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Increased Biting Rate of Insecticide-Resistant *Culex* Mosquitoes and Community Adherence to IRS for Malaria Control in Urban Malabo, Bioko Island, Equatorial Guinea

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Abstract

Sustaining high levels of indoor residual spraying (IRS) coverage (≥85%) for community protection against malaria remains a challenge for IRS campaigns. We examined biting rates and insecticide resistance in *Culex* species and *Anopheles gambiae* s.l., and their potential effect on community adherence to IRS. The average IRS coverage in urban Malabo between 2015 and 2017 remained at 80%. *Culex* biting rate increased 6.0-fold ($P < 0.001$) between 2014 and 2017, reaching 8.08 bites per person per night, whereas that of *An. gambiae* s.l. remained steady at around 0.68. Although *An. gambiae* s.l. was susceptible to carbamates and organophosphates insecticides, *Culex* spp. were phenotypically resistant to all four main classes of WHO-recommended IRS insecticides. Similarly, the residual activity of the organophosphate insecticide used since 2017, ACTELLIC 300CS, was 8 mo for *An. gambiae* s.l., but was almost absent against *Culex* for 2 mo post-spray. A survey conducted in 2018 within urban Malabo indicated that 77.0% of respondents related IRS as means of protection against mosquito bites, but only 3.2% knew that only *Anopheles* mosquitoes transmit malaria. Therefore, the increasing biting rates of *culicines* in urban Malabo, and their resistance to all IRS insecticides, is raising concern that a growing number of people may refuse to participate in IRS as result of its perceived failure in controlling mosquitoes. Although this is not yet the case on Bioko Island, communication strategies need refining to sensitize communities about the effectiveness of IRS in controlling malaria vectors in the midst of insecticide resistance in nonmalaria vector mosquitoes.

Key words: *Culex*, insecticide resistance, indoor residual spraying adherence, malaria control, Bioko Island

One of the key pillars of the strategic framework for Global Technical Strategy for malaria 2016–2030 (GTS) is to ensure universal access to core malaria interventions. Indoor residual spraying (IRS) and insecticide-treated nets remain the primary vector control tools in the GTS (WHO 2015a). These two control interventions have accounted for almost 60% of global investment in malaria control in recent times (WHO 2015b). Globally, malaria interventions through vector control and effective treatment have reduced malaria mortality by 62% between 2000 and 2015 (WHO 2016).

The Bioko Island Malaria Control Project (BIMCP) has deployed IRS and long-lasting insecticidal nets (LLINs) at large-scale interventions since 2004. Together with effective case management, these interventions have reduced malaria parasite prevalence on Bioko Island from 43.3% at baseline of the interventions in 2004 to 10.5% in 2016 (Bradley et al. 2015; Cook et al. 2018). In addition, two primary malaria vectors, *Anopheles funestus* (Giles, Diptera, Culicidae) and *Anopheles gambiae* s.s. (Giles, Diptera, Culicidae), disappeared from the Island, leaving *Anopheles coluzzii* (Coetzee, Diptera, Culicidae) and *Anopheles melas* (Theobald, Diptera, Culicidae) (Overgaard et al. 2012). Entomological inoculation rates have dropped from over 1,000 infective bites per person per annum to 13 between 2004 and 2017 (Cano et al. 2004, Sharp et al. 2007, G.F. et al., unpublished data).

For interventions to provide a high level of community protection, sustained universal coverage is required. In the case of IRS, 85% or more of all structures in the community that are potential resting places of the vectors should be sprayed (WHO 2015b). On Bioko Island of Equatorial Guinea, the average IRS coverage for malaria vector control in urban Malabo has reached ~80% from 2015 through 2017. The coverage in rural Bioko has risen even higher from 86% in 2015 to 92% in 2016 and 2017. Sustaining high coverage in urban Malabo, however, has become an ongoing challenge for the IRS campaign. Low community adherence, which is one key factor that determines the impact of IRS on malaria transmission (Montgomery et al. 2010, Munguambe et al. 2011), is influenced by a community's knowledge about malaria transmission and prevention.

