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Dynamics of pyrethroid resistance in *Anopheles gambiae* sensu lato from the south-north transect Benin, West Africa

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Abstract

The current study was aimed to study the dynamics of deltamethrin resistance in *Anopheles gambiae* sensu lato from the south-north transect Benin, West Africa. Larvae and pupae of *Anopheles gambiae s.l.* populations were collected from the breeding sites in Atlantic, collines and Borgou departments in 2015 and 2020. WHO susceptibility tests were conducted on unfed female mosquitoes aged 2-5 days old. WHO bioassays were performed with impregnated papers of deltamethrin (0.05%). *An. gambiae* mosquitoes were identified to species using PCR techniques in 2015. Molecular assays were also carried out to identify *kdr* mutations in individual mosquitoes. *An. gambiae s.l.* populations from Allada, Dassa-Zoume and Parakou were resistant to deltamethrin in 2015 and still remained resistant to this product in 2020. PCR revealed 100% of mosquitoes tested were *Anopheles gambiae s.s*. The *L1014F kdr* mutation was found in *An. gambiae s.s.* Allada, Dassa-Zoumè and Parakou at various allelic frequencies. Female *An. gambiae s.l.* populations from the south-north transect Benin were resistant to deltamethrin with high allelic frequencies of *L1014F kdr* mutation. With the rapid spread of pyrethroid resistance in the malaria vectors from the main ecological settings and the various resistance mechanisms involved, the geographic distribution of vector susceptibility to pyrethroids is critically needed as it will provide baseline information for vector control. In order to guide future malaria vector control interventions in Benin, the presence though at high frequency of the West African *kdr* mutation in *Anopheles gambiae* populations from the main ecological settings needs to be carefully monitored in the country.

Keywords: Dynamics, Malaria vectors, Resistance, deltamethrin, *Kdr* mutation, Benin

Introduction

In 2012, World Health Organization (WHO) estimated that 207 million case of malaria occurred worldwide with 627000 deaths. Most cases (80%) and deaths (90%) occurred in Africa, and most deaths (77%) were in children under 5years of age (WHO, 2013a).

In Benin, Malaria is a major health problem. It is the main cause of morbidity and mortality particularly among children under five and pregnant women. It is transmitted by *Anopheles* mosquitoes, and because there is currently no vaccine available, vector control is one of the most important means of malaria prevention. This vector control is generally done with insecticides.

In this country as across Africa, malaria control relies heavily on vector control through the use of insecticide-treated nets (ITN) and indoor residual spraying (IRS). In West Africa, the main mechanism involved in pyrethroid-resistance in *Anopheles gambiae* is caused by target site insensitivity through a knockdown resistance (*kdr*)-like mutation caused by a single point mutation (Leu-Phe) in the para-sodium channel gene (Chandre *et al*., 1999). Malaria vector resistance to insecticides in Benin is conferred by two main mechanisms: (1) alterations at site of action in the sodium channel, viz the *kdr* mutations and (2) an increase of detoxification and/or metabolism through high levels of multi-function oxidases (MFOs), non-specific esterases (NSEs) (Corbel *et al*., 2007; Djogbénou *et al*., 2009; Djègbé *et al*., 2011; Aïzoun *et al*., 2013a; Aïzoun *et al*., 2013b).

Beninese National Malaria Control Programme has implemented large-scale and free distribution of long-lasting insecticidal nets (LLINs) recently through the entire country to increase coverage of LLINs. It is crucial that information on current status of *An. gambiae s.l.* resistance to pyrethroid being investigated. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies. In this study, we report the assessment of the susceptibility status, insecticide resistance levels in *Anopheles gambiae s.l.* to deltamethrin to evaluate the presence and extent of the distribution of the *kdr* mutation within and among these *An. gambiae s.l.* populations in the south-north transect Benin, where pyrethroid resistance was also recently reported in *An. gambiae* (Djègbé *et al*., 2011; Aïzoun *et al.*, 2013a; Aïzoun *et al.*, 2013b).

