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Evaluation of the residual effectiveness of Fludora™ fusion WP-SB, a combination of clothianidin and deltamethrin, for the control of pyrethroid-resistant malaria vectors on Bioko Island, Equatorial Guinea

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ABSTRACT

Over the past decade, insecticide resistance to malaria vectors has been identified in 71 malaria endemic countries. This has posed a major global health challenge in the fight against malaria, with declining rates of indoor residual spraying coverage attributed to pyrethroid-resistance. As part of its vector control monitoring strategies, the Bioko Island Malaria Control Project (BIMCP) in Equatorial Guinea conducted routine insecticide resistance bioassays using the WHO's standard susceptibility tests from 2013 to 2018. During the same period, the frequency of the target-site knockdown resistance allele (*kdr*) in the local vector population was also determined via PCR for detection of the L1014 F mutation. Biochemical analysis for metabolic resistance was also conducted in 2015. From 2016–2017, Fludora™ fusion, a formulated combination of clothianidin (a neonicotinoid) and deltamethrin (a pyrethroid) was evaluated for 9 months on Bioko Island, using the WHO's standard test procedure for determining residual effectiveness of insecticides on sprayed surfaces. In 2016, the mortality rate of the vectors on 0.05% deltamethrin was as low as 38%. The frequency of the West African form of knockdown resistance allele, L1014 F, in the vector population was as high as 80%, and metabolic resistance analysis indicated high upregulated cytochrome P450 s. However, the residual effectiveness of Fludora™ fusion recorded mortalities above 80% after 72 h of exposure for 8 months. Although both target-site knockdown resistance and metabolic resistance to pyrethroids were implicated in the local malaria vector population, Fludora™ fusion was effective under field conditions in controlling the resistant vectors for a period of 8 months on wooden surfaces on Bioko Island and represents a valuable addition to IRS programs, especially in regions with high levels of pyrethroid resistance.

1. Introduction

From 2000–2015, roughly 75% of the global reduction in malaria parasite prevalence among children aged 2–10 years can be attributed to malaria control interventions (WHO, 2016). Using insecticides either through indoor residual spraying (IRS) or insecticide-treated mosquito nets (ITNs) remain the primary tools for vector control and are still considered effective in most malaria-endemic settings. In the 2000s, the use of insecticide-treated mosquito nets was credited with reducing malaria mortality by 55% in children under 5 years of age in sub-Saharan Africa (WHO, 2014). Other findings in different epidemiological settings have shown IRS and ITNs have substantially reduced all-cause infant and child mortality (Sharp et al., 2007; Akogbeto et al., 2011; Mashauri et al., 2013; Oxborough et al., 2014). On Bioko Island in the Republic of Equatorial Guinea, malaria vector control strategies rely primarily on IRS and the use of long-lasting insecticidal nets (LLINs), which have reduced malaria parasite prevalence in children between 2–14 years old from 45% in 2004 to 12.5% in 2018 in 18 sentinel sites, and 10.9 in the whole Island (Kleinschmidt et al., 2007; Bradley et al., 2015; BIMCP, 2018 Report).

The World Health Organization (WHO) recommends the use of IRS and ITNs as the primary vector control interventions for reducing and interrupting malaria transmission, and states that they should both be part of national malaria control strategies (WHO, 2006). However, realizing the full potential of IRS and ITNs in reducing malaria transmission depends largely on the effectiveness of the insecticides used for

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controlling malaria vectors (Najera and Zaim, 2003). The development and extensive spread of insecticide resistance in malaria vectors is a threat to the effectiveness of such vector control tools in sub-Saharan Africa which has been well documented (Coleman et al., 2006; N'Guessan et al., 2007a; Ranson et al., 2011; Coleman et al., 2017), and of particular relevance as 93% of the global malaria deaths occur in the region (WHO, 2018). Ongoing resistance monitoring results indicate that the malaria vectors are resistant to the four classes of WHO-recommended insecticides (carbamates, organochlorines, organophosphates, and pyrethroids) (WHO, 2012), and in 2016 resistance was reported in 71 malaria-endemic countries, with pyrethroid resistance being the most commonly reported (Coleman et al., 2017). Presently, pyrethroids are the only insecticide recommended by WHO to use in ITNs, and a have accounted for a large share of the insecticide used for IRS programs worldwide (Coleman et al., 2017). Furthermore, the decline of IRS coverage in sub-Saharan Africa, decreasing from 80 million people at risk in 2010, to 64 million in 2017, has largely been attributed to pyrethroid-resistance and the need to change or rotate to more expensive classes of insecticide (WHO, 2018).

