

Full Length Research Paper

Relationship between the presence of *kdr* and *Ace-1* mutations and the infection with *Plasmodium falciparum* in *Anopheles gambiae* s.s. in Benin

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Several published reports have been devoted to insecticide resistance in *Anopheles gambiae* in Africa. However, there are still not enough published reports about the impact of insecticide resistance on malaria transmission. In this study, we proposed to investigate the presence of the circum-sporozoite antigen of *Plasmodium falciparum* in *A. gambiae* carrying *kdr* and *Ace-1* genes. *A. gambiae* (3,112) were analyzed using the enzyme-linked immunosorbent assay (ELISA), circum-sporozoite protein (CSP) test. The DNA from these samples was extracted and a polymerase chain reaction (PCR) was performed using *kdr* and *Ace-1* markers. We used Chi-square statistical test to determine the relationship between the resistance genotypes and the rates of infections with *P. falciparum*. The results showed that the *kdr* allele was found at a very high frequency (0.80 on average) in the 14 populations of *A. gambiae* s.s. sampled in spite of their molecular forms (M or S). The *Ace-1* gene was also observed in some localities, but at a low frequency (0.3 on average). The ELISA-CSP tests performed on parous females carrying the *kdr* gene showed that there was no significant difference between the allele frequencies of infected and non-infected females. *kdr* and *Ace-1* resistance genes did not affect the *A. gambiae* s.s. infection with *P. falciparum*.

Key words: Resistance, mutation, *P. falciparum*, circum-sporozoite protein (CSP).

INTRODUCTION

Malaria is regarded as the most important parasitic diseases responsible for high morbidity and mortality (Gentilini, 1991). The recent WHO world malaria report 2011 (WHO, 2011) showed an estimated 216 million malaria cases worldwide in 2010 of which 81% occurred in Africa. In Benin, malaria is endemic and seasonal throughout the year. Overall malaria represents 37% of the hospital consultations and it is one of the first causes of morbidity and mortality recorded in hospitals. Pregnant women and children under five are the most vulnerable

persons at risk (PNLP, 2007). Malaria is mainly transmitted in tropical Africa by *Anopheles gambiae* s.l. and *Anopheles funestus* (Louise et al., 2009; Djènontin et al., 2010a). In the past, *A. gambiae* s.l. was considered as a unique species, but cytogenetic studies based on chromosomal inversions showed that it is rather a complex made of seven species presenting the same morphological patterns (Hunt et al., 1998). Among the seven sibling species, *A. gambiae* s.s. and *A. arabiensis* are responsible for 90% of malaria transmission in the African tropics (Mouchet et al., 2004). *Plasmodium falciparum* (Pf) is the most virulent parasite species which causes severe and complicated malaria. Infection by *P. falciparum* is reported worldwide in Central and Latin America, Asia and South East Asia, Pacific Islands and

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Sub-Saharan Africa where *P. falciparum* is predominant (WHO, 2009).

To date, there has not been any available vaccine against malaria, yet the possible anti-malaria chemoprophylaxis still remain absolutely restrictive; therefore, vector control would essentially be the primary means of malaria prevention. The two main control methods against *Anopheles* vectors are Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Treated Nets (LLINs) (OMS, 2006). But the longstanding use of insecticides, especially in agricultural areas, has led to the development of insecticide-resistant strains in the midst of the vector populations (Akogbéto and Yakoubou, 1999; Chandre et al., 1999a; Diabaté et al., 2001). The resistance mechanism developed by *Anopheles* vectors to insecticides is mainly due to a target genetic mutation called *kdr* mutation. The mutation results in both the reduction of "knockdown" and the lethal effect of pyrethroids used for mosquito net treatment (Chandre et al., 1999b; Corbel et al., 2004). The reports of Fanello et al. (2001) and Weill et al. (2000) in West Africa showed the presence of *kdr* mutation in the S molecular form of *A. gambiae* s.s. In Benin, and later in many West African countries, the *kdr* mutation was detected in both the S and M molecular forms of *A. gambiae* s.s. (Akogbéto, 2001; Djèntonin et al., 2010b) at a very high frequency (Djègbé et al., 2011). Resistance to carbamates and organophosphates, due to the glycine to serine substitution at position 119 (G119S) in the gene that encodes for acetylcholinesterase 1 (*Ace-1*) (Weill et al., 2003) was also reported in *A. gambiae* s.s. from West Africa (Djogbénou et al., 2008a; Weill et al., 2004). This G119S mutation is found in association with resistance in the M and S molecular forms (Ahoua et al., 2010), and sequence comparison between forms at this locus suggests a unique mutational event that co-occurs in both forms through introgression from the S form (Djogbénou et al., 2008b).

