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Multiple insecticide resistance and first evidence of *V410L* kdr mutation in *Aedes (Stegomyia) aegypti* (Linnaeus) from Burkina Faso

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Abstract

The response to recent dengue outbreaks in Burkina Faso was insecticide-based, despite poor knowledge of the vector population's susceptibility to the insecticides used. Here, we report on the susceptibility to the main insecticide classes and identify important underlying mechanisms in *Aedes aegypti* populations in Ouagadougou and Banfora, in 2019 and 2020. Wild *Ae. aegypti* were tested as adults in WHO bioassays and then screened in real time melting curve qPCR analyses to genotype the F1534C, V1016I, and V410L *Aedes* kdr mutations. *Ae. aegypti* showed moderate resistance to 0.1% bendiocarb (80–95% survival post-exposure), 0.8% Malathion (60–100%), 0.21% pirimiphos-methyl (75% – 97%), and high resistance to 0.03% deltamethrin (20–70%). PBO pre-exposure partially restored pyrethroid susceptibility. Genotyping detected high frequency of 1534C allele (0.92) and moderate 1016I (0.1–0.32). The V410L mutation was detected in Burkina Faso for the first time (frequency 0.1–0.36). Mosquitoes surviving 4 h exposure to 0.03% deltamethrin had significantly higher frequencies of the F1534C mutation than dead mosquitoes (0.70 vs. 0.96, $p < 0.0001$) and mosquitoes surviving 2 - 4 h exposure had a significantly reduced life span. *Ae. aegypti* from Burkina Faso are resistant to multiple insecticide classes with multiple mechanisms involved, demonstrating the essential role of insecticide resistance monitoring within national dengue control programmes.

KEYWORDS
Aedes aegypti, arbovirus, Burkina Faso, dengue, insecticide resistance, kdr mutations, vector control

INTRODUCTION

The *Aedes*-borne arboviruses that cause dengue, Zika, Chikungunya, and Yellow fever are being reported worldwide at increasing intensity and frequency. Today, they are a serious global health concern across all tropical and sub-tropical regions. In Africa in 2015, over 800 million persons were at risk from at least one of these arboviruses infection, with the dengue viruses alone accounting for 750 million (Weetman et al., 2018). During the two last decades, there have been increasingly high reports of dengue outbreaks in many sub-Saharan Africa

countries (Simo et al., 2019, Mwanyika et al., 2021) including Burkina Faso where two major dengue outbreaks were reported in 2013 and 2017 dengue (Tarnagda et al., 2018, Im et al., 2020) with *Ae. aegypti* as the preliminary vector involved in the transmission. In 2017, 15,096 dengue cases with 30 deaths were reported with the capital city Ouagadougou accounting for 63% of cases and 83% of deaths (Ministry of Health).

Use of insecticide based-control strategies is a common approach in response to dengue outbreaks in order to quickly drop the vector density and impact the ongoing transmission. Actellic® (pirimiphos-methyl) and malathion were used in space spray in response to the 2017 dengue outbreaks in Ouagadougou (Ministry of Health). The effectiveness of insecticide-based approach to control the main dengue vectors *Aedes* during outbreaks depends on the susceptibility of the vector to the class of insecticides used. As Burkina Faso is one of the sub-Saharan African countries where resistance is seriously compromising malaria control (Toe et al., 2014), it is important to investigate the susceptibility of dengue vectors in the country.

Resistance to the four common public health insecticide classes (carbamates, organophosphates, organochlorines, and pyrethroids) is widespread in *Ae. aegypti* populations worldwide (Smith et al., 2016, Moyes et al., 2017) and though it is well studied in Asia and Latin America, Africa lags behind (Moyes et al., 2017, Ranson et al., 2010). For dengue control, insecticides are used typically for space spraying (fogging) to target adult mosquitoes during outbreaks, and as larvicides applied directly to container habitats to target immature stages. Indoor residual spraying (IRS) and insecticide-treated screens have also been shown to be effective (Manrique-Saide et al., 2015, Paredes-Esquivel et al., 2016, Dunbar et al., 2019) but are not yet widely used.

The evidence for insecticide resistance compromising dengue control has been widely reported (Vontas et al., 2012, Ranson et al., 2010, Dusfour et al., 2011, Marcombe et al., 2011). In the island of Martinique, pyrethroids resistance has been demonstrated to reduce space spray for control (Marcombe et al., 2011). Temephos applied in water holding containers at field dose found its effectiveness compromised against Brazilian, Colombian and Caribbean *Aedes* resistance populations (Montella et al., 2007, Grisales et al., 2013, Rawlins, 1998). Management of resistance in *Ae. aegypti* requires understanding of the major resistance mechanisms and patterns of cross resistance. Two major mechanisms have been described in *Ae. aegypti* globally: alteration of the insecticide target sites and decreased insecticide uptake through metabolic mechanisms (Bariami et al., 2012, Li et al., 2007, Stevenson et al., 2012, Taylor and Feyereisen, 1996).

The target sites mutations are the 'knock down resistance' (kdr) mutations in the voltage sensitive sodium channel (Vgsc). They are responsible for conferring cross resistance between pyrethroids and Dichlorodiphenyltrichloroethane (DDT) and are well-known and studied (Williamson et al., 1996). In *Ae. aegypti*, eleven mutation events at nine codon positions have been identified in the VGSC II-IV subunits with various implications for pyrethroid/DDT resistance (Moyes et al., 2017, Haddi et al., 2017). The most commonly encountered *Ae. aegypti* kdr mutations, either alone or in association, are F1534C, V1016G, I1011M, S989P, and V1016I (Moyes et al., 2017). The most recently reported is the V410L mutation, first reported in Brasilia less than five years ago (Haddi et al., 2017) and seems to be strongly associated with pyrethroid resistance (Saavedra-Rodriguez et al., 2018). In Africa, the few studies that have been done have identified F1534C, V1016I as the main circulating kdr mutations (Moyes et al., 2017) but V410L is now spreading and has been reported from Angola (Ayres et al., 2020) and from Cote d'Ivoire (Konan et al., 2021). In Burkina Faso, the F1534C was found to be almost fixed in populations of *Ae. aegypti* from Ouagadougou (Somgandé, 1200 logements, Tabtenga) collected in 2016–2017 while the V1016I was reported at low frequency (Badolo et al., 2019, Sombie et al., 2019).