Despite the remarkable reduction in malaria transmission compared with pre-intervention levels on Bioko Island, decreases in malaria transmission in recent times have stagnated and remain heterogeneous on the Island. The current goal of the BIMCP is to accelerate toward malaria elimination on the Island by refining the core interventions, and taking into consideration the introduction of malaria vaccines. Focalized IRS with more than 85% coverage in communities with parasite prevalence of ≥10% is one of the objectives for the vector control interventions. Maintaining high IRS coverage on Bioko Island, specifically in urban areas, calls for investigations into the factors determining community nonadherence to IRS.

A short-term goal of the Global Plan for Insecticide Resistance Management (GPIRM) in malaria vectors, developed in 2012 by the WHO, is to preserve the effectiveness of current vector control interventions (WHO 2012). The plan provides global and country level strategies with insecticide resistance monitoring and surveillance playing an integral part in the malaria vector control programs. Unsurprisingly, insecticide resistance surveillance in malaria control programs focuses mainly on *Anopheles* vector mosquitoes. However, a meta-analysis of studies concerning the motivations for using LLINs at the household level indicated that reducing mosquito nuisance, rather than preventing malaria transmission, can be an important motivation (Pulford et al. 2011). Furthermore, it is unlikely that the general population is well versed in differentiating between anophelines and culicines. If nuisance reduction is indeed an important motivation for participating in IRS programs, failure of IRS to successfully control nuisance mosquitoes may lead to reduced household participation. Therefore, this study examined *Culex* biting density on Bioko Island in recent years between 2014 and 2017, as well as their current insecticide resistance status. Finally, we discussed if failure of IRS in controlling *Culex* mosquitoes could potentially affect IRS participation.

Materials and Methods

Study Site

This study was conducted in the Republic of Equatorial Guinea on Bioko Island. The island has a population of approximately 250,000 people who are at risk through year-round malaria transmission. Malabo, the capital city of Equatorial Guinea, lies on the northern coast and contains roughly 90% of the Island population. In 2004, Marathon Oil and its business partners, Noble Energy, GEPetrol, and SONAGAS, teamed up with the government of Equatorial Guinea to develop the Bioko Island Malaria Control Project (BIMCP), which is implemented by the nonprofit organization, Medical Care Development International (MCDI). From 2004 to 2014, IRS was conducted Island-wide using either pyrethroids or carbamates. Since 2015, a stratified approach has been used with IRS deployed in areas with high parasite prevalence and in remote areas that are difficult to reach and far from health facilities. ACTELLIC 300CS, an organophosphate insecticide, was introduced in 2017. Mass LLIN distribution campaigns were performed Island-wide in 2008/2009 using PermaNet 2.0 and again in 2014/2015 with PermaNet 3.0 (Vestergaard, Switzerland). Olyset plus (Sumitomo, Japan) LLINs were used during a 2018 mass distribution. Continuous distributions are carried out through routine antenatal care in health facilities and through primary schools.

Annual Malaria Indicator Survey

The BIMCP has conducted annual Malaria Indicator Surveys (MIS) on Bioko Island since 2004 using a nearly identical study protocol throughout at sentinel sites. Since 2015, the MIS sampling procedure, however, included all communities on the Island with the historical sentinel sites maintained for comparison over time. Within the communities, households were randomly sampled using systemic sampling. Heads of households were asked whether they received IRS within the previous 12 mo, and any individuals who refused IRS were asked their reasons for doing so. The methodology of the MIS surveys and the trends in parasite prevalence following 13 yr of malaria interventions on the Island have been recently published (Cook et al. 2018).

Rapid Knowledge, Attitude, and Practice Survey

In 2018, the BIMCP embarked on a door-to-door mass distribution campaign of LLIN Island-wide. A rapid bed net distribution follow-up survey was conducted 2 wk after distribution in two subdistricts in urban Malabo. In total, 400 households were randomly selected (200 per subdistrict). Interviews were conducted by trained enumerators on household members above 18 yr. Communities that were targeted for spraying in 2018 were included in the survey. The survey, among other things, sought to find out whether the respondents could relate the type of mosquitoes responsible for malaria transmission.

