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Does household use of mosquito coil influence insecticide susceptibility in vector mosquitoes? A laboratory investigation with *Aedes aegypti* population

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Key Words

Mosquitoes, insecticide Resistance, mosquito coil, *Aedes aegypti*, F1534C mutation, enzyme activity, PCR, Kdr.

Abstract

Aedes aegypti mosquitoes are vectors of enormous public health importance, because of their ability to transmit different serotypes of viral pathogens in West Africa and the continent. Insecticide based control strategies for these vectors have led to the rapid spread of resistance constituting point mutations (Knock-down resistance or *kdr*), and the upregulation of detoxifying enzymes to nullify insecticide effects. This study was conducted to assess the influence effected by one of the paramount tools in controlling mosquito vectors – the pyrethroid based mosquito coil. Susceptibility to deltamethrin, leading to the development of *kdr* mutations from interaction to pyrethroid containing was determined: the influence of continuous pickup of sublethal insecticide doses by *Aedes aegypti* on the enzyme activities leading to insecticide resistance were also elucidated. The exposure in the laboratory constituted a determination of a lethal time for 50% of vector population to attain mortality (LT_{50}) following exposure to insecticide in a Peet Grady chamber and the continuous generational exposure of the laboratory colony along a generational path to the 16^{th} generation with LT_{50} determined at every 6^{th} generation. Running concurrently was the determination of susceptibility profile of *Aedes aegypti* colonies along the coastal areas of Ghana. The prior determination of the LT_{50} rose from 8 minutes (95% CI; 6–9) at F0 to 28 minutes (95% CI; 23–34) at F6. Along the coast of Ghana, the resistance to deltamethrin was coupled frequencies of V1016I, V410L and F1534C *kdr* mutations. There was evidence of higher frequencies of 1016I mutations, which also had a corresponding increase in alpha esterase activity. This study brought to light the increased level of deltamethrin resistance and the implicitness of the pyrethroid-based mosquito coil in resistance selection.

List of abbreviations

Kdr	Knockdown resistance
WHO	World Health Organisation
ITN	Insecticide treated nets
LLIN	Long Lasting Insecticidal Nets
IRS	Indoor Residual Spraying
LT_{50}	Lethal time for 50% of test elements to attain full mortality
DDT	Dichlorodiphenyltrichloroethane
qPCR	quantitative Polymerase Chain Reaction
CTAB	Cetyl trimethylammonium bromide
VSSC	Voltage Sensitive Sodium Channels

List of publications

Paper A

Ablorde A, Ayettey J, Kroidl I, Wieser A, Kudom AA. *Co-occurrence of multiple kdr mutations (F1534C, V1016I, V410L) in Aedes aegypti from coastal areas in Ghana and assessment of the role of mosquito coil in causing pyrethroid resistance.* Acta Trop. 2023 Jul;243:106937. doi: 10.1016/j.actatropica.2023.106937. Epub 2023 May 3. PMID: 37146863.

Paper B

Ablorde A, Kroidl I, Wieser A, Kudom AA**.** Impact of the exposure of sublethal dose of mosquito coil on the development of insecticide resistance in Aedes aegypti (L.) (Diptera: Culicidae) Medical and Veterinary Entomology (**Accepted – yet to be published)**

1. My contribution to the publications

1.1 Contribution to paper A

Investigation: I was solely responsible for conducting the research. I specifically performed all experimentations and personally performed data collection activities. All undertakings leading to the production of all the research data was personally handled. The preparation and creation of the original draft were all personally conducted.

1.2 Contribution to paper B

Investigation: I was solely responsible for conducting the research. I specifically performed all experimentations and personally performed data collection activities. All undertakings leading to the production of all the research data was personally handled. The preparation and creation of the original draft were all personally conducted.

2. Introductory summary

2.1 Background

Chikungunya, dengue fever, yellow fever, and other arboviral illnesses are all primarily spread by the vector mosquito *Aedes aegypti*. The primary means of control for this vector are currently chemical substances known as insecticides. Nevertheless, most insecticide-based protocols are becoming less effective due to an increase in resistance in the African *Aedes aegypti* population. It has been discovered that the *Aedes aegypti* population in Ghana is highly resistant to DDT, carbamates, pyrethroid insecticides, and organophosphate insecticides (Ablorde et al., 2023; Kawada et al., 2016; Kudom, 2020; Kwame Amlalo et al., 2022). The Ghanaian populations have shown evidence of target mutations, including F1534C, V1016I, and V410I, as well as metabolic resistance mechanisms (Ablorde et al., 2023; Kawada et al., 2016; Kudom, 2020; Kwame Amlalo et al., 2022; Owusu-Asenso et al., 2022). There have been reports of an equivalent degree of pyrethroid resistance in *Aedes aegypti* from other parts of Africa, notably, Cape Verde and Angola (Ayres et al., 2020), Cote d'Ivoire (Konan et al., 2021) and Cameroon (Yougang et al., 2020). In mosquito vectors, overuse of chemical insecticides has been the primary cause of resistance. Insecticides, especially pyrethroids, are heavily used in organised vector control programmes against *Ae. aegypti*, for example in dengue control programmes. According to Kawada et al. (Kawada et al., 2009), this is thought to be a primary cause of pyrethroid-resistant vector populations in numerous South American and Asian nations. For example, the IRS programme against dengue vectors employed about 21,000 litres of pyrethroid pesticides in the southern sector of Vietnam in 2007 alone (Pasteur Institute, 2008). Thus, it was not shocking when Kawada et al. (Kawada et al., 2009) demonstrated that IRS was the primary cause of the F1534C mutation in Vietnam. In *Ae. aegypti*, the mutation is the main cause for pyrethroid resistance. The rise of resistance in mosquito vectors has also been linked to urbanisation and the pollution that goes along with it. (Huber et al., 2003) proved dengue vectors at large urban areas encompassing diverse inhabitants acquired higher resistance levels than those in rural areas. According to Satoto et al.(Satoto et al., 2019), urbanisation in Indonesia is the main factor contributing to the rise of resistance in Magelang. It's interesting to note the precise reason why African populations of *Ae. aegypti* are resistant to insecticides is yet unknown (Weetman et al., 2018). Most African nations lack structured vector control initiatives that specifically target *Ae. aegypti*. Despite this, several studies have linked the use of insecticides in homes as a primary selection pressure on *Ae. aegypti* because of the species' rather close relationship to human dwellings (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). (Gray et al., 2018), discovered that pyrethroid-resistant *Ae. aegypti* field strains showed considerably lower mortality rates than the unexposed strains following exposure to household aerosol insecticidal products containing pyrethroid, as

active components. Results from our earlier investigation demonstrated that after six generations of exposure to sublethal dosages of mosquito coil fumes, *Ae. aegypti* developed a two-fold increase in resistance to meperfluthrin-based mosquito coils (Ablorde et al., 2023). Nonetheless, the mosquito population's level of resistance to the insecticide deltamethrin was not significantly impacted by the tolerance to the coil. A very particular widely utilised insecticidal tool for household in several African nations is the mosquito coil. Over 2 billion individuals use 45 to 50 billion mosquito coils annually to avoid mosquito bites, according to estimates (Zhang et al., 2010). According to reports, over 40% of Ghanaian families use a mosquito coil every day to avoid mosquito bites (Boakye et al., 2009; Kudom et al., 2013). In addition to local habitations, using a coil to ward off houseflies and other bothersome insects is increasingly widespread in the neighbourhood marketplaces. An intriguing question is, could sublethal exposure to mosquito coil result in widespread insecticide resistance in the vector mosquito population: since our recent findings, showed a twofold tolerance increase of *Ae. aegypti* to mosquito coil (Ablorde et al., 2023). Semivolatile pyrethroids, which are frequently used as mosquito coil active ingredients, typically diffuse over open areas, and repel or kill mosquitoes that come into contact with the cloud (Ritchie & Devine, 2013; Xue et al., 2012). A mosquito's ability to successfully establish contact with its host may be hindered by this mechanism of action (WHO, 2009). Nonetheless this form of action also runs the risk of exposing the mosquito population to sublethal concentrations of pyrethroid pesticides, which could eventually lead to complete resistance in the vector population. Thus, the work of this investigation is to assess sublethal mosquito coil exposure on the development insecticide resistance in *Ae. aegypti.* A deeper comprehension of any potential elements that may contribute to the development of insecticide resistance in mosquito vectors is necessary considering the emergence of insecticide resistance in *Ae. aegypti* from Africa.

2.2 Statement of the problem

Uncertainty surrounds the precise cause of *Ae. aegypti* resistance in Africa. Nevertheless, given the vector's proximity to homes, household insecticide use, such as mosquito coils, may significantly contribute to *Ae. aegypti* resistance (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). Several countries across Africa, Asia and South America depend on mosquito coil in controlling vector mosquitoes. For example, in Ghana, about 40% of the population depends on mosquito coil as a personal protective measure against vector mosquitoes. The onset and quick spreading of kdr mutations (F1534C, V1016I, V410L) within *Aedes* populations in Africa, demands much elucidation of one of the main vector control tools.

2.3 Objectives

This study provides the present-day frequencies and distribution of knock-down resistant mutations within local *Ae. aegypti* population along the coast of Ghana and the role of mosquito coil in the increase of pyrethroid resistance in *Aedes aegypti*.

The main questions investigated within this study are:

- The frequency of the main *kdr* mutations (V1016I, V410L and F1534C,) implicated in resistance in *Ae. aegypti.*
- The change in deltamethrin resistance in local *Aedes aegypti* populations along the coast of Ghana on after exposure to pyrethroid based mosquito coils.
- The lethal time (LT_{50}) of deltamethrin exposed *Aedes aegypti* populations.
- Investigate the role of biochemical enzymes in the loss of susceptibility in local populations.
- Investigate the associations between the kdr mutations and the impact of their co-occurrence.

2.4 Methods

2.4.1 Methods used in Paper A

2.4.1.1 Study sites

Four urban centers in Ghana's coastal regions—Aflao, Accra, Tema, and Elmina—were the sites of the study. Between 2020 and 2022, mosquito larvae were gathered from abandoned automobile tyres. The larvae were raised into adults in a lab after being transported there. Additionally, the mosquito coil experiment's laboratory colony was formed in 2019 using field data collected from Elmina. The colony was raised under laboratory settings (Temp: 29±1 °C / RH: 75±10%) to the 7th generation before experimentations begun.

2.4.1.2 WHO susceptibility test against *Aedes aegypti* from coastal areas in Ghana

The WHO susceptibility test kit with insecticide impregnated papers, which contain deltamethrin (0.05%) as the main ingredient were used with 3-to-5-day old adult females for the susceptibility test. For another set for replications, an exposure to a synergist (4% PBO) for one hour, preceded the exposure to the active ingredient (deltamethrin). Following exposure, 10% sugar solution were administered to mosquitoes. Knockdown and mortalities were read after 60 minutes and 24 hours respectively. Mosquitoes that were slated for biomolecular assays were stored at -20°C.

