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## Assessment of anti mosquito measures in households and resistance status of *Culex* species in urban areas in southern Ghana: Implications for the sustainability of ITN use

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### ABSTRACT

**Objective:** To determine resistance status of *Culex* species to different class of insecticides and assess the major anti-mosquito strategies employed by urban households and their possible effects on malaria acquisition. **Methods:** Structured questionnaires were randomly administered to obtain information on demographic characteristics, measures that people use to prevent mosquito bites and their perception of where mosquitoes breed in their communities. Adult susceptibility assays were also carried out using WHO discriminating dosages of four insecticides from different chemical classes against *Culex* species. **Results:** Majority of respondents (54.75%) preferred the use of domestic insecticides in the form of aerosols and coils. Among households that used domestic insecticides, the use of coil was most frequent (62.9%) with a mean (95% CI) of 9.18 (8.99, 9.37) coils per week. Strong level of pyrethroid-resistance and multiple insecticide resistance in *Culex* species were also detected in some of the study sites. **Conclusions:** The excessive use of domestic insecticides and high level of resistance in *Culex* species observed in the study area has implications for the ITN component of the nation's malaria control program in more subtle ways. People will lose interest in the use of ITN when it fails to protect users from bites of resistant *Culex* species. Excessive use of domestic insecticides may also select resistance in both malaria vectors and *Culex* species. On this account we recommend that nuisance mosquitoes must be controlled as part of malaria control programs to improve acceptance and utilization of ITN.

## 1. Introduction

The rapid rate of urbanisation in Ghana has come with its attendant sanitation and public health problems, including the creation of numerous anthropogenic habitats. These problems have arisen as a result of poor sanitation and improper solid and liquid waste management, among many others. The situation has not received the needed attention partly because majority of the mosquitoes that breed in these habitats are *Culex* species, which are of little or no importance as disease vectors in the country.

*Culex* species are important vectors for lymphatic filariasis

in some geographic areas including East Africa but in Ghana and other west African countries only *Anopheles* and *Mansonia* species are reported vectors of the disease[1–3], making *Culex* species less important in disease transmission in the country. Nonetheless, such mosquitoes can cause considerable annoyance through their bites. Hence they are considered as nuisance or pest mosquitoes.

The current strategy of the Ghana National Malaria Control Programme involves the use of insecticide-treated nets (ITN) and indoor residual spraying (IRS). Over the past decade, there has been a tremendous increase in number of households protected by ITN and IRS. Percentage of households owning at least one ITN increased from 2% in 2000 to 47% in 2010 but there is no evidence of a reduction in malaria cases or a decrease in the number of reported deaths[4,5]. This continuing problematic situation calls for a

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re-evaluation of the strategies for effective and sustainable vector management.

In most cases, malaria prevention strategies target the vectors; however there are some concerns that nuisance mosquitoes can interfere with such strategies in more subtle ways. For example, there are serious concerns that the use of insecticide net can be disregarded by the local people, if the nets fail to protect users from nuisance mosquitoes. Pyrethroid resistance among mosquito populations can reduce the efficacy of ITN against such mosquitoes. However, most of the reports on insecticide resistance in Ghana have mainly been on malaria vectors<sup>[6–8]</sup>, leaving out nuisance mosquitoes such as *Culex* species.

Apart from ITN, there are other anti-mosquito strategies, such as the use of insecticides, employed by people to avoid mosquito bites. However the continuous increase in malaria admissions give reasons to assess, at the household level, what strategies people are really using and their effectiveness in preventing malaria in the country. Boakye and colleagues<sup>[9]</sup> reported high level of use of insecticide as the major anti mosquito strategies in some communities in Accra with a minimal use of ITN. In the present study, similar assessment was carried out in different areas in order to ascertain the level of such practise in the country and the possible effect on malaria prevention.

Coetzee and colleagues<sup>[8]</sup> also shown different level of insecticide usage among households depending on the season. In that study there was lower mosquito coil usage in the dry season than in the rainy season, which was attributed to the drying up of most mosquito breeding sites, especially transitory breeding sites. Therefore, in this study data was collected during the dry season with the consideration that most mosquitoes may be breeding in permanent or anthropogenic habitats. The main objectives of the study was to determined resistance status of *Culex* species to different classes of insecticides and assess major anti-mosquito measures employed by people to prevent mosquito bites.