To preserve the susceptibility and slow the spread of insecticide resistance, the Global Plan for Insecticide Resistance Management (GPIRM) has called for the development of new active ingredients for IRS and ITNs in medium to long-term strategies for malaria vector control (WHO, 2012). The GPIRM has outlined strategies to preserve the effectiveness of current vector control tools and at the same time develop new and innovative vector control tools for the future, to maintain progress towards malaria elimination. Mosaic spraying, rotational use of insecticides, integrated control strategies, and the use of combinations of two or more compounds of insecticides with different modes of action are currently recommended for vector control. Whereas some of these recommendations can be applied at the field level, the combination of compounds as a single product or formulation is only possible to achieve at the product development level. Following the GPIRM guidelines and the clear need to develop new insecticide classes, the WHO provided interim approval to a new insecticide class, chlorfenapyr (pyrolles), to be used for malaria vector control (WHO, 2017); through the recommendation of using the new ITN, the Interceptor G2. However, the recommendation does not extend to the use of IRS at this time.

This study describes the results of a field trial of Fludora™ fusion on Bioko Island, through the collaboration between the Bioko Island Malaria Control Project (BIMCP) and Bayer SAS. Bayer's Fludora™ fusion, is a combination of clothianidin (a neonicotinoid) and deltamethrin (a pyrethroid) with a new mode of action against malaria vectors. Whereas the mode of action for pyrethroids involves the opening of the sodium channels leading to continuous nerve excitation, paralysis and the eventual death of the vector, neonicotinoids act on the acetylcholine receptors (nAChRs) as agonists, inducing hyper-excitation of the nervous system (Soderlund and Bloomquist, 1989; Narahashi, 1992). Hyper-excitation of the nervous system also leads to overstimulation of the voltage-sensitive sodium channels (Na-Channels) (Bodereau-Dubois et al., 2012). A formulated combination of neonicotinoid (imidacloprid) and pyrethroid (flumethrin) has shown significant synergism in an efficacy and mortality study with various species of ticks and fleas for up to eight months (Stanneck et al., 2012). Initial experimental huts trials of the insecticide product in Benin have demonstrated residual efficacy lasting 8–9 months on mud and cement against pyrethroid-resistant malaria vectors (Ngufor et al., 2017). As of 2018, the World Health Organization Prequalification Team (WHO-PQT) added Fludora™ fusion to the pre-qualified list of insecticides to be used for IRS (WHO Prequalified Lists, 2019).

2. Material and methods

2.1. Study setting

The study was conducted on Bioko Island of the Republic of Equatorial Guinea, with a population of approximately 339,695 (Equatorial Guinea Population Census, 2015), where malaria transmission occurs throughout the year. In 2004, Marathon Oil and partners, Noble Energy, GEPetrol, Atlantic Methanol Production Company (AMPCO) and SONAGAS, teamed up with the government of Equatorial Guinea and the National Malaria Control Program to develop the Bioko Island Malaria Control Project. The BIMCP is implemented by Medical Care Development International (MCDI), a non-profit organization, in collaboration with the London School of Hygiene and Tropical Medicine (LSTHM), Texas A&M University, and the Liverpool School of Tropical Medicine (LSTM). The first round of IRS was carried out on Bioko in 2004 using Alpha-cypermethrin (Fendona™ BASF SE). This was followed by biannual rounds of the carbamate insecticide, bendiocarb (Ficam™, Bayer) from 2005 to 2012. In 2013, a single round of a long-lasting polymer-enhanced formulation of deltamethrin (K Othrine PolyZone, Bayer) was carried out. In 2014, a randomized control trial to compare the effectiveness of bendiocarb and deltamethrin (K Othrine PolyZone, Bayer) for use in IRS on Bioko was conducted (Bradley et al., 2016). Bendiocarb was used again in 2015 and 2016 using a targeted strategy for focal spraying. In 2017 and 2018, Pirimimos-methyl, an Organophosphate (Actellic 300 CS, Syngenta) was used to conduct IRS. Additionally, large-scale distributions of LLINs were carried out in 2007/2008, 2014/2015 and 2018 (Sharp et al., 2007; Cook et al., 2018; BIMCP, 2018 Report).