2.4.1.3 Laboratory evaluation of the effect of sublethal exposure to mosquito coil on multiple generations of *Aedes aegypti*

With reference to the WHO (WHO, 2009) guidelines, the Peety-Grady chamber was used in conducting the mosquito coil bioassays. The Peety-Grady chamber is a box of perfect square measuring 180cm on all sides with each of the four upright standing sides having a glass observation window (Kudom, 2020). Four holding cages containing female mosquitoes, were then introduced into the chamber. Following exposure, mosquitoes were withdrawn from the cage and fed on 10% sugar solution. The above process was run for 5-, 10-, 15- and 20-minutes exposure times to establish the LT_{50}. The LT_{50} value (7 minutes) served as a the sublethal time for the entire study. Fully lab established mosquitoes begun the serial exposure, where surviving populations of the female mosquitoes exposed in the Peety-Grady chamber, were blood fed, into another generation. This cycle was repeated till the end of the sixth generation when another LT_{50} was determined. For both selected and control colonies, susceptibility levels to deltamethrin (0.05%), deltamethrin (0.05%) plus PBO (4%), and the mosquito coil during the whole diagnostic exposure time (1 hour) were assessed. For every bioassay, knockdown was noted at 60 minutes, and the ultimate mortality was measured at 24 hours.

2.4.1.4 qPCR screening for F1534C, V1016I, and V410L *Kdr* mutation among the field and laboratory populations (DNA extraction and kdr genotyping)

About 200 female mosquitoes (50 from every study site – both experimental and control colonies) were collected. These mosquitoes were sequenced and genotyped for V1016I, V410L and F1534C *kdr mutations.* Using 2% CTAB, DNA was extracted from each mosquito identical to the method by(Healey et al., 2014)(Healey et al., 2014). Using qPCR melting curve analysis described by (Estep et al., 2018) V1016 and F1534C and (Saavedra-Rodriguez et al., 2018)V410L, the *kdr* mutations were screened. Melting curve analysis followed PCR cycle with mutant and wild type V410L mutations peaking at 83°C and 86.5°C respectively. For V1016I and F1534C, their wild types peaked at 83°C and 78°C respectively whilst their mutant types peaked at 76°C and 82°C respectively.

2.4.1.5 Data Analysis

Percentages of mosquito coil and deltamethrin (0.05%) based mortalities were expressed. Using GeneCalc, a chi-test of ascertaining Hardy-Weinberg equilibrium was elucidated (Bin´kowski & Miks, 2018). An association between phenotypes (both susceptible/resistant) and genotypes were elucidated (Fisher's test). GraphPad Prism was used. The two-sided test was employed with p-value < 0.05, considered to be significant.

2.4.2 Methods used in Paper B

2.4.2.1 WHO susceptibility bioassay against the control and exposed colonies

In ascertaining levels of resistance among *Ae. aegypti* population, female mosquitoes were exposed to (0.05% and 0.25%) Deltamethrin and (0.75%, and 3.75%) Permethrin, DDT, Fenitrothion and carbamates. WHO test kits and insecticide-treated papers were used for the test (World Health Organization, 2016) which comprised of four replications of 25 female mosquitoes in each replication for each insecticide. After experimentation, mosquitoes were fed on glucose solution (10% sugar). Rates of knockdown and mortality were taken 60 minutes and 24 hours respectively after each experiment.

2.4.2.2 Laboratory evaluation of the effect of full exposure time of mosquito coil on control and exposed colonies of *Aedes aegypti*

Samples of both the experimental and control colonies, were exposed to the mosquito coil for one hour. Four cages, with 100 mosquitoes each were introduced into the Peety Grady chamber for 1 hour. 10% sugar solution was fed to mosquitoes after exposure. Knockdown and mortalities were read 60 minutes and 24 hours following the end of the exposure. This was done for both the experimental and control colonies alike.

2.4.2.3 Biochemical assay

Two-to-five-day old mosquitoes that had not been exposed to any insecticide were used in the biochemical microplate analysis in detecting elevated metabolic enzyme activities (Leong et al., 2018). 50 mosquitoes each from the experimental and control groups were sampled and assayed for non-specific elevated (α- and β) esterase, Mixed Function Oxidase, Glutathione-S-Transferases (GST) and insensitive acetylcholinesterase. Susceptible reference strain from Cote d'Ivoire was also investigated as an outgroup. The Spectra Max 340PC spectrophotometer with microplate reader and the SoftMax Pro 5.0 software were used for measuring the absorbance.

2.4.2.4 Data analysis

GraphPad prism was used to compute Fisher's exact test to ascertain a correlation that exist among phenotypes(resistant) and alleles. Gene-Calc (Bińkowski & Miks, 2018) was used to ascertain deviation from Hardy-Weinberg equilibrium. As a second line of checks, Man-Whitney test was used to compare enzyme activities because the dataset failed Shapiro Wilks test for normality.

2.4.3 Ethical Considerations

There were no ethical considerations in the carrying out of this study because there were no human subjects involved. However, proposals were submitted to the Institutional Review Board (IRB) of the University of Cape Coast for ethical approval. The ethical approval from the University of Cape Coast was submitted to the Munich Medical Research School (MMRS) for further approval. The Munich Medical Research School issued the final approval on 03.03.2022 (Project Nr: 21-1290) for the study as well.

2.5 Results

2.5.1 Paper A

Mosquito population from study location.

The three main mutations, namely V1016I, V410L and F1534C were discovered as mostly spread along coastal areas of southern Ghana as well as in the exposed laboratory bred population. It was discovered that the F1534C mutant type was widely fixated in nearly all colonies including the laboratory populations. At codon positions 410, 1534 and 1016 the allelic frequencies were 31.6%, 85.5% and 12.9% respectively. Identified from 185 mosquitoes across the four study sites, were twelve different genotypes. For full *kdr* homozygosity (II/LL/CC), only 1 mosquito was identified just as in the case of 1 mosquito been identified as homozygous susceptible (VV/VV/FF). The most observed genotypic combination was the VV/VV/CC (27%, n = 185).

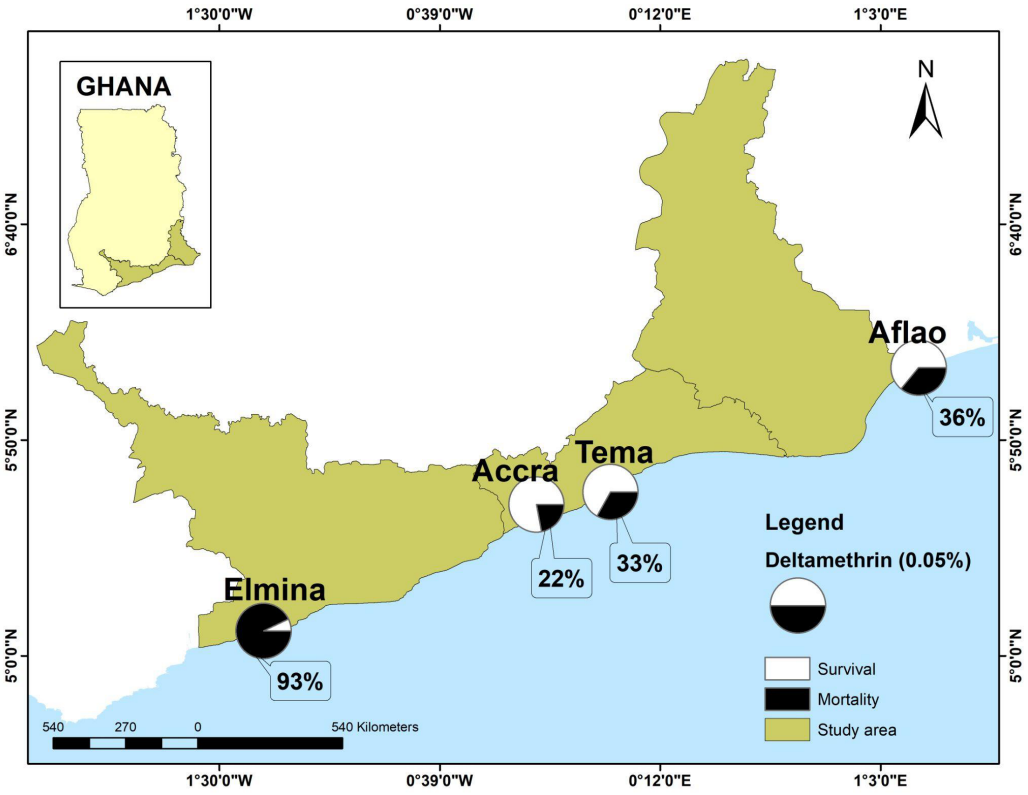

Figure 2.1: Susceptibility to deltamethrin (0.05%) of *Aedes aegypti* populations from four urban towns along the coastal areas in Ghana

Laboratory colonies

Hardy-Weinberg equilibrium was established with respect to V1016I distribution in both colonies but in the reference to F1534C and V410L, there was a divergence from the equilibrium. The selected colony showed higher mutant allelic frequencies for codon 1016 than the control. For both colonies, allelic frequencies of F1534C and V410L were much alike. The control colony had five genotypic combinations whereas there were only three genotypic combinations in the selected one.

2.5.2 Paper B

WHO susceptibility Test

Both exposed and control colonies had high resistance to the insecticides (carbamates, deltamethrin and DDT) with that of Fenitrothion been moderate (Fig 1). However, mortality caused by deltamethrin was extreme for the control colony but not the exposed colony (T-test, p = .03) although the knockdown effect of the insecticide was similar in both colonies (T-test, p = .09). Within the exposed colony, the knockdown

effect was higher than the final mortality (T-test, p = .01). However, the mortalities caused by DDT, carbamate and fenitrothion were similar in both exposed and control colonies. For the mosquito coil bioassay, the one-hour exposure to both mosquito colonies caused 100% knockdown. Nevertheless, complete mortality was observed in amongst the controls whereas a lower 68% mortality was recorded for the exposed.

Distribution of F1534C, V1016I and V410L and metabolic resistance

A total of 100 individual female mosquitoes each from the exposed and control colonise got screened for V1016I, V410L and F1534C mutations. The distribution of resistant alleles of the kdr mutations, F1534C and V410L, were similar for both colonies. However, the frequency of 1016I resistant allele were not elevated for controls like the exposed (X2 = 7.7, p = .006) (Table 1). The enzyme activities of alpha and beta esterase, MFO, ACT and GST for both colonies had been summarised in Fig 5. Alpha esterase activity was elevated for the exposed colony (Dunn's test, p = .0008). However, the other four enzyme activities were similar in both colonies.

