillabery (0.70), while the lowest was in Boboye (0.46). The investigation of the presence of *kdr*-e showed the presence of the mutation at frequencies varying between 0.41 in Tessaoua to 0.87 in Gaya. The *ace-1* mutation remained low within the populations tested with the highest frequency found in Madarounfa (0.21), followed by Niamey V (0.20) (Table 3).

Table 2: Number of mosquitoes tested and frequency of target site resistance markers cross the sites in 2019.

Localities	# Tested	*kdr*-w Mutation				*ace*-1 Mutation			
		RR	RS	SS	freq	RR	RS	SS	Freq
Agadez	**117**	21	10	86	***0.22***	4	7	106	***0.06***
Balleyara	**118**	39	8	71	***0.36***	10	2	106	***0.09***
Fararrat	**118**	31	8	79	***0.3***	3	8	107	***0.06***
Gaya	**117**	35	13	69	***0.35***	6	6	105	***0.08***
Guidimouni	**117**	40	10	67	***0.38***	4	7	106	***0.06***
Niamey V	**113**	22	9	92	***0.23***	6	8	99	***0.09***
Say	**118**	24	8	86	***0.24***	7	2	109	***0.07***
Tessaoua	**119**	30	23	66	***0.35***	4	10	105	***0.08***
Zindarou	**119**	33	9	77	***0.32***	2	9	108	***0.05***
Total	**1056**	275	98	693	***0.31***	46	59	951	***0.07***

freq=frequency; RR=homozygous resistant; RS=heterozygous resistant; SS=homozygous susceptible

Table 3: Number of mosquitoes tested and frequency of target site insecticide resistance alleles across the sites in 2020

Localities	*Kdr*-w Mutation					*Kdr*-e Mutation					*ace*-1 Mutation				
	# Tested	RR	RS	SS	freq	# Tested	RR	RS	SS	freq	# Tested	RR	RS	SS	freq
Agadez	114	65	11	38	**0.62**	118	71	11	36	**0.65**	114	19	2	93	**0.17**
Balleyara	108	58	25	25	**0.65**	115	59	28	28	**0.63**	108	12	1	95	**0.12**
Boboye	113	16	72	25	**0.46**	115	86	14	15	**0.81**	114	19	0	95	**0.17**
Gaya	116	88	15	13	**0.81**	120	99	11	10	**0.87**	112	8	1	103	**0.08**
Guidimouni	115	62	24	29	**0.64**	115	81	17	17	**0.78**	111	14	0	97	**0.13**
Keita	116	50	42	25	**0.61**	120	93	7	20	**0.80**	115	15	1	99	**0.13**
Madaoua	119	46	39	34	**0.55**	120	97	12	11	**0.86**	118	10	0	108	**0.08**
Madarounfa	113	57	15	41	**0.57**	113	60	13	40	**0.59**	113	24	0	89	**0.21**
Magaria	120	66	29	25	**0.67**	120	71	18	31	**0.67**	119	10	2	107	**0.09**
Matamey	115	59	23	33	**0.61**	114	82	16	22	**0.79**	114	18	0	96	**0.16**
Niamey V	116	57	32	27	**0.63**	120	57	32	25	**0.61**	114	18	9	87	**0.20**
Say	105	50	29	26	**0.61**	107	71	15	21	**0.73**	106	7	3	96	**0.08**
Tchintabaraden	108	41	34	33	**0.54**	115	54	6	55	**0.50**	109	18	0	91	**0.16**
Tessaoua	114	65	14	35	**0.63**	117	36	25	56	**0.41**	114	15	5	94	**0.15**
Tillabery	116	70	22	24	**0.70**	118	78	2	38	**0.67**	111	13	2	96	**0.13**
Total	**1708**	**850**	**426**	**433**	***0.62***	**1747**	**1095**	**227**	**425**	***0.69***	**1692**	**220**	**26**	**1446**	***0.14***

= number, RR = homozygous resistant, RS = heterozygous, SS = homozygous susceptible, Freq = resistant allele frequency