2.2. Insecticide resistance monitoring

WHO's standard insecticide susceptibility cone bioassays were used for the routine monitoring of the phenotypic resistance profile of the local vector population from 2013 to 2018 (WHO, 2013a). Since then, the susceptibility for all four classes of insecticides has been monitored on a yearly basis using impregnated papers: deltamethrin 0.5%, bendiocarb 0.1%, malathion 5%, and DDT 4%. Mosquito larvae were collected at different locations on Bioko Island and raised to F1 adults (wild progeny) for the bioassays. *Anopheles coluzzii,* captured using human landing catches throughout the years at sentinel sites on the island from 2013 to 2016 were genotyped for L1014 F (*kdr-w*) and AChE G119S (Ace-1) mutations using a polymerase chain reaction (PCR) based assay (Martinez-Torres et al.,1998). Biochemical tests (enzyme activity assays) for metabolic resistance were conducted in 2012 and 2015, after susceptibility test results indicated the presence of phenotypic resistance with a high frequency of *kdr-w* allele in the local vector population (Vontas et al., 2018)

2.3. Evaluation of fludora™ fusion on Bioko Island

A sample of Fludora™ fusion WP-SB was sent to the BIMCP for a small-scale field trial in 2016. Using the BIMCP mapping system to uniquely identify houses, 10 houses made of wood were randomly selected and sprayed in Sampaka, a community within the Malabo Urban district that was not part of the 2016 IRS spray round with bendiocarb. Houses with different substrates other than wood were not selected for this study. The selected wooden houses were at least 800 m away from the nearest bendiocarb sprayed households and separated from one another by at least 50 m away. Trained spray operators were supervised to deposit the correct concentration of the insecticide (200 mg clothianidin and 25 mg deltamethrin per square metre) on indoor walls using the 15-liter capacity Hudson expert pumps (H.D Hudson Manufacturing Company, Chicago IL) (WHO, 2015). Sprayed houses were monitored using the WHO's standard test procedure for determining residual efficacy of insecticides on sprayed surfaces (WHO,

Fig. 1. Standard WHO cone bioassay with Fludora™ fusion insecticide on wooden surface on Bioko Island.

2013a).

Wild *Anopheles* species larvae were collected from the field at different locations on the Island and bred in the insectary to F1 adults (wild progeny). Unfed female *Anopheles* mosquitoes 3–5 days old were used for the tests. The insectary had a temperature-controlled environment of between 26 °C ± 2 °C and humidity of about 75%–90%. The number of houses tested in a given month depended on the numbers of F1 adult female *Anopheles* reared in that particular month. A minimum of six sampling sites and a maximum of 12 sites were monitored throughout the study period. Temperature conditions in the houses during the period ranged between 27 °C–30 °C, with humidity ranging from 60% to 80%. WHO cones were placed at three different levels on the sprayed walls: the upper part of the walls at a height of ˜70 cm from the ceiling or roof, the middle portions, and the bottom parts of the walls at about 50–70 cm from the floor (Fig. 1.). Ten F1 adult females were placed in each cone and exposed to the insecticide for 30 min. After 30 min, the mosquitoes were aspirated and placed in labeled paper cups with 8% sugar solution and transported to the insectary. Two control tests for six sampling sites that were not exposed to Fludora™ fusion were also monitored. Verbal consents were obtained from homeowners before proceeding with the tests. The National Malaria Control Program (NMCP) of the Ministry of Health and Social Welfare, Equatorial Guinea, granted the ethical approval for the study.

Mosquito mortality was recorded 24 h after exposure and observed daily for up to 72 h for delayed mortality. Where the control mortalities were within the range of 5%–20%, the average observed mortalities were corrected using Abbott's formula (WHO, 2013b). Spraying with the Fludora™ fusion sample was carried out in June 2016 and the evaluation took place from August 2016 to March 2017.

3. Results

3.1. Susceptibility and target-site tests

The main malaria vectors on Bioko Island consist of *An. coluzzii* and *An. melas* (Meyer et al., 2016). Susceptibility profiles for deltamethrin were monitored on 0.05% deltamethrin impregnated papers, with results indicating a downward trend in vector mortality rate from 2013 (97%) to 2016 (38%). The mortality rate for deltamethrin in 2013 was just close to the 98% efficacy threshold (97.5%). However, in 2014, the vector mortality rate dropped to 79.5%, followed by a significant drop in 2015 and 2016 of 29% and 38%. While the susceptibility of the vectors to deltamethrin decreased from 2013 to 2016, the frequency of *kdr-w* allele (L1014 F) increased during the same period (Fig. 2). The percentage frequency of *kdr-w* in the vector population was 48.6% in 2013 and increased to 68.1% in 2014, followed by 78%, 80.9% and 87.0% in 2015, 2016 and 2017 respectively.

The results of the susceptibility tests carried out from 2013 to 2017 using impregnated papers with 0.01% bendiocarb (carbamate), 4% DDT (organochlorine) and 5% malathion (organophosphate) are summarized in Table 1.