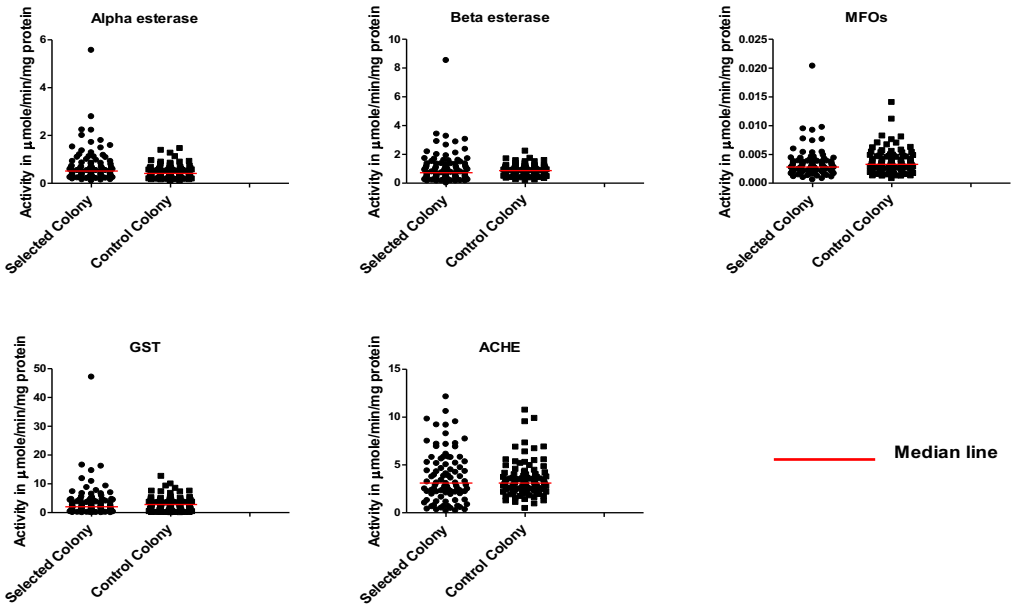

Figure 2.2: Median distribution of activity of the various enzymes

2.6 Discussion

Excessive use of insecticides to control mosquito vectors can cause the evolution of resistance in the vector populations reducing the efficacy of interventions based on these substances. The paper of Kawada (Kawada et al., 2016) was the first to report kdr in *Aedes aegypti* in Ghana. This has similarly been followed by other studies on the continent (Ayres et al., 2020; Konan et al., 2021). The study explored the how implicit a pyrethroid containing coil, got in driving resistance with respect to V1016I, V410L and F1534C mutations. On the field work along the coast of Ghana, the indication of a higher frequencies of mutations than any preceding works was evidenced. This study elucidated a broader range of frequencies and occurrences in terms of kdr mutations than from a previous study by Amlalo (Kwame Amlalo et al., 2022) reported in the past. For instance, in the work by Amlalo there was evidence of the presence of double mutant (II/CC) in two mosquitoes whereas in this study, about half of the total population in the study were totally susceptible (homozygous) to V1016I and of total resistance (homozygous) to F1534C. This was represented as (VV/CC). These extensive changes in mutations, are clear evidence of widespread variations along the coast of Ghana. Due to this, increased attention needs to be given to resistance management before these mutants become fully fixated in the local population in Ghana. The laboratory experimentations were encompassed with the colonies being exposed to sublethal levels of meperfluthrin-based mosquito coils. These effects were mainly hormetic. These included flight distractions, distorted behaviour and to some extent, insect fitness was bargained (Ablorde et al., 2023; Boonyuan et al., 2011) just like in the case of the larvae of Colorado potato beetle (*Leptinotarsa decemlineata),* where adults from larvae previously exposed to insecticide, had higher body mass and higher survival. It was discovered that, the sublethal levels increased the mosquitoes' resistance to deltamethrin. Just like the work by Wagman (Wagman et al., 2015) where susceptibility of *Aedes aegypti* was considerably lower on exposure to transfluthrin. This increase in loss of susceptibility to deltamethrin is evident through similar knockdowns recorded for both control and exposed colonies. This is associated with the suggestion that such an increase in resistance to deltamethrin is mediated by metabolic regulation phenotypes. For instance, in this study, there was limited alpha-esterase activity in the control in comparison to experimental colonies, indicating a possible role of deltamethrin resistance. This suggests a major problem because of the propensity of cross resistance to other insecticides. Other examples, are the study by Cuamba and Wondji a decade ago (Cuamba et al., 2010; Wondji et al., 2012) who found increased esterase activity in pyrethroid and carbamate resistant *An. funestus* populations from Mozambique and Malawi.

2.7 Conclusion

A persistent exposure of the household used mosquito coil results in the development of resistance to the coil and an increase in deltamethrin resistance. Moreover, the continual exposing of *Aedes aegypti* to sublethal quantities of this household mosquito coil revealed a greater tolerance to the coil but deltamethrin resistant levels remained about equal. Nonetheless, its dominating role as the principal tool for vector control in Ghanaian households makes it a candidate for vector control that requires a widescale probing. The higher esterase activity of the V1016I without increased deltamethrin resistance should be considered widely due to the endangerment of cross resistance.

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4. Publications

4.1 Paper A

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Co-occurrence of multiple *kdr* mutations (F1534C, V1016I, V410L) in *Aedes aegypti* from coastal areas in Ghana and assessment of the role of mosquito coil in causing pyrethroid resistance

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ABSTRACT

The rapid spread of knockdown-resistance (*kdr*) mutations in Africa calls for monitoring and investigation into the cause of pyrethroid resistance to inform management strategies. This study investigated the pyrethroid resistance profile of *Aedes aegypti* from coastal towns in Ghana and the impact of mosquito coil, a popular household pyrethroid-based anti-mosquito tool, on the development of pyrethroid resistance. Susceptibility to deltamethrin and the presence of *kdr* mutations was determined in adult female mosquitoes reared from larvae. Furthermore, the LT_{50} of a mosquito coil (0.08% meperfluthrin) against a laboratory colony was determined, and the value was used as a sublethal dose in an experimental study. The laboratory colony of *Ae. aegypti* was exposed to the sublethal dose of the coil once per generation for six generations (F6). The susceptibility of the exposed colony to deltamethrin (0.05%) was determined. The *Ae. aegypti* populations from the coastal towns were resistant to deltamethrin with co-occurrence of F1534C, V1016I and V410L *kdr* mutations. In the experimental study, the LT_{50} (95% CI) of the selected colony against the coil rose from 8 minutes (95% CI; 6–9) at F0 to 28 minutes (95% CI; 23–34) at F6. Nonetheless, deltamethrin caused similar mortalities in the selected and control colonies. The mutant allele frequencies of 1534C and 410L were similar but 1016I was higher in the selected colony (17%) than in the control (5%). However, the increased tolerance to the coil and high mutant allele frequency of 1016I in the selected colony did not affect the mosquito's resistance level to deltamethrin insecticide. Further study is needed to elucidate the role of pyrethroid-based mosquito coils in the development of insecticide resistance in mosquito vectors.

Introduction

Aedes aegypti is an important vector of yellow fever, dengue, Zika and chikungunya viruses. Several epidemics of *Ae. aegypti*-borne diseases have been reported in many countries within the last decade. For example, in West Africa, there were confirmed cases in Ghana (Amoako et al., 2018) as well as outbreaks of dengue in Burkina Faso (Tarnagda et al., 2018), Cote d'Ivoire (Fofana et al., 2019) and Senegal (Dieng et al., 2021). Emergency control measures against this vector depend on the use of chemicals, particularly, pyrethroid insecticides. The major vector control tools including insecticide-treated bed nets and indoor residual spraying, until recently, mainly depended on pyrethroid insecticides. However, the emergence of pyrethroid resistance in this vector population threatens the efficacy of such control tools.

Pyrethroid insecticides target the voltage-gated sodium channel (VGSC) in the nervous system of the mosquito (Liu et al., 2006). The insecticide acts in disrupting the sodium channel by depolarizing neurones, paralysing and eventually killing the mosquito (Narahashi, 1996). However, a single substitution in the amino acid sequence at key positions within the sodium channel proteins can cause a resistance phenotype known as knockdown resistance (*kdr*) (Liu et al., 2006). About ten major *kdr* mutations have been detected in *Ae. aegypti* (Moyes

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et al., 2017). These mutations vary in frequency, geographical distribution, and impact on resistance phenotype (Moyes et al., 2017). For instance, the F1534C mutation has a worldwide distribution with at least two independent origins (Cosme et al., 2020) whereas V1016G is found in Asia and V1016I in the Americas and Africa (Moyes et al., 2017). Furthermore, F1534C is known to confer a low resistance phenotype but in combination with V1016I confers higher pyrethroid resistance (Vera-Maloof et al., 2015). It has been postulated that knowledge of the frequencies of both F1534C and V1016I mutations is important to predict the potential of a population to evolve a pyrethroid resistance phenotype (Vera-Maloof et al., 2015).

The most widespread *kdr* mutation in *Ae. aegypti* populations from Africa is the F1534C mutation, which together with V1016I was first detected in Africa from *Ae. aegypti* populations from Ghana (Kawada et al., 2016). The mutation has since been found in Cote d'Ivoire (Konan et al., 2021), Cameroon (Yougang et al., 2020), Angola and Cape Verde (Ayres et al., 2020). In Burkina Faso, the F1534C was found to be almost fixed in the populations of *Ae. aegypti* from the capital city, Ouagadougou (Sombié et al., 2019). A third mutation V410L, which was discovered in Brazil (Haddi et al., 2017), is now spreading in Africa with reported cases from Ghana (Amlalo et al., 2022), Angola (Ayres et al., 2020), Cote d'Ivoire (Konan et al., 2021) and Burkina Faso (Toé et al., 2022). The exact cause of resistance in *Ae. aegypti* in Africa remains unclear. However, owing to the vector's close association to houses, domestic use of insecticides including mosquito coils could be a very important cause of resistance in *Ae. aegypti* (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). The emergence and rapid spread of *kdr* mutations in Africa call for an investigation into the cause of resistance to inform resistance management strategies. In this study, we provide the current distribution of *kdr* mutations in the *Ae. aegypti* population from coastal areas in Ghana and the impact of mosquito coil on the development of pyrethroid resistance in the vector population.

Materials and Methods

Study site

The study was conducted in four urban towns in the coastal areas of Ghana, namely Aflao, Accra, Tema and Elmina. Aflao is in the Volta region of Ghana and is a border town with Togo to the east of the country. Accra is the capital of Ghana and together with Tema are in the Greater Accra region. Elmina is an important tourist destination located in the Central region towards the western part of Ghana. These areas constitute the coastal parts of Ghana which were not entirely captured in the previous studies in Ghana. The previous study assessed the insecticide resistance status of the vector from northern part of Ghana to Accra (Kawada et al. 2016). Mosquito larvae were collected from abandoned car tires from April to September 2020 in Accra and Tema and September 2021 to February 2022 in Aflao and Elmina. The larvae were brought to the laboratory and reared into adults. Furthermore, the laboratory colony used for the mosquito coil experiment was established in 2019 from field collection from Elmina. The colony was raised to the 7th generation and subsequently used for the experiments conducted in this study. The adults were fed on 10% sugar solution. The laboratory conditions for breeding were maintained at 29±1°C with a relative humidity of 75±10%.