Transcriptomic studies have identified putative candidate resistance genes in *Ae. aegypti* that are switched on in response to selection by, or exposure to, insecticides. Several cytochrome P450 genes have been found overexpressed in field or laboratory pyrethroid resistance strains, with evidence indicating a role in pyrethroids metabolism by CYP9J28, CYP9J10, CYP9J26 CYP6BB2 and CYP6P12 (Xu et al., 2016, Kasai et al., 2014, Stevenson et al., 2012, Ishak et al., 2016, Pavlidi et al., 2012). Resistance to the organophosphate temephos is associated with the P450s genes CYP6Z8, CYP6N12 and the carboxylesterases genes CCEAE3A in multiple studies (Grigoraki et al., 2016).

Recent reports investigating the susceptibility of *Ae. aegypti* in Burkina Faso, suggested that they are highly resistance to pyrethroids, moderately resistance to carbamates but remain susceptible to organophosphate insecticides (Badolo et al., 2019, Namountougou et al., 2020, Sombie et al., 2019). Here, we provide recent data on resistance to the main insecticide classes status in *Ae. aegypti* populations from two cities in Burkina Faso, following its evolution over a two year period and we identify the underlying insecticide resistance mechanisms in *Ae. aegypti* in Burkina Faso.

MATERIALS AND METHODS

Study sites

The study was conducted in two cities of Burkina Faso: Ouagadougou and Banfora.

Ouagadougou (12°21′58″ N, 1°31′05″ W): the capital city is the largest city of the country and located in the central region with an arid savannah (soudano-sahalian) climate, annual rainfall of 780 mm, and mean temperature of 28°C. During the 2017 dengue outbreak, more than 60% of the national dengue cases have been recorded in Ouagadougou (Ministry of Health, 2018). In the city of Ouagadougou, two sites were selected. The first, 1200 logements, comprises modern concrete buildings, has piped water, electricity, sanitation and waste management systems. The second site, named Tabtenga, comprises predominantly single storey traditional style adobe housing at high density. It is located approximately 5 km east of 1200 logements but it has no centralized water supply, no electricity, and no waste management systems.

Banfora (10°″37′36″ N, 4°45′29″ W) 440 Km from Ouagadougou, Banfora is located in the Cascades region of south west Burkina Faso

in the Soudan savannah climatic zone. The annual rainfall exceeds 1200 mm yearly. Despite its position as a transit city route to Mali and Cote d'Ivoire, few dengue cases were reported during the 2016 outbreak. The study was carried out in two sites: an urban area ("District 1 &7") and a peri-urban area ("Bounouna").

Mosquito sampling and WHO susceptibility bioassays

Aedes mosquitoes were collected as eggs using oviposition traps or as larvae in water holding containers constituted by used tires, discarded domestic containers at the field sites. Several days (at least five days per site) collection were performed to have mixed and big size population. Mosquitoes were reared to adults for use in laboratory tests, under controlled conditions of temperature (27 ± 2°C) and relative humidity (75 ± 10%). The samples were collected in the wet season (July – November) in 2019 and 2020 in both cities of Ouagadougou and Banfora. All material from each sampling site was reared and tested separately. F1 females were obtained F0 mosquitoes collected as eggs or larvae. They were blood fed on rabbit prior to be allowed for egg-laying. Adults were provided access to 10% glucose solution.

For each bioassay, one hundred mosquitoes (four replicates of 25 mosquitoes) of 3 to 5 days old F0 or F1 non-blood fed female mosquitoes were exposed to the insecticide-treated paper at *Aedes* diagnostic dose for an hour following the WHO tube bioassay procedure (WHO, 2016). The following insecticides and concentration were tested: the pyrethroids deltamethrin and alpha-cypermethrin, both at 0.03%; the organophosphates, 0.8% malathion, and 0.21% pirimiphos methyl and the carbamate bendiocarb at *Anopheles gambiae* diagnostic concentration of 0.1%. The susceptibility profile was determined per insecticide based on the mortality rate recorded 24 h after exposure to the insecticide treated paper, and classified as resistant, probable resistant, or susceptible following WHO criteria (WHO, 2016). Control, dead, and alive mosquitoes after each test were stored over silica gel in 15 ml tubes.

Resistance intensity and PBO assays

Bioassays were performed using 3–5 days unfed F1 adults. Mosquitoes were exposed to 4% PBO paper or insecticide-free paper for 1 h before been exposed to 0.03% deltamethrin or 0.03% alpha-cypermethrin. The mortality rates were recorded 24 h after exposure. For the intensity bioassays, F1 adults from the same larvae collection were exposed to 0.03%, 0.25% and 0.5% deltamethrin for an hour and the mortalities recorded 24 h after exposure.

Longevity following deltamethrin exposure mortality assessment

Using mosquitoes reared from wild larvae, three to five days old non-blood fed female F1 *Ae. aegypti* were exposed to 0.03% deltamethrin for 60, 120, and 240 min. Mortality at 24 h post-exposure was recorded and any remaining live mosquitoes were maintained in the insectary until death; mortality was recorded daily.