Whereas the vectors were completely susceptible (100% mortality) to malathion and bendiocarb between 2013 and 2017, they were resistant to DDT since 2014. Acetylcholinesterase target-site mutation (AChE G119S) that confers resistance to carbamates and organophosphates have not been detected in the local vector population.

3.2. Results of the evaluation of Fludura™ fusion

The field evaluation of Fludora™ fusion commenced in August 2016, two months after spraying in June. Mortality rates were determined at 24 h after exposure. Delayed mortalities were also determined at 48 h and 72 h after exposure. In Fig. 3, mortality rates of 100% were recorded for up to three months after spraying at 24 h, 48 h and 72 h of observation. Evaluation was not carried out in the fourth month. In the fifth month, the mortality rates for all three observation periods were above the 80% threshold. Although the mortality rate after 24 h of observation in the sixth month was slightly below 80% (78%), the delayed mortalities at 48 h and 72 h were above 80%. In the seventh month however, the mortality rate after 24 h of observation went slightly above 80% (81%). In the eighth month, only the delayed mortalities at 72 h of observation were above 80%. The test was extended into the ninth month that observed both immediate and delayed mortalities below 80%. Thus, on Bioko Island, the residual efficacy on wooden surfaces of the insecticide is within the range of 7–8 months.

4. Discussion

The use of IRS and LLINs on Bioko Island as an integrated malaria vector control strategy has significantly reduced malaria parasite prevalence in 2 to 14-year-olds from 45% at baseline of the interventions in 2004 to 12.5% in 2018 (Cook et al., 2018). The EIR has also dropped from 425 infective bites per person per annum in 2009 to as low as 4 infective bites per person per annum in 2016 (Overgaard et al., 2012, Meyers et al., 2016). Additionally, all-cause under-five mortality declined from 152 per 1000 live births to 55 per 1000 live births within the first four years of the intervention (Kleinschmidt et al., 2009; Bradley et al., 2015).

From 2004–2018, three classes of insecticides (pyrethroids, carbamates, and organophosphates) were used extensively for malaria vector

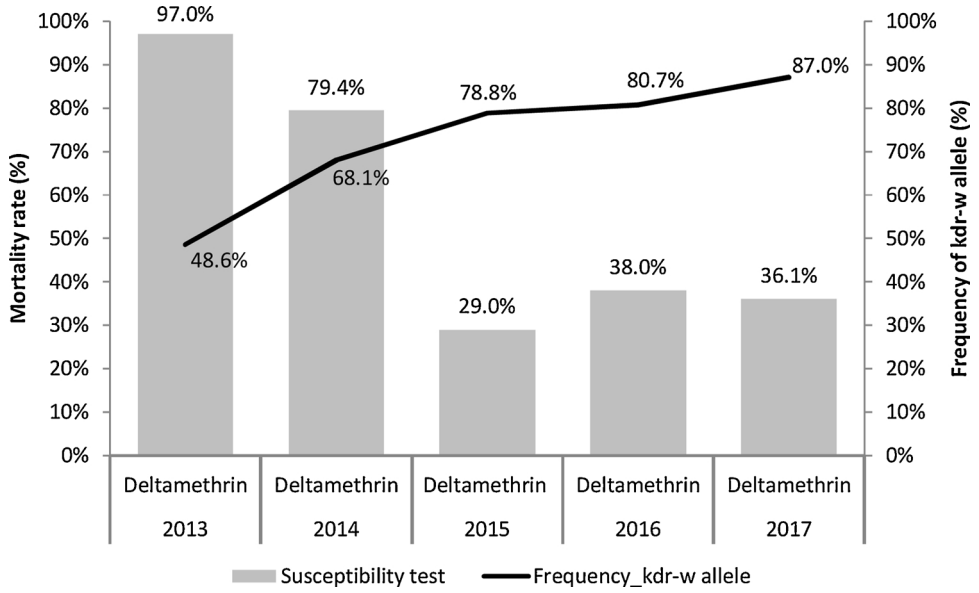

Fig. 2. Mortality rate of Anopheles vectors exposed on deltamethrin and the frequency of *kdr-w* allele.

Table 1
Phenotypic resistance profile of *Anopheles gambiae s.l.* to WHO insecticide-impregnated papers.