Entomological Surveillance

Since 2009, entomological monitoring included human landing catches (HLCs) at sentinel sites in both rural and urban Bioko (Meyers et al. 2016). Within a sentinel site, mosquitoes were collected by trained volunteers from 7 p.m. to 6 a.m. in three houses approximately 100 m apart. In each collection house, two HLC collectors were located inside and two outside. The indoor and outdoor collectors switched positions at midnight to limit collector bias. Two entomology field supervisors oversaw the collections to ensure that the volunteer collectors remained active during the night. From 2010, HLCs were conducted monthly in each sentinel site throughout the year. For this study, HLC data from 2014 to 2017 from a community in urban Malabo (Sumco) that received IRS under the stratification policy were analyzed. Mosquitoes were collected and identified based on morphology (Gillies and de Meillon 1968, Gillies and Coetzee 1987).

WHO standard insecticide susceptibility bioassays were used to monitor the phenotypic resistance profile of both *Culex* and *Anopheles* mosquitoes (WHO 2013). All four classes of insecticides were tested: 0.05% deltamethrin, 0.1% bendiocarb, 5% malathion, and 4% DDT. Table 1 shows the number of mosquitoes (n) exposed to each insecticide-impregnated paper for the recommended time period in hours. In 2017, BIMCP deployed a microencapsulated organophosphate insecticide formulation (CS) of pirimiphos-methyl, ACTELLIC 300CS (Syngenta, Switzerland), for IRS. The residual effectiveness of the insecticide was evaluated using the WHO's standard cone wall bioassays 2 mo post-spray and followed up monthly to 9 mo (WHO 2013). Mosquito larvae were collected at different locations within urban Malabo and reared to 3- to 5-d-old

Table 1. Exposure time of *Anopheles gambiae* s.l. and *Culex* spp. to WHO insecticide-impregnated papers

Insecticide (conc.)	*An. gambiae* s.l. (n)		*Culex* spp. (n)	
	Exposure (h)	Mortality at 24 h (%)	Exposure (h)	Mortality at 24 h (%)
DDT (4%)	1 (n = 82)	2.5	4 (n = 89)	0
Deltamethrin (0.05%)	1 (n = 81)	40	1 (n = 86)	7
Malathion (5%)	1 (n = 83)	100	1 (n = 83)	4
Bendiocarb (0.1%)	1 (n = 82)	100	2 (n = 81)	12

WHO/VBC/81.806: Tentative diagnostic concentration and exposure times for adult mosquitoes.

adults (wild progeny) in insectary conditions (temperature 27°C ± 2 and relative humidity 70 ± 10%) for the bioassays. Both culicines and anophelines were exposed on cement-plastered surface for 30 min with 24-h recovery period.

Molecular Analyses

The collected mosquitoes were identified as *Anopheles* versus *Culex* based on morphology. Mosquitoes were stored in 80% ethanol prior to shipping to Texas A&M University for molecular species diagnostic analyses. The QIAGEN tissue extraction kit was used to perform DNA extractions on the head and thorax (*Anopheles*) or whole body (*Culex*) on a QIAGEN Biosprint (QIAGEN Sciences Inc., Germantown, MD). Diagnostic PCR followed by a Hha1 restriction enzyme digestion was performed to identify the mosquito species of *An. gambiae* s.l. (Fanello et al. 2002). Molecular identification of *Culex quinquefasciatus* (Say, Diptera, Culicidae) was conducted according the protocol provided by Crabtree et al. (1995).

Statistical Analysis

For this study, we examined the biting rates of *Anopheles* and *Culex* mosquitoes in urban Malabo where the phenotypic resistance testing for both mosquitoes was conducted. Changes in the number of *Anopheles* and *Culex* mosquitoes collected by HLC from 2014 to 2017 were analyzed by generalized linear mixed models using R software (R Core Team 2015). The response variable of mosquitoes collected per collector per night was modeled by the fixed variable of collection year. The month of collection and number of collectors were modeled as random variables to account for sampling variation. The data were fit to a negative binomial distribution using the package *glmmADMB*, with a single-inflation parameter to fit the data because of the relatively large number of collections that had zero mosquitoes. Abundance ratios were calculated as the exponentiated model coefficients and 95% confidence intervals to represent the relative annual change in the biting rate.