Discussion

Resistance of *An. gambiae* s.l. to the diagnostic dose of the three pyrethroids tested (deltamethrin, permethrin, and alpha-cypermethrin) was observed in 2019 and 2020 in all sites in Niger. Both consecutive monitoring years recorded similar resistance status on *Anopheles gambiae* s.l. at each site surveyed twice. The presence and increase in insecticide resistance alleles is a known trend in most African countries [25, 26,

27, 28, 29, 30, 31, 32, 33, 34]. Most sub-Saharan African countries including Niger have reported resistance, particularly to pyrethroid insecticides [15, 16]. As further reported by several authors, the spread and increasing insecticide resistance of malaria vectors is partly due to the use of insecticide-based tools for public health and partly due to agricultural applications of pesticides [29, 35, 36, 37]. Niger has conducted four mass ITN distribution campaigns using pyrethroid-only ITNs in high malaria burden districts to gradually cover the entire country with ITNs. Mass ITN distributions targeting those districts started in 2005, while mass distribution and universal coverage of selected regions started in 2014, using pyrethroid-only treated ITNs.Furthermore, most of the sites selected for insecticide resistance monitoring in Niger are in areas of intensive agriculture including rice and cotton cultivation, with intensive pesticide use,which may have contributed to the moderate or high pyrethroid resistance intensity recorded in most of the sites. This presents an obvious challenge for malaria control as it limits the country's options for efficient insecticide-based vector control interventions [10, 30].

Synergist assays showed the impact of PBO in substantial increased of mortality for all pyrethroids in nearly all sites, indicating the involvement of P450 enzymes as an important resistance mechanism. Per WHO recommendations, the deployment of PBO ITNs is recommended when a vector population is highly resistant to pyrethroids, and a significant increase of mortality is observed when those vectors are pre exposed to PBO [38]. As several PBO ITNs are now available, the decision to use them should be evidence-based and driven by the percentage increment of mortality in the presence of PBO. Therefore, PBO ITNs could be procured and distributed strategically in Niger. The data gathered across the country showed substantially higher mosquito mortality against alpha-cypermethrin and deltamethrin than permethrin after pre-exposure to PBO in most of the sites. This trend could support for the choice of alphacypermethrin or deltamethrin-based PBO ITNs to be prioritized in most of the sites. The use of PBO ITNs with type II pyrethroids (deltamethrin or alpha-cypermethrin) have shown higher performance than those with the type I pyrethroid permethrin, as reported by several authors over studies particularly conducted in Western African countries where the vector populations are highly resistant to pyrethroids [26, 39]. Given the observed effect of PBO at all sites, the NMCP could prioritize PBO-based ITNs in the country's malaria vector control management plan.

Susceptibility to chlorfenapyr in all sites in 2019 and in 2020 with the doses of 100 and 200 µg/bottles confirms the suitability of chlorfenapyr as an option for controlling highly pyrethroid-resistant vector populations as previously recorded [40, 41, 42]. The overall data on chlorfenapyr susceptibility was similar to several reports from studies conducted in Sub-Saharan Africa [26, 43]. Kouassi *et al*. [26] reported in 2020 that higher mortality of mosquitoes could be observed using chlorfenapyr, particularly in areas where insecticide detoxification was the main resistance mechanism, suggesting that ITNs with chlorfenapyr may be appropriate in Niger.