In a recent study from Centre de Recherche Entomologique de Cotonou (CREC) (Gnanguenon et al., 2013), it was shown that the *A. gambiae* specimens carrying *kdr* resistance gene, especially the RR genotypes (resistance homozygotes), were more likely to pass through the holes of LLINs better than the sensitive SS (susceptible homozygotes). Actually, the number of mosquitoes analyzed through this study was only indicative. Hence, the author suggested that the study will need to be carried out on a more significant sample size from several localities. Previous reports have shown that *kdr* resistant mosquitoes stay longer in contact with the nets despite the excito-repellent effect of the insecticide treatment. This fact appears to be an advantage in tackling *kdr* resistant mosquitoes for further control measures of this particular strain.

In addition, the impact of insecticide resistance in malaria vectors on the efficacy of Insecticidal Treated

Nets (ITNs) and IRS has been reported in experimental huts in South Benin by N'guessan et al. (2007). This report showed a high survival rate of resistant *A. gambiae* from huts with either ITNs or an IRS treatment. Contrastingly in the susceptible area of North Benin, the mortality rate of *A. gambiae* was above 90% in treated huts. Similarly, a most recent report by Asidi et al. (2012) confirmed a loss of household protection from ITNs in areas of South Benin where *A. gambiae* is pyrethroid resistant. To date, the impact of vector resistance at operational level has not yet been observed where ITNs and IRS are being widely used (Henry et al., 2006). If the insecticide resistance has an impact on the vector control operations, then resistant mosquitoes would be more infected than susceptible mosquitoes in areas where the selection pressure by LLINs or IRS is high. As a matter of fact, the resistant mosquitoes were supposed to be older since the susceptible mosquitoes were more exposed to the lethal dose of impregnated materials and get killed subsequently.

In this study, we evaluated the strength of association between mosquitoes carrying a resistance allele and the presence of circum-sporozoite protein (CSP) within the two molecular forms M and S of *A. gambiae* s.s. We then investigated the presence of circum-sporozoite antigens of *P. falciparum* in some females of *A. gambiae* collected from several localities of Benin. The females carrying sporozoites were then genotyped and compared to uninfected control samples from the same population. A contingency table helped to show if there was a strong association between the risk of infection in both molecular forms (M and S) and the resistance genotype.

METHODOLOGY

Study areas

This study was conducted from 2009 to 2011 during both dry and rainy season in 14 localities selected from the transect south to north which includes the major ecological systems and various agricultural practices throughout Benin: Sèmè, Dangbo, Adjohoun, Adjara, Misséréte and Tori-Bossito located in a cereal zone in the South of Benin; Bamè located in a rice zone in the center of Benin; Pehunco, Kouandé, Cobly, Boukoumbé, Tanguiéta and Toukountouna located in a cotton and cereal growing area in high altitude of northwest Benin; Malanville, a rice growing zone in North Benin at the boarder of Niger Republic (Figure 1).

The diversity of ecological facies was taken into account in order to collect both M and S molecular forms of *A. gambiae* with related frequencies of RR, RS and SS resistance genotypes for comparison. Moreover, it was not obvious at the beginning to find a great number of *A. gambiae* infected with *P. falciparum* in the current context of widespread use of treated nets by the populations. But we thought we could increase our chance of finding an area where *A. gambiae* infection rate would be relatively high by varying the ecological areas.