We used the same model as described previously to analyze the change in biting rate for indoor and outdoor caught *Culex* mosquitoes. In addition, changes in the number of *Culex* mosquitoes collected by indoor/outdoor HLC from 2014 to 2017 were also analyzed by generalized linear models (GLMs) using a binomial distribution to obtain the odds ratio of mosquito's host-seeking preference (indoor vs outdoor). The response variable of mosquitoes collected outdoors versus indoors per collector was modeled by the fixed variable of year of collection. A random variable was conservatively designed that treated each paired indoor/outdoor collection at each house as an independent collection unit. The random variable individually coded every sampling event by time and location. This included collection year, month, and house to best account for sampling bias caused by changes in housing condition, collector identity, and meteorological conditions over the 4-year study period.

Results

The densities of *Culex* mosquitoes in urban Malabo as measured by the biting rate showed a dramatic increase from 1.35 (SE = 0.19) bites/person/night in 2014 to 8.08 (SE = 0.46) bites/person/night in 2017. In contrast, *An. gambiae* s.l. biting rates in urban Malabo remained relatively stable between 0.61 (SE = 0.11) and 1.19 (SE = 0.15) bites/person/night (Fig. 1). Therefore, by 2017, the *Culex* biting rate was 6.8-fold that of *Anopheles*. A GLM using a negative binomial distribution fit the mosquito collection data well, as the predicted number of mosquitoes collected per person by year closely followed the observed data. The model showed that increase in the number of collected *Culex* mosquitoes by HLC was significant (abundance ratio: 1.61 [1.42, 1.83] per year, $P < 0.001$). On the other hand, there was no significant change in the number of *Anopheles* mosquitoes collected per person (abundance ratio: 1.34 [0.96, 1.73], $P = 0.071$; Fig. 1).

In total, 350 *An. gambiae* s.l. were collected across 2014–2017 in Sumco, of which 230 were subjected to molecular species diagnostics. Of these, 99.1% belonged to the Bioko's primary malaria vector *An. coluzzii*, with the remainder belonging to *An. melas*. Two specimens of this species were collected in 2017 (n = 90). *Culex* mosquitoes collected as part of the malaria vector monitoring efforts are not analyzed or stored. Therefore, no *Culex* species diagnostic data are available for the study period. However, 184 *Culex* specimens collected in November 2018 in Sumco and nearby Ela Nguema, both in urban Malabo, were subjected to a molecular species diagnostic PCR. Of the 184 analyzed *Culex* specimens, 167 (90.7%) were confirmed to be *Cx. quinquefasciatus*. The remainder probably belonged to an unidentified *Culex* species, although PCR failures cannot be ruled out.

To determine whether there was a change in the biting behavior (indoor vs outdoor) of *Culex* mosquitoes from 2014 to 2017, a GLM using the binomial distribution was used. The number of mosquitoes caught host-seeking indoors and outdoors both increased from 2014 to 2017 (indoor: 1.57 [1.18, 1.91], $P < 0.001$; outdoor: 1.76 [1.45, 2.20], $P < 0.001$). Overall, the outdoor biting rate was a slightly higher than the indoor biting rate, but there was no change in the proportion of *Culex* collected indoor versus outdoor from 2014 to 2017 (odds of host-seeking outdoors vs indoors: 0.90 [0.80, 1.01], $P = 0.08$; Supp Fig. 1 [online only]). The hourly biting periods of *Culex* mosquitoes from 2014 to 2017 are presented in Fig. 2. Generally, *Culex* mosquitoes were actively biting at the start of the collection period and remained largely the same until the early morning.

The phenotypic resistance profiles of both *Culex* spp. and *An. gambiae* s.l. determined using the standard WHO's susceptibility bioassay are summarized in Table 2. *Anopheles gambiae* s.l. were phenotypically resistant to both deltamethrin (40% mortality) and DDT (2.5% mortality), but susceptible to bendiocarb (100% mortality) and malathion (100% mortality). In contrast, *Culex*