Investigation of resistance mechanisms

Kdr genotyping

In 2019, a sub set of 50 *Ae. aegypti* mosquitoes from each of the four sites, and no exposure to insecticide, were screened for the kdr mutations. We first screened for the F1534C and V1016I mutations as they had already been reported (Badolo et al., 2019, Sombie et al., 2019). We extended the screening to V410L as this mutation has been recently reported in neighbouring Cote d'Ivoire (Konan et al., 2021). In 2020, dead and living mosquitoes from 4 h exposure to 0.03% deltamethrin were screened for the three mutations: F1534C, V1016I, and V410L. Genomic DNA was extracted from whole mosquitoes using genomic DNA isolation reagent from DNAzol® Reagent (Invitrogen by Thermo Fisher Scientific) according to the manufacturer procedure. Real-time melting curve qPCR analyses were performed to genotype the F1534C V1016I and V410L *Aedes* kdr mutations. For the detection of F1534C kdr mutation according to Saavedra-Rodriguez et al, (Saavedra-Rodriguez et al., 2007), a PCR reaction (10 µl) consisting of 2 µl of SolisFAST® SolisGreen® qPCR Mix (from SOLIS BIOBYNE), 0.4 µl of each of the three primers (**Cys1534+**: 5′-GCG GGC AGG GCG GCG GGG GCG GGG CCT CTA CTT TGT GTT CTT CAT CAT GTG-3′; **Phe1534+:** 5′-GCG GGC TCT ACT TTG TGT TCT TCA TCA TAT T-3′ and **1534-**: 5′-TCT GCT CGT TGA AGT TGT CGA T-3′), 5.8 µl of H_20 and 1 µl of DNA was run in the following cycling conditions: 95°C for 3 min, 37 cycles at 95°C for 10 sec, 57°C for 30 sec, 72°C for 30 s with the final extension of 95°C for 10 s following by the melting curve on a gradient of temperature from 65°C to 95°C with an increment of 0.5°C every 5 s. The presence of the V1016I mutation was detected as described by Saavedra-Rodriguez et al. (Saavedra-Rodriguez et al., 2007) using a 10 µl PCR mix reaction of 2 µl of SolisFAST® SolisGreen® qPCR Mix (from SOLIS BIOBYNE), 0.4 µl of each of the three primers (**Val1016f**: 5′-GCG GGC AGG GCG GCG GGG GCG GGG CCA CAA ATT GTT TCC CAC CCG CAC CGG3′; **Ile1016f**: 5′-GCG GGC ACA AAT TGT TTC CCA CCC GCA CTG A-3′ and **Ile1016r**: 5′-TGA TGA ACC SGA ATT GGA CAA AAG C-3′), 5.8 µl of H_20 and 1 µl of DNA. The cycling conditions were the following: 95°C for 3 min, 35 cycles at 95°C for 10 sec, 60°C for 10 sec, and 72°C for 30 s with the final extension of 95°C for 10 s following by the melting curve on a gradient of temperature from 65°C to 95°C with an increment of 0.2°C every 10 s. The V410L was detected using the assays described by Saavedra-Rodriguez et al. in 2018 (Saavedra-Rodriguez et al., 2018). A PCR mix reaction of 10 µl consisting of 2 µl of SolisFAST® SolisGreen® qPCR Mix (from SOLIS BIOBYNE), 0.4 µl of each of the three primers (**Val410: 5**′-GCG GGC AGG GCG GCG GGG GCG GGG CCA TCT TCT TGG GTT CGT TCT ACC GTG-3′; **Leu410**: 5′-GCG GGC ATC TTC TTG GGT TCG TTC TAC CAT T-3′ and **Rev410**: 5′-TTC TTC CTC GGC GGC CTC TT-3′), 5.8 µl of H20