In the localities of the south and center, the climate is characterized by a low temperature (23°C). The average annual temperature is 27°C with an annual rainfall mean varying from 820

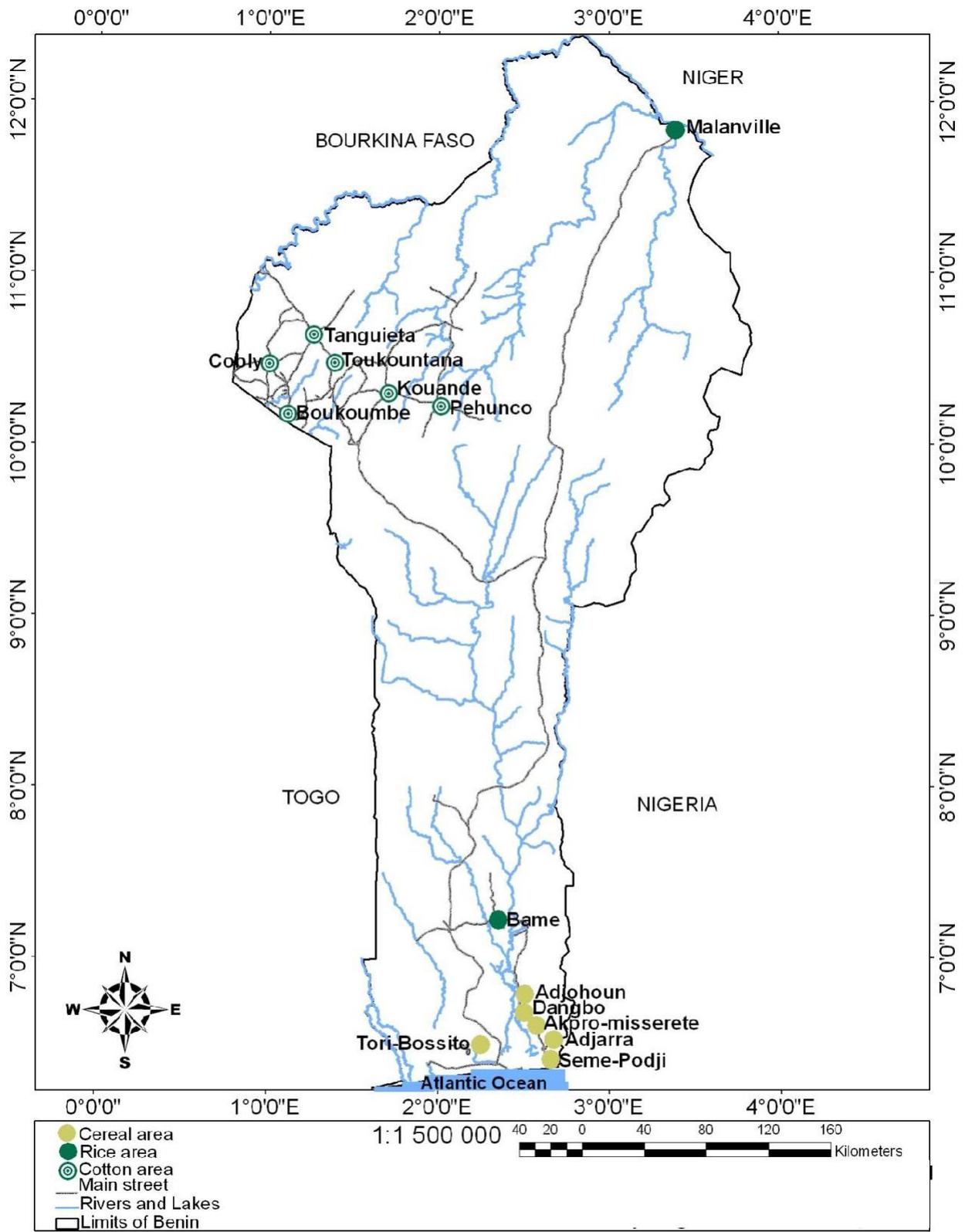


Figure 1. Map of Benin showing the localities in the northern and the southern sites of the study area.
 Source: Fond topographique IGN 2004, Benin, Razaki OSSE and Virgile Gnanguenon.

Table 1. Frequency of *kdr* alleles and genotypes observed in the M and S forms of *A. gambiae* s.s. in North and South Benin.