## 2. Materials and methods

### 2.1. Assessment of anti mosquito measures

Data were collected from February to March, 2011 during the dry season, from households in peri-urban areas close to rice fields (Okyereko and Asutsuare) and urban communities (Jamestown and Bakaekyir) in Southern Ghana. Jamestown and Bakaekyir are high-poverty pocket areas in Accra Metropolis and Sekondi-Takoradi Metropolis respectively. These areas have poor waste management practices, thus creating many anthropogenic habitats. Asutsuare and Okyereko are peri-urban areas close to irrigated rice paddies and there is cultivation of rice throughout the year.

Most transitory mosquito habitats dry up during the dry season, making anthropogenic habitats important mosquito breeding sites in that season.

Structured questionnaires were randomly administered to obtain information on demographic characteristics, strategies that people use to prevent mosquito bites and their perception of where mosquitoes breed in their communities. Furthermore, households that used mosquito coils were quantified; the number of coils used in the previous week was recorded. The questionnaires were answered by family heads. A response rate of 60% and 80% for urban areas and peri-urban areas, respectively, were estimated from a pre-trial. Hence, with 80% power, 5% significance level and a relative effect size of 0.25 (Cohen's *d*), 200 questionnaires were administered in each of the urban areas and 150 questionnaires in each of the peri-urban areas. The use of mosquito-proof screen was not considered in this study because it is a cultural practice in most urban houses in the country.

The questionnaires were analyzed with SPSS (version 16). Kruskal-Wallis tests, as well as multiple comparisons by ranks, were used to test the significance of differences between the numbers of mosquito coils used in each of the communities.

### 2.2. Insecticide susceptibility test

Mosquito larvae were collected from ponds, polluted drains, choked gutters and rice fields from seven localities in southern Ghana. Collection sites were selected from different settings (Figure 1); urban and peri-urban, urban agricultural and commercial. Larvae were brought to the laboratory for emergence and testing of adults.



**Figure 1.** Map of Ghana showing sites where mosquitoes were collected for the insecticide susceptibility test.

Adult susceptibility assays were carried out using WHO discriminating dosages of four insecticides from different chemical classes: Permethrin 0.75%, DDT 4%, Malathion 5%

and Bendiocarb 0.1%. Mortality resulting from tarsal contact with treated filter paper was measured using WHO test kits. Four batches of 25 unfed females, aged 2–5 d, were exposed to papers impregnated with malathion and bendiocarb for 1h, permethrin for 2 h and DDT for 4 h. Tests with untreated papers were run in parallel and served as control. The number knocked down were recorded every 15 min for permethrin and DDT and mortality rate was recorded after 24 h for all the insecticides[10].

2.3. Ethical consideration

Informed consent was sought and obtained from all study participants after a standard explanation of the study objectives had been clearly spelled out and confidentiality was assured. All protocols followed were in line with the ethics requirements of the University of Cape Coast.

3. Results

3.1. Assessment of anti mosquito measures

A total 695 households answered the questionnaire and their demographic characteristics are summarized in Table 1. Major anti-mosquito strategies were the use of insecticide-treated nets and domestic insecticides in the form of aerosols, coils and repellents. The percentage of people that claimed ITN as their anti-mosquito strategy was higher in the peri-urban areas [Okyereko (48.3%), Asutsuare (32.5%)] than in the urban communities (Table 2). However majority of respondents (54.75%) preferred the use of domestic insecticides in the form of aerosols and coils (Figure 2).

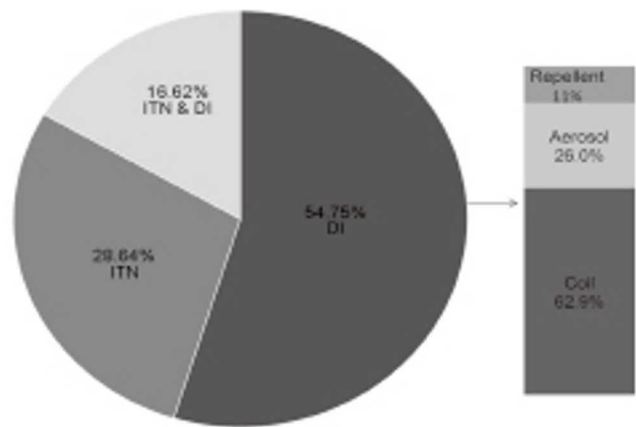


Figure 2. Preference for different anti-mosquito strategies employed by households in southern Ghana (DI: domestic insecticide, ITN: insecticide treated net).