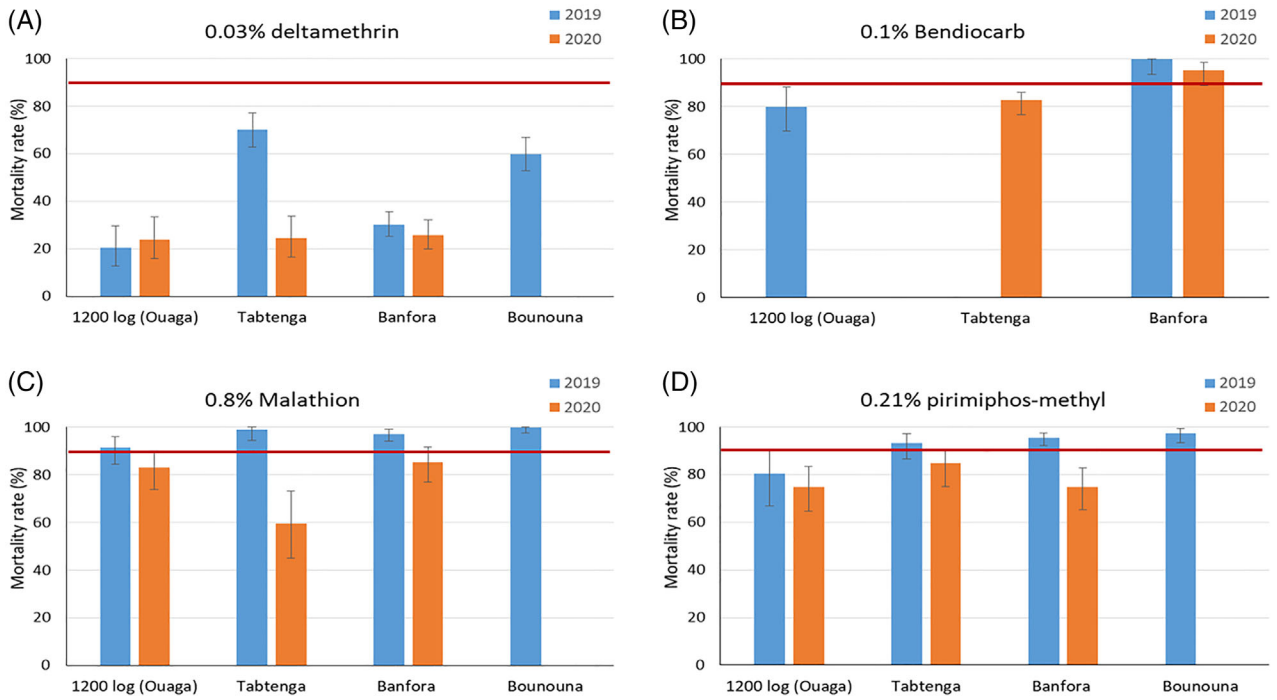

FIGURE 1 Mortality rates at 24 h post-exposure to deltamethrin, bendiocarb, malathion, and pirimiphos-methyl insecticides as shown, in adult female *Ae. aegypti* from the sampling sites in Ouagadougou (1200 logements, Tabtenga) and Banfora (Banfora District 1&7, Bounouna) in 2019 and 2020 (Bounouna was not tested in 2020, bendiocarb insecticide was not tested in Tabtenga in 2019)

and 1 µl of DNA was run in the following cycling conditions: 95°C for 3 min, 40 cycles at 95°C for 10 sec, 60°C for 10 sec, 72°C for 30 s with a final extension of 95°C for 10 s following by the melting curve on a gradient of temperature from 65°C to 95°C with an increment of 0.2°C every 10s. All the PCRs were run on the Stratagene Mx3005P qPCR machine (Agilent Technologies).

Statistical analyses

The susceptibility status of an *Ae. aegypti* mosquito population was determined according to the WHO criteria (WHO, 2016) which is based on the 24 h mortality rate. Abbot's correction was not required as the mortality rate in the controls was below 5%. The mortality rates before and after pre-exposure to PBO, as well mortality rates following exposure to different concentration or times were compared by Fisher's exact test using R software. The frequencies of F1534C, V1016I, and V410L kdr mutations were determined in each unexposed population for 2019 collection and in dead and live mosquitoes following 240 min deltamethrin exposure and compared two by two using Pearson's Chi-squared test.

To estimate the impact of deltamethrin exposure on mosquito survival, we performed a Cox proportional hazards regression model using the R package 'survival'. The number of mosquitoes that died per day was used as a response variable and an interaction between treatment (control vs. deltamethrin) and exposure duration (1, 2, and 4 h) was included as an explanatory variable. Replicate was included as frailty to account for variation among test batches. This full model was then compared using ANOVAs to models with the independent effect of treatment and exposure (i.e., without interaction and single variables). The full model was retained as the best model.

RESULTS

Aedes aegypti insecticide susceptibility profile

Susceptibility to pyrethroids

Mortality rates recorded at 24 h following exposure to the *Ae. aegypti* diagnostic dose of deltamethrin showed mortality under the threshold of 90% over the two years of monitoring. The mortalities ranged from 20% to 70% (Figure 1a), thus confirming that the vector population is resistant to the class II pyrethroid deltamethrin in the urban and peri-urban areas of Banfora and Ouagadougou. Moreover, resistance increased over the two year period as shown by the significant decrease in mortality rates between 2019 and 2020 in Tabtenga, Ouagadougou (70% vs. 24%, $p < 0.0001$).

Susceptibility to carbamates

Moderate resistance to the carbamate bendiocarb (0.1%) was observed at 1200 logements in Ouagadougou and in the peri-urban

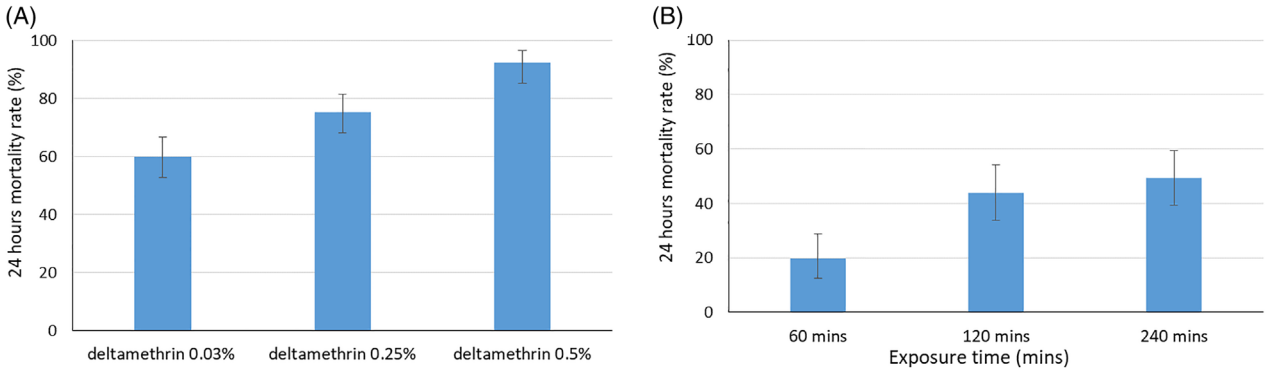

FIGURE 2 Twenty-four hours mortality rates (with binominal confidence interval) to 0.03% (1x), 0.25% (8.3x), and 0.5% (16.6x) deltamethrin in *Ae. aegypti* from Bounouna in 2020 (a), and to 60, 120, and 240 min exposure to 0.03% deltamethrin in Banfora urban site in 2020 (b)

site Bounouna, in Banfora. Twenty-four hour mortality rates of 80% and 82% were recorded in 1200 Logements in 2019 and in Bounouna, respectively, in 2020 (Figure 1b). *Ae. aegypti* at District 1&7 of the urban site in Banfora urban site were fully susceptible in 2019 (mortality = 100%), but the mortality rate fell to 95% in 2020, suggesting the beginning of the emergence of resistance.