WHO susceptibility test against Aedes aegypti from coastal areas in Ghana

The susceptibility of adult females from the four urban coastal areas was determined using a WHO susceptibility test kit and deltamethrin-impregnated papers at the diagnostic concentration of 0.05%. The experiment was conducted according to the protocol described by WHO (WHO/ZIKV/VC/16.1). About 25 unfed 3-to-5-day-old female *Ae. aegypti*, were released into the susceptibility test tubes containing the impregnated papers. The untreated paper was used as a control. The experiment was replicated four times for each location. The synergist bioassay was conducted for the *Ae. aegypti* populations from Aflao, Tema and Elmina. The 3-to-5-day-old female mosquitoes were exposed to 4% piperonyl butoxide (PBO) paper or untreated paper for 1 h before being exposed to 0.05% deltamethrin. The laboratory conditions for the bioassay were 29 ± 1°C and a relative humidity of 75 ± 10%. After the exposure, mosquitoes were transferred to holding tubes and fed with 10% sugar solution. Knockdown was read after one hour and total death or mortality was recorded after 24 hours. Mosquitoes were stored at -20°C for the molecular assay.

Laboratory evaluation of the effect of sublethal exposure to mosquito coil on multiple generations of Aedes aegypti

Mosquito coil bioassays were conducted in a Peet-Grady chamber according to the protocol described by WHO (WHO/HTM/NTD/WHOPES/2009.3). The Peet-Grady chamber was constructed with wood with a dimension of 180 cm x 180 cm x 180 cm with four glass observation windows as described in Kudom (2020). A single coil was fitted to its metal holder and lit. The coil was allowed to smoulder for five minutes and then placed in the chamber on a flat platform. The mosquitoes in four holding cages were suspended 10 cm from the sides of the chamber and 80 cm from the ceiling. A small electric fan was placed inside the chamber to ensure uniform dispersion of the smoke emitted from the coil inside the chamber.

Ae. aegypti colony, originally collected from Elmina (Ghana) were severally exposed to a sublethal dose of a mosquito coil (Sasso®, 0.08% meperfluthrin, Samara company Ltd); once per generation for six generations. The LT_{50} of the coil against the *Ae. aegypti* Elmina colony was determined and the value was used as a sub-lethal exposure time for the experiment.

In determining the LT50, four experiments were conducted with the following exposure time: 5, 10, 15 and 20 minutes. Each experiment was conducted with 2-5 day old 100 females (25 females each in a cage). After each experiment, the door to the Peet-Grady chamber was open to allowed for ventilation for two days before the next experiment was set-up. After the exposure, mosquitoes were transferred from the cage to plastic cups and given 10% glucose solution. Knockdown was recorded at 60 minutes and the final mortality was read at 24 hours.

For the exposure of the colony to sublethal doses, four hundred female mosquitoes were randomly collected from the colony using an aspirator. Hundred female mosquitoes each were placed in the four different cages and exposed to the coil for 7 minutes, the LT_{50} exposure time that was previously determined. The mosquitoes that survived were blood-fed to lay eggs. The eggs were raised into adults to form the F1 generation of the experimental colony. From the F1 generation, another set of 400 females was randomly collected and exposed to the sub-lethal dose of the mosquito coil in the Peet-Grady chamber. Again, the survivors were blood-fed to start the next generation. This cycle was repeated to the 6th generation. At the end of the 6th generation, LT_{50} of the mosquito coil against the selected colony was determined again. Susceptibility levels to deltamethrin (0.05%) and deltamethrin (0.05%) plus PBO (4%), as well as the susceptibility level to the mosquito coil at full diagnostic exposure time (1 hour), were evaluated for the selected and control colonies. Knockdown was recorded at 60 minutes and the final mortality was read at 24 hours for all the bioassays.

qPCR screening for F1534C, V1016I, and V410L Kdr mutation among the field and laboratory populations

DNA extraction

Fifty female mosquitoes each from the four locations as well as the selected and the control colonies were randomly taken from the mosquitoes used for the susceptibility bioassay and stored in the freezer. If available 25 each from the dead and survivors were taken from each location and were genotyped for the F1534C, V1016I, and V410L *kdr*

mutations. DNA from each mosquito was extracted using 2% CTAB (Cetyltrimethyl Ammonium Bromide) as described by Healey et al. (2014). The *kdr* mutations were screened using qPCR melting curve analysis based on the protocols described by Saavedra-Rodriguez et al. (2018) (V410L) and Estep et al. (2018) (V1016 and F1534C). V410fw and L410fw primers and 0.1μM of primer 410rev, 9.5μL of 2x Sybr Hi-Rox Mix (Bioline), 1μL of genomic DNA and DNase-free water. V1016I detection was done in a 20μL reaction consisting of 8.2μL of 2x Sybr Hi-Rox Mix (Bioline), 0.15μM of Val1016f primer, 0.2μM each of lle1016f and lle1016r primers, 2μL DNA template and DNase-free water. The cycling condition for this procedure is 95ºC for 3 minutes, 40x (95 ºC :10sec, 60 ºC:10sec,72 ºC :30sec) 95 ºC with the melting condition of 65º-95º inc 0.2 ºC per 10sec. Each 20μL reaction for V1016I consisted of 8μL of 2x Sybr Hi-Rox Mix (Bioline), 0.15μM of Val1016f primer, 0.2μM each of lle1016f and lle1016r primers, 2μL DNA template and DNase-free water, with the same cycling conditions as the V410L. F1534C was screened in a reaction volume of 20μL containing 8μL of 2x Sybr Hi-Rox Mix (Bioline), 0.3μM of Cys1534+ primer, 0.3μM each of Phe1534+ and 1534- primers, 2μL DNA and DNase-free water. The cycling conditions for F1534C included 3min at 95°C, 37 cycles of (10 sec at 95°C, 10 sec at 57°C, 30 sec at 72°C) and 95 ºC for 10 seconds. A melting curve analysis was performed at the end of the PCR cycle at 65°C - 95 ºC. The mutant gene (resistant) and wild type (susceptible) respectively produced melting curve peaks at 83°C and 86°C for V410L, 80°C and 86°C for V1016I, and 86°C and 82°C for F1534C. The negative controls consisted of master mix with DNase-free water, whilst the positive controls were positive DNA samples from a previous study (Amlalo et al. 2022).

Data analysis

The mortalities caused by deltamethrin (0.05%), and the mosquito coil were expressed in percentages. A chi-square test for deviation from Hardy-Weinberg equilibrium was performed using Gene-Calc (Bińkowski and Miks, 2018). Fisher's exact test was computed for an association between genotype and the resistance/susceptible phenotype using GraphPad Prism. Tests were two-sided, p-value < 0.05 was considered significant.

Results

Susceptibility to deltamethrin among field populations

The female *Ae. aegypti* populations from the coastal areas were resistant to deltamethrin (Fig. 1). The level of resistance was higher in the population from Accra, Tema and Aflao (22-36% mortality) than in the population from Elmina (93% mortality). However, pre-exposure of the Tema and Aflao populations to PBO increased the mortality from 32% to 66% and from 36% to 96% respectively while mortality for the Elmina population was restored to 100%.

Mosquito coil bioassay on the control and experimental colony

The LT_{50} (95% CI) of the coil against the selected colony rose from 7.8 (95% CI; 6.14 – 9.41) minutes from the beginning of the experiment (F0 generation) to 28.3 minutes (95% CI; 23.11 – 33.58) at the 6th generation of the colony. Mortality resulting from the sub-lethal exposure time chosen in this study (7 mins) decreased from 41% of the F0 generation to 20% at the 6th (F6) generation.

The exposure of the control colony and the selected colony to a mosquito coil for 1 hour caused a mean mortality (± SD) of 95.8% (± 8.3) and 55% (± 24.5) respectively and the difference was significant (Mann-Whitney U = 0.00, P = 0.018) (Fig. 2). Exposure of the selected and control colonies to deltamethrin (0.05%) caused a mortality of 72% and 68% respectively. However, pre-exposure to PBO restored their susceptibility to 100% for both colonies.

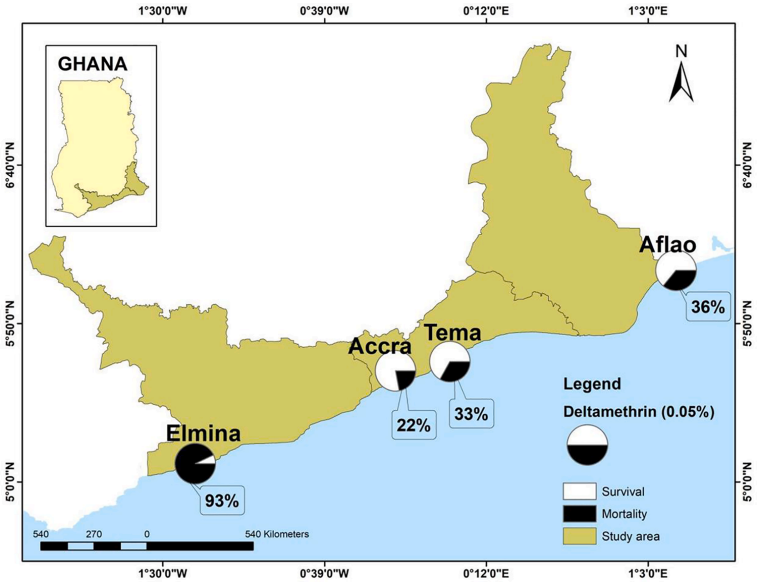

Fig. 1. Susceptibility to deltamethrin (0.05%) of *Aedes aegypti* populations from four urban towns along the coastal areas in Ghana from April to September 2020 (Accra, Tema) and from September 2021 to February 2022 (Aflao, Elmina).

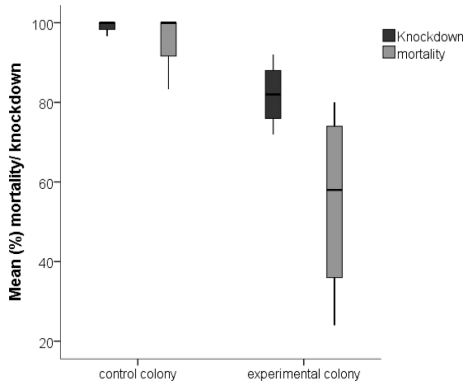

Fig. 2. A box plot of (%) knockdown and mortality of the control or selected (experimental) laboratory strain of *Aedes aegypti* exposed to a diagnostic dosage (1-hour exposure time) of mosquito coil in a Peet-Grady chamber.

Distribution of F1534C, V1016I, and V410L Kdr in coastal areas of Ghana

Using the allele-specific RT-PCR, a total of 197, 198, and 200 wild mosquitoes were genotyped for the V1016I, V410L and F1534C mutations respectively. The genotypic frequencies of the F1534C, V1016I, and V410L have been summarised in Table 1. The three *kdr* alleles were present in *Ae. aegypti* population from the four populations with a high frequency of F1534C. However, only a few wild types (about 3.5%) of the F1534C were detected for the four locations. A homozygous mutant was found in a single mosquito for codon 410 and two mosquitoes for codon 1016. Overall, 12.9%, 31.6% and 85.5% of the *kdr* allele frequencies were recorded for codons 1016, 410 and 1534 respectively.

Twelve genotypes across the three *kdr* mutations were identified from the 185 *Ae. aegypti* from the four locations (Fig 4). Only one individual was homozygous for the three *kdr* mutations (II/LL/CC) and another single mosquito was found to be homozygous susceptible for all three mutations (VV/VV/FF). The VV/VV/CC genotype combination was the most frequently observed genotype (27%, n = 185). However, the genotype combinations differed among the four locations. Accra had the least variable genotype combinations (4) whereas the most variable genotype combinations were recorded at Elmina (9). Only two of the genotype combinations (VV/VL/CC and VV/VV/CC) were common among the four locations (Fig. 3). The VV/VV/CC was the dominant genotype combination in both Accra and Tema, VI/VL/CC also dominated in Aflao whiles VV/VL/FC was the dominant genotype combination in Elmina.