	Mortality rates at 24h (%)		
Year	DDT (4%)	Malathion (5%)	Bendiocarb (0.1%)
2013	*	100%	100%
2014	45.7%	100%	100%
2015	5.4%	100%	100%
2016	15.2%	100%	100%
2017	2.2%	100%	100%

control. It is well documented that extensive and repeated use of insecticides leads to selection pressure of resistance genes in malaria vector populations (Vulule et al., 1994; N'Guessan et al., 2007b), the BIMCP conducts phenotypic, target-site, and metabolic resistance monitoring analysis as an integral component of the vector control activities on Bioko (Reimer et al., 2005; Hemingway et al., 2013; Meyer et al., 2016; Vontas et al., 2018). Metabolic resistance analysis conducted by LSTM in 2012 found a low level of up-regulation in a number of oxidative stress genes tested in sampled vector mosquitoes from the island. The low level up-regulation was observed to have no IRS operational significance of metabolic resistance of the vectors to pyrethroids (Hemingway et al., 2013). The following year, in 2013 WHO susceptibility tests showed that the malaria vectors on Bioko were phenotypically susceptible to carbamates, organophosphates, and pyrethroids. Additionally, AChE G119S mutations were not detected but the frequency of kdr-w was about 48.6% (Hemingway et al., 2013). Therefore, the BIMCP switched to deltamethrin for IRS in 2013, as (1) there was no evidence that P450-based metabolic resistance mechanisms were present (Hemingway et al., 2013); (2) following the WHO recommendation on rotation of insecticides to manage resistance; and (3) the formulation of deltamethrin was long acting as compared to the short residual life of bendiocarb, which reduced costs of spraying by conducting only one spray round per year. In 2014, a cluster randomized trial to compare the effectiveness of deltamethrin versus bendiocarb for IRS on Bioko, suggested that bendiocarb was more effective at preventing malaria (Bradley et al., 2016). However, the difference between the two insecticides was not significant and consequent susceptibility tests confirmed that mortality with deltamethrin was only (79.4%) and PCR-based target-site knockdown resistance (68.1%). These results suggested further investigation into the mechanism of the

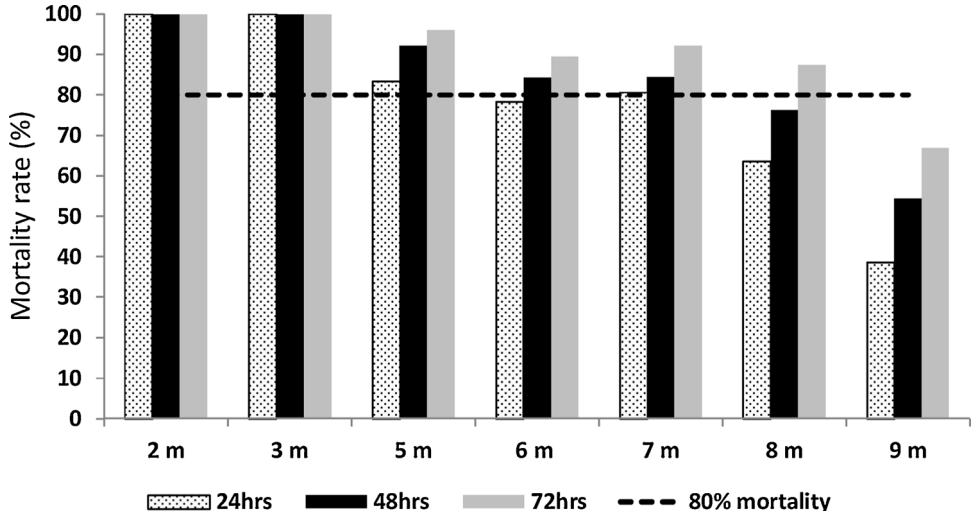

Fig. 3. Mortality rates of *Anopheles* vectors exposed on to Fludora™ fusion on wooden surface.

resistance was needed, resulting in another round of metabolic resistance analysis carried out by LSTM on local malaria vectors in 2015. These new results indicated that all *An. gambiae s.l.* that survived the bioassay test after 24 h of observation were *An. coluzzii*, and all were homozygous for *kdr-w* mutations. Furthermore, survivors had several upregulated cytochrome P450 s. Of the 48 genes that were significantly overexpressed, four were P450s: Cyp6P4, Cyp9K1, Cyp6Z1, and Cyp6Z2. For instance, CYP9K1 that was over-expressed by 6–10-fold and was able to metabolize 32% of the deltamethrin applied following a 2 -h incubation period. However, bendiocarb was not metabolized by CYP9K1, with no substrate depletion after 20 min incubation (Vontas et al., 2018). Thus, the malaria vector population on Bioko had developed both target-site and metabolic resistance to deltamethrin, and could no longer be used for IRS.