Materials and Methods

Study area

The study was carried out in some localities following a south-north transect Benin. Three contrasting localities of Benin were selected for mosquito collection on the basis of variation in agricultural production, use of insecticides and/or ecological settings. The localities were: Dassa-Zoumè a rice growing area located in the middle part of the country. Allada is a cereal growing area (maize, ground-nut and so on) located in the south part of the country. Parakou, an urban vegetable growing area located in the north of Benin. The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have a direct impact on the development of insecticide resistance in the local mosquito vectors.

The southern region is characterized by a tropical Guinean climate with two rainy seasons (April to July and September to November) with a mean annual rainfall over 1,500 mm. The middle part of the country is characterized by a Sudano Guinean climate with two rainy seasons (March to July and August) with an average rainfall of 1,000 mm per year. The northern zone is characterized by a Sudanian climate with only one rainy season per year (May to October) and one dry season (November to April). The temperature ranged from 22 to 33°C with the annual mean rainfall which is 1,300 mm.

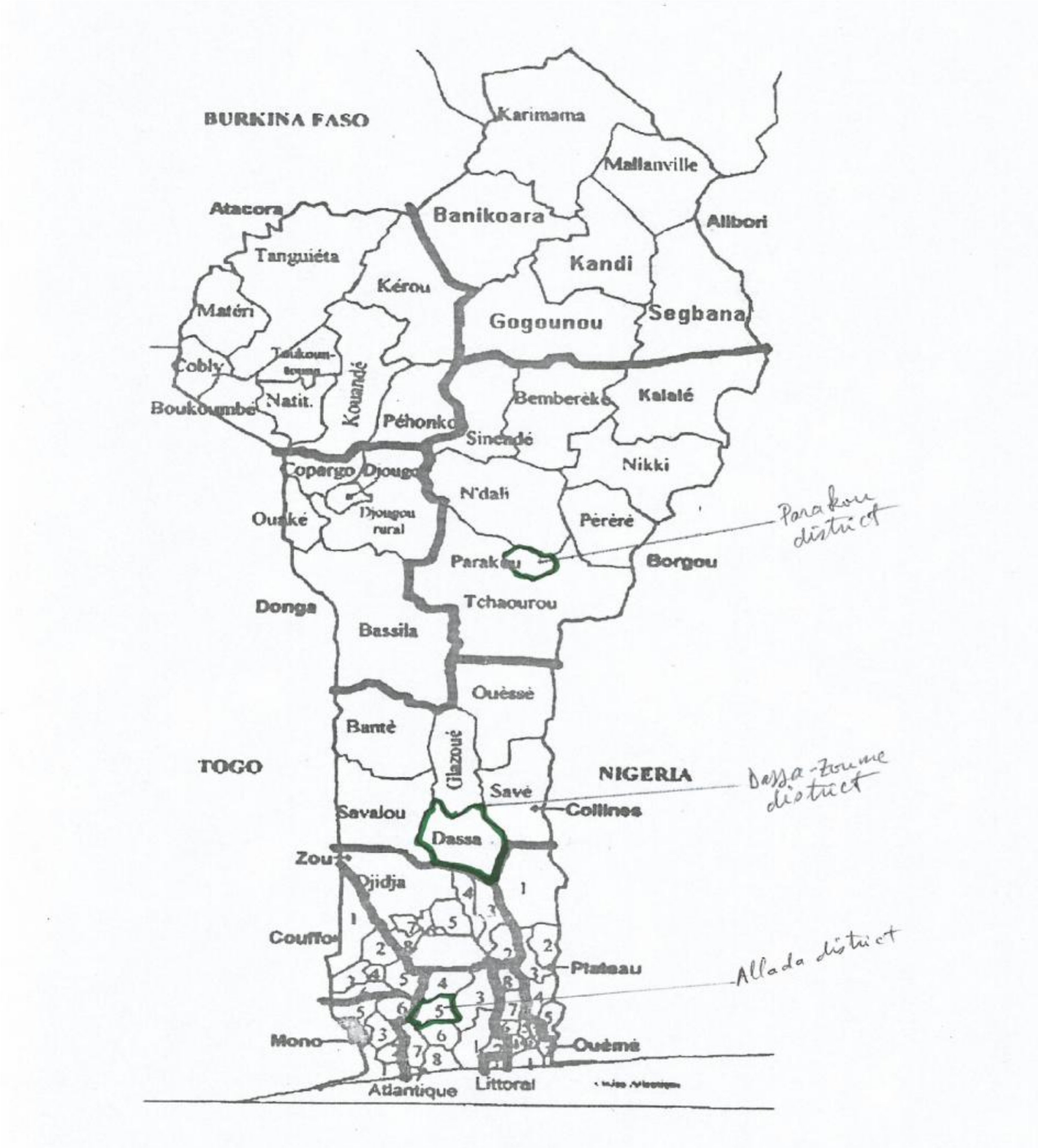

Figure 1 Map of Republic of Benin showing districts surveyed

Mosquito sampling

An. gambiae s.l. populations were collected from April to July and September to November 2015 and 2020 during the rainy season in Allada locality selected in south Benin. Larvae and pupae were collected in Allada district within both padding and village using the dipping method on several breeding sites (brick pits, pools, marshes, streams, ditches, pits dug for plastering traditional huts, puddles of water, water pockets caused by the gutters).

An. gambiae s.l. populations were also collected from July to November 2015 and 2020 in Dassa-Zoumè district selected in the centre part of the country. *Anopheles* pre-imaginal stages (L1 to L4 instars) were collected via ladles within rice farms, Lema, from Dassa- Zoumè. Due to that the farms are irrigated, breeding sites are present throughout the year and we therefore assumed that the larvae collected in the study period were representative of the population that could be found during other periods of the year.

An. gambiae s.l. populations were also collected from May to October 2015 and 2020 during the rainy season in Parakou district selected in north Benin. *Anopheles* pre-imaginal stages (L1 to L4 instars) were collected via ladles within vegetable farms from Parakou. In these three districts, larvae and pupae were collected using the dipping on breeding sites and then kept in separated labeled bottles related to each locality. Otherwise, larvae collected from multiple breeding sites were pooled together related to each locality then re-distributed evenly in development trays containing tap water.