Seven genotype combinations were found in the dead and alive mosquitoes that were exposed to deltamethrin insecticides (Fig. 4). However, VI/VL/CC, VV/VV/CC and VV/VL/CC constituted more than 80% of the combinations in each group. There was no association

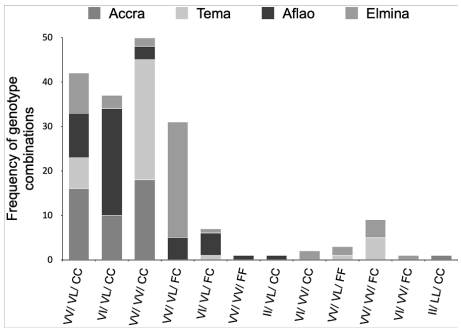

Fig. 3. Frequency of the combined V410L, V1016I and F1534C genotypes from the four coastal areas in Ghana (Accra, Tema, Aflao and Elmina)

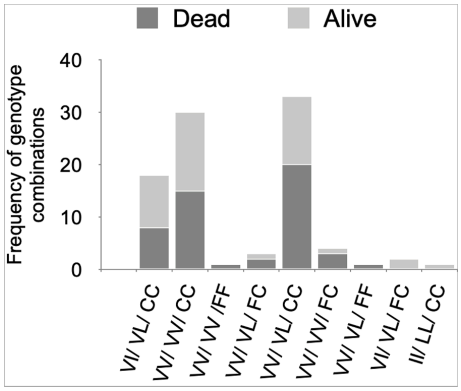

Fig. 4. Frequency of the combined V410L, V1016I and F1534C genotypes from the mosquitoes that died or survived the exposure to deltamethrin (0.05%) insecticide from the four urban areas.

Table 2
Chi-test of association between alleles and resistant phenotypes for dead and alive *Aedes aegypti* mosquitoes after exposure to deltamethrin (0.05%).

Delta	F1534C			V410L			V1016I		
(0.05%)	F	C	X(*p*)	V	L	X(*p*)	V	I	X(*p*)
Dead	9	93	1.23	69	31	0.05	94	8	1.389
Alive	5	97	(*0.27*)	58	28	(*0.819*)	86	14	(*0.238*)

*p-values in brackets

Table 1
The frequencies of V1016I, V410L and F1534C *kdr* genotypes in *Aedes aegypti* from four urban areas and experimental populations.

Location	V1016I				V410L				F1534C			
	SS	RS	RR	I (freq%)	SS	RS	RR	L (freq%)	SS	RS	RR	C (freq%)
Field populations												
Accra	38	10	1	10	22	27	1	30	0	1	49	99
Aflao	20	29	1	31	5	45	0	45	1	10	39	88
Elmina	43	7	0	7	9	41	0	41	2	33	15	64
Tema	47	1	0	1	38	10	0	11	1	6	43	92
Laboratory populations												
unexposed	45	5	0	5	6	44	0	44	0	1	49	99
exposed	31	16	0	17	6	40	0	43	0	0	46	100

between each of the mutant genotypes and the resistant phenotype for each of the mutations (Table 2).

Distribution of F1534C, V1016I, and V410L Kdr in the experimental and control colonies coastal

Overall, 97, 96, and 96 mosquitoes were genotyped for V1016I, V410L and F1534C respectively. The genotypic frequencies of the F1534C, V1016I, and V410L of the selected and control colonies have been summarised in Table 1. For both colonies, the distribution of V1016I genotypes was consistent with Hardy-Weinberg equilibrium whereas the distribution of V410L and F1534C genotypes deviated from Hardy-Weinberg equilibrium. The mutant allelic frequencies for the codon 1016 were higher in the selected colony compared to the control. However, allelic frequencies of V410L and F1534C in both the selected and the control colonies were similar.

Five genotype combinations were found in the control colony compared to three found in the selected colony. The VV/VL/CC was the dominant genotype combination in the colonies.

Discussion

The first *kdr* mutation in *Ae. aegypti* population from Africa was reported in Ghana about half a decade ago (Kawada et al., 2016). Since then, different studies have reported multiple *kdr* mutations in *Ae. aegypti* populations from several African countries (Ayres et al., 2020; Konan et al., 2021; Sombié et al., 2019; Toé et al., 2022). In this study, we report the detection and co-occurrence of F1534C, V1016I and V410L *kdr* mutations in *Ae. aegypti* population from coastal areas in Ghana. These three mutations were recently detected in *Ae. aegypti* populations from Accra (Amlalo et al., 2022). However, this study shows a wider distribution of the multiple mutations in Ghana than in any previous study. The first report on the detection of V1016I mutation in Africa in 2016 found the mutation in a single *Ae. aegypti* mosquito from Accra (Ghana). It is alarming to observe a high frequency of the mutation in Accra and other coastal towns within this short time. In this study, we found a high frequency of V1016I in the *Ae. aegypti* population from Aflao, which is 187 km from Accra and shares a border with Lomé the capital town of Togo. Both *Ae. aegypti* populations from Aflao and Accra also recorded the highest mutant allele frequency for the V410L. However, F1534C was the most widely spread *kdr* mutation in *Ae. aegypti* from the four study locations, which is consistent with the previous studies in the country (Kawada et al., 2016; Kudom, 2020; Amlalo et al., 2022).

Single or multiple of the *kdr* mutations detected in this study have been associated with resistance to different pyrethroid insecticides and DDT (Toé et al., 2022; Vera-Maloof et al., 2015; Yanola et al., 2011). However, our present study did not find any association between each of the mutations and the deltamethrin-resistant phenotype. In a similar study in the city of Ouagadougou in Burkina Faso, the double mutant genotype II/CC (V1016I and F1534C) was associated with permethrin resistance but not deltamethrin resistance (Sombié et al., 2019). Metabolic resistance mechanisms were not determined in this study but the result from the PBO synergist bioassay indicates a possible role of the mechanism in the deltamethrin resistance in the study population. For instance, after pre-exposure to PBO, the mortality to deltamethrin rose from 36% to 96% for the Aflao population and mortality for the Elmina population was restored to 100%, suggesting that the multiple *kdr* mutations did not have sufficient influence on the deltamethrin resistance phenotype in the study population. On the other hand, it could be inferred from the result that the study population is at its early stages of evolution of pyrethroid resistance. Vera-Maloof et al. (2015) postulated that high pyrethroid resistance in *Ae. aegypti* requires the sequential evolution of F1534C and V1016I mutations where the F1534C mutation evolved first but conferred only a low level of resistance but appears to enable the V1016I mutation to survive. The double mutants confer higher pyrethroid resistance in the vector population than the single F1534C mutation. In this study, only two individual mosquitoes had the double mutants (II/CC) whereas 50% were homozygote susceptible to V1016I but homozygote resistant to F1534C (VV/CC). This calls for resistance management measures before the evolution reaches advanced stages and fully compromises the efficacy of pyrethroid insecticides.

The main cause of resistance in *Ae. aegypti* in Ghana has not been fully established. This could partly be a result of a lack of accurate data on the general use of pesticides in the country. Notwithstanding, household use of insecticide has been cited as a potential cause of resistance among the vector population. Mosquito coil is one of the most popular insecticide-based tools used in Ghanaian households. About 40% of households in the country have been reported to use the coil daily as their main personal anti-mosquito measure and each household could use up to 2 coils per night (Boakye et al., 2009; Kudom et al., 2013). It has been suggested that such a high level of mosquito coil usage could cause the development of pyrethroid resistance in mosquitoes (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022).

From the results of this study, the persistent exposure of *Ae. aegypti* to sublethal doses of mosquito coil resulted in a twofold tolerance to the coil after six generations but did not affect the mosquito's resistance to deltamethrin substantially. Moreover, pre-exposure to the synergist PBO before deltamethrin caused complete mortality in both selected and control colonies. This shows that the three *kdr* mutations detected in both the selected and control colonies did not have much influence on the deltamethrin resistance observed in the colonies. The role of F1534C/S in deltamethrin resistance among *Aedes* species is yet to be fully elucidated. While Kasai et al. (2019) found that an *Ae. albopictus* strain homozygous for the F1534C mutation was 11-fold resistance to deltamethrin plus PBO, Stenhouse et al. (2013), found that F1534C mutation was not associated with deltamethrin resistance in *Ae. aegypti.* However, Yang et al. (2021) linked the high levels of resistance in *Ae. albopictus* to deltamethrin to detoxification metabolism but not the F1534S mutation, which is consistent with the results from this study. Pyrethroid insecticide are grouped into two; type I and type II, based on their structures. There are also new generation of pyrethroids that are known to be semi-volatile in nature and mainly used in household insecticide products. They are largely polyfluorinated benzyl compounds (transfluthrin, metofluthrin, meperfluthrin etc.). The structural differences among pyrethroids could influence the level of resistance caused by a particular resistance mechanism. For instance, some studies have showed that pyrethroid insecticides with polyfluorobenzyl alcohols can withstand P450 metabolism (Tan and McCaffery 2007, Horstmann and Sonneck 2016). Thus, P450-mediated metabolically resistant mosquitoes could be susceptible to polyfluorobenzyl based pyrethroid insecticides but resistant to deltamethrin. These findings suggest that pyrethroid resistance status determined for the selected colony in this study based on single insecticide (deltamethrin) may not give a true picture of the situation. The use of multiple pyrethroid insecticide from the different groups may give a better understanding of the pyrethroid resistance status of the selected colony.

A major limitation of this study is that the exact contact time of mosquitoes to insecticide fumes from mosquito coil in real life is not known. Thus, the sublethal exposure time used in this study as well as the condition in the exposure chamber simulated in this study may not reflect the true condition in real-life situations for the mosquitoes. Furthermore, the sublethal exposure selection experiment should have been done on multiple lines to rule out stochastic effects. Thus, care should be taken in interpreting the results outside laboratory settings. A more robust testing in a future study should include exposure condition closer to a real-life situation and selection of experimental colonies in multiple mosquito lines. Notwithstanding, the study has provided some valuable insight into the interaction between *Ae. aegypti* and mosquito coil usage. As spatial repellents, mosquitoes are likely to be exposed to sublethal dosages of insecticides from the coil in real life. The study has shown that *Ae. aegypti* that survived exposure to a mosquito coil could

still produce viable offspring and may develop tolerance to the coil under continuous exposure of the coil to the subsequent generations. But the question as to whether the mosquito's tolerance to the coil could lead to insecticide resistance under continuous exposure may require further studies to answer it. Perhaps, the six generations used in this study were not long enough to see a significant effect on pyrethroid resistance. Owing to the excessive use of mosquito coils in Ghana and many other countries, it is important to have a full understanding of the coil's impact on the development of resistance in mosquito vectors.