Among households that used domestic insecticides, the use of coil was most frequent (62.9%) with a mean (95% CI) of 9.18 (8.99, 9.37) coils per week. The number of coils used per week was highest in Okyereko but not different from that for other study sites except Bakaekyir ( $\chi^2 = 22.2, df = 1, P < 0.001$ ).

About 50% of respondents in the two urban communities perceived mosquitoes to be breeding in gutters (Table 3). A high percentage of respondents (70.7%) in Okyereko thought rice farms were the major mosquito breeding sites but only 10% of respondents in Asutsuare had the same perception. A number of respondents in all the study sites perceived bushes to be breeding sites for mosquitoes (Table 3).

Table 1

Characteristics of respondents in four communities in southern Ghana.

Demographic parameters		Community			
		Jamestown (%)	Bakaekyir (%)	Okyereko (%)	Asutsuare (%)
Educational Level	Basic	30.0	15.3	16.7	30.0
	Secondary	40.5	36.7	35.3	55.0
	Tertiary	1.5	30.7	18.7	11.5
	No formal	28.0	17.3	29.3	3.5
Occupation	Public	2.5	18.5	14.7	13.0
	Private	18.5	37.7	38.0	14.5
	Self employed	77.0	43.8	47.3	46.5
	Others	2.0	–	–	26.0

Table 2

Pattern of anti-mosquito strategies employed by households in four communities in southern Ghana (n = number of household).

Community (n)	% anti-mosquito strategies				Mean no. of coils used per week (95% CI) N <sup>†</sup>
	ITN	DI	ITN & DI	Other	
Asutsuare (200)	32.5	35.0	31.0	1.5	9.4 (9.1, 9.8)a 81
Okyereko (147)	48.3	29.9	18.4	3.5	9.9 (9.7, 10.1)a 44
James town (200)	19.5	72.0	2.0	6.5	9.4 (9.1, 9.7)a 107
Bakaekyir (148)	12.2	75.0	12.8	0.0	8.2 (7.7, 8.7)b 72

†Values in columns not sharing the same letters are significantly different at 5% level.

**Table 3**

Perception of people on where mosquitoes breed in their communities in four communities in southern Ghana.

Perceived breeding sites	Community			
	Jamestown (%)	Bakaekyir (%)	Okyereko (%)	Asutsuare (%)
Bush	5.5	16.1	3.3	14.5
Gutter	54.0	49.0	–	27.0
Stagnant water	25.0	9.8	11.3	24.5
River/lagoon	7.0	14.0	8.7	14.5
Rice farm	–	–	70.7	10.0
Others	8.5	11.1	6.0	9.5

**3.2. Insecticide susceptibility test**

A total of 2 864 *Culex* mosquitoes from seven different locations were tested for resistance to permethrin, DDT, malathion and bendiocarb. Figure 3a–d shows resistance status of *Culex* species in southern Ghana. Resistance to DDT was evident at all study sites.

At Dzorwulu, an urban agricultural area and Dansoman, a commercial area in Accra, there were low mortality rates of *Culex* species to DDT and permethrin. Apart from DDT to which resistance is suspected, the mosquito population sampled from a swampy area in Abokobi was susceptible to the rest of the insecticides.