Locality	<i>kdr</i> mutation L1014F									
	M Form			Frequency		S Form			Frequency	
	RR	RS	SS	R (%)	S (%)	RR	RS	SS	R (%)	S (%)
Sèmè	64	3	0	97.76	2.24	-	-	-	-	-
Dangbo	120	3	0	98.78	1.22	-	-	-	-	-
Adjohoun	117	55	13	78.1	21.9	4	10	0	64.28	35.72
Adjara	148	3	0	99	1	-	-	-	-	-
Missérété	41	2	0	97.67	2.33	-	-	-	-	-
Tori-Bossito	247	78	45	77.29	22.71	27	16	29	48.61	51.39
Bamè	175	62	2	86.19	13.81	2	0	0	100	0
Pehunco	-	-	-	-	-	10	14	0	70.83	29.17
Kouandé	-	-	-	-	-	24	2	0	96.15	3.85
Cobly	-	-	-	-	-	15	4	0	89.47	10.53
Boukoumbé	-	-	-	-	-	9	16	0	68	32
Tanguiéta	-	-	-	-	-	22	3	1	90.38	9.62
Toukountouna	-	-	-	-	-	12	9	3	68.75	31.25
Malanville	33	27	18	59.61	40.39	4	0	0	100	0
Total	945	233	78	84.51	15.49	129	74	33	70.33	29.67

to 1,200 mm. By contrast in the north, the climate is characterized by a long dry season from November to April and one rainy season from May to September. The average temperature is about 27°C with an annual rainfall of 750 mm.

Mosquito collection

The mosquitoes analyzed were collected using three sampling methods: (1) human landing catches (HLC), (2) indoor pyrethrum spray catches (PSC), and (3) window-traps (WT) installed at the windows of selected houses.

For the HLC collection, two villages were selected per site and two houses per village to collect mosquitoes. The collection was carried out twice a month by adult volunteers who have given their informed consent. In each house, a collector was positioned inside and another outside. They caught any mosquitoes that landed on their legs with an aspirator from 9.00 p.m to 5.00 a.m. Considering the risk of malaria transmission, the collectors were given an antimalarial prophylaxis as a prevention against malaria. During the course of this study, all mosquito collectors were monitored for any malaria symptom noticed, an immediate parasitological test would take place followed by an antimalarial treatment if necessary.

For the indoor pyrethrum spray catches, we used pyrethrum spray and white canvas spread on the floor to collect knocked down mosquitoes from 7 to 9 a.m.

Regarding to the WT collection mentioned earlier, four bedrooms were selected in each of the villages chosen for the study. The window-traps used were made of a mesh of terylene (synthetic fiber) mounted on a cubical iron frame with an edge measuring 30 cm, with 1 side drawn into a funnel to direct mosquitoes into the trap (Bar-Zeev and Self, 1966). The trap was fixed to a plywood sheet that could be fitted to window frames of differing sizes. The traps were placed before dusk and emptied of mosquitoes at 7 a.m.

Laboratory processing of mosquitoes collected

The collected mosquitoes were identified using a morphological

identification key of Coluzzi (1964). The specimens of *A. gambiae* were stored separately according to the locations they were collected from, then labeled and conserved in Eppendorf tubes containing silica gel. The whole Eppendorf tubes were stored in a freezer at -20°C before any further analyses.

Any mosquito that was to undergo the enzyme-linked immunosorbent assay-CSP test (ELISA-CSP) as well as the polymerase chain reaction (PCR) test was divided in two parts: the head-thorax was kept to process ELISA-CSP and the abdomen left to perform PCR. The search for the circum-sporozoite antigen of *P. falciparum* was carried out following the method of Wirtz et al. (1987). The carcasses (abdomen, legs and wings) of each mosquito were used to identify molecular forms and genotypes of *kdr* and *Ace-1* mutations according to the protocols described by Favia et al. (2001), Martinez-Torres et al. (1998) and Weill et al. (2004), respectively.

Data analysis

The genotype differentiation between the vectors that tested positive and negative to the ELISA-CSP test regarding *kdr* and *Ace-1* mutations was tested using Fisher's exact test with GENEPOP software. Chi square test was used to reveal if there was any possible association between *A. gambiae* s.s. that tested positive to CSP and the resistant genotypes for *kdr* and *Ace-1* mutations.

RESULTS

Allele frequency of *kdr* and *Ace-1* mutations in the M and S molecular forms

A total of 3,112 females of *A. gambiae* from different localities were analyzed. The two M and S molecular forms of *A. gambiae* s.s. were identified with a complete predominance of the M form (93.04%, n=1,975) in the

Table 2. Positivity indexes in CS of *P. falciparum* regarding the M and S forms of *A. gambiae* s.s.