Conclusion

In effect, F1534C, V1016I and V410L *kdr* mutations were found to be widely distributed in the urban coastal areas in Ghana. However, the influence of the mutations on deltamethrin needs better investigation, particularly, since the concentration used in this study (0.05%) is 1.6 times higher than the WHO diagnostic concentration (0.03%). A nationwide survey is needed to know the extent of the spread of these mutations to inform a resistance management plan. Furthermore, the persistent exposure of sublethal dosage of mosquito coil to *Ae. aegypti* showed an increase in their tolerance to the coil but did not change their resistance status to a pyrethroid insecticide. Further studies are needed to elucidate the impact of mosquito coil usage on insecticide resistance development in mosquito vectors, particularly in countries where households excessively use the coil.

CRediT authorship contribution statement

Aikins Ablorde: Investigation, Data curation, Writing – original draft. **Joana Ayettey:** Investigation. **Inge Kroidl:** Writing – review & editing. **Andreas Wieser:** Writing – original draft, Methodology. **Andreas A. Kudom:** Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2023.106937.

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Paper B

Impact of the exposure of sublethal dose of mosquito coil on the development of insecticide resistance in *Aedes aegypti* (L.) (Diptera: Culicidae)

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Abstract

Mosquito coil is commonly used in many African households for protection against mosquito bites. The coil usually has semi-volatile pyrethroids as an active ingredient, which usually diffuse across open space, and the cloud either kills mosquitoes that are exposed, or mosquitoes can be exposed to sublethal doses of the insecticides. This study was conducted to assess the impact of sublethal doses of mosquito coil on the development of insecticide resistance in *Aedes aegypti*, a major vector for dengue fever and several other arboviral diseases. A laboratory colony of *Ae. aegypti* was exposed to sublethal doses of a meperfluthrin-based mosquito coil in a Peet-Grady chamber once per generation for 16 generations. The susceptibility of the exposed colony to a diagnostic dose of the mosquito coil as well as to three other insecticides were determined. Three different *kdr* mutations and five enzyme activities were evaluated in both the exposed and control colonies. After 16 generations of sublethal exposure to mosquito coils, the full diagnostic dose of the coil caused 68% mortality to the exposed colony compared to 100% mortality in the control colony. Mortality caused by deltamethrin (0.05%) was also significantly lower in the exposed colony. The frequency of 1016I *kdr* mutation as well as MFO and alpha esterase activities were higher in the exposed colony compared to the control colony. This study provides evidence of the development of pyrethroid resistance in an *Ae. aegypti* population due to sublethal exposure to mosquito coil for 16 generations. Given the large-scale use of mosquito coils in many African households, its role as a pyrethroid resistance selection source should be taken into consideration when designing resistance management strategies.

Keywords: *Aedes* mosquitoes, insecticide resistance, *kdr*-mutation, metabolic resistance, meperfluthrin, pyrethroid resistance, semi-volatile pyrethroid, vector control

Introduction

Aedes aegypti is a major vector for several arboviral diseases including yellow fever, dengue fever and chikungunya. Insecticides are the main control measures against this vector. However, insecticide resistance is increasing in *Ae. aegypti* populations worldwide, threatening the effectiveness of most insecticide-based tools. In Ghana, *Ae. aegypti* populations are highly resistant to pyrethroid insecticides, DDT, carbamates, and organophosphate insecticides (Ablorde et al., 2023; Kawada et al., 2016; Kudom, 2020; Amlalo et al., 2022). Target mutations comprising F1534C, V1016I and V410I as well as metabolic mechanisms have been detected in the Ghanaian populations (Ablorde et al., 2023; Kawada et al., 2016; Kudom, 2020; Amlalo et al., 2022; Owusu-Asenso et al., 2022). Similar levels of pyrethroid resistance have been reported in *Ae. aegypti* populations from different African countries including Cote d'Ivoire (Konan et al., 2021), Cameroon (Yougang et al., 2020) Angola, and Cape Verde (Ayres et al., 2020).

Excessive use of insecticides has been the main drive of resistance in mosquito vectors. Organised vector control programs targeting *Ae. aegypti* such as dengue control programs depend on heavy use of insecticides, particularly pyrethroids. This is suspected to be a major driver of pyrethroid-resistant vector populations in many countries in Asia and South America (Kawada et al. 2009). For instance, about 21,000 litres of pyrethroid insecticides were used in the southern sector of Vietnam in 2007 alone for the Indoor Residual Spraying (IRS) program against dengue vectors (Pasteur Institute, 2008). It was therefore not surprising that Kawada et al., (2009) showed that IRS was the driving factor behind the F1534C mutation in Vietnam, a mutation linked to pyrethroid resistance in *Ae. aegypti*. Urbanization with its associated pollution has also been linked to the emergence of resistance in vector mosquitoes. Huber et al., (2003) showed that the dengue vectors in huge cities with cosmopolitan populations developed elevated levels of resistance in

comparison to the countryside. In Indonesia, Satoto et al., (2019) identified urbanization as the principal cause of the emergence of resistance in the city of Magelang. Interestingly, the exact cause of insecticide resistance in *Ae. aegypti* populations from Africa remains unclear (Weetman et al., 2018). Most African countries do not have organized vector control programs that target *Ae. aegypti*. Notwithstanding, many studies have implicated household use of insecticides as a major selection pressure on *Ae. aegypti* owing to this species' close association with human habitation (Boakye et al., 2009; Kudom et al., 2013; Toé et al., 2022). Gray et al., (2018) found that after exposure to household aerosol insecticide products containing pyrethroid active ingredients, pyrethroid-resistant *Ae. aegypti* field strains had significantly lower mortality rates compared to the unexposed strains. Findings from our previous study showed a two-fold increase in tolerance to meperfluthrin based mosquito coil by *Ae. aegypti* after being exposed to a sublethal doses of the mosquito coil for six generations (Ablorde et al., 2023). However, the tolerance to the coil did not substantially affect the level of resistance of the mosquito population to the insecticide deltamethrin.

Mosquito coil is one of the most popular insecticide-based tools used in households in many African countries. It is estimated that 45 to 50 billion mosquito coils are used annually by over 2 billion people worldwide in preventing mosquito bites (Zhang et al., 2010). In Ghana, about 40% of households have been reported to use the coil daily in preventing mosquito bites (Boakye et al., 2009; Kudom et al., 2013). Besides residential areas, it is becoming common for local markets to use mosquito coils to drive away houseflies and other nuisance insects. Based on our previous findings that found a twofold tolerance increase in *Ae. aegypti* to sublethal doses of mosquito coil (Ablorde et al., 2023), the question is, could sublethal exposure of mosquito coil lead to broader insecticide resistance in the vector mosquito population?

Mosquito coils commonly have semi-volatile pyrethroids as active ingredients, which usually diffuse across open space, and the cloud either repels or kills mosquitoes that are exposed (Ritchie & Devine, 2013; Xue et al., 2012). While the insecticidal cloud can prevent mosquitoes from successfully making contact with their host (WHO, 2009), the outer edges of the plume could expose mosquitoes to sublethal doses of pyrethroid insecticides. This formulation has the potential to contribute to the development of pyrethroid resistance in the vector population. This study was therefore conducted to assess the impact of such sublethal exposure to mosquito coil on the development of insecticide resistance in *Ae. aegypti* populations. The emergence of insecticide resistance in *Ae. aegypti* from Africa call for a better understanding of any potential factors that could contribute to the development of insecticide resistance in the mosquito vectors.

Materials and methods

Laboratory exposure of *Aedes aegypti to* sublethal doses of mosquito coil

The *Ae. aegypti* colony, originally collected from abandoned car tires within the Elmina municipality in the Central region of Ghana was established in 2019 in the Vector Biology and Control Insectary in the University of Cape Coast. The mosquito population is resistant to various pyrethroid insecticides (Kudom, 2020) as well as to DDT (organochlorine), bendiocarb (carbamate) and shows moderate resistance to fenitrothion (organophosphate) insecticides. Three target-site mutations (F1534C, V1016I and V410I) as well as metabolic mechanisms have been detected in the population (Ayettey et al., 2023; Kudom, 2020). The colony was raised to the 7th generation and subsequently used for the test in this study. The adults were fed on 10% sugar

115 solution. The laboratory conditions for breeding were maintained at 29±1◦C with a relative
116 humidity of 75±10%.
117 From the colony, two separate populations were established. One population was exposed to the
118 coil (exposed colony) and the other population served as a control (control colony). Sasso®
119 mosquito coil (0.08% meperfluthrin, Samara company Ltd) was used for the experiment. The
120 WHO diagnostic time (full lethal dose) of mosquito coil against mosquito population in a Peet-
121 Grady chamber is 60 minutes (WHO/HTM/NTD/ WHOPES/2009.3). Thus, any time below 60
122 minutes becomes a sublethal dose. In this study, a 7-minute exposure time was used as a sublethal
123 dose. The sublethal dose exposure time was based on results from a previous experiment on the
124 colony that established the LT_{50} from Sasso mosquito coil exposure to be 7 minutes (Ablorde et
125 al., 2023).
126 The mosquito coil bioassay was conducted in a Peet-Grady chamber according to the protocol
127 described by WHO (WHO/HTM/NTD/ WHOPES/2009.3). The Peet-Grady chamber was
128 constructed of wood and had a dimension of 180 cm x 180 cm x 180 cm with four observation
129 glass windows as described in Kudom (2020). A single coil was fitted to its metal holder and lit
130 and allowed to smoulder for five minutes before it was placed in the chamber on a flat platform.
131 The mosquitoes in four holding cages (30 cm x 30cm x 30 cm BugDorm-1 cages) were suspended
132 10 cm from the sides of the chamber and 80 cm from the ceiling. An electric fan was placed on
133 the floor inside the chamber to ensure uniform dispersion of the smoke emitted from the coil inside
134 the chamber.
135 For the exposure of sublethal doses of coil to the colony, four hundred, 2–5-day old female
136 mosquitoes were randomly collected from the colony using an aspirator into the BugDorm-1 cages.
137 One hundred female mosquitoes were placed in each of the four cages and exposed to the coil for

7 minutes in the Peet-Grady chamber. After the exposure, mosquitoes were transferred from the cage to new cages and given 10% glucose solution. Knockdown was recorded at 60 minutes and the final mortality was read at 24 hours. The mosquitoes that survived were blood-fed to lay eggs. The eggs were raised into adults to form the next generation of the exposed colony. This cycle was repeated for 16 generations. In parallel, the same procedure was carried out for the control colony without the exposure to the coil for 16 generations. At the end of the 16th generation, susceptibility of the female mosquitoes in each of the colony to three insecticides and the diagnostic dose of the coil was determined. Furthermore, the frequencies of three *kdr*-mutations as well as the level of activities of five metabolic enzymes were also evaluated in the individual female mosquitoes from each colony.

