Assessing Insecticide Susceptibility and Resistance Intensity of *Anopheles gambiae* s.l. Populations From Some Districts of Benin Republic, West Africa

Casimir Dossou Kpanou,1,2,7 Hermann W. Sagbohan,1,2 Arthur Sovi,1,3,4 Razaki Osse,1,5 Gil G. Padonou,1,2 Albert Salako,1 Filémon Tokponnon,1 Arsène Jacques Fassinou,1 Boulaïs Yoovogan,1,2 Udoka C. Nwangwu,6 Constantin J. Adoha,1,2 Esdras Mahoutin Odjo,1,2 Idolphonse Ahogni,1 Aboubakar Sidick,1 Lamine Saïd Baba-Moussa,2 and Martin Akogbéto1,2

1Département de Biologie des Vecteurs, Centre de Recherche entomologique de Cotonou (CREC), 06 BP 2604 Cotonou, Benin, 2Département de Zoologie, Faculté des Sciences et Techniques, University of Abomey-Calavi, 01 BP 526 Abomey-Calavi, Benin, 3Department of Sciences and Techniques for Animal and Fisheries Production, Faculty of Agronomy, University of Parakou, BP 123 Parakou, Benin, 4Department of Disease Control, Faculty of Infectious and Tropical Diseases, The London School of Hygiene and Tropical Medicine, Keppel St, London WC1E 7HT, UK, 5Département des Sciences Animales et Halieutiques, École de gestion et d’exploitation des systèmes d’élevage, Université Nationale d’Agriculture de Porto-Novo, BP 43 Kétou, Bénin, 6Department of Disease Surveillance, National Arbovirus and Vectors Research Centre (NAVRC), 4 Park Ave, GRA 400102, Enugu, Nigeria, and 7Corresponding author, e-mail: casimirkpanou@yahoo.com

**Abstract**

Pyrethroid resistance is widespread in sub-Saharan Africa. The objective of this study was to assess the insecticide resistance intensity in *Anopheles gambiae* s.l. (Diptera: Culicidae) in four districts of Benin in order to better understand how pyrethroid-only nets are likely to be effective. Thus, adult females of *An. gambiae* s.l., reared from field-collected larvae were used for assessing resistance intensity to permethrin and deltamethrin. They were tested at 1×, 5×, and 10× the diagnostic dose, using both WHO susceptibility tube testing and CDC bottle bioassays. Identification of molecular species, as well as of L1014F *Kdr* and *Ace*-1 *R* mutations was performed using the PCR. The level of expression of biochemical enzymes was also evaluated. Overall, moderate to high resistance intensity to permethrin and deltamethrin was observed, irrespective of the testing method. While the L1014F *Kdr* frequency was high (>75%), *Ace*-1 *R* was low (≤6%) in *An. gambiae* s.s. and *Anopheles coluzzii*, the two predominant species [52% (95% CI: 44.8–59.1) and 45% (95% CI: 38.0–52.2), respectively]. *Anopheles arabiensis* was found at very low frequency (3%, 95%CI: 1.1–6.4). For Biochemical analyses, α and β-esterases were over-expressed in all four districts, while mixed-function oxidases (MFOs) were over-expressed in only one. Overall, the two testing methods led to comparable conclusions, though there were a few inconsistencies between them. The moderate-high resistance intensity observed in the study area suggests that dual active-ingredient (AI) long-lasting insecticidal nets (LLINs) may provide better control of insecticide-resistant mosquitoes.

**Key words:** pyrethroid, resistance intensity, WHO susceptibility tube testing, CDC bottle bioassay

**Background**

In sub-Saharan Africa, malaria remains a major public health problem. It is transmitted by *Anopheles gambiae* s.l., the main vector of the disease in the region (Mouchet et al. 2004). To date, the main vector control tools include long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS).

However, in recent years, Benin, like several other sub-Saharan Africa countries, has faced the issue of vector resistance to insecticides (WHO 2020). Thus, insecticides to which vectors are resistant include pyrethroids such as permethrin, deltamethrin, and alphacypermethrin commonly incorporated in mosquito nets (Ngufor et al. 2015, Salako et al. 2018, Sovi et al. 2020a), as well as carbamates like bendiocarb
used for IRS (Aïkpon et al. 2014, Gnanguenon et al. 2015, Salako et al. 2018). Organophosphates are also not spared due to a decrease of susceptibility of An. gambiae s.l. to fenitrothion and propoxur (Aïkpon et al. 2014). However, there is no record of resistance to pirimiphos-methyl used for IRS in the Atacora region so far, though this cannot be ruled out in future, given the dynamic nature of the phenomenon (Grau-Bové et al. 2021). As a result, it is important that regular monitoring is carried out to detect the early occurrence of resistance in malaria vectors. This will better guide National Malaria Control Programs (NMCP) in their choice of insecticide-based tools.