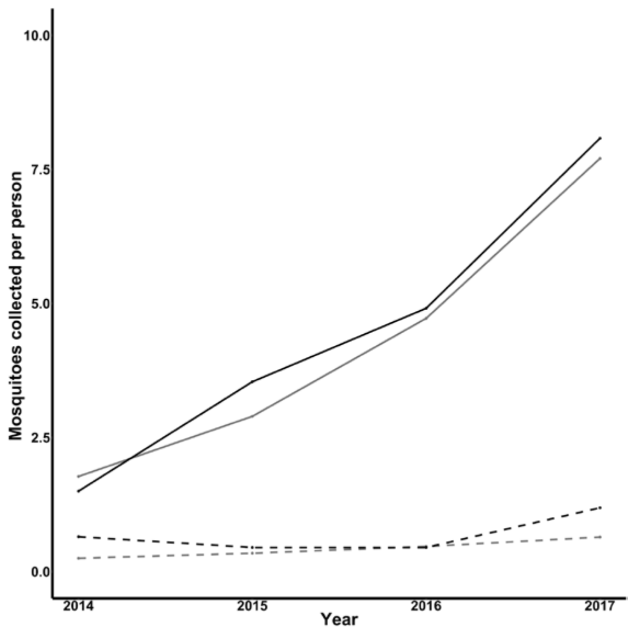

Fig. 1. Comparison of observed biting rate of *Anopheles gambiae* s.l. (gray dotted line) and *Culex* spp. (gray line) in urban Malabo with model predicted data (*An. gambiae* s.l.: black dotted line; *Culex* spp.: black line).

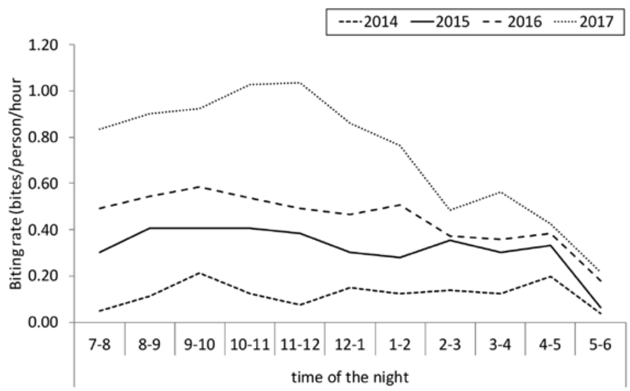

Fig. 2. The hourly biting rates of *Culex* mosquitoes in urban Malabo between 2014 and 2017.

Table 2. Resistant status of *Anopheles gambiae* s.l. and *Culex* spp. to WHO-recommended IRS insecticides

Insecticide	Class	Mosquito spp.	Status
Deltamethrin	Pyrethroid	*An. gambiae* s.l.	Resistance
DDT	Organochlorine	*An. gambiae* s.l.	Resistance
Bendiocarb	Carbamates	*An. gambiae* s.l.	Susceptible
Malathion	Organophosphate	*An. gambiae* s.l.	Susceptible
Deltamethrin	Pyrethroid	*Culex* spp.	Resistance
DDT	Organochlorine	*Culex* spp.	Resistance
Bendiocarb	Carbamates	*Culex* spp.	Resistance
Malathion	Organophosphate	*Culex* spp.	Resistance

spp. were phenotypically resistant not only to deltamethrin (7% mortality) and DDT (0% mortality), but also to bendiocarb (4% mortality) and malathion (12% mortality). Therefore, resistance is present in both *Culex* and *Anopheles* mosquitoes, but *Culex* mosquitoes are resistant against more insecticide classes.

Mortality of *Anopheles* and *Culex* mosquitoes following exposure to walls sprayed with ACTELLIC 300CS, introduced by the BIMCP IRS campaign in 2017, was evaluated between 2 and 9 mo post-spraying using a cone assay. The results show a striking difference in the effect of walls sprayed with ACTELLIC 300CS on *Culex* versus *Anopheles* mortality (Fig. 3). Whereas the mortality of *An. gambiae* s.l., which is completely susceptible to organophosphate, remained above 80% up to 8 mo after spraying, mortality of the resistant *Culex* spp. was only 5.4% at the first time point included in the study (2 mo).

In 2014, when the IRS was conducted island-wide, the MIS showed that 2.6% of the people in urban Malabo who refused IRS perceived it was not effective. When the Island was stratified between 2015 and 2017 and IRS was focalized in targeted communities, the percentage of respondents who rejected IRS because they perceived it was not effective dropped to an average of 1.03%. In addition, a survey conducted in April 2018 that followed up on a mass distribution of LLINs within urban Malabo revealed that 77.0% of respondents considered IRS a means to protect against mosquitoes bites, but only 3.2% knew that only *Anopheles* mosquitoes transmit malaria.