Multiple resistance mechanisms are associated with insecticide resistance in mosquitoes, including target-site mutations and markers of metabolic and cuticular resistance [44, 45]. Among these, resistance to pyrethroids and dichlorodiphenyltrichloroethane (DDT) is associated with a substitution of the amino acid leucine with either phenylalanine (L1014F, referred to as *kdr*-West) or serine (L1014S, referred to as *kdr*-East) at position 1014 on the voltage-gated sodium channel gene [28, 34, 44, 45, 46, 47, 48]. For organophosphate and carbamate insecticides, a target site mechanism, known as *ace*-1, represents the substitution of an amino acid glycine to serine at position 119 on the acetylcholinesterase 1 gene [23]. All of these mutations can occur within a single mosquito, contributing to resistance to multiple classes of insecticides [49]. In Niger, the frequencies of *kdr*-w mutations observed by sites increased over the two study years. While we cannot definitively determine the reason for the drastic increase in the *kdr*-w mutation from one year to the other, the increase observed in 2020 could be related to the variability of larval habitats visited every year. Czeher *et al*. [15] reported similar trends in Niger through studies conducted after the first ITN distribution of 2005, highlighting the variable presence of the *kdr*-w mutation within the population of *An. coluzzii*, which also represented the predominant vector in our current study sites. Also, limited entomological data were available in Niger before recent works conducted by Soumaila *et al*. [16] and Ibrahim *et al.* [17] confirming the presence of the *kdr*-w mutation in the country following resistance observed in selected sites. However, the *kdr*-e mutation had not previously been investigated in the country. This study characterized the presence of the allele for the first time in Niger, and surprisingly, the frequency of the mutation was already high within the local populations, showing that the mutation may have been occurring in the country for an unknown period, in contrast to other countries where only a few specimens carrying this allele have been recorded [50, 51]. Similar to the *kdr* mutations, the *ace*-1 resistance allele was also detected within the mosquito populations in all sites, but at a low frequency. Even though carbamate and organophosphate insecticides are not reported in this study, the frequency of the *ace*-1 mutation suggests that the vectors are under selective pressure from carbamate or organophosphate insecticides, though the mutation frequency and phenotypic status are not directly correlated [52].

Conclusion

Findings from this study showed malaria vector resistance to pyrethroids and susceptibility to chlorfenapyr, in addition to a positive effect of PBO pre-exposure on vector mortality in all sites. As prevention measures to control malaria in Niger focus on the universal coverage of ITNs, the threat of insecticide resistance and multiple resistance mechanisms could be overcome by strategic management of the issue using available tools. The data collected over the two years showed that PBO-based ITNs and ITNs that include chlorfenapyr could be used for effective malaria vector control.

Abbreviations

ace-1
Acetylcholinesterase
CDC
Centers for Disease Control and Prevention
IRS
Indoor Residual Spraying
ITN
Insecticide-treated net
kdr
Knocked-down resistance
NMCP
National Malaria Control Programme
PBO
Piperonyl Butoxide
PCR
Polymerase Chain Reaction
SINE
Short Interspersed Element
WHO
World Health Organization

Declarations

Ethics approval and consent to participate

None required.

Consent for publication

This manuscript was formally cleared through CDC and PMI approval system for external publications.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HS and JC drafted the manuscript. BH; IIA; SA; AD; IS; SG; MSZA; IM; SK; NMS; WH and BM supported and supervised field collections. RL; HJ; SI; EC; ZT; EM; JC, ED, SAT and CF revised the manuscript for improvement. All corresponding authors reviewed the draft, provided inputs, which were collated and incorporated by JC. All authors read and approved the final manuscript.

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Figures

Figure 1

Map of Niger showing selected insecticide resistance monitoring sites across the country, 2019 to 2020

Figure 2

Susceptibility and synergism bioassay results of alpha-cypermethrin against *An. gambiae* s.l. in 2019 and 2020 across all sites surveyed

Figure 3

Susceptibility and synergism bioassay results of deltamethrin against *An. gambiae* s.l. recorded in 2019 and 2020 across all sites surveyed