WHO insecticide susceptibility bioassay against exposed and control colonies

Standard WHO susceptibility bioassays comprising exposure tubes and WHO impregnated papers of deltamethrin (5%), bendiocarb (0.1%) and fenitrothion (1%) were conducted on 100 female mosquitoes for each insecticide for each colony respectively. The susceptibility of bendiocarb (carbamate) and fenitrothion (organophosphate) insecticides were performed to check for cross resistance with the pyrethroid insecticides. The impregnated papers and the tubes were purchased from a WHO distributor (Vector Control Research Unit, University of Malaysia, Malaysia). The experimental procedure followed the WHO protocol described in WHO/ZIKV/VC/ 16.1. In summary, 3–5-day-old, non-blood fed females were used for the bioassay. Up to 25 individuals were exposed to the impregnated paper in the exposure tube for 60 minutes. After the exposure, the mosquitoes were transferred into a holding tube and fed with 10% sugar solution. Four replicates were done for each of the insecticides. The experiment set-up was run in parallel with an untreated paper as a control.

For the full diagnostic mosquito coil bioassay, twenty-five female mosquitoes each were placed in four BugDorm-1 cages and exposed to the coil for 60 minutes in the Peet-Grady chamber. After the exposure, mosquitoes were transferred from the cage to new cages and given 10% glucose solution. The experiment was repeated once for each colony.

For both insecticide and coil bioassays, the knockdown and total mortality were recorded 60 minutes and 24 hrs after exposure to the insecticide or the coil respectively.

qPCR screening for F1534C, V1016I, and V410L *Kdr* mutation among the field and laboratory populations

One hundred female mosquitoes each from the exposed and the control colonies were randomly sampled and genotyped for the F1534C, V1016I, and V410L *kdr* mutations. DNA from each mosquito was extracted using 2% CTAB (Cetyltrimethyl Ammonium Bromide) as described by Healey et al. (2014). The *kdr* mutations were screened using qPCR melting curve analysis based on the protocols described by Saavedra-Rodriguez et al., (2018) for V410L target-site mutation and Estep et al. (2018) for V1016 and F1534C target-site mutations. The detection of target site mutation V410L was done with each reaction containing 50µM of each forward primers, V410 fw and L410 fw, and 100 µM of the reverse primer, 410 rev, 9.5µL of 2x Sybr Hi-Rox Mix (Bioline), 1µL of genomic DNA and 7µL of DNase-free water. The V1016I target-site mutation detection was done in a 20µL reaction consisting of 8.2µL of 2x Sybr Hi-Rox Mix (Bioline), 0.15µM of Val1016f primer, 0.2µM each of lle1016f and lle1016r primers, 2µL DNA template and DNase-free water. The cycling conditions for this procedure were 95ºC for 3 minutes, 40x (95 ºC :10sec, 60 ºC:10sec, 72 ºC :30sec) 95 ºC with the melting condition of 65-95ºC in 0.2 ºC per 10 sec. Each 20 µL reaction for V1016I consisted of 8µL of 2x Sybr Hi-Rox Mix (Bioline), 0.15µM of Val1016f

primer, 0.2 µM each of lle1016f and lle1016r primers, 2µL DNA template and DNase-free water, with the same cycling conditions as the V410L. F1534C was screened in a reaction volume of 20µL containing 8µL of 2x Sybr Hi-Rox Mix (Bioline), 0.3µM of Cys1534+ primer, 0.3µM each of Phe1534+ and 1534- primers, 2µL DNA and DNase-free water. The cycling conditions for F1534C included 3min at 95°C, 37 cycles of (10 sec at 95°C, 10 sec at 57°C, 30 sec at 72°C) and 95°C for 10 seconds. A melting curve analysis was performed at the end of the PCR cycle at 65°C - 95 °C. The mutant gene (resistant) and wild type (susceptible) respectively produced melting curve peaks at 83°C and 86°C for V410L, 80°C and 86°C for V1016I, and 86°C and 82°C for F1534C. The negative controls consisted of master mix with DNase-free water, whilst the positive controls were positive DNA samples from a previous study (Ablorde et al., 2023).

Assessment of the levels of enzyme activities in the exposed and control colonies.

Five enzyme activities (alpha- and beta-esterases (NSEs), glutathione S- transferase (GST), mixed function monooxygenase (MFO) and insensitive acetylcholinesterase were assessed in 100 individual females each from the exposed and control colonies. The mosquitoes were randomly selected from each colony at about 3-5-days of age and non-blood fed. Biochemical microplate assays were used to assess the metabolic enzyme activities as described by Leong et al., (2019). Each mosquito was homogenised in 1000 µl of (K_3PO_4) buffer and 100 µl of the homogenates were aliquoted on to a 96-well microplate in triplicates of 30 mosquitoes on each plate with two adjacent homogenate buffers for controls. Alpha- and beta-naphthyl were used as the substrates for NSEs; for the GSTs, reduced glutathione and cDNB (1-Chloro-2,4-dinitrobenzene) were used as substrate. TMBZ (3,3'-,5,5'-Tetramethylbenzidine,) was used as a substrate for the oxidase assay and DTNB (5,5-dithio-bis-(2-nitrobenzoic acid) was used for insensitive acetylcholinesterase.

Protein content for individual mosquitoes was determined with Coomassie Protein assay reagent® (Thermo Scientific) following the protocol described by the manufacturer. Absorbance was read using the SpectraMax 340PC microplate reader.

Data Analysis

The mortalities from the WHO susceptibility tests for both the control and experimental colonies, were analyzed in percentages. A chi-test of association between alleles and resistant phenotypes was conducted using GraphPad prism. Gene-Calc (Bin´kowski & Miks, 2018) was used to ascertain deviations from the Hardy-Weinberg equilibrium. The median enzyme activities significantly above that of the reference mosquito colony were considered elevated. The Mann-Whitney test was used to compare mortalities caused by both insecticide and the mosquito coil between the exposed and control colonies. The Dunn test was also used to compare the enzyme activities between the exposed and control colonies.

Results

WHO susceptibility test against exposed and control colonies of *Aedes aegypti*

Both exposed and control colonies were highly resistant to deltamethrin, and carbamate with moderate resistance to fenitrothion (Fig 1, 2). However, mortality caused by deltamethrin was significantly higher in the control colony than in the exposed colony (Mann-Whitney test, $p = 0.04$). The knockdown effect of the insecticide was similar in both colonies (Mann-Whitney test, $p = 0.1$). Within the exposed colony, the knockdown effect was higher than the final mortality (Mann-Whitney test, $p = 0.02$). However, the mortalities caused by carbamate and fenitrothion were similar in both exposed and control colonies. For the mosquito coil bioassay, the one-hour exposure to both mosquito colonies caused 100% knockdown. However, complete mortality was

observed in the control colony whereas the exposed colony had only 68% (± 4.6) mean percentage mortality (± SD).

Distribution of F1534C, V1016I and V410L and metabolic resistance

A total of 100 individual female mosquitoes each from the exposed and control colonies were genotyped for F1534C, V1016I and V410L mutations (Table 1). The distribution of resistant alleles of V410L and F1534C *kdr* mutations were similar in both colonies. However, the frequency of the 1016I resistant allele was higher in the exposed colony than in the control colony ($X2 = 7.7$, $p = 0.006$) (Table 2). The enzyme activities of alpha- and beta-esterase, MFO, ACT and GST for both colonies had been summarised in Fig 3. Alpha-esterase (Dunn's test, $p = 0.01$) and MFO (Dunn's test, $p = 0.04$) activities were significantly higher in the exposed colony than in the control colony. However, the other three enzyme activities were similar in both colonies.

Discussion

Based on the WHO guidelines on the assessment of biological efficacy of a mosquito coil, an exposure of mosquito populations to a coil for 60 minutes in a Peet Grady chamber should cause 98-100% mortality to susceptible populations (WHO/HTM/NTD/WHOPES/2009.3). In this study, while the control colony had complete mortality to the coil, the exposed colony recorded only 68% (± 4.6) mean percentage mortality (± SD), suggesting the development of increased tolerance or resistance to the coil. Although, this is a first laboratory-based study showing that the continuous exposure to mosquito coil could cause resistance in *Ae. aegypti*, resistance to different pyrethroid based mosquito coils have been reported in both *Ae. aegypti* and other mosquito vectors before (Amelia-Yap et al., 2018; Avicor et al., 2017; Lukwa et al., 2014). *Ae. aegypti* populations from

Indonesia were found to be resistant to d-allethrin, transfluthrin, and metofluthrin based mosquito coils (Amelia-Yap et al., 2018). Also, *Anopheles gambiae* populations from Ghana and Tanzania have shown resistance to d-allethrin (Avicor et al., 2017), metofluthrin, esbiothrin (Lukwa et al, 2008) and dimefluthrin based mosquito coils. Many households in Africa and Asia countries depend on mosquito coil for the protection against mosquito bites, particularly outdoor biting mosquitoes such *Ae. aegypti*. Thus, the potential decrease in effectiveness of the coil against mosquito vectors due to resistance could have a major public health implication. On the other hand, development of insecticide resistance in the vectors due to excessive use of mosquito coil could also affect the efficacy of other insecticide-based mosquito control tools including insecticide treated bed net.

The effect of the exposure of sublethal doses of insecticides on insects has not been fully elucidated. Nonetheless, several studies have shown that such exposure could have differential effect on insect's fitness, behaviour and tolerance to insecticides (Ablorde et al., 2023; Boonyuan et al., 2011; S. Ritchie et al., 1997). For example, the sublethal exposure of pyrethroid insecticides on the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) caused higher adult survival and higher adult body mass than those not exposed to the insecticide (Margus et al., 2019). Also, sublethal exposure to metofluthrin, a semi-volatile pyrethroid did not affect the fitness of *Ae. aegypti*. On the contrary, chronic exposure to sublethal concentrations of chlorpyrifos or imidacloprid insecticide had an adverse effect on the development and reproductive fitness of *Culex pipiens* (Naggar et al., 2019). In this study, exposure of *Ae. aegypti* to a sublethal dose of a meperfluthrin based mosquito coil did not only cause resistance to the coil but increased the mosquito's resistance to deltamethrin. In a related experiment, sublethal exposure to transfluthrin,

also a semi-volatile pyrethroid, resulted in a decrease of the susceptibility of *Ae. aegypti* to the insecticide after nine generations (Wagman et al., 2015).

Target-site mutations F1534C, V1016I and V410I have been identified in pyrethroid resistant *Ae. aegypti* populations from Ghana. The F1534C mutation is the most widely distributed *kdr* mutation and close to fixation in many parts of the country (Ablorde et al., 2023; Ayettey et al., 2023; Amlalo et al., 2022). A combination of F1534C and V1016I have been reported to confer higher resistance to pyrethroid insecticides than F1534C alone (Moyes et al., 2017). In this study, the frequency of F1534C *kdr* mutation were high in the exposed and control colonies, which is consistent with what is found in the wild population in Ghana (Ablorde et al., 2023; Ayettey et al., 2023; Kudom, 2020). However, the frequency of the 1016I resistant allele was higher in the exposed colony than the control. In Wagman et al. (2015), the decrease in susceptibility to pyrethroid insecticide of the transfluthrin-selected colony had a higher frequency of the 1016I *kdr* allele than the control group. Also, application of household aerosol sprays led to a significant increase in the frequency of 1016I *kdr* homozygotes in the surviving *Ae. aegypti* population from Mexico (Gray et al., 2018). Results from this study and previous studies (Gray et al., 2018; Wagman et al., 2015) suggest a strong selection pressure for the 1016I resistant allele from pyrethroid-based household insecticide products.