Larvae were provided access to powdered TetraFin® fish food, and were reared to adults under insectary conditions of 25+/- 2°C and 70 to 80% relative humidity at Center of Entomological Researches of Cotonou (CREC) located in Akpakpa, in Cotonou district in 2015 and in insectary of Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin in 2020. *An. gambiae s.l.* Kisumu, a reference susceptible strain was used as a control for the bioassay tests.

Susceptibility tests were done following WHO protocol on unfed females mosquitoes aged 2-5 days old reared from larval and pupal collections. All susceptibility tests were conducted in 2015 in the CREC laboratory at 25+/-2°C and 70 to 80% relative humidity whereas in 2020, susceptibility tests were conducted in Laboratory of Applied Entomology and Vector Control (LAEVC).

Testing insecticide susceptibility

The principle of the WHO bioassay is to expose insects to a given dose of insecticide for a given time to assess susceptibility or resistance. The standard WHO discriminating dosages are twice the experimentally derived 100% lethal concentration (LC100 value) of a reference susceptible strain (WHO, 1998a). In this study, the insecticide tested was deltamethrin (0.05%). The choice of deltamethrin was justified by its use on Long Lasting Insecticidal Nets which were used by National Malaria Control Programme for implementation of large-scale and free distribution through the entire country to increase coverage.

An aspirator was used to introduce 20 to 25 unfed female mosquitoes aged 2–5 days into five WHO holding tubes (four tests and one control) that contained untreated papers. They were then gently blown into the exposure tubes containing the insecticide impregnated papers. After one-hour exposure, mosquitoes were transferred back into holding tubes and provided with cotton wool moistened with a 10% honey solution. The number of mosquitoes "knocked down" at 60 minutes and mortalities at 24 hours were recorded following the WHO protocol (WHO, 1998a).

PCR detection of species and the *kdr* mutation

At the end of WHO bioassays in 2015, a polymerase chain reaction test for species identification (Scott *et al*., 1993) was performed to identify the members of *An. gambiae* complex collected from each site. PCR for the detection of the *kdr* Leu-phe mutation was carried out on alive *An. gambiae* mosquitoes as described by Martinez-Torres *et al.* (1998).