Locality	Total tested	M form		S form	
		CS+	CS+ (%)	CS+	CS+ (%)
Sèmè	175	9	5.14	-	-
Dangbo	207	17	8.21	-	-
Adjohoun	226	10	4.42	-	-
Adjara	334	13	3.89	-	-
Misséréété	75	4	5.33	-	-
Tori-Bossito	534	35	6.55	-	-
Bamè	424	29	6.84	-	-
Pehunco	105	-	-	3	2.86
Kouandé	76	-	-	9	11.84
Cobly	352	-	-	15	4.26
Boukoumbé	180	-	-	3	1.67
Tanguiéta	46	-	-	4	8.7
Toukountouna	56	-	-	4	7.14
Malanville	322	14	4.35	1	0.31
Total	3,112	131	5.7	39	4.77

localities of Southern Benin, while the S form (94.87%, n=815) was found to be more frequent in the northern localities, except Malanville which showed a higher rate of the M form (95.12%, n=322) (Table 1). A sample of 1,492 individuals belonging to the two molecular forms was analyzed with PCR for *kdr* mutation. The allele frequency of this mutation varied from 59.61 (Malanville, n=78) to 99% (Adjara, n=151). The *kdr* frequency was 84.51% regarding the M molecular form specimens from the southern sites of Benin and 70.33% of the S form was mainly recorded in the specimens from the northern sites (Table 1). The susceptibility of each of the two forms for *kdr* mutation was calculated using the proportion *kdr* frequency in the M and S forms relative to the localities where the two populations were found in sympatry. Thus, in Malanville (Northern Benin), the frequency was higher in the S form (100%, n=4) than the M form (59.61%, n=78), which is in agreement with the results of a large number of published reports from West Africa (Dabiré et al., 2008). On the other hand, it was contrastingly different in the South Benin sites of Adjohoun where 78.10% of the M form (n=185) and 64.28% of the S form (n=14) ($P<0.05$) was recorded. Similarly, in Tori-Bossito (Southern Benin), 77.29% of the M form (n= 70) and 48.61% for the S form (n=72) ($P<0.05$) were recorded (Table 1). The *Ace-1* mutation was only observed in the localities of Adjara, Sèmè, Pehunco, Cobly and Toukountouna at a very low frequency varying from 2.63 (Cobly, n=19) to 10.41% (Toukountouna, n=24). The average frequency for the M form was 3% and 3.71% for the S form.

Positivity index of the circum-sporozoite antigen of *P. falciparum* in the M and S forms of *A. gambiae*

The results of the ELISA-CSP tests (Table 2) showed a mean positivity index of 5.46% for *An. gambiae* s.s. which varies from one locality to another. It was 1.67% in Boukoumbé against 11.84% in Kouandé. The positivity index in *A. gambiae* s.s. with M molecular form reached 5.70% varying within localities where 3.89% was recorded in Adjara and 8.21% in Dangbo. In the S molecular form individuals, the mean positivity index of CSP from the northern sites was 4.77% varying from 0.31% in Malanville to 11.84% in Kouandé. There was no significant difference in sensitivity between the M and S forms for CSP *P. falciparum* ($P>0.05$).

Genotypes and “presumed phenotype” of *kdr* and *Ace-1* genes in the *A. gambiae* s.s. tested either positive or negative for the CS antigen of *P. falciparum*

Table 3 of the results show the relationship between the presence of *kdr* and *Ace-1* resistance genes and the positive-testing for the CS antigen of *P. falciparum* in *A. gambiae* s.s. The results in this table help to compare the genotypes and the presumed phenotypes (susceptible or resistant) for *kdr* and *Ace-1* mutations among *A. gambiae* s.s. individuals that tested positive or negative for the CSP antigen of *P. falciparum*. No association was found between the resistant (or sensitive) genotype and the

Table 3. Genotypes and presumed phenotypes ([R], [S]) of *kdr* and *Ace-1* mutations in the *A. gambiae* s.s tested positive and negative for the CS antigen of *P. falciparum*.