Vector resistance to insecticides is mainly assessed by two methods, namely, WHO susceptibility tube testing and US Centers for diseases Control and Prevention (CDC) bottle bioassay. While the ranges of mortality rates defined by the WHO (WHO 2013b) after exposure of mosquitoes to the diagnostic doses provide information on the resistance status of mosquitoes, they do not provide details on their resistance intensity level. There was therefore a need to develop a tool that could assess the intensity of resistance in order to stratify the degree of resistance of the different mosquito populations tested. Indeed, measuring the intensity of vector resistance to insecticides is very important because it allows for a better assessment of its potential impact on the effectiveness of insecticide-based vector control tools. This is why the relatively new notion of resistance intensity testing involving exposure of mosquitoes to increasing doses of insecticides was initiated and first introduced in the WHO guideline in 2016 (WHO 2016). The present study aimed at assessing the pyrethroid resistance intensity of populations of An. gambiae s.l. from some southern and northern districts of Benin, using the WHO susceptibility tube testing and the CDC bottle bioassays. The comparability of the two methods as well as the resistance mechanisms involved were also evaluated.

Materials and Methods

Study Area

The study was conducted between September and November 2017 in two districts (Cotonou and Porto-Novo) of southern Benin where urban market gardening is practiced, and two others (Parakou and Kandi) of northern Benin where cotton cultivation is intensive (Fig. 1). These different agricultural practices are characterized by strong use of insecticides for the control of crop pests. In addition, the populations of these study districts are characterized by a strong culture of LLIN usage (Tokponnon et al. 2013), to protect themselves against mosquito bites.

The district of Cotonou (6°21′36″N, 2°26′24″E) covers an area of 79 km². It is located on a coastal strand made up of a strip of alluvial sand that stretches for about 200 km. Its relief is homogeneous enough. Its climate is composed of two rainy seasons (mid-July to mid-September and mid-November to mid-March) and two dry seasons (mid-July to mid-September and mid-November to mid-July). The heaviest rainfall is recorded in June, reaching 300 to 400 mm. Overall, the temperature varies between 18°C and 35°C. October). Generally, the average annual rainfall is between 800 and 1,300 mm. The temperature of this district fluctuates between 17°C and 39°C depending on the season. The prevailing winds observed there are the harmattan and the monsoon. The district of Kandi is located at an altitude of approximately 288 m.

Mosquito larvae collection Mosquito larvae were collected from various breeding sites of the four study districts, using dippers. These larvae kept in different trays labeled by collection site, were taken to the insectary of the Center for Research in Entomology of Cotonou (CREC) for rearing to the adult stage. The adult mosquitoes that emerged were caged, fed with a 10% sweetened juice, and maintained at a temperature of 27 ± 2°C and relative humidity of 75 ± 5%. Females of the An. gambiae s.l., morphologically identified using the identification key of Gillies and de Meillon (1968), were used for the insecticide susceptibility testing.

Resistance Intensity Testing

WHO Susceptibility Tube Testing

WHO susceptibility tube testing was performed according to the WHO protocol (WHO 2016), using females An. gambiae s.l. that were 2 to 5 d old. These mosquitoes were exposed to papers impregnated with permethrin0.75% (1x), 3.75% (5x) and, 7.5% (10x) as well as, deltamethrin 0.05% (1x), 0.25 (5x), and 0.5% (10x).

About 20 to 25 mosquitoes were introduced into 4 tubes lined with WHO test papers, treated with a given dose of an insecticide, for a 1-h exposure period. In parallel, the same number of mosquitoes was introduced into two tubes, each lined with an untreated paper serving as control. During the exposure, the number of mosquitoes knocked down was recorded at different time intervals (0, 10, 15, 20, 30, 45, 60 min). Postexposure, the mosquitoes were transferred to observation tubes at a temperature of 27 ± 2°C and relative humidity of 75 ± 5%, with free access to a 10% sweetened juice. Mortality after 24 h was determined and interpreted according to the WHO ranges (WHO 2016).