Statistical analysis and data interpretation

The resistance status of mosquito samples was determined according to the WHO criteria (WHO, 2013b) as follows:

-Mortality rates between 98%-100% indicate full susceptibility
-Mortality rates between 90%-97% require further investigation
-Mortality rates < 90%, the population is considered resistant to the tested insecticides.

Abbott s formula was not used in this study for the correction of mortality rates in test tubes because the mortality rates in control tube were less than 5% (Abbott, 1987).

The correlation between the results of insecticide susceptibility and molecular results (*kdr* frequencies) was also assessed for each of the districts surveyed.

ANOVA test was performed with mortality rate as the dependent variable and the localities as a covariate. ANOVA test was also performed with *kdr* frequency as the dependent variable and the localities as a covariate.

Results and Discussion

Evolution of *Anopheles gambiae s.l.* populations resistance to deltamethrin in Allada, Dassa-Zoumè and Parakou districts from 2015 to 2020

Kisumu strain (control) confirmed its susceptibility status as a reference strain. The 24 hours mortality recording shows that female *Anopheles gambiae* Kisumu which were exposed to WHO papers impregnated with deltamethrin 0.05% were fully susceptible to this product. They were dead and none of them could fly after 24 h mortality recording required by WHO (Table 1).

Regarding field collected female *Anopheles gambiae s.l.* populations from Allada, Dassa-Zoumè and Parakou, they were resistant to deltamethrin with the mortality rates of 53%, 61% and 86% respectively in 2015. These *Anopheles gambiae s.l.* populations still remained resistant to the same product in 2020 with the mortality rates of 47%, 58% and 82% respectively (Table 1).

Univariate logistic regression, performed with mortality rate as the dependent variable and localities as a covariate with ANOVA test showed that the phenotypic resistance to deltamethrin was associated with the localities ($p < 0.05$). In addition, univariate logistic regression, performed with *kdr* frequency as the dependent variable and localities as a covariate with ANOVA test, showed that high *kdr* frequency was associated with the localities ($p < 0.05$).

Table-1: Mortality of *An. gambiae s.l.* populations from Allada, Dassa-Zoumè and Parakou districts after one hour exposure to WHO impregnated papers with deltamethrin (0.05%) in 2015 and 2020

Locations	Years	Insecticide	Number tested	%Mortality	Resistance status
Kisumu (Control)	2015	Deltamethrin	100	100	S
	2020	Deltamethrin	100	100	S
Allada	2015	Deltamethrin	100	53	R
	2020	Deltamethrin	100	47	R
Dassa-Zoumè	2015	Deltarmethrin	100	61	R
	2020	Deltamethrin	100	58	R
Parakou	2015	Deltamethrin	100	86	R
	2020	Deltamethrin	100	82	R

Mosquito species identification

PCR revealed that 100% of mosquitoes tested were *Anopheles gambiae s.s.* (Table 2).

Table-2: *Kdr* frequency in surviving *An. gambiae* populations from Allada, Dassa-Zoumè and Parakou districts 24 h post-exposure to WHO impregnated papers with deltamethrin in 2015

			Kdr mutation			
Locations	Number tested	Species Ag	RR	RS	SS	F(*Kdr*)
Allada	25	25	15	6	4	0.72
Dassa-Zoumè	22	22	18	4	0	0.91
Parakou	21	21	15	5	1	0.83

Ag: *An. gambiae s.s.*

Detection of the *Kdr* mutation

The results of molecular tests performed on *Anopheles gambiae* populations from Allada, Dassa-Zoumè and Parakou districts revealed very high frequencies of *Kdr* mutation. These various allelic frequencies of *L1014F kdr* mutation were 72%, 91% and 83% respectively (Table 2).

The monitoring of insecticide resistance in malaria vectors is of prime importance especially where control programmes are planned or already running in order to assess potential selection effects of insecticidal compounds on vector populations, and to take appropriate measures such as switching to other classes of compounds.