Susceptibility to organophosphates

In 2019, all populations with one exception were nearly susceptible to both organophosphates, malathion and pirimiphos-methyl with mortalities over the 90% (probable susceptibility) threshold. The exception was the population at 1200 logements where a mortality of 80% was recorded for pirimiphos-methyl. However, by 2020, all field collected *Ae. aegypti* tested were resistant to both pirimiphos-methyl and malathion with mortality rates ranging from 74% to 85% and from 60% to 85% respectively (Figure 1c,d).

Pyrethroid resistance intensity

Very strong resistance to deltamethrin was detected in both urban and peri-urban sites in Banfora as indicated by the deltamethrin intensity bioassays. In the peri-urban site, Bounouna mortality rates of 75% and 92% were obtained after exposure to 8 times and 16 times *Aedes* diagnostic dose (Figure 2). In the urban site, exposure time, rather than insecticide concentration was varied; here, increasing the exposure time from 60 min to 120 min and 240 min, increased the mortality rate but even after 4 h exposure, 24 h mortality was just 50% (Figure 2).

Resistance mechanisms

Monooxygenase metabolic-based mechanism

Results from the PBO synergist assay in the field showed a significant increase in deltamethrin and alpha-cypermethrin mortality rates, from 47% to 95% and 56% to 86% ($p < 0.0001$), respectively, with PBO pre-exposure though full susceptibility to pyrethroids was not restored (Figure 3). This partial recovery of susceptibility suggests that the cytochrome P450 monooxygenases are also playing a role in pyrethroid resistance in this population.

Kdr target site mutations-based mechanism

To investigate mechanisms based on the target sites mutations, a subset of 50 unexposed *Aedes* mosquitoes collected in 2019 from all four sites were genotyped for the *Aedes* kdr mutations. The genotypic frequencies of the F1534C, V1016I, and V410L are shown in the Table 1.

The 1534C allele was at found at frequency ranging from 0.45 to 0.92 with a significantly higher frequency in Ouagadougou than Banfora. When comparing between urban and peri-urban area, a slightly high frequency was observed in the urban area, with a significant difference between Banfora urban and Banfora peri-urban Bounouna (0.68 vs. 0.45, $p = 0.001$).

A low frequency of the 1016I allele was recorded, with only four individual mutant homozygotes (I/I). The mean frequency ranged from 0.1 to 0.32 and a significantly higher frequency was observed in both areas of Ouagadougou compared to Banfora city areas.

The V410L mutation was recorded for the first time in Burkina Faso, at frequencies ranging from 0.1 to 0.36 in *Ae. aegypti* from Burkina Faso. The frequencies of this V410L mutation were similar to the V1016I mutation (Table 1).

Overall, there is a significant variation in the frequency of the three mutations, between the cities of Ouagadougou and Banfora.

The screening of the F1534C, V1016I, and V410L kdr mutations in dead and alive *Ae. aegypti* following 4 h exposure to 0.03% deltamethrin revealed higher frequencies of the three mutations in alive mosquitoes (Table 2) but the difference was significant only for the F1534C mutation. All the alive mosquitoes carried the 1534C mutant allele, 44 individuals were homozygote C/C while only eight were heterozygotes F/C. The frequencies of the three mutations F1534C, V1016I and V410L were increased from 0.68 to 0.81, 0.1 to 0.28 and

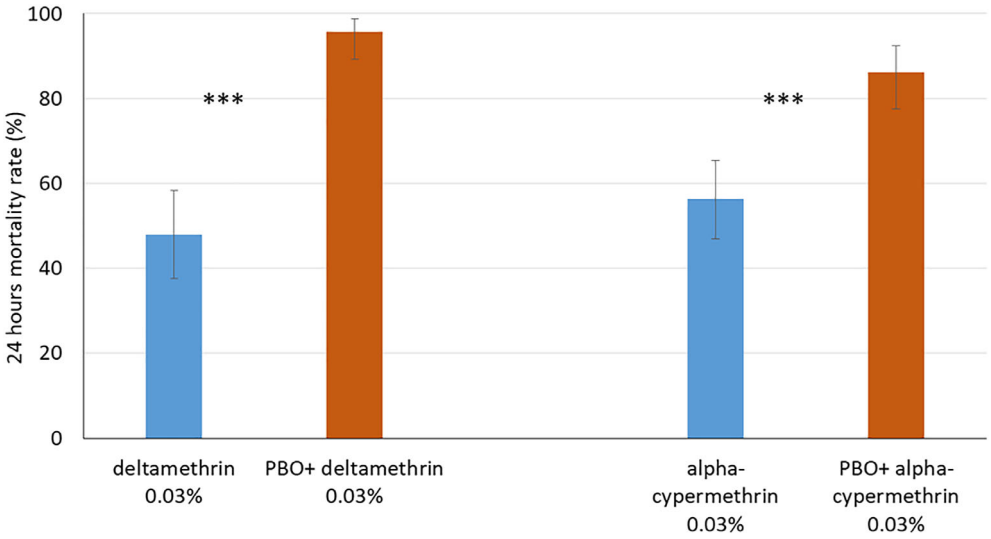

FIGURE 3 Twenty-four hours mortality rate (with binominal confidence interval) after exposure to 0.03% deltamethrin and 0.03 alpha-cypermethrin with or without pre-exposure to piperonyl butoxide (PBO (A), in Bounouna, the peri-urban site of Banfora in 2019. * indicates the level of significant difference, ***$p < 0.0001$ (Fisher's exact test)