After testing, a subset of mosquitoes was used for molecular analyses.

CDC Bottle Bioassay

Insecticide resistance intensity was also assessed through the CDC bottle bioassay method. Thus, female specimens of An. gambiae s.l. aged 2 to 5 d were exposed to 250 ml Wheaton glass bottles coated with pyrethroid insecticides at 1x, 5x, and 10x the diagnostic doses (permethrin 21.5 μg, 107.5 μg, 215 μg active ingredient (ai)/ bottle, and deltamethrin 12.5, 62.5, 125 μg ai/ bottle) over 30 min exposure period. For each dose of the insecticide, 20–25 mosquitoes were introduced into each of the 4 coated bottles. A bottle coated with acetone only was used as control. After exposure, mortality was recorded at the diagnostic time of 30 min, according to the CDC guidelines (WHO 2016).

Identification of the Molecular Species of the An. gambiae Complex Species and Characterization of the L1014F Kdr and G119S Ace-1R Resistance Mutation

In each study district, 50 mosquitoes from the WHO susceptibility tube testing performed with permethrin 0.75% (1x), were analyzed
by PCR following the protocol of Santolamazza et al. (2008) to identify the sibling species of the An. gambiae complex.

The genotypes of the L1014F Kdr and G119S Ace-1R mutations were determined according to the protocols of Martinez-Torres et al. (1998) and Weill et al. (2004), respectively. The allelic frequency of these two mutations was evaluated in the molecular species identified within the An. gambiae complex in each study district.

Biochemical Analysis
Thirty to fifty An. gambiae s.l. females from the study districts, aged 2–5 d but not previously used for any test, were used for biochemical analyses. Prior to these analyses, the mosquito specimens were stored at –80°C in dry Eppendorf tubes. The level of expression of biochemical enzymes [mixed function oxidases (MFO), nonspecific esterases (α and β-esterases), and glutathione S-transferases (GST)] in the populations of An. gambiae s.l. from the four districts surveyed, as well as the Kisumu susceptible strain of An. gambiae s.s., was evaluated using the protocol of Hemingway et al. (1998).

Data Analysis
To determine the level of pyrethroid resistance intensity, mortality rates obtained 24 h post-WHO susceptibility testing, and at 30 min diagnostic time for the CDC bottle bioassays were interpreted according to the following WHO criteria (WHO 2016):

- ≤90% mortality at 1× dose and, 98–100% mortality at 5× dose: low resistance intensity.
- ≤98% mortality at 5× dose and, 98–100% mortality at 10× dose: moderate resistance intensity.
- ≤98% mortality at 10×: high resistance intensity.

Fig. 1. Map of the study area.
Confidence intervals of the mortality rates as well as of frequencies of the L1014F Kdr and G119S Ace-1R mutations were determined using the exact binomial test.

Logistic regression was performed to assess whether there is a spatial variation in the distribution of the molecular species of the An. gambiae complex between the southern and the northern districts.

The comparison of the activity of metabolic enzymes between populations of An. gambiae s.l. collected from the four study districts and the Kisumu susceptible laboratory strain was performed using the Mann-Whitney U test.

R statistical software, version 3.6.2 was used to perform all statistical analyzes.

Results

WHO Susceptibility Tube Testing for Evaluating Pyrethroid Resistance Intensity in An. gambiae s.l.

Pyrethroid resistance was observed in all four surveyed districts. Indeed, with permethrin 1x, the mortality rates ranged between 14.1% (95% CI: 7.5–23.4) and 52.1% (95% CI: 41.6–62.4). For deltamethrin 1x, they varied from 14.9% (95% CI: 8.4–23.7) to 40.4% (95% CI: 30.2–51.4) (Table 1).

Overall, with higher doses (5x and 10x), permethrin and deltamethrin mortality rates increased in all four districts (Table 1).

When considering the 10x diagnostic dose of the two tested insecticides, mortality rates ≥ 98% were observed only with permethrin in Parakou (99%, 95% CI: 94.5–99.9) and Kandi (99%, 95% CI: 94.5–99.9). This indicates moderate-high pyrethroid resistance intensity in An. gambiae s.l. (Table 1).

CDC Bottle Bioassay for Assessing Pyrethroid Resistance Intensity in An. gambiae s.l.