Furthermore, the susceptibility testing on DDT 4% also revealed the vectors were resistant to organochlorines. Previous findings have suggested that knockdown resistance confers cross-resistance to DDT and pyrethroids (Soderlund and Knipple, 2003). Additionally, widespread metabolic resistance to pyrethroids and knockdown resistance have been observed in *An. gambiae s.l.* in West Africa (Chandre et al., 1999; Etang et al., 2006; Pinto et al., 2006). Based on these results, carbamates and organophosphates, with similar mode of action, were the only available insecticide options for IRS on Bioko.

For the distribution of LLINs on Bioko, the BIMCP is currently using Olyset Plus nets (Sumitomo Chemical Co., Ltd) that contain permethrin and Piperonyl Butoxide (PBO) for its mass net distribution campaigns. Although a recent multi-country study indicated that nets provided protection against malaria infection regardless of resistance frequency (Kleinschmidt et al., 2018), there have been reported vector control failure associated with insecticide resistance and the use of IRS (Coetzee et al., 2013). In 2012, The BIMCP and the Ministry of Health of Social Welfare (MOHSW) of Equatorial Guinea developed the National Operational Plan for Insecticide Resistance Management for Bioko Island, which originally recommended the rotational use of pyrethroids and carbamates (Hemingway et al., 2013). The Plan was updated in 2015 based on the updated resistance monitoring data and recommended to rotate the use of carbamates and organophosphates for IRS with bendiocarb and pirimiphos-methyl (Actellic 300CS) being the insecticides of choice. However, due to the short residual life of bendiocarb, which required two spray rounds a year; and while Actellic 300CS has a longer residual life, requiring only one spray round a year, the rotation of these insecticides, with a similar mode of action, represents a major cost for vector control programs. It has been estimated that the rotational use of carbamates and organophosphates as a result of pyrethroids' failure may increase the cost of IRS by 30–70% (WHO, 2012). A recent study on the susceptibility of the mosquito population on Bioko has shown that even though *Anopheles* vectors are susceptible to the two classes of insecticide, carbamates and organophosphates, *Culex* mosquitos, mainly *Culex quiquefasciatus (90.7%)* are resistant to all classes of insecticide (Fuseini et al., 2019). Therefore, the BIMCP seeks an additional class of WHO approved insecticide with a different mode of action and potentially effective against *Culex* mosquitoes for use in rotation with carbamates and organophosphates for IRS.

This study has demonstrated that the new formulation of the Fludora™ fusion combination can control pyrethroid-resistant malaria vectors on Bioko Island for a period of up to 8 months on wooden surfaces. Using the threshold of 80% mortality, the residual efficacy of the formulation was seven months for immediate mortality at 24 h of observation. However, the residual efficacy of the combination was extended up to 8 months for delayed mortality at 72 h of observation. Despite these promising results, the beneficial effect of the combination compared to the effect of clothianidin alone was not evaluated in this study. Experimental huts trial of the combination in Benin has demonstrated considerably high induced mortality (60–80%) and long residual mortality against wild pyrethroid resistant mosquitoes on mud walls for 9 months compared to cement and wooden walls between 6–8 months. The Benin study also showed that when clothianidin was applied alone or in combination with deltamethrin, mortality was induced with a full effect only after 5 days of observation, while we only observed delayed mortality for up to 3 days post-exposure. However, delayed mortality was not strongly expressed with deltamethrin alone (Ngufor et al., 2017).

5. Conclusion

The Bioko Island Malaria Control Project (BIMCP) through the NMCP of Equatorial Guinea, like other malaria programs around the world, is under increasing threat of target mosquitoes developing resistance to the WHO recommended insecticides. Insecticide resistance is exerting major costs on vector control, thereby forcing programs to scale-down IRS coverage. Fludora™ fusion, a combination of clothianidin and deltamethrin with a different mode of action, has been shown to have residual efficacy on Bioko Island lasting for a period of 8 months and, if given WHO recommendation, may be used as a potential insecticide to rotate with carbamates and organophosphates to control pyrethroid-resistant mosquitoes. The combination insecticide has been listed by the WHO-PQT for IRS and its use could help preserve the effectiveness of pyrethroids and make a significant contribution to the overall mission of malaria elimination in Equatorial Guinea and other malaria-endemic countries globally.

Declarations of interest

None.

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References

Akogbeto, M., Padonou, G.G., Bankole, H.S., et al., 2011. Dramatic decrease in malaria transmission after large-scale indoor residual spraying with bendiocarb in Benin, an area of high resistance of *Anopheles gambiae* to pyrethroids. Am. J. Trop. Med. Hyg. 85 (4), 586–593.

BIMCP. Report, 2018. The Government of Equatorial Guinea & Medical Care Development International (MCDI). Bioko Island Malaria Control Project (BIMCP) Malaria Indicator survey Report. Tech. Rep., Medical Care Development International.