Locality	CSP	<i>kdr</i> mutation L1014F					P	<i>Ace-1</i> mutation G119S					P
		RR	RS	SS	[R]	[S]		RR	RS	SS	[R]	[S]	
Sèmè	(+)	8	1	0	8	1	0.87	0	0	9	0	9	0.67
	(-)	56	2	-	56	2		0	3	10	3	10	
Dangbo	(+)	17	0	-	17	0	0.93	0	0	7	0	7	0.93
	(-)	103	3	-	103	3		0	3	68	3	68	
Adjohoun	(+)	6	1	3	6	4	0.97	0	0	2	0	2	1
	(-)	115	64	10	115	74		0	0	8	0	8	
Adjarra	(+)	12	1	0	12	1	0.87	0	1	12	1	12	0.87
	(-)	136	2	-	136	2		0	4	23	4	23	
Misséréké	(+)	3	1	-	3	1	0.74	0	0	4	0	4	1
	(-)	38	1	-	38	1		0	0	39	0	39	
Tori-Bossito	(+)	15	10	10	15	20	0.21	-	-	-	-	0	-
	(-)	259	84	64	259	148		-	-	-	-	0	
Bamè	(+)	26	3	0	26	0	0.25	-	-	-	-	0	-
	(-)	151	59	2	151	61		-	-	-	-	0	
Pehunco	(+)	2	1	0	2	1	0.57	0	2	0	2	0	-
	(-)	8	13	-	8	13		0	2	22	2	22	
Kouandé	(+)	3	1	-	3	1	0.76	0	0	4	0	4	1
	(-)	21	1	-	21	1		0	0	22	0	22	
Cobly	(+)	8	2	-	8	2	0.96	0	1	9	1	9	0.87
	(-)	7	2	-	7	2		0	0	9	0	9	
Boukoubé	(+)	0	2	-	0	2	-	0	0	2	0	2	1
	(-)	9	14	-	9	14		0	0	23	0	23	
Tanguiéta	(+)	3	0	-	3	0	0.86	0	0	3	0	3	1
	(-)	19	3	1	19	3		0	0	17	0	17	
Toukountouna	(+)	1	1	0	1	1	1	0	1	1	1	1	0.69
	(-)	11	8	3	11	11		0	4	18	4	18	
Malanville	(+)	10	4	2	10	6	0.37	-	-	-	-	0	-
	(-)	27	23	16	27	38		-	-	-	-	0	
Total	(+)	114	28	15	114	15	0.87	-	5	53	5		1
	(-)	960	279	96	960	96		-	16	237	16		

presumed phenotype of the vectors and their positive-tests to CSP ($P>0.05$).

With the *Ace-1* gene, there was no significant difference

between the resistant (or sensitive) genotype and the presumed phenotype of the vectors and their positive-tests to CSP ($P>0.05$).

DISCUSSION

The study of mosquitoes as vectors and the transmission process were an important preliminary not only to understand the epidemiology of malaria but also to implement an efficient and targeted control of these vectors (Omumbo et al., 2005).

The results of this research have shown that *kdr* gene (*Leu-Phe*) was found at a very high frequency (0.80 on average) in *A. gambiae* s.s. populations regardless of the forms (M or S). The development of the resistance to different insecticides used in public health, particularly, the class of pyrethroids, has been reported in Côte d'Ivoire (Elissa et al., 1993), Kenya (Vulule et al., 1999), Nigeria (Awolola et al., 2008), Cameroun (Etang et al., 2003) and Benin (Takken et al., 2003; Djènonatin et al., 2009; Yadouleton et al., 2011) during the last decade. The *kdr* mutation plays a tremendous role in the development of *A. gambiae* resistance to dichlorodiphenyl trichloroethane (DDT) and pyrethroid insecticides in Africa as reported by numerous authors (Ranson et al., 2000; N'Guessan et al., 2010). The development of resistance mechanisms in malaria vectors is well reported (Enayati et al., 2003), but the factors leading to the selection of resistance remain unknown especially in rural areas where the production of cereals does not require the use of insecticides. However, it is obvious that the development of resistance is related to both the bio-ecology of mosquito vectors and the intensity of the selection pressure exerted by the use of insecticides in public health and agriculture. In our opinion, any pressure on a mosquito, be it a larvae or an adult, could trigger off a defensive reaction from the mosquito that could create a mechanism to control such a pressure in a far future.