In the four districts surveyed, bottle bioassays performed with the diagnostic dose (1x) of permethrin and deltamethrin displayed mortality rates < 90%, which indicates resistance to these two pyrethroid insecticides. Also, mortality increased with insecticide dose (Table 2).

For permethrin 10x, mortality rates ≥ 98% were observed in Cotonou (98.9%, 95% CI: 94.0–99.9), Parakou (98.7%, 95% CI: 93.1–99.9), and Kandi (98.7%, 95% CI: 92.9–99.9). For deltamethrin 10x, a mortality rate higher than 98% was observed only in Parakou (98.8%, 95% CI: 93.6–99.9) (Table 2). Overall, these results confirm that the pyrethroid resistance intensity was moderate to high.

Frequency of the L1014F kdr and G119S Ace-1R Mutations in Molecular Species Identified Within the An. gambiae Complex From the 4 Districts Surveyed

Overall, in the study area, molecular species identification revealed that An. gambiae s.s. (52%, 95% confidence interval (CI): 44.8–59.1) and Anopheles coluzzii (45%, 95% CI: 38.0–52.2) were the two predominant species, followed by Anopheles arabiensis (3%, 95% CI: 1.1–6.4) which was found at very low frequency. In the southern districts, An. coluzzii predominated [100% (95% CI: 92.8–100) in Cotonou and, 74% (95% CI: 59.6–85.4) in Porto-Novo], while in the northern districts, An. gambiae s.s. was the main species [94% (95% CI: 83.5–98.7) in Parakou, and 88% (95% CI: 75.7–95.5) in Kandi]. An. arabiensis was only found in the northern part of the country, precisely in the Kandi district (Table 3). Results from the logistic regression analysis clearly show a significant spatial variation in the prevalence of the two major molecular species between the southern and northern districts of the country (P < 0.001) (Table 4).

In the study area, the L1014F Kdr mutation was near fixation in the An. gambiae complex, with similar mean frequencies between An. coluzzii (80.6%, 95% CI: 74.0–86.1) and An. gambiae s.s. (78.8%, 95% CI: 72.7–84.2). A similar trend was observed at the district level (Table 3).
The G119S Ace-1R mutation had a very low frequency in the study area. Its frequency in An. coluzzii ranged from 0% (95% CI: 0.0–45.9) in Parakou to 5% (95% CI: 1.6–11.3) in Cotonou, with a mean of 4.4% (95% CI: 1.9–8.6) for the whole study area. In An. gambiae s.s., it varied between 3.4% (95% CI: 0.7–9.6) in Kandi and 5.3% (95% CI: 1.7–11.9) in Parakou, with an average of 4.3% (95% CI: 1.9–8.1) in all four districts combined (Table 3). Overall, there was no significant difference between the mean frequencies of this mutation observed in An. coluzzii and An. gambiae s.s.

The low number of mosquitoes tested for some molecular species in some districts (3 individuals of An. coluzzii in Parakou, and 6 of An. arabiensis in Kandi) did not allow for an accurate estimate of the frequency of the two mutations.
Biochemical Analysis

Biochemical tests revealed an over-expression of the activity of α-esterases in populations of An. gambiae s.l. collected from Porto-Nov, Parakou, and Kandi (P < 0.05) (Fig. 2a). The over-expression of β-esterases occurred in all four study districts (P < 0.05) (Fig. 2b).

The activity of MFOs was over-expressed in the An. gambiae s.l. population from Kandi, when compared with the Kisumu susceptible strain (P = 0.0002) (Fig. 2c).

Regarding the GSTs, an over-expression of their activity was observed in An. gambiae s.l. populations from Cotonou (P < 0.0001), Parakou (P = 0.0212), and Kandi (P = 0.026), compared to the Kisumu susceptible strain (Fig. 2d).

Table 4. Logistic regression assessing the spatial variation of the proportion of Anopheles culuzii and Anopheles gambiae s.s. between the southern and the northern districts

| Molecular species | N(%) | Coeff OR (95%CI) | P (wald test) | P (LR test) |
|-------------------|------|-----------------|--------------|-------------|
| An. coluzzii | 87 (96.7%) | 3 (3.3%) | 3.3673 | < 0.001 | < 0.001 |
| An. gambiae s.s. | 13 (12.5%) | 91 (87.5%) | -5.3132 | 0 (0–0.02) | |

N, number of molecular species; %, percentage of molecular species; Coeff, coefficient; CI, confidence intervals

Discussion

Through two testing methods (WHO susceptibility tube testing and CDC bottle bioassay), this study provides information on the resistance intensity of An. gambiae s.l. to deltamethrin and permethrin, two insecticides commonly incorporated into mosquito nets used in Benin. The trial also enabled determination through PCR of the presence of different mechanisms of resistance to insecticides, as well as the frequency of molecular species of the An. gambiae complex in some southern and northern districts of the country.