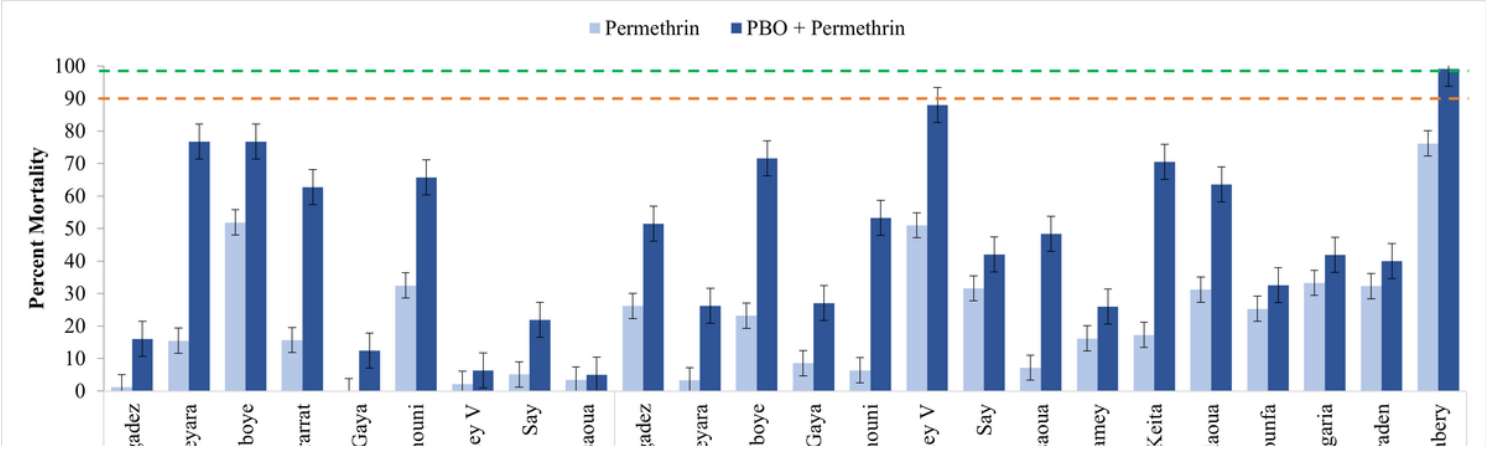

Figure 4

Susceptibility and synergism bioassay results of permethrin against *An. gambiae* s.l. recorded in 2019 and 2020 across all sites surveyed

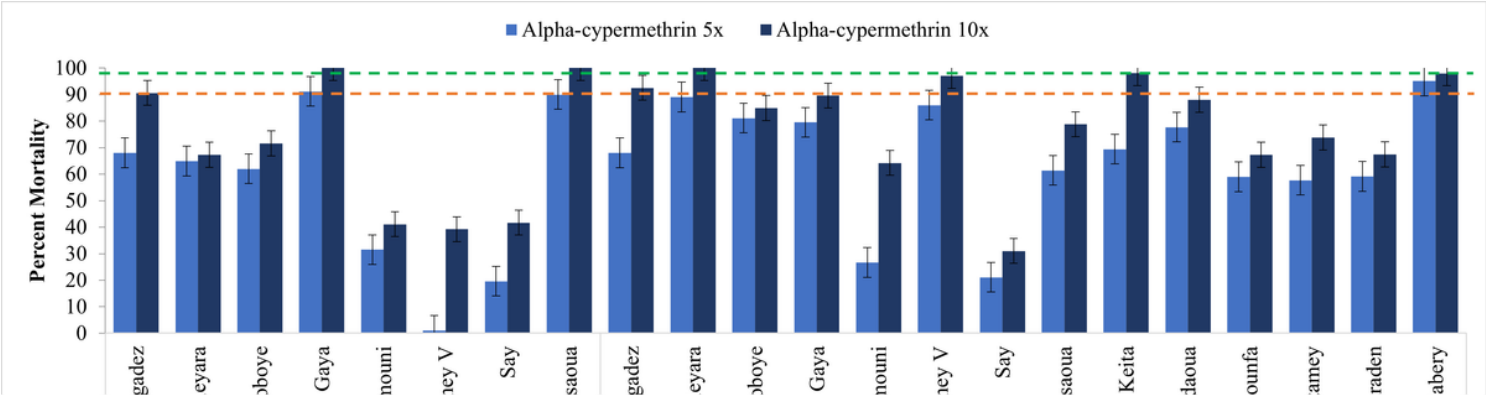

Figure 5

Intensity assays of alpha-cypermethrin (0.25% and 0.5%) against *An. gambiae* s.l. in 2019 and 2020 across all sites surveyed