Discussion

The remarkable gains made by the BIMCP are faced with some challenges toward the elimination of malaria on Bioko Island, including lower than desirable community adherence to IRS and very low LLIN use (Cook et al. 2018). One of the current strategies is to refine the vector control interventions by identifying factors that prevent universal coverage of LLINs and IRS. LLIN usage has generally remained low at about 40% (Cook et al. 2018), and IRS coverage, though relatively high (80.0%), is below the 85% coverage needed to provide a high level of community protection (WHO 2013) in the urban set-up where 90% of the population resides. Nonetheless, these core interventions remain largely effective in protecting individuals against infections (Cook et al. 2018).

The BIMCP annual MIS and the 2018 cross-sectional survey have identified several factors for nonadherence to LLINs and IRS. These included net availability, inconvenience of using nets, itching, increased temperature within nets, and poor net condition (Romay-Barja et al. 2016). With regard to IRS, reasons such as not being contacted by the project team, inconvenience, attitude of spray operators, IRS being distractive, IRS causes ill health, and not being effective were also given for rejecting IRS. Several malaria control programs have reported similar reasons for nonadherence to LLINs and IRS (Montgomery et al. 2010, Baume and Koh 2011, Munguambe et al. 2011, Ingabire et al. 2015). However, the motivations for using LLINs (Alaii et al. 2003, Pulford et al. 2011) and accepting IRS (Rodriguez et al. 2003) are not exclusively to obtain protection against malaria vectors, but also against the nuisance biting mosquitoes as well.

This study showed that although a large number of respondents (77%) think of IRS as a measure that provides protection against mosquitoes bites, only 3.2% knew that only *Anopheles* mosquitoes transmit malaria. This is consistent with a study conducted in South Mexico that revealed that even though 48% of the respondents associated malaria with mosquito bites, only 3% directly linked IRS with the prevention of malaria transmission (Rodriguez et al. 2003). Thus, the perceived benefit of IRS to the majority of this group was to reduce mosquito abundance. Therefore, the increased biting rate of *Culex* mosquitoes over time, and the ineffectiveness of each spray round at killing *Culex* mosquitoes could be perceived as IRS not

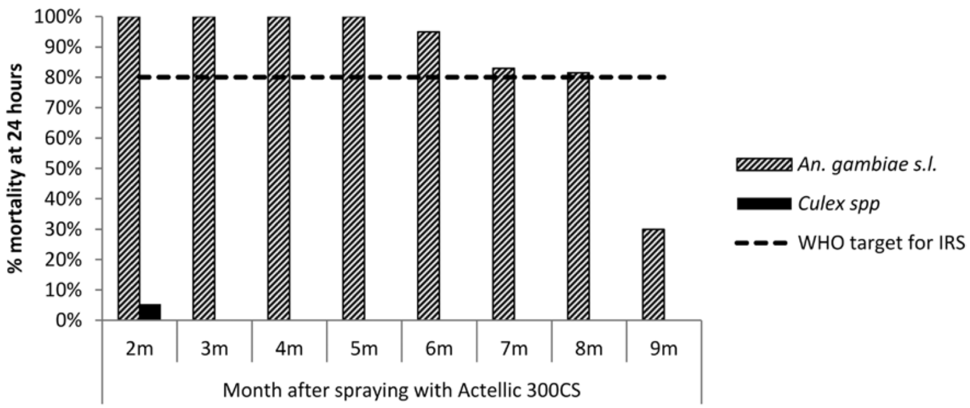

Fig. 3. Residual effectiveness of ACTELLIC 300CS organophosphate against mosquitoes from urban Malabo, Bioko Island.

Fig. 4. Vehicle ruts with clean water that provides breeding sites for *Anopheles* larvae.

being effective against malaria vectors. The MIS showed that only a small proportion of the population in urban Malabo currently rejects IRS because they perceive the IRS was not effective. Although this is encouraging, there is reason to be concerned that this could change in the future. Specifically, only a small proportion of people are aware of the difference between malaria vectors and nuisance mosquitoes. Combined with the dramatic increase in the biting rate of nuisance mosquitoes, this may eventually result in the perception that IRS is largely ineffective, even if it effectively controls malaria vectors. Unless efforts are undertaken to increase awareness among the population about the impact of IRS on *Anopheles* mosquitoes specifically, this could result in lower community adherence and reduced IRS coverage.