Overall, with the two susceptibility testing methods used, the four populations of An. gambiae s.l. were all resistant to the diagnostic doses of permethrin and deltamethrin (mortality <90%). This
confirms the widespread resistance of malaria vectors to pyrethroids that has been previously observed by several authors in Benin (Corbel et al. 2007, Yadouleton et al. 2009, Djogbenou et al. 2010, Salako et al. 2018).

The tests conducted with insecticides at 5x and 10x the diagnostic dose showed that the intensity of resistance varied from moderate to high. Overall, in terms of resistance intensity, the two methods used had generally led to comparable conclusions except in a few cases (high resistance intensity through WHO susceptibility tube testing, versus moderate resistance intensity through CDC bottle bioassay with permethrin in Cotonou, and deltamethrin in Kandi). This is due to the mortality rates being generally higher with CDC bottle bioassay compared to WHO susceptible tube testing. The opposite trend was observed in Mali by Sovi et al. (2020b). This inconsistency could be due to the self-coating of the Wheaton glass bottles, contrary to the WHO-treated papers that are standardized. Indeed, the self-coating of bottles is more likely to cause technician-induced mortality rate variations. This is in line with works by Owusu et al. (2015), which showed that the two testing methods can help detect resistance but display a number of inconsistencies.

Moderate to high resistance intensity observed in the study area, could be due to the massive pyrethroid-only LLINs distribution performed nationwide every three years since 2011 and the strong culture of net usage of the population (Tokponnon et al. 2013). Other factors contributing to the selection of highly resistant individuals within the natural population of mosquitoes could be the uncontrolled use of insecticides for agricultural purposes as well as the domestic use of aerosol insecticides.

The predominance of An. coluzzi and An. gambiae s.s. respectively in the southern and the northern parts of the country, concur with data from previous studies (Salako et al. 2018, Sovi et al. 2020a). To clarify this trend, a further scrutiny investigation on the distribution of the different types (temporary, permanent, and semi-permanent) of larval habitats and other factors that might have contributed to that deserve to be conducted in these two parts of the country.

Overall, the L1014F Kdr frequency was high in the An. gambiae complex. At the molecular species level, the frequency of this mutation was similar between An. coluzzi and An. gambiae s.s., which suggests that the two species might have been under similar selection pressure. In addition, the over-expression of MFOs in mosquito populations from Kandi is worrisome as this enzyme detoxifies pyrethroid insecticides in insects. This is reminiscent of results obtained by Aïzoun et al. (2013) in the same district. Due to the oxidative stress, the over-expression of GST observed in most districts of the study area might have played a nonsignificant role in the resistance of An. gambiae s.l.to pyrethroids (Hemingway et al. 2004). Findings from previous studies performed in Benin showed involvement of the N1575Y Kdr mutation (Jones et al. 2012) and of the over-expression of cytochrome P450s genes (Ngufor et al. 2015) detected through the taqman qPCR assays in resistance of populations of An. gambiae s.l. to pyrethroids. The fact that the present study did not look for these resistance mechanisms constitutes limitations.

The over-expression of α and β-estersases, as well as the presence of a few homozygous (RS) individuals for the G119S Ace-1s mutation in all four districts surveyed, stress the need for close monitoring of phenotypic resistance to organophosphate and carbamate insecticides.

According to the WHO, an operational failure is likely to occur when there is confirmed resistance at the 5x or at the 10x the diagnostic dose (WHO 2016). Given that this was observed in natural populations of An. gambiae s.l. of the four districts surveyed, pyrethroid-only LLINs may not provide optimal protection against the vectors. Thus, dual active-ingredient LLINs such as: Piperonyl Butoxide (PBO)-LLINs [Olyset Plus (PBO + Permethrin), VEERALIN (PBO + Alphacypermethrin), Permanet 3.0 (PBO + Deltamethrin)] could be considered as an alternative option, though synergistic PBO bioassays were not carried out in the present study, which is a limitation. Indeed, following a randomized controlled trial that showed a better performance of PBO-LLINs over standard ones in Tanzania (Protopopoff et al. 2018), WHO has recommended PBO-LLINs for use in areas with moderate-high resistance intensity at least partly due to oxidoases (WHO 2017). Other viable options could also be pyriproxyfen (PfP)-LLINs [Royal Guard (PfP + Alphacypermethrin), Olyset Duo (PfP + Permethrin)], or Interceptor G2 (Chlorfenapyr + Alphacypermethrin). However, the higher per-unit cost of these LLINs as well as the lack of evidence of epidemiological performance for some of them over pyrethroid-only LLINs, limit their deployment in the communities.

