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Vector resistance to pyrethroids and impact on the efficacy of insecticide-treated mosquito nets in Southern Benin

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The use of Long Lasting Insecticidal Nets (LLINs) to protect population from malaria is one of the main strategies which decline the malaria morbidity and mortality in several trials carried out in different countries. However, resistance to pyrethroids is one of some problems which compromise the LLINs effectiveness. This study compares the effectiveness of LLINs in two areas, low and high resistance areas. Mosquitoes were collected in 8 clusters of Plateau department from July 2011 to June 2013 using human landing catch. The heads and thorax of *Anopheles gambiae* collected were analyzed using the ELISA Circum- Sporozoite Protein test. The human biting rates and entomological inoculation rate (EIR) were compared between areas. On the total of 4228 vectors tested, 220 were positive for *Plasmodium falciparum*. The EIR obtained in the high resistance zones were not significantly higher (p >0.05) than those obtained in the low resistance zones. In fact, the EIR ranged from 66.69 infected bites/human/6months to 89.67 infected bites/h/24 months in the high resistance zones compared to 184.89 infected bites/h/6 months to 357.53 infected bites/h/24 months in the low resistance zones. This study has not shown evidence of an impact of resistance on the efficacy of LLINs.

Key words: Resistance, long lasting insecticidal nets effectiveness, malaria, *An. gambiae*.

INTRODUCTION

The fight against malaria is a priority for health authorities in endemic countries. Among the tools used in the prevention of this disease, vector control is a very important and closely monitored axis. LLINs constitute

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the main means of struggle and much effort has been made to increase their accessibility to populations, especially children under five and pregnant women. Thanks to the universal access campaign of July 2011, the coverage and use of LLINs in Benin has been more than 80% (EDSB IV, 2012). The success of LLINs in malaria prevention is that they provide both effective individual protections for users and community protection if the net coverage rate is large enough to reduce vectorial capacity (WHO, 2005; WHO, 2009). Unfortunately, pyrethroid resistance in malaria vectors is steadily increasing in Africa (Ranson et al., 2011) and could therefore compromise or limit the effectiveness of LLINs in preventing malaria.

In Benin, this reduction in the protective effect of LLINs in vector resistance zones to insecticides was highlighted as well in experimental huts (N'Guessan et al., 2007) and in the community (Asidi et al., 2012). But it should be noted that few data exist to show the real impact of vector resistance to insecticides on malaria transmission indicators. The purpose of this study is to test whether vector resistance to pyrethroids has an impact on the efficacy of insecticide-treated mosquito nets. Initially, it was planned to carry out the study in two areas, an area where malaria vectors are resistant to pyrethroids, and the other where they are sensitive (control zone). However, in the absence of anopheles' susceptibility zone to pyrethroids in Benin (Yadouleton et al., 2010, Djègbè et al., 2011, Aïkpon et al., 2013), the susceptibility zone has been replaced by a zone of low resistance that we denominate for the convenience of understanding and the "R + zone" in contrast to the zone of high resistance called "R +++ zone". On this basis, in 2012 and 2013, the entomological indicators of transmission (Human biting rate, sporozoite index, entomological inoculation rate) of malaria were analyzed according to the two zones.

MATERIALS AND METHODS

Study areas

The study took place in the Plateau department located in south-eastern Benin and more specifically in Ifangni, Sakété, Pobè and Kétou districts. Before the study began, WHO susceptibility tests were performed on *Anopheles gambiae* using deltamethrin to select the villages where activities were held. Figure 1 shows the distribution of the villages based on the mortalities observed with deltamethrin in 2011. Due to the absence of an area where *An. gambiae* are fully susceptible to pyrethroids in Benin, criteria were used to categorize the level of resistance (Sovi et al., 2014). “R+++ area” was called an area where the observed mortality was between 0 and 60% and “R + area” an area where the observed mortality ranged 80 to 100%. These two areas were identified based on baseline resistance data collected in Plateau department.

Thus, 4 villages of high resistance and 4 villages of low resistance were selected for monitoring malaria transmission. They are: Itakpako (R+), Ko-koumolou (R+) and Itassumba (R+) at Ifangni, Igbola (R+++) and Djohounkollé (R+) at Sakété, Idena 2 (R+++) and Mowodani (R+++) at Kétou, Okoofi (R+++) at Pobè (Figure 1).

Sampling of mosquito populations

To measure transmission, we collected *An. gambiae* in each cluster from July 2011 to June 2013 and evaluated the frequency of infected bites that one man receives in a night. Mosquito catches were carried out on voluntary human who, beforehand, gave their favorable consent for the conduct of the activity in 2 houses, inside and outside, using mouth aspirators. The mosquito catches were carried out from 9 pm to 5 am.