Female *Anopheles gambiae s.l.* populations from Allada, Dassa-Zoumè and Parakou, were resistant to deltamethrin in 2015. These *Anopheles gambiae s.l.* populations still remained resistant to the same product in 2020. So, *Anopheles gambiae s.l.* natural populations have developed resistance to deltamethrin in the different bio-climatic areas surveyed in the south-north transect Benin. The management of insecticide resistance is a major issue, which must interest the different National Malaria Control Programmes. This management requires two kinds of information: sound knowledge of the mechanisms of resistance and a thorough resistance monitoring programme (Aïzoun *et al.*, 2013a).

The resistance levels to deltamethrin observed with *Anopheles gambiae s.l.* populations from Allada and Dassa-Zoumè were higher than the one observed with *Anopheles gambiae s.l.* populations from Parakou. In fact, Allada, a cereal growing area and Dassa-Zoumè, a rice growing area are two localities where no insecticidal products are generally used to control agricultural pests comparatively to the vegetable growing area of Parakou where various insecticidal products are used for this purpose. According to Corbel *et al.* (2007), in the vegetable growing area of Parakou, the low level of pyrethroid-resistance observed in *An. gambiae s.l.* was explained by the presence of (i) a relatively high proportion of susceptible *An. arabiensis* mosquitoes, (ii) a rather low *kdr* allelic frequency in *An. gambiae s.s.* and (iii) the absence of metabolic-based resistance. But, in the current study, all *Anopheles gambiae* specimens from Parakou tested were *Anopheles gambiae s.s.* No *An. arabiensis* mosquitoes were found. This result showed that *An. arabiensis* populations from vegetable growing area of Parakou tend to decline. The main reason of this decrease in *An. arabiensis* populations from Parakou was that the proportion of *An. arabiensis* mosquitoes from Parakou found by Corbel *et al.* (2007) was almost susceptible.

The presence and frequency of the *kdr* mutations constitute a valuable and useful resistance marker for two main reasons. First, it provides an early warning of resistance development as the mutation arises well before any effect on phenotype can be detected in a population (Kelly-Hope *et al.*, 2008). Indeed, the expression of the 24h-survival diagnostic phenotype (WHO, 1998b) appear to be recessive (Chandre *et al*., 2000; Corbel *et al*., 2004).

The *kdr* frequency recorded in *Anopheles gambiae* populations from Parakou was 0.83 in the current study. The *kdr* frequency recorded in the same *Anopheles gambiae* populations in 2007 by Corbel *et al.*, (2007) was 0.20. In similar way, the *kdr* frequencies in *Anopheles gambiae* populations from Dassa-Zoumè and Allada in 2008 were 0.46 and 0.33 respectively (Djogbenou *et al.*, unpublished data). In the current study, the *kdr* frequencies recorded in these same *Anopheles gambiae* populations were 0.91 and 0.72 respectively. These results showed that *kdr* frequency in these *Anopheles gambiae* populations has significantly increased after five years. This is consistent with previous observations reporting an increase of the *kdr L1014F* frequency in *An. gambiae* following a nationwide distribution of long-lasting insecticide-treated nets in Niger (Czeher *et al*., 2008).

The *kdr* frequency in *Anopheles gambiae* populations from Dassa-Zoumè recorded in this study was higher than the one observed in *Anopheles gambiae* populations from Parakou. By contrast to the finding of Diabaté *et al*. (2002) in Burkina Faso, the agricultural use of insecticide was not always a source of selection pressure for resistance in *An. gambiae s.l.* as *kdr* frequency in *Anopheles gambiae* Dassa-Zoume was higher than the one observed in *Anopheles gambiae* Parakou. In fact, Dassa-Zoume is a rice growing area where no insecticidal products are generally used to control agricultural pests comparatively to the vegetable growing area of Parakou where various insecticidal products are used for this purpose.