TABLE 1 Frequency of the F1534C, V1016I, and V410L in unexposed *Ae. aegypti* from the urban and peri-urban sites of the cities of Ouagadougou and Banfora in 2019

	F1534C			V1016I			V410L		
	Genotype	*n*	f(1534C)	Genotype	*n*	f(1016I)	Genotype	*n*	f(410 L)
1200 logements (Ouagadougou peri-urban)	CC	42	0.92	II	1	0.29	LL	6	0.31
	FC	8		VI	27		VL	19	
	FF	0		VV	22		VV	25	
Tabtenga (Ougadougou urban)	CC	37	0.87	II	1	0.32	LL	5	0.36
	FC	13		VI	30		VL	26	
	FF	0		VV	19		VV	19	
Banfora urban	CC	19	0.68	II	2	0.1	LL	1	0.1
	FC	29		VI	6		VL	8	
	FF	1		VV	42		VV	41	
Bounouna (Banfora peri-urban)	CC	4	0.45	II	0	0.12	LL	0	0.11
	FC	37		VI	12		VL	11	
	FF	9		VV	38		VV	39	

TABLE 2 Frequency of the F1534C, V1016I, and V410L mutations from dead and alive *ae. Aegypti* originating from the Banfora urban sampling site in 2020 following 4 h exposure to 0.03% deltamethrin

	F1534C*			V1016I			V410L		
	Genotype	*n*	f(1534C)	Genotype	*n*	f(1016I)	Genotype	*n*	f(410 L)
Banfora, delta dead	CC	21	0.70	II	1	0.24	LL	1	0.23
	FC	27		VI	22		VL	21	
	FF	2		VV	28		VV	29	
Banfora, delta alive	CC	44	0.96	II	4	0.34	LL	2	0.31
	FC	8		VI	26		VL	27	
	FF	0		VV	22		VV	22	

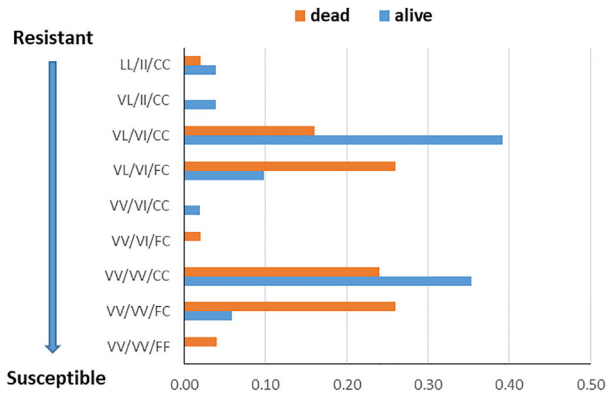

FIGURE 4 Distribution of the three loci (in order V410L-V1016I-F1534C) haplotypes among dead and alive female mosquitoes following 4 h exposure to 0.03% deltamethrin; mosquitoes are *Aedes aegypti* reared from wild eggs or larvae originating in the urban site of Banfora in 2020

from 0.1 to 0.26, respectively, between 2019 and 2020 in Banfora urban site, suggesting an increase in pyrethroids resistance.

Figure 4 shows the proportion of the loci haplotypes (V410L-V1016I-F1534C) when comparing the three kdr mutations between dead and alive. Mosquitoes carrying wild haplotypes (V410/V1016/F1534) were found only in dead mosquitoes. The two most prevalent haplotypes in alive mosquitoes were VL/VI/CC (39%) and VV/VV/CC (35%) and all alive individuals were homozygotes for the 1534C allele. The two most prevalent haplotypes in dead mosquitoes were VV/VV/FC (26%) and VL/VI/FC (26%). Interestingly, within the three loci haplotype combinations, haplotypes where individuals were homozygous for the 1534C allele had high frequency in alive mosquitoes. Individuals that were homozygous for this mutation, both, alone or in association with the other two mutations, were significantly more likely to survive 4 h deltamethrin exposure.

Impact of resistance on longevity

The Cox proportional hazard model showed that mosquitoes exposed to 0.03% deltamethrin treated paper for 2 and 4 h die significantly faster compared to mosquitoes exposed to untreated paper (by 1.7x $p = 0.02$ and and 1.9x 0.002 respectivelly). This led to a reduction in mean lifetime of approximately 15 days for mosquitoes exposed to deltamethrin for 2 h or more, relative to controls (Figure 4). However, exposure for 1 h only did not impact on mosquito survival (Figure 5, Table S2).

DISCUSSION

The last decade was marked by several large outbreaks of dengue in Sub-Saharan Africa and it is clear that further outbreaks are inevitable. The development of a dengue/arbovirus control program has become a priority for countries where previously, dengue was of little concern. More recently, it has been shown that sustained mass releases of Wolbachia-infected *Ae. aegypti* can reduce dengue transmission by 77% (Utarini et al., 2021), which is an unprecedented achievement for vector control of dengue. However, it seems certain that in Burkina Faso and many other low and middle income countries, vector control will continue to rely on insecticides for some time. Hence, our report on the susceptibility profile of a number of different *Ae. aegypti* populations to the main public health insecticide classes, together with insight into the mechanisms that may be involved in resistance,