Bodereau-Dubois, B., List, O., Calas-List, D., et al., 2012. Transmembrane potential polarization, calcium influx, and receptor conformational state modulate the sensitivity of the imidacloprid-insensitive neuronal insect nicotinic acetylcholine receptor to neonicotinoid insecticides. J. Pharmacol. Exp. Ther. 341 (2), 326–339.

Bradley, J., Lines, J., Fuseini, G., et al., 2015. Outdoor biting by Anopheles mosquito on Bioko Island does not currently impact on malaria control. Malar. J. 14, 170.

Bradley, J., Hergott, D., Garcia, G., et al., 2016. A cluster randomized trial comparing deltamethrin and bendiocarb as insecticides for indoor residual spraying to control malaria on Bioko Island, Equatorial Guinea. Malar. J. 15, 1.

Chandre, F., Darrier, F., Manga, L., et al., 1999. Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. Bull. World Health Organ. 77 (3), 230–234.

Coetzee, M., Kruger, P., Hunt, R.H., et al., 2013. Malaria in South Africa: 110 years of learning to control the disease. S. Afr. Med. J. 103, 770–778.

Coleman, M., Sharp, B., Seocharan, I., et al., 2006. Developing an evidence-based decision support system for rational insecticide choice in the control of African malaria vectors. J. Med. Entomol. 43, 663–668.

Coleman, M., Hemingway, J., Gleave, K.A., et al., 2017. Developing global maps of insecticide resistance risk to improve vector control. Malar. J. 16, 86.

Cook, J., Hergott, D., Phiri, W., et al., 2018. Trends in parasite prevalence following 13 years of malaria interventions on Bioko island, Equatorial Guinea: 2004–2016. Malar.