Conclusion

Female *An. gambiae s.l.* populations from the south-north transect Benin were resistant to deltamethrin with high allelic frequencies of *L1014F kdr* mutation. With the rapid spread of pyrethroid resistance in the malaria vectors from the main ecological settings and the various resistance mechanisms involved, the geographic distribution of vector susceptibility to pyrethroids is critically needed as it will provide baseline information for vector control. In order to guide future malaria vector control interventions in Benin, the presence though at high frequency of the West African *kdr* mutation in *Anopheles gambiae* populations from the main ecological settings needs to be carefully monitored in the country.

References

1: Abbott, W.S. (1987): A method of computing the effectiveness of an insecticide. *J. Am. Mosq. Control Assoc*, 3(2): 302 303.

2: Aïzoun, N., Ossè, R., Azondekon, R., Alia, R., Oussou, O., Gnanguenon, V., Aïkpon,, R., Padonou , G.G., Akogbéto, M. (2013a): Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. *Parasit. Vectors*, 6: 147.

3: Aïzoun, N., Aïkpon, R., Padonou, G.G., Oussou, O., Oké-Agbo, F., Gnanguenon, V., Ossè, R., Akogbéto, M. (2013b): Mixed function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in *Anopheles gambiae s.l.* in the south-north transect Benin, West Africa. *Parasit. Vectors*, 6: 223.

4: Chandre, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J., Guillet, P., (1999): Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bull. World Health Organ*, 77(3)**:** 230 234.

5: Chandre, F., Darriet, F., Duchon, S., Finot, L., Manguin, S. *et al*. (2000): Modifications of pyrethroid effects associated with kdr mutation in Anopheles gambiae. Med. Vet. Entomol, 14**:** 81-88.

6: Corbel, V., Chandre, F., Brengues, C., Akogbéto, M., Lardeux, F., *et al*. (2004): Dosage-dependent effects of permethrin-treated nets on the behaviour of Anopheles gambiae and the selection of pyrethroid resistance. Malar. J, 3**:** 22.

7: Corbel, V., N'Guessan, R., Brengues, C., Chandre, F., Djogbenou, L., Martin, T., Akogbeto, M., Hougard, J-M., Rowland, M. (2007): Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. *Acta Tropica*, 101: 207 216.

8: Czeher, C., Labbo, R., Arzika, I., Duchemin, J.B. (2008): Evidence of increasing Leu-Phe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticidetreated nets implementation. *Malar. J*, 7: 189.

9: Diabaté, A., Baldet, T., Chandre, F., Akogbeto, M., Darriet, F., Brengues, C. *et al*. (2002): The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae sl* in Burkina Faso. *Am. J. Trop. Med. Hyg*, 67: 617 622.

10: Djègbé, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., Akogbéto, M., and Corbel, V. (2011): Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S *kdr* mutation in *Anopheles gambiae* from West Africa. *Malar. J*, 10: 261.

11: Djogbénou, L., Pasteur, N., Akogbéto, M., Weill, M., Chandre, F. (2009): Insecticide resistance in the *Anopheles gambiae* complex in Benin: a nationwide survey. *Med. Vet. Entomology*, 69: 160 164.

12: Kelly-Hope, L., Ranson, H., and Hemingway, J. (2008): Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. Lancet Infect Dis, 8**:** 387-389.

13: Martinez-Torres, D., Chandre, F., Williamson, M.S., Darriet, F., Berge, J.B., Devonshire, A.L., Guillet, P., Pasteur, N., Pauron, D. (1998): Molecular characterization of pyrethroid knockdown resistance (*kdr*) in major malaria vector *An. gambiae s.s*. *Insect Mol. Bio*, 7: 179 184.

14: Scott, J.A., Brogdon, W.G., Collins, F.H. (1993): Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am. J. Trop. Med. Hyg*, 49: 520 529.

15: WHO (1998a): Tests procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces. Report of the WHO Informal

Consultation. WHO/MAL/98.12, World Health Organization, Geneva.
16: WHO (1998b): Report of the WHO Informal Consultation. *Tests procedures for insecticide esistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces*. Geneva: World Health Organization: Parasitic Diseases and Vector Control (PVC)/Communicable Disease Control, Prevention and Eradication (CPE); 1998:43.
17: WHO (2013a): *World Malaria Report*. Geneva, World Health Organization.
18: WHO (2013b): Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization.

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