The *kdr* mutation was initially detected in the cotton areas, first in the midst of populations including the S molecular form of *A. gambiae* s.s. before spreading in the M molecular form by introgression (Weill et al., 2000). The results obtained in Malanville, Northern Benin where *kdr* frequency was higher in S form than in M form, were consistent with this hypothesis even if the number of S form is low. This recalls the work of Djogbenou et al. (2010) and Djègbé et al. (2011) in Benin who showed the predominance of M form in Malanville, probably because of the abundance of rice fields irrigated by the Niger River. However, if this hypothesis is true, then the contrasting data showing the highest frequency of *kdr* gene in the M form in Adjohoun and Tori-Bossito in the south would still be unexplained. Presumably it could have occurred by a long term effect of adaptation to bioecological changes further to the appearance of *kdr* mutation in Southern Benin. Owing to the short period of its life cycle and the quickness of mosquito reproducibility, the transfer of *kdr* gene into the M form could have developed and spread very quickly (Yadouleton et al., 2010).

Besides the resistance to pyrethroids, a low resistance of *A. gambiae* s.s. to carbamates (*Ace-1*) was also noticed. A similar level of resistance was reported by Djogbénou et al. (2008b) and Yadouleton et al. (2010). This early onset of *Ace-1* gene is to be carefully considered with great interest since National Malaria Control Program in Benin has been using Bendiocarb for IRS during the past 5 years as part of its malaria control strategies. This situation remains a serious concern as the exertion of selection pressure could increase and spread the level of *Ace-1* gene later in the near future. Therefore, it appears urgent to set up an in country resistance monitoring framework where WHO bioassay kits could be performed. The baseline data would be initially collected and compared later on with those from areas treated with Bendiocarb to monitor the development of *Ace-1* gene and vectors susceptibility. This monitoring will greatly contribute a better management of insecticide resistance in malaria vectors and a correct choice of products for an effective malaria transmission control.

Our research showed that *A. gambiae* s.s is the main malaria vector in Benin with a positivity index in CS of *P. falciparum* of 11.84 varying from one locality to another.

In a recent field study (Gnanguênon et al., 2013), it was shown that the *kdr* gene enables *A. gambiae* populations carrying this mutation to pass through the holes of treated nets. Similarly, it is probable that the *A. gambiae* s.s. carrying *kdr* mutation are more likely to transmit malaria since they are resistant to insecticides and live longer than susceptible mosquitoes which do not carry the mutation (Rivero et al., 2010). It is important to mention that susceptible mosquitoes are theoretically considered more vulnerable whenever in contact with external aggressions (LLINs, IRS). Yet, the ELISA-CSP tests performed on parous females carrying the *kdr* mutation and the ones not carrying it showed the same level of *P. falciparum* infection. This result is in agreement with the report by Koffi et al. (2002) in Côte d'Ivoire. These results corroborate also with those of Vézilier et al. (2010) who showed in specific experimental conditions that esterase and acetylcholinesterase-based insecticide resistance did not have a clear effect on the infection rate of *Plasmodium relictum* or oocyst burden in *Culex pipiens* mosquitoes. Moreover, recent works showed that insecticide resistance levels in wild *Culex quinquefasciatus* mosquitoes are negatively correlated with the density of the filarial parasite *Wuchereria bancrofti*, and the parasite development is blocked at the L1 stage in laboratory mosquitoes selected for artificially high levels of insecticide resistance (McCarroll and Hemingway, 2002; Curtis, 2001).

Conclusion

Malaria is transmitted by two molecular forms of *A. gambiae* s.s populations in Benin where the M form is

mostly located in southern forest and humid savanna areas and the S form in the northern dried savanna areas. The two forms are resistant to insecticides especially DDT and pyrethroids. The main resistance mechanism identified in both forms is the *kdr* mutation with a high frequency recorded in the S form as well as the M form conversely to reports from Côte d'Ivoire, Burkina Faso, Mali and other countries of the region.

The hypothesis stating that *A. gambiae* s.s. populations carrying *kdr* mutation are more likely to transmit malaria, was not verified in the present study. Therefore, there was no significant difference in positive-testing for CSP of *P. falciparum* in the *A. gambiae* with RR, RS and SS genotypes ($P>0.05$). Our findings showed that there is no association between the presence of *kdr* gene and *P. falciparum* infectivity. In addition, the M and S molecular forms have the same sensitivity towards the malaria parasite.

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