Conclusion

In terms of resistance intensity, WHO susceptibility tube testing and CDC bottle bioassay provide comparable conclusions even though in some cases the two testing methods display some inconsistencies. The moderate to high pyrethroid resistance intensity observed in An. gambiae s.l. with involvement of the L1014F Kdr mutation and over-expression of the MFOs suggest that dual active-ingredient LLINs that manufacturers are currently developing may provide better control of insecticide-resistant malaria vectors.

Acknowledgments

We thank the US President's Malaria Initiative that financially supported the present study through the United State Agency for International Development (Implementation Letter ≠37). We also acknowledge the US Centers for Diseases Control and Prevention for supplying reagents, and the technicians who conducted the insecticide susceptibility testing and the molecular work.

References Cited

Aïkpon, R., M. Sézonlin, R. Ossé, and M. Akogbétò. 2014. Evidence of multiple mechanisms providing carbamate and organophosphate resistance in field An. gambiae population from Atacora in Benin. Parasit Vectors. 7:368.

Aïzoun, N., R. Aïkpon, V. Gnangueno, O. Oussou, F. Agossa, G. Padonou, and M. Akogbétò. 2013. Status of organophosphate and carbamate resistance in Anopheles gambiae s.s. from southern and north Benin, West Africa. Parasit Vectors. 6:274.

Corbel, V., R. N’Guessan, C. Brengues, F. Chandre, L. Djogbenou, T. Martin, M. Akogbétò, J. M. Hougourd, and M. Rowland. 2007. Multiple insecticide resistance mechanisms in Anopheles gambiae and Culex quinquefasciatus from Benin, West Africa. Acta Trop. 101: 207–216.

Djogbéniou, L., N. Pasteur, S. Bio-Bangana, T. Baldet, S. R. Irish, M. Akogbétò, M. Weill, and F. Chandre. 2010. Malaria vectors in the Republic of Benin: distribution of species and molecular forms of the Anopheles gambiae complex. Acta Trop. 114: 116–122.

Gilles, M.T., and B. de Meillon. 1968. The anopheline of Africa South of the Sahara. South African Institute for Medical Research. 54: 343.

Gnanguenou, V., F. R. Agossa, K. Badiro, R. Govoetchan, R. Anagounou, F. Oke-Agbo, R. Azondekon, Y. R. Agbanrin, R. Attolou, F. T. Tokponnon, et al. 2015. Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved. Parasit Vectors. 8: 223.

Grau-Bové, X., E. Lucas, D. Pipist, E. Rippon, A. E. van ’t Hof, E. Constant, S. Dadzie, A. Egyir-Yawson, J. Essamb, J. Chahri, et al.; Anopheles gambiae 1000 Genomes Consortium. 2021. Resistance to pirimiphos-methyl in
West African Anopheles is spreading via duplication and introgression of the Ace1 locus. Plos Genet. 17: e1009253.

Hemingway, J., N. Hawkes, L. Prapanthadara, K. G. Jayawardenal, and H. Ranson. 1998. The role of gene splicing, gene amplification and regulation in mosquito insecticide resistance. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 353: 1695–1699.

Hemingway, J., N. J. Hawkes, L. McCarroll, and H. Ranson. 2004. The molecular basis of insecticide resistance in mosquitoes. Insect Biochem. Mol. Biol. 34: 653–665.

INSAE. 2013. Population size of villages and city districts in Benin (RGPH-4, 2013). National Institute of Statistics and Economic Analysis, Porto-Novo, Benin.

Jones, C. M., M. Liyanapathirana, F. R. Agossa, D. Weetman, H. Ranson, M. J. Donnelly, and C. S. Wilding. 2012. Footprints of positive selection associated with a mutation (N1575Y) in the voltage-gated sodium channel of Anopheles gambiae. Proc. Natl. Acad. Sci. U. S. A. 109: 6614–6619.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Bergé, A. L. Devons, P. Guillen, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Insect Mol. Biol. 7: 179–184.