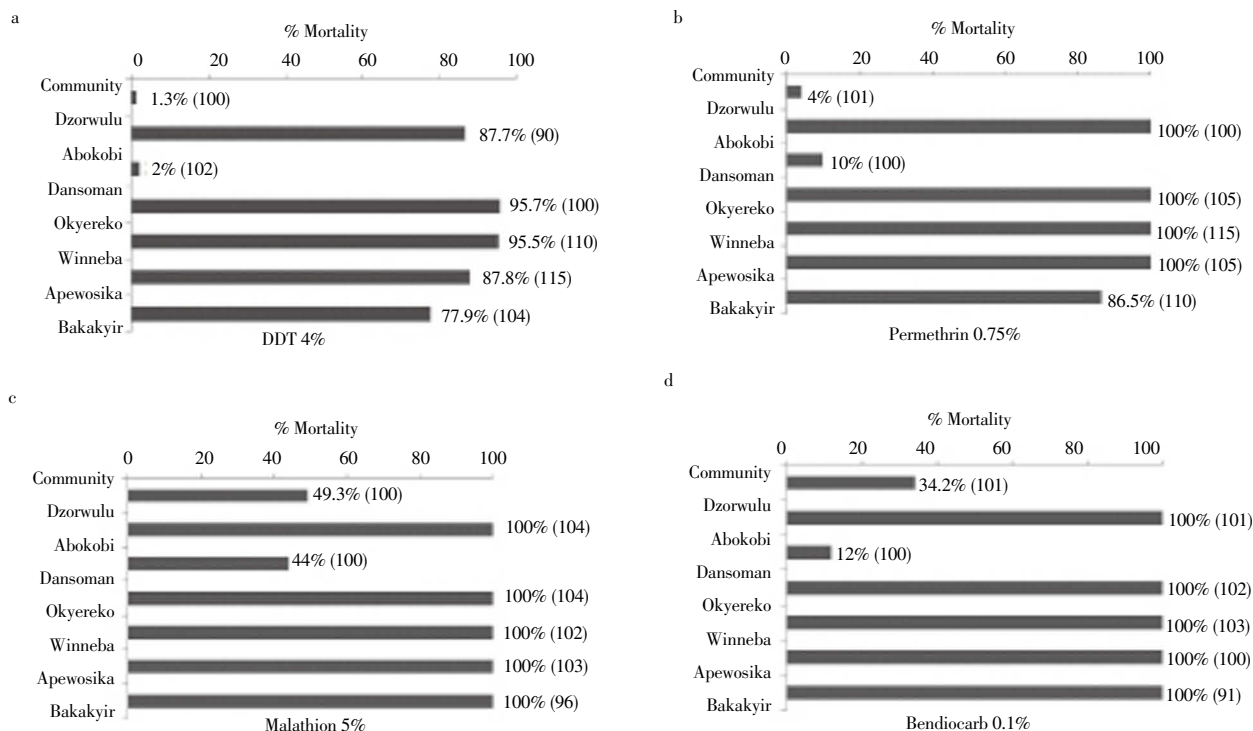
Mosquitoes from a rice field in Okyereko were susceptible to almost all the insecticides. A mortality rate of 95.5% was recorded after exposure to DDT; this is an evidence to suspect resistance in the area. Similar results we obtained from Winneba, an urban area close to Okyereko. *Culex* population from Bakakyir was susceptible to malathion and bendiocarb. However resistance to DDT and Permethrin is

suspected.

**4. Discussion**

Most of the respondents protected themselves from mosquito bites by using either insecticide treated net (ITN) or domestic insecticide in the form of aerosol and coils or in combination. This is in agreement with other studies carried out in other parts of the country<sup>[9,11]</sup> except that there was high ITN ownership or ITN use among respondents in this study. Strong level of pyrethroid–resistance and multiple insecticide resistance in *Culex* species were also detected in some of the study sites and this is the first report of multiple insecticide resistance in *Culex* species in Ghana.

The most frequent strategy was the use of mosquito coils. Ahorlu *et al*<sup>[11]</sup> found burning coils to be a response to nuisance from mosquitoes. Several controlled trials have provided consistent evidence that burning coils inhibits nuisance biting by various mosquito species<sup>[12]</sup>. However, it



**Figure 3.** Resistance status of *Cx. quinquefasciatus* in Southern Ghana against discriminating dosages of a) DDT (4%); b) permethrin (0.75%); c) malathion (5%) and d) bendiocarb (0.1%).

is not evident that burning mosquito coils prevents malaria acquisition, though a reduction in the number of mosquito bites would seem likely to reduce malaria transmission. Nevertheless, Lawrance and Croft<sup>[12]</sup> suggested that since just one infective anopheline is needed to transmit malaria, a modest reduction in bite numbers could very well have no clinical impact at all. Therefore, mosquito coils might be effective in reducing mosquito bites but may not be effective as a malaria control strategy. Pyrethroid resistance seen in some mosquito populations, including malaria vectors, might even reduce the efficacy of coils against them. Mortality rate of less than 50% was observed after a susceptibility test was carried out on *Anopheles gambiae* (*An. gambiae*) using different mosquito coils used in Ghana<sup>[13]</sup>.

The use of domestic insecticides can also have adverse effects on the health of users<sup>[14–19]</sup> and serious economic implications on households. For example, the mean number of coils used per week was about nine, which may have an average cost of \$1 (market price). Bakaakyir, a community in Sekondi–Takoradi Metropolis, has a median household income of \$4.91 per day<sup>[20]</sup>. Therefore a household in that area may spend approximately 2.9% on its entire weekly income on mosquito coils.