In our study, the knockdown effect of the coil and deltamethrin insecticide on both mosquito colonies was not substantially affected. Mosquitoes were completely knocked down after exposure to the coil and the knockdown observed after exposure to deltamethrin was similar in both exposed and control colonies. This may suggest that the increase in deltamethrin resistance and the resistance to the coil in the exposed colony may be largely mediated by metabolic resistance mechanisms or other unknown mechanisms, which were not determined in this study. The

monooxygenase, esterase, and glutathione-S-transferase activities have all been shown to be associated with pyrethroid-resistance in *Ae. aegypti* (Pimsamarn et al., 2009). Several PBO synergists and enzyme activity assays give evidence of the role of metabolic resistance mechanisms in pyrethroid resistance in the *Ae. aegypti* population from Ghana (Abdulai et al., 2023; Ablorde et al., 2023; Ayettey et al., 2023; Kudom, 2020; Amlalo et al., 2022). However, the two studies that performed enzyme activity assays with *Ae. aegypti* populations from the country did not compare the field caught mosquitoes to an appropriate susceptible *Ae. aegypti* reference strain (Kudom, 2020; Amlalo et al., 2022). Thus, the specific enzyme(s) responsible for the pyrethroid resistance in Ghanaian *Ae. aegypti* population remain unclear. However, in the present study, alpha-esterase and MFO activities were higher in the exposed colony than in the control colony, suggesting their possible role in the increased deltamethrin resistance as well as resistance to the coil. The high activity of alpha-esterase in the exposed colony is a major cause for concern since it can quickly lead to cross resistance in other classes of insecticides. For instance, increased esterase activity had been recorded in pyrethroid and carbamate resistant *Anopheles funestus* populations from Mozambique and Malawi (Cuamba et al., 2010; Wondji et al., 2012). However, resistance to bendiocarb and fenitrothion were not affected substantially in the exposed colony compared to the control colony in this study.

In Ghana, households use mosquito coils by placing them outdoors or indoors with open windows. In such situations, the exposure concentration for mosquitoes could vary greatly, with sublethal doses being the most encountered concentration. However, the degree to which natural mosquito populations would experience the selective pressure observed in this experiment may differ under natural settings. For example, the behaviour of mosquitoes under restrain in cages during the experiment may not be the same as free flying mosquitoes under natural conditions. Also,

environmental conditions under laboratory setup may differ from natural conditions. All these factors could affect the amount and duration a mosquito is exposed to an insecticide from a mosquito coil. Hence, care should be taken in interpreting the results from this study outside laboratory settings. Also, we exposed female mosquitoes mainly under the assumption that due to host seeking behaviour they would be drawn towards the human hosts and thus closer to the coils, whereas the exposure of males is likely less intense. However, a co-exposure of males might speed up the generation of genetic resistance by removing susceptible males and thus facilitating homogeneous offspring. The amount of exposure of male mosquitoes to mosquito coil vapours however is even more unclear than the female exposure and was thus neglected in this study to remove confounding.

Despite this limitation, the result from this study gives an insight into the interaction between *Ae. aegypti* mosquitoes and mosquito coil as well as other semi-volatile pyrethroids commonly used in domestic anti-mosquito products. More work is needed to define the observations from this study within the larger landscape of pyrethroid use. Future studies should investigate how prolonged exposure to sub-lethal doses of semi-volatile pyrethroids might impact insecticide resistance in natural vector populations and how existing pyrethroid-resistant populations might respond to mosquito coils or a given spatial repellent product in real life field settings.

Conclusion

This study shows that under laboratory settings, a persistent exposure of meperfluthrin-based mosquito coil to *Ae. aegypti* for 16 generations resulted in 1) the development of resistance to the coil as well as an increased resistance to deltamethrin insecticide, 2) higher frequency of V1016I *kdr* mutation as well as higher alpha-esterase and MFO activities in the exposed population

compared to the control. Given the large-scale use of mosquito coil in many African households, its role as a pyrethroid resistance selection source should be taken into consideration when designing resistance management strategies.

Conflict of interest disclosure

All the authors of this manuscript have no conflict of interest to declare. We also confirm that there are no disputes over the ownership of the data presented in the paper and all contributions have been attributed appropriately, via co-authorship.

Data Availability Statement

Data available in the results section of the manuscript.

Authors' Contribution statement

Aikins Ablorde: Investigation; Writing - original draft; Formal analysis; Methodology; Visualization; Data curation. Inge Kroidl: Supervision; Writing - review & editing. Andreas Wieser: Supervision; Writing - review & editing; Resources; Methodology; Validation. Andreas A. Kudom: Conceptualization; Writing - original draft; Writing - review & editing; Supervision; Resources; Project administration; Methodology; Validation.

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Figures

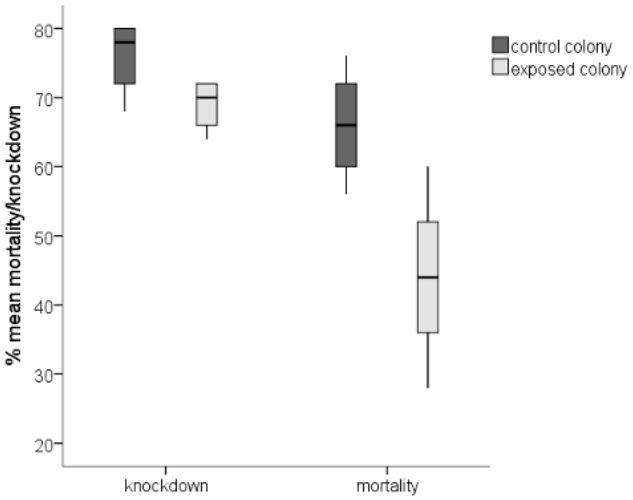

Figure 1: Percentage mean mortality or knockdown after exposure of exposed and control colonies to deltamethrin (0.05%).

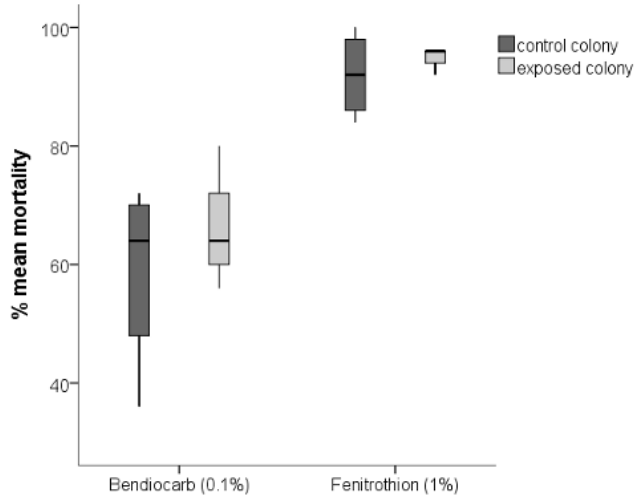

Figure 2: Percentage mean mortality after exposure of exposed and control colonies to bendiocarb (0.1%) and fenitrothion (1%).

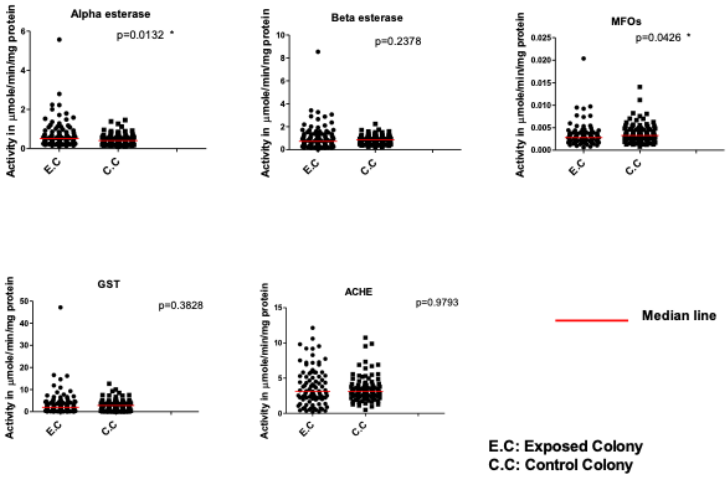

Figure 3: The enzyme activities of alpha- and beta-esterase, glutathione S- transferase (GST), mixed function monooxygenase (MFO) and insensitive acetylcholinesterase (ACHE) for exposed (E.C) and control colonies (C.C) (the red line denote the median enzyme activity).

Tables

Table 1: Genotype and allele frequencies for the exposed and control colonies of *Aedes aegypti*

Colony	F1534C				V410L				V10161			
	SS	RS	RR	C (freq%)	SS	RS	RR	L (freq%)	SS	RS	RR	I (freq%)
control	0	4	46	96	2	48	0	48	48	2	0	2
exposed	1	0	48	96	1	49	0	49	38	12	0	12

535 **Table 2**: Chi-test association between allelic frequencies of three *kdr* mutations of exposed and

536 control colonies of *Aedes aegypti*

Colony	F1534C			V410L			V10161		
	F	C	$X^2(p)$	V	L	$X^2(p)$	V	I	$X^2(p)$
control	4	96	0.687(P=*0.407*)	52	48	0.02 (P=*0.8875*)	98	2	7.68 (P=*0.0056*)
exposed	2	98		51	49		88	12	

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Curriculum vitae

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Education

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May 2012 – December 2015, **Senior Research Assistant, NMIMR – University of Ghana**

September 2010 – August 2011, **National service**, **37 Military Hospital**

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10th – 14th February 2014 *Anopheles gambiae* Species Identification and Insecticide Resistance Detection Using Molecular Techniques by U.S. Naval Medical Research Unit No. 3 Cairo, Egypt.

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3rd – 5th March 2012 Vector Biology Research Training Program

Publications

1. Ablorde A, Ayettey J, Kroidl I, Wieser A, Kudom AA. **Co-occurrence of multiple kdr mutations (F1534C, V1016I, V410L) in Aedes aegypti from coastal areas in Ghana and assessment of the role of mosquito coil in causing pyrethroid resistance.** Acta Trop. 2023 Jul; 243:106937. doi: 10.1016/j.actatropica.2023.106937. Epub 2023 May 3. PMID: 37146863.

2. Insecticide susceptibility of natural populations of *Anopheles coluzzii and Anopheles gambiae* (*sensu stricto*) from Okyereko irrigation site, Ghana, West Africa - Parasites & Vectors

Joseph Chabi, Philip K. Baidoo, Alex K. Datsomor, Dora Okyere, **Aikins Ablorde**, Alidu Iddrisu, Michael D. Wilson, Samuel K. Dadzie, Helen P. Jamet and Joseph W. DiclaroII

3. Ovipositional Behavior of *Anopheles gambiae* Mosquitoes - Japanese Society of Tropical Medicine.

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References

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Complete list of my publications

1. Ablorde A, Kroidl I, Wieser A, Kudom AA**.** Impact of the exposure of sublethal dose of mosquito coil on the development of insecticide resistance in Aedes aegypti (L.) (Diptera: Culicidae) Medical and Veterinary Entomology (**Accepted – yet to be published)**

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