Mouchet, J., P. Carnevale, M. Roosemans, J. Juvelz, S. Manguin, D. Richard Lenoir, J. Sircoulon. 2004. Biodiversity of Malaria Worldwide. Editions John Libbey Eurotext, Montrouge, France.

Ngufor, C., R. N’Guessan, J. Fagbohoun, K. Subramaniam, A. Odjo, A. Fongnkin, M. Akogbeto, D. Weetman, and M. Rowland. 2015. Insecticide resistance profile of Anopheles gambiae from a phase II field station in Covi, southern Benin: implications for the evaluation of novel vector control products. Malar. J. 14: 464.

Owuwa, H. F., D. Janáčkoyová, D. Malone, and P. Müller. 2015. Comparability between insecticide resistance bioassays for mosquito vectors: time to review current methodology? Parasit. Vectors 8: 337.

Protopopoff, N., J. F. Mosha, E. Lukole, J. D. Charlwood, A. Wright, C. D. Mwalimu, A. Manjurano, F. W. Mosha, W. Kisinza, I. Kleinschmidt, et al. 2018. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. Lancet. 391: 1577–1588.

Salako, A. S., I. Ahogni, R. Aikpon, A. Sidick, F. Dagnon, A. Sovi, A. Sominahouf, F. Agossa, L. Iyike, and M. C. Akogbeto. 2018. Insecticide resistance status, frequency of L1014F Kdr and G119S Ace-1 mutations, and expression of detoxification enzymes in Anopheles gambiae (s.l.) in two regions of northern Benin in preparation for indoor residual spraying. Parasit. Vectors. 11: 618.

Santolamazzza, F., M. Calzetta, J. Etang, E. Barrese, I. Dia, A. Caccone, M. J. Donnelly, V. Petrarca, F. Simard, J. Pinto, et al. 2008. Distribution of knock-down resistance mutations in Anopheles gambiae molecular forms in west and west-central Africa. Malar. J. 7: 74.

Sovi, A., R. Govoetchan, R. Osté, C. Z. Koukpo, A. S. Salako, T. Syme, R. Anagonou, A. Fongnkin, U. C. Nwangwu, F. Oké-Agbo, et al. 2020a. Resistance status of Anopheles gambiae s.l. to insecticides following the 2011 mass distribution campaign of long-lasting insecticidal nets (LLINs) in the Plateau Department, south-eastern Benin. Malar. J. 19: 26.

Sovi, A., C. Keita, Y. Sina, A. Dicco, I. Traore, M. B. M. Cisse, O. Kosta, D. Dengela, C. Flatley, E. Bankineza, et al. 2020b. Anopheles gambiae (s.l.) exhibit high intensity pyrethroid resistance throughout Southern and Central Mali (2016-2018): PBO or next generation LLINs may provide greater control. Parasit. Vectors. 13:239 b.

Tokponnon, F. T., B. Aholoukpe, E. Y. Denon, V. Gnanguenon, A. Bokossa, R. N’guessan, M. Oke, D. K. Hazard, and M. C. Akogbeto. 2013. Evaluation of the coverage and effective use rate of long-lasting insecticidal nets after nation-wide scale up of their distribution in Benin. Parasit. Vectors. 6: 265.

Weill, M., C. Malcolm, F. Chandre, K. Mogensen, A. Berthomieu, M. Marquine, and M. Raymond. 2004. The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. Insect Mol. Biol. 13: 1–7.

World Health Organization. 2013b. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva.

World Health Organization. 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes – 2nd ed. World Health Organization, Geneva. https://apps.who.int/malaria/publications/a9789241511575/en/. Accessed 4 August 2021.

World Health Organization. 2017. Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide: recommendations. World Health Organization. https://apps.who.int/iris/handle/10665/258939. Accessed 19 August 2021. Licence: CC BY-NC-SA 3.0 IGO.

World Health Organization. 2020. World Malaria Report. World Health Organization, Geneva.

Yadouleton, A. W., A. Asidi, R. F. Djouaka, J. Braima, C. D. Agossou, and M. C. Akogbeto. 2009. Development of vegetable farming: a cause of the emergence of insecticide resistance in populations of Anopheles gambiae in urban areas of Benin. Malar. J. 8: 103.