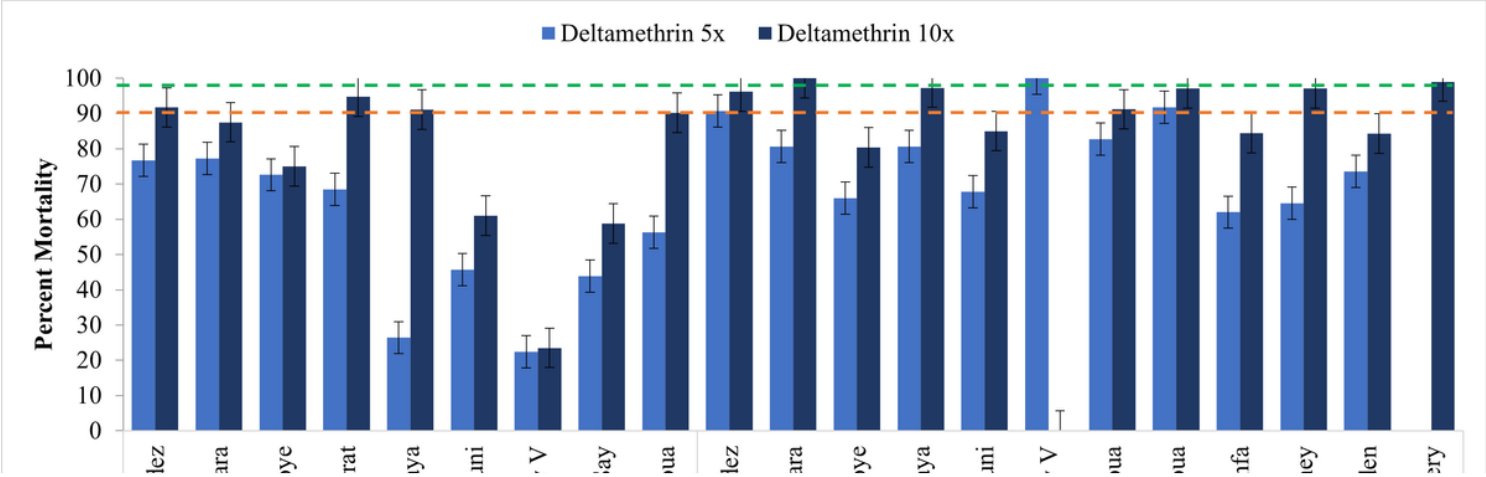

Figure 6

Intensity assays of deltamethrin (0.25% and 0.5%) against *An. gambiae* s.l. in 2019 and 2020 across all sites surveyed

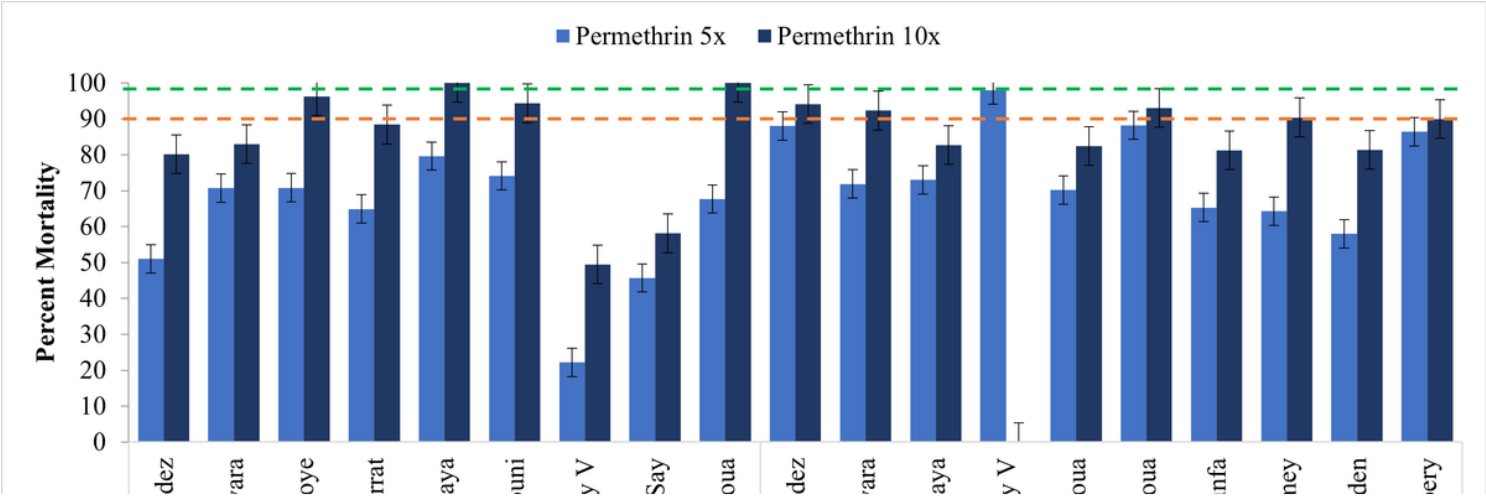

Figure 7

Intensity assays of permethrin (3.75% and 7.5%) against *An. gambiae* s.l. in 2019 and 2020 across all sites surveyed