Two night catch sessions were conducted per month in each cluster, for a total of 64 night / month catches for all 8 clusters. The recorded data evaluated the number of bites received by one man per night for each zone, the longevity and the entomological inoculation rate (EIR) of *An. gambiae*.

Mosquito treatment

After each night of capture, the captured mosquitoes were identified the following morning according to the genus and the species from the key of Gillies and Meillon (1968) using the binocular loupe. The ovaries of part of the anopheles vectors were dissected to determine the rate of partition, observing the degree of winding of the tracheoles (Detinova et al., 1964). The heads and thorax of these anopheleses were stored separately at the place of catch in labeled Eppendorf tubes containing silica gel at -20°C for enzymatic immunoassay (ELISA-CSP) infection.

ELISA processing of mosquitoes collected

To determine the infection rate of *P. falciparum* female anopheles, the Enzyme Linked Immunosorbent Assay (ELISA) was used. It is a colorimetric technique that consists of looking for plasmodium membrane protein antibodies in the head and thorax of mosquitoes. This immunological procedure is based on the antibody-antigen reaction to detect the presence of the sporozoite parasite in the mosquito (Burkot et al., 1984, Lombardi et al., 1987).

Data analysis

The entomological inoculation rate (EIR) representing the number of infective bites received per man and per time unit (day, month or year) was calculated for each zone to measure the intensity of malaria transmission. It was calculated by making the product of the human biting rate (Hbr) by the sporozoite index (Is). Human biting rates and EIR were compared between areas using the Poisson test. All analyses were performed with R-2.15.2 statistical software (R Development Core Team, 2011). Human biting rate and entomological inoculation rate were estimated with their 95% confidence interval.

RESULTS

Variation of the aggressivity of *An. gambiae* (Hbr) 24 months after the establishment of the LLINs in the zones of low and high resistances

Table 1 shows the average number of bites received by one man overnight, 6, 12, 18, and 24 months after the establishment of LLINs in areas of high and low vector resistance to insecticides. During the 24 months, the number of bites received by one man per night was

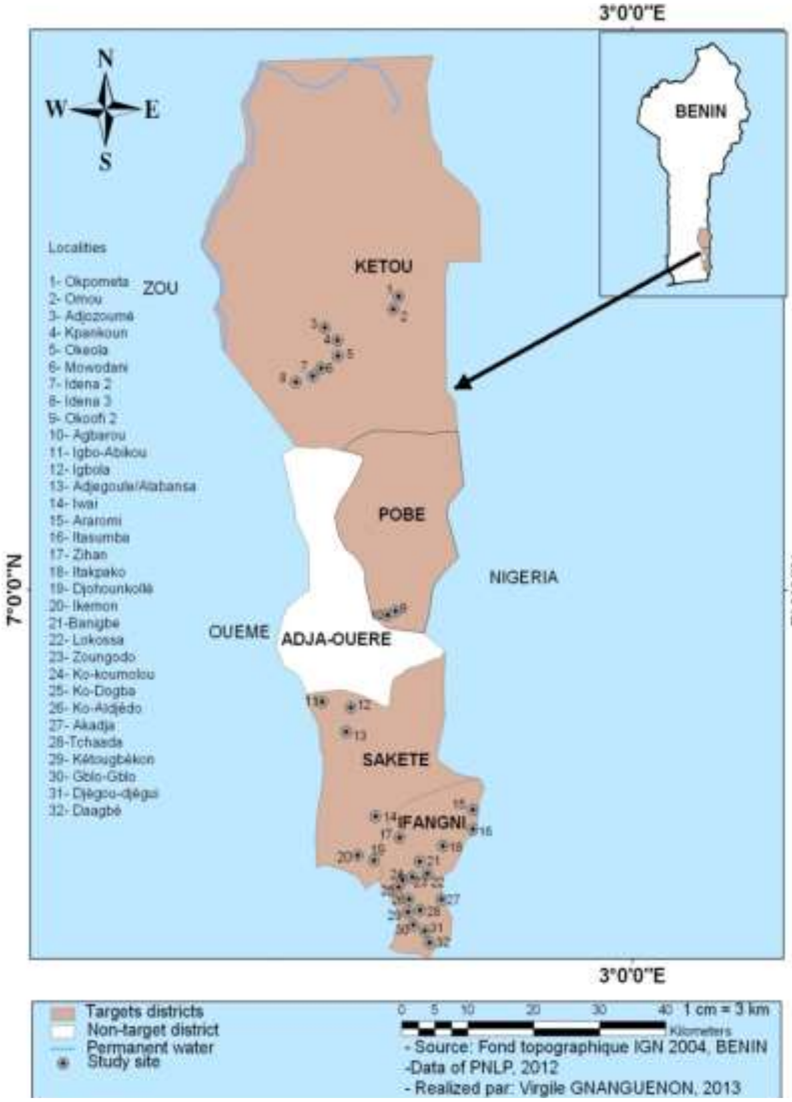

Figure 1. Map showing the study villages in Plateau department.

significantly higher (p <0.0001) in areas of high resistance (R +++) than in areas of low resistance without Itassoumba (R + - Itassoumba).