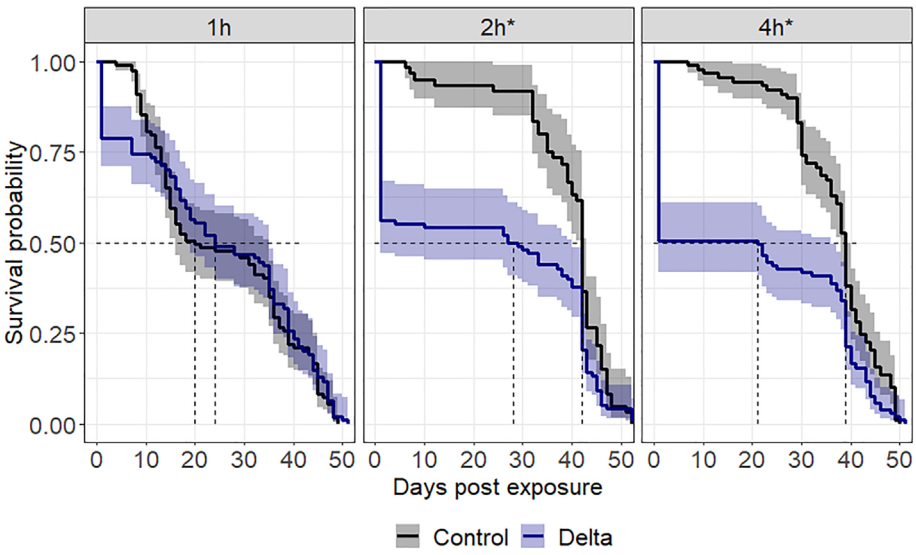

FIGURE 5 Survival curves from the cox proportional hazard regression model. Panels show the mean and 95% confidence intervals for daily survival probability of mosquitoes exposed to 0.03% deltamethrin (blue line and shade) or untreated control (black line, grey shade) for 1, 2 and 4 h dashed lines correspond to the mean lifetime, that is, L50; and * denotes a significant difference between control and treatment survival curves ($p < 0.05$). Day 0 was the day of exposure to the insecticide

provides essential evidence for any future national dengue/arbovirus control program.

Multiple insecticide resistance associated to high pyrethroids in Ae. egypti from Burkina Faso

Following the standard WHO tube bioassays method, *Ae. aegypti* from peri-urban and urban sites of Banfora and Ouagadougou showed phenotypic resistance to multiple insecticide classes including pyrethroids, carbamates and organophosphates.

We recorded an exceptional high resistance to deltamethrin with mortality rate below 30% after one hour exposure in 2020 and mortality around 50% following 4 h exposure. Pyrethroid resistance in *Ae. aegypti* is known to be occur worldwide (Moyes et al., 2017) but its arrival in West Africa is relatively recent in Senegal (Sene et al., 2021), in Ghana (Kawada et al., 2016), and Cote d'Ivoire (Konan et al., 2021). Recent studies by Badolo et al. (Badolo et al., 2019), Namountougou et al. (Namountougou et al., 2020), and Sombie (Sombie et al., 2019) have reported resistance to both pyrethroid I and II types in the two largest cities, Ouagadougou and Bobo-Dioulasso. Burkina Faso has no history of pyrethroids used against in *Ae. aegypti*, yet the resistance observed seems to have increased yearly, suggesting other permanent source of resistance selection pressure. Selection of pyrethroid resistance in the malaria vector from Burkina Faso has been linked to the agriculture used of insecticide (Diabate et al., 2002, Hien et al., 2017) with additional selection pressure from the wide scale deployment of Insecticide Treated Nets (Toe et al., 2014). ITNS have been shown to impact on *Ae. aegypti*, but not at levels likely to select for resistance (Lenhart et al., 2008). Many over-the-counter personal protection methods commonly used against nocturnal nuisance biting by *Culex quinquefasciatus*, such as mosquito coils or aerosols contain pyrethroids. Together, exposure via these routes presumably exerted strong selection pressure on *Ae. aegypti* populations, even if this species was not the intended target. Clearly, the level of pyrethroid resistance is a major cause for concern in Burkina Faso as the pyrethroids are the first line of defence for vector control in the event of a dengue outbreak.

As with the pyrethroid insecticides, the *Ae. aegypti* mosquitoes from both urban and peri-urban sites of Banfora and Ouagadougou were resistant to the carbamate insecticide bendiocarb but the level of resistance was moderate. A 2019 report reported resistance to 0.1% bendiocarb in *Ae. aegypti* collected from drums and tires in from the same study sites in Ouagadougou as in this study (Badolo et al., 2019) and in the city of Bobo-Dioulasso (Namountougou et al., 2020). All studies reporting bendiocarb resistance to date, have reported it as being relatively moderate. *Ae. aegypti* resistance to 0.1% bendiocarb has recently been reported in Senegal (Sene et al., 2021) and Cote d'Ivoire (Konan et al., 2021).

The *Ae. aegypti* populations tested were resistant at moderate level to pirimiphos-methyl and malathion insecticide at diagnostic doses of 0.21% and 0.8%, respectively. During the dengue outbreak of 2017, malathion and pirimiphos-methyl were used for outdoor space-spraying only in Ouagadougou (Ministry of Health). It seems unlikely that this activity was responsible for or contributed significantly to the emergence of organophosphate resistance, partly because of the short period of spraying (less than a month) and partly because despite the absence of spraying in Banfora, resistance to malathion and pirimiphos-methyl were also reported there. This is the first report of organophosphate resistance in Burkina Faso as previous studies that tested malathion or fenitrothion and did not report resistance to these organophosphates class insecticides (Badolo et al., 2019, Ouattara et al., 2019). However, those earlier studies screened using the *Anopheles* diagnostic dose for Malathion (5%), which is higher than the dose used for *Ae. aegypti*, 0.8%, which would explain the different results between studies. Nevertheless studies from Cote d'Ivoire (Konan et al., 2021) and Senegal (Sene et al., 2021) used the *Anopheles* diagnostic dose of fenitrothion and malathion and reported resistance to organophosphates.