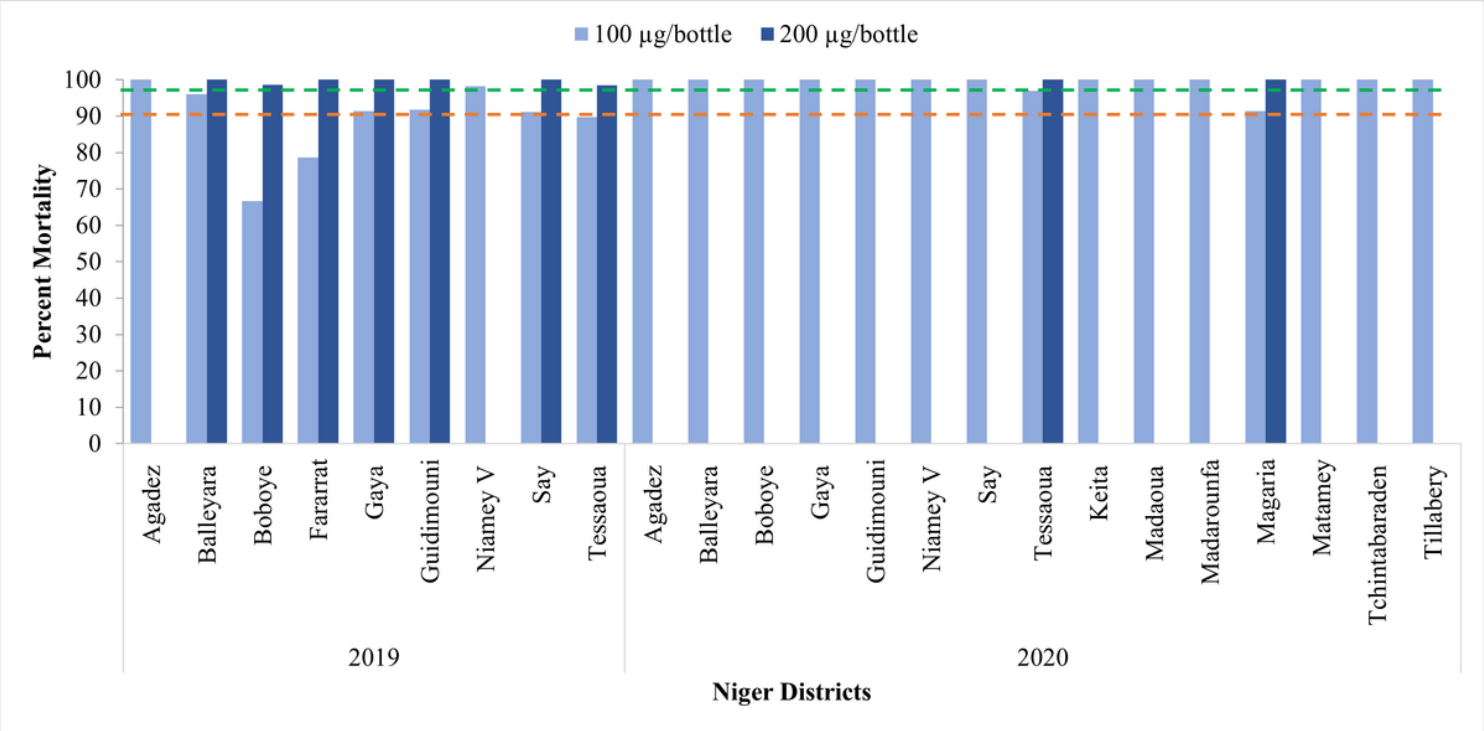

Figure 8

Susceptibility of *An. gambiae* s.l. to chlorfenapyr 100µg/bottle and 200µg/bottle in 2019 and 2020 across all sites surveyed

Figure 9

Species composition of *An. gambiae* s.l. of mosquitoes tested for insecticide susceptibility in 2019 and 2020 across all sites surveyed

Supplementary Files

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