The evidence in support of ITNs as a malaria control strategy is impressive. Besides the personal benefit to users, non-users living within an area with high ITN coverage can also benefit from reduced risk of infection, due to the resulting reduction in overall malaria transmission<sup>[21,22]</sup>. However, mosquitoes that bite outdoors immediately after sunset and before sunrise may escape the protective effect of ITN and this can threaten the sustainable use of ITN. Peak biting rate of *An. gambiae* sl and *Culex* species was reported to be around 2 am and 12 midnight respectively in some communities in Accra<sup>[23]</sup> and this coincides with the time most people are in bed; however, their results also showed a significant number of bites from *Culex* as early as 6 pm<sup>[23]</sup>. Bites from *Culex* will not cause malaria, but they can threaten the sustainability of ITN use. For instance, mosquito bites before bed time have been shown to be one of the reasons why people refuse to use ITN<sup>[24]</sup>.

The level of pyrethroid resistance observed has implications for the ITN component of the nation's malaria control program. For instance, with strong pyrethroid-resistance in *Culex* species, people may not perceive the personal protective effect of ITNs (if they do not kill *Culex*) and this can be an obstacle to ITN use and malaria prevention. There have been reported cases of failure of IRS<sup>[25]</sup> and reduced efficacy of ITN and IRS<sup>[26]</sup> for malaria control due to pyrethroid resistance.

Resistance to other chemical classes observed in the study area can endanger novel strategies or resistance management strategies that combine different classes of insecticides. The treatment of nets with mixtures or mosaics of insecticides that have different target sites has been demonstrated both in laboratory and field conditions<sup>[27,28]</sup> as a potential resistant management strategy. This makes resistance of *Culex* species to malathion and bendiocarb worrying and needing immediate attention. Further studies are on-going to establish the type of mechanism involved

in the resistance of the mosquitoes against different insecticides in the study area, which may be useful in planning resistance management strategies.

With the excessive use of domestic insecticides and high level of resistance in *Culex* species observed in the study area, it is imperative to supplement the ITN component of malaria control with effective management of anthropogenic habitats. Although *Culex* species is not a vector of malaria, it needs to be controlled as part of a malaria control program. People will lose interest in the use of ITN when it fails to protect users from bites of both resistant malaria vectors and *Culex* species. High density of *Culex* species will also compel people to use domestic insecticides excessively, which may select for resistance in both malaria vectors and *Culex* species. Excessive use of domestic insecticide has already been reported to be partly responsible in the development of insecticide resistance in *An. gambiae* in the study area and other areas<sup>[9,29,30]</sup>. We have the opinion that insecticide resistance observed among *Culex* species in this study is partly as a result of excessive use of domestic use of insecticide.

Choked gutters were most important *Culex* breeding sites during dry season in Bakaakyir<sup>[30]</sup>, one of the study areas. Many studies in Ghana as well as other African countries have shown choked gutters and other anthropogenic habitats to be very important breeding sites for *Culex* species<sup>[23,31,32]</sup>. From the results, it is evident that majority of local people have basic information on the importance of choked gutters in the life history of mosquitoes. This relevant previous knowledge will make it easier for them to get involved in managing those breeding sites. There is a need for a vigorous campaign to educate communities on the effect of poor sanitation on mosquito life history, the consequences for their health and the economic burden it could place on their households. Similar effort, commitment and political will that was used to persuade people to accept the use of ITN can also be used to get people involved in improving the sanitation condition in their area.

A limitation of this study was that it did not take into account the number of people that sleep under ITN in households that mentioned ITN as their anti-mosquito strategy. Hence, it is possible that not all the members of the household may enjoy ITN protection in the household. This may partly explain why some of the households employed ITN in combination with other strategies. Most of the households in the study area periodically received free ITN; hence there is high ownership of ITN. It is possible that people that owned ITN might mention it as one of their anti-mosquito strategy, though they might not be using it regularly.

In summary, our study found that though ITN was used in some households, major anti mosquito strategy was the use of mosquito coil. However this strategy may not be very effect against malaria prevention and this may be one of the reasons why malaria admission has not decrease in the country. Also high levels of insecticide resistance were observed in some *Culex* populations and this has the tendency to reduce the motivation or efficacy to the use of ITN. We have the opinion that the vector control component



of malaria control programs should be broadened to include the control of nuisance mosquitoes or management of anthropogenic habitats, especially in urban areas. This may reduce mosquito bites before bed time and improve the acceptance and utilization of ITN among the local people. It may also reduce the health threats or economic burden placed on households as a result of the use of domestic insecticides. For now ITN remains the major tool for malaria control and therefore should be protected from any factors that can threaten the sustainability of its use.

### Conflict of interest statement

We declare that we have no conflict of interest

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