Our results showed an increased biting rate of *Culex* mosquitoes, both indoor and outdoor, in Urban Malabo, in the last several years despite ongoing spraying. These nuisance mosquitoes bite both indoors and outdoors and largely throughout the night. In addition, we found a high level of insecticide resistance in the *Culex* mosquitoes in 2017. Although we do not have insecticide resistance data on *Culex* mosquitoes from Urban Malabo from years prior to 2017, one possible explanation for the increased biting rate is a recent emergence/increase of insecticide resistance against the various classes of insecticide used in IRS in urban Malabo. A similar observation has been reported in Tanzania where *Culex* mosquitoes were highly resistant to all the classes of IRS insecticides (Khayrandish and Wood 1993, Tungu et al. 2010).

The breeding habitats of *Anopheles* mosquitoes in Bioko range from puddles, vehicle ruts (Fig. 4), roadside ditches, construction sites, and swampy areas. Although anophelines and culicines are sympatric in a number of these habitats, in urban Malabo, culicines breed exclusively in highly polluted wastewater and sewage systems in discarded tires, water storage containers, choked gutters, abandon swimming pools (Fig. 5), and pit latrines (Toto et al. 2003, Jones et al. 2012). Therefore, *Culex* mosquitoes could experience insecticide

Fig. 5. Abandoned swimming pool that provides breeding site for *Culex* larva.

exposure from a variety of sources including agricultural, industrial, and domestic uses. This might partially explain the difference in insecticide resistance observed between *Culex* and *Anopheles* mosquitoes (Vatandoost et al. 2004, Nalwanga and Sempebwa 2011). In addition, prior exposure of mosquito larvae to high levels of fertilizers, herbicides, oil compounds, and detergents can result in increased tolerance to insecticide due to the higher expression of a wide variety of detoxification enzymes (Suwanchaichinda and Brattsten 2001, Strode et al. 2006, Poupardin et al. 2008).

Recent field evaluations have demonstrated the long-lasting residual activity of the microencapsulated formulation (CS) of pirimiphos-methyl (Actellic CS, Syngenta, Switzerland) against *An. gambiae* s.l. for periods between 5 and 10 mo on a variety of surfaces (Chanda et al. 2013, Rowland et al. 2013). This study established 8-mo residual effectiveness of ACTELLIC 300CS against *An. gambiae* s.l. on cement-plastered surface. However, it was not effective against *Culex* mosquitoes 2 mo post-spraying. Evaluation of residual activity of pirimiphos-methyl CS in Benin also showed that *Cx. quinquefasciatus* mortality was consistently lower than that of *An. gambiae* (Rowland et al. 2013).

Culex mosquitoes are widely distributed in the African continent and are said to be the predominant mosquitoes in the cities and urban areas in West Africa where they breed in high levels polluted water bodies (Barr 1967, Subra 1981, de Souza et al. 2014). Furthermore, they are known vectors of lymphatic filariasis and a variety of arboviruses in East Africa (Simonsen et al. 2010, Braack et al. 2018). The high biting rate of *Culex* mosquitoes in urban Malabo (roughly eightfold that of *Anopheles* mosquitoes), the fact that these mosquitoes bite both indoors and outdoors throughout the night, combined with a failure of IRS to effectively control culicines, may not only negatively affect community adherence of IRS, but could potentially allow diseases transmitted by *Culex* mosquitoes to spread. Therefore, malaria vector control programs, in addition to monitoring insecticide resistance profile of the *Anopheles* vector mosquitoes, should also consider monitoring the resistance profile of culicines. This will aid in providing timely information for communication strategies discussing the effectiveness of IRS in controlling malaria vectors in the midst of insecticide resistance in *Culex* mosquitoes. Furthermore, where possible, larval source management should be considered as supplementary to IRS and LLINs in vector control programs in areas with resistant *Culex* populations. Follow-up study is needed to determine the vectorial capacity of *Culex* mosquitoes in transmitting filariasis and arboviruses on Bioko Island.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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