As with the pyrethroids, the source of selection pressure for resistance in both carbamates of organophosphates is unknown although malathion and pirimiphos methyl were used for outdoor spray during the dengue outbreak in 2016 and 2017. The moderate level of the resistance suggests that insecticides from these classes may still be suitable for use in arbovirus outbreaks although clearly, continual monitoring of their resistance status will be essential.

Mechanisms of insecticide resistance conferring resistance to pyrethroids

In this study, we found high pyrethroid resistance in all *Ae. aegypti* populations and two main mechanisms were identified, overexpression of detoxification enzymes families and kdr mutations. Several kdr point mutations in the vgsc gene have been reported in *Ae. aegypti* but few are associated with pyrethroid resistance. We screened for the F1534C, V1016I, and V410L and tested for their association with deltamethrin resistance. We recorded high frequency of the 1534C allele in the urban site of Ouagadougou and found this allele significantly associated with the deltamethrin resistance phenotype strongly implicating it in pyrethroid type II resistance. This mutation has previously been reported in the same locality at a frequency around 0.94 (Badolo et al., 2019). Elsewhere in Sub-Saharan Africa, it has been reported since 2016 in Ghana (Kawada et al., 2016) and recently in Cote d'Ivoire (Konan et al., 2021), Cameroon (Yougang et al., 2020), Angola and Cape Verde (Ayres et al., 2020). This mutation is the most widespread and associated with both pyrethroid type I and II resistance (Moyes et al., 2017). The V1016I mutation was found at relatively moderate frequency as reported in the previous study from Ouagadougou (Badolo et al., 2019, Sombie et al., 2019). Its frequency had increased over time and could increase the strength of pyrethroid resistance in Burkina Faso as the haplotype 1016I/1534C is strongly associated with pyrethroid resistance (Dusfour et al., 2015) and both mutations are thought to be co-evolved (Saavedra-Rodriguez et al., 2018). We reported for the first time in Burkina Faso the V410L kdr mutation at relatively low frequencies that were different in the

two cities. We did not clearly establish its involvement in deltamethrin resistance although a slightly higher frequency was obtained in *Ae. aegypti* surviving four hours exposure. But this mutation alone or in combination with F1534C has been demonstrated to reduce the sensitivity of mosquitos sodium channel expressed in Xenopus oocystes to both type I and I pyrethroids (Haddi et al., 2017). In West Africa, this mutation has been reported in the neighbouring country of in Cote d'Ivoire (Konan et al., 2021). However, the low frequency of the V410L mutation recorded does not allow assessment of its implications alone or in association with pyrethroid resistance.

Impact of the highly pyrethroids resistance on mosquitoes life span

We found that highly resistance *Ae. Aegypti* mosquitoes surviving 2 and 4 h 0.03% deltamethrin died faster than exposed mosquitoes while one hour exposure did not affect survivor probability. Mosquitoes surviving these long exposures are likely to have a high frequency of kdr mutations. The 1534C allele has been found to affect the larval development time and female fecundity but not adult longevity in selected laboratory kdr *Ae. aegypti* mosquitoes (Brito et al., 2013). Other *Aedes* kdr mutations such as V1016G and S89P have been reported to shorten *Ae. aegypti* life span (Rigby et al., 2020). All these studies used selected laboratory resistant strains to assess the fitness cost and compared the results with laboratory susceptible kdr free mosquitoes. Using wild mosquitoes to assess the impact of insecticide resistance on fitness cost will provide important insights for the management of resistance.

CONCLUSION

Our study detected resistance to multiple insecticide classes in *Ae. aegypti* populations from Burkina Faso. This resistance is variable according to the insecticide class, the city, and the site (urban and periurban) with an exceptional high pyrethroid resistance. During our survey, we reported for the first time resistance to organophosphate and evidence of the V410L kdr mutation in Burkina Faso. Although this study was limited only to the cities of Ouagadougou and Banfora, this reveals the magnitude of the challenge of insecticide resistance for dengue control in Burkina Faso. The resistance observed might involve multiple mechanisms which could exert a fitness cost on the vector, elucidation of which require future investigation. Nationwide monitoring of the insecticide susceptible profile and underlying resistance mechanisms should be implemented to assess the scale of the resistance in dengue vector mosquitoes from Burkina Faso. The recent outbreaks of dengue have demonstrated the need for nationwide monitoring programme for the management of insecticide resistance.

AUTHOR CONTRIBUTIONS

Hyacinthe K. Toé, Moussa W. Guelbeogo, Basile Kamgang, Philip J. McCall, and N'Falé Sagnon conception and design of the study; Hyacinthe K. Toé, Soumanaba Zongo, Madou Tapsoba, and Alphonse Traoré acquisition of data; Hyacinthe K. Toé, Soumanaba Zongo, Mafalda Viana, and Antoine Sanou analysis and interpretation of data; Hyacinthe K. Toé, Moussa W. Guelbeogo, Basile Kamgang, and Philip J. McCall drafting the manuscript and revising it critically for important intellectual content, All the authors: final approval of the version to be submitted.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Raw standard tube bioassays data including the locality where samples were collected, the year of the test, the insecticide and concentration tested, the total exposed and the number of dead and alive mosquitoes.

Table S2. Summary of results obtained from the Cox proportional hazard regression model. Coefficients and associated standard error are given for each retained variable and are relative to the [reference]. Hazard ratios are estimated as the exponential of the coefficients. Bold indicates significance with p-value <0.05.

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