J. 17, 62.
Equatorial Guinea Population Census, 2015. Government of Equatorial Guinea Presents the 2015 Population Census. Available at: (Accessed: 4 March 2019). http://embassyofequatorialguinea.co.uk/government-of-equatorial-guinea-presents-the-2015-population-census/.
Etang, J., Fondjo, E., Chandre, F., 2006. First report of knockdown mutations in the malaria vector *Anopheles gambiae* from Cameroon. Am. J. Trop. Med. Hyg. 74, 795–797.
Fuseini, G., Ncogo Nguema, R., Phiri, W.P., et al., 2019. Increased biting rate of insecticide-resistant Culex mosquitoes and community adherence to IRS for malaria control in urban malabo, Bioko Island. Equatorial Guinea. J. Med. Entomol. https://doi.org/10.1093/jme/tjz025. tjz025.
Hemingway, J., Vontas, J., Raman, J., et al., 2013. Country-level operational implementation of the global plan for the insecticide resistance management plan. Proc. Natl. Acad. Sci. U. S. A. 110 (23), 9397–9402 2013.
Kleinschmidt, I., Torrez, M., Schwabe, C., et al., 2007. Factors influencing the effectiveness of malaria control in bioko island, Equatorial Guinea. Am. J. Trop. Med. Hyg. 76 (6), 1027–1032.
Kleinschmidt, I., Schwabe, C., Benavente, L., et al., 2009. Marked increase in child survival after 4 years of intensive malaria control. Am. J. Trop. Med. Hyg. 80, 882–888.
Kleinschmidt, I., Bradley, J., Knox, T.B., et al., 2018. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. Lancet Infect. Dis. 18, 640–649.
Martinez-Torres, D., Chandre, F., Williamson, M.S., et al., 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s.* Insect Mol. Biol. 7, 179–184.
Mashauri, F.M., Kinung'hi, S.M., Kaatano, G.M., et al., 2013. Impact of indoor residual spraying of lambda-cyhalothrin on malaria prevalence and anemia in an epidemic-prone district of Muleba, north-western Tanzania. Am. J. Trop. Med. Hyg. 88 (5), 841–849.
Meyer, J.L., Fuseini, G., Schwabe, C., et al., 2016. Increasing outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. Malar. J. 15, 239.
N'Guessan, R., Corbel, V., Akogbéto, M., et al., 2007a. Reduced efficacy of insecticide treated nets and indoor residual spraying for malaria control in pyrethroid resistance area. Benin. Emerg. Infect. Dis 13 (2), 199–206.
N'Guessan, R., Corbel, V., Akogbeto, M., et al., 2007b. Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistance area. Benin. Emerg. Inf. Dis 13 (2), 199–206.
Najera, J.A., Zaim, M., 2003. Malaria Vector Control: Decision Making Criteria and Procedure for Judicious Use of Insecticide. WHO/CDS/WHOPES/2002.5. Rev.1. World Health Organization, Geneva, Switzerland.
Narahashi, T., 1992. Nerve membrane Na+ channels as targets of insecticides. Trends Pharmacol. Sci. 13, 236–241.
Ngufor, C., Fongnikin, A., Rowland, M., N'Guessan, R., et al., 2017. Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant Anopheles gambiae sl in Southern Benin. PLoS One 12 (12), e0189575 [https://doi.org/10.1371/journal.pone.0189575].
Overgaard, H.J., Reddy, V.P., Reddy, M.R., et al., 2012. Entomological inoculation rates in 2009 on Bioko Island, Equatorial Guinea. Mal. J. 11, 88.
Oxborough, R.M., Kitau, J., Jones, R., et al., 2014. Long-lasting control of Anopheles arabiensis by a single spray application of micro-encapsulated pirimiphos-methyl (Actellic® 300 CS). Malar. J. 13, 37.
Pinto, J., Lynd, A., Elissa, N., et al., 2006. Co-occurrence of East and West African kdr mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville. Gabon. Med. Vet. Entomol. 20, 27–32.
Ranson, H., N'Guessan, R., Lines, J., et al., 2011. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 27, 91–98.
Reimer, L.J., Tripet, F., Slotman, M., et al., 2005. An unusual distribution of the kdr gene among populations of *Anopheles gambiae* on the Island of Bioko, Equatorial Guinea. Ins. Mol. Bio. 14, 683–688.
Sharp, B.L., Ridl, F.C., Govender, D., et al., 2007. Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. Malar. J. 6, 52.
Soderlund, D.M., Bloomquist, J.R., 1989. Neurotoxic actions of pyrethroid insecticides. Annu. Rev. Entomol. 34, 77–96.
Soderlund, D.M., Knipple, D.C., 2003. The molecular biology of knockdown resistance to pyrethroid insecticides. Inse. Bio-Chem. Mol. BioL. 33, 563–577.
Stanneck, D., Ebbinghaus-Kintscher, U., Schoenhense, E., et al., 2012. The synergistic action of imidacloprid and flumethrin and their release kinetics from collars applied for ectoparasite control in dogs and cats. Parasit. Vectors 5, 73.
Vontas, J., Grigorakia, L., Hemingway, J., et al., 2018. Rapid selection of a pyrethroid metabolic enzyme CYP9K1 by operational malaria control activities. Proc. Natl. Acad. Sci. U.S.A [www.pnas.org/cgi/doi/10.1073/pnas.1719663115].
Vulule, J.M., Beach, R.F., Atieli, F.K., et al., 1994. Reduced susceptibility of *Anopheles gambiae* to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. Med. Vet. Entomol. 8, 71–75.
WHO, 2006. Indoor Residual Spraying: Use of Indoor Residual Spraying for Scaling Global Malaria Control and Elimination. WHO/HTM/MAL/2006.1112 Edn. World Health Organization, Geneva, Switzerland. http://www.who.int/malaria/publications/atoz/htm_mal_2006_1112/en/.
WHO, 2012. Global Plan for Insecticide Resistance Management in Malaria Vectors. World Health Organization, Geneva, Switzerland. https://www.who.int/malaria/publications/atoz/gpirm/en/.
WHO, 2013a. Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. Sec. Edn. World Health Organization, Geneva, Switzerland.
WHO, 2013b. Malaria Entomology and Vector Control: Guide for Participants. World Health Organization, Geneva Switzerland.
WHO, 2014. World Malaria Report 2014. World Health Organization, Geneva, Switzerland. http://www.who.int/malaria/publications/world_malaria_report_2014/en/.
WHO, 2015. Indoor Residual Spraying: an Operational Manual for Indoor Residual Spraying (IRS) for Malaria Transmission Control and Elimination. SeC. Edn. World Health Organization, Geneva, Switzerland.
WHO, 2016. World Malaria Report 2016. World Health Organization, Geneva, Switzerland. http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/.
WHO, 2017. Report of the Twentieth WHOPES Working Group Meeting. Review of Interceptor G2LN, DawaPlus 3.0 LN, DawaPlus 4.0 LN, SumiLarv 2 MR, Chlorfenapyr 240 SC. World Health Organization, Geneva, Switzerland.
WHO, 2018. World Malaria Report. World Health Organization, Geneva, Switzerland. https://www.who.int/malaria/publications/world-malaria-report-2018/en/.
WHO, 2019. List of WHO Prequalified Vector Control Products. World Health Organization, Geneva, Switzerland. https://www.who.int/pq-vector-control/prequalified-lists/LOPrequalifiedProducts20190411.pdf?ua=1.