Indeed, the biting rate was 3.22; 2.73; 2.13 and 1.77 bites / man / night in the R +++ zones against 0.36; 0.43; 0.40 and 0.39 in the R + - Itassoumba zones, respectively 6, 12, 18 and 24 months after the establishment of the LLINs. On the other hand, in the zones R + + Itassoumba the situation is contrary (Table 1).

Impact of the effectiveness of the LLINs on the transmission of malaria in the low and high resistance zone of *An. gambiae* to pyrethroids

A total of 4228 *An. gambiae* heads-thorax were submitted to the CSP ELISA, including 724 for areas of high resistance and 3504 for areas of low resistance (Table 2). On the total tested, 220 were positive for *P. falciparum*. The average sporozoite index is 0.052.

6, 12 and 18 months after the establishment of LLINs, mosquito infectivity is similar (p> 0.05) in areas of high (R +++) and low resistance (R + - Itassoumba; R + + Itassoumba). 24 months after intervention, the sporozoite index was significantly higher in the R +++ zones than in the R + + Itassoumba zones. On the other hand, no significant difference was observed between the sporozoite index of R +++ and R + - Itassoumba zones 24 months after the implementation of the intervention.

The EIR obtained in the R +++ zones were significantly higher (p <0.0001) than those obtained in the R + - Itassoumba zones during the evaluation periods. In fact, the EIR ranged from 66.69 infected bites/human/6 months to 89.67 infected bites/h/ 24 months in the R +++

Table 1. *An. gambiae* biting rate (Hbr) in low and high resistant areas 2 years after the establishment of LLINs in the Plateau department.

Periods	Areas	Total vector	Human night	Hbr (bit/human/night)	RR	IC-95%	p
6 months (July 2011-December 2011)	R^{+++}	566	176	3.22	1	-	-
	R^{+} + Itassoumba	1502	176	8.53	2.65	[2.41-2.92]	<0.0001
	R^{+} - Itassoumba	47	132	0.36	0.11	[0.08-0.15]	<0.0001
12 months (July 2011-June 2012)	R^{+++}	1004	368	2.73	1.00	-	-
	R^{+} + Itassoumba	4113	368	11.18	43.94	[41.27-46.77]	<0.0001
	R^{+} - Itassoumba	119	276	0.43	0.16	[0,13-0,19]	<0.0001
18 months (July 2011-December 2012)	R^{+++}	1190	560	2.13	1.00	-	-
	R^{+} + Itassoumba	5769	560	10.30	4.85	[4.55-5.16]	<0.0001
	R^{+} - Itassoumba	168	420	0.40	0.19	[0.16-0.22]	<0.0001
24 months (July 2011-June 2013)	R+++	1273	720	1.77	1.00	-	-
	R^{+} + Itassoumba	7415	720	10.30	5.82	[5.49-6.18]	<0.0001
	R^{+} - Itassoumba	211	540	0.39	0.22	[0.19-0.26]	<0.0001

Table 2. Entomological inoculation rate (EIR) observed in *An. gambiae* in low and high resistant zones 2 years after the establishment of LLINs in Plateau department.

Periods	Areas	Head-thoraxtested	Thorax (+)	Is (%)	Hbr (Bit/human/night)	Infectivebiting/human/ night	EIR/ period	RR	IC 95% (RR)	p
6 months (July 2011-December 2011)	R^{+++}	269	31	11.52	3.22	0.37	66.69	1.00	-	-
	R^{+} + Itassoumba	358	43	12.01	8.53	1.02	184.49	2.77	[2.09-3.68]	<0.0001
	R^{+} - Itassoumba	33	5	15.15	0.36	0.05	9.71	0.14	[0.07-0.31]	<0.0001
12 months (July 2011-June 2012)	R^{+++}	488	39	7.99	2.73	0.22	78.49	1.00		-
	R^{+} + Itassoumba	1633	120	7.35	11.18	0.82	295.67	3.78	[2.95-4.83]	<0.0001
	R^{+} - Itassoumba	114	9	7.89	0.43	0.03	12.25	0.15	[0.08-0.30]	<0.0001
18 months (July 2011-December 2012)	R^{+++}	646	48	7.43	2.13	0.16	85.26	1.00	-	-
	R^{+} + Itassoumba	2546	140	5.50	10.30	0.57	305.90	3.60	[2.84-4.56]	<0.0001
	R^{+} - Itassoumba	194	13	6.70	0.40	0.03	14.47	0.17	[0.09-0.31]	<0.0001
24 months (July 2011-June 2013)	R+++	724	51	7.04	1.77	0.12	89.67	1.00	-	-
	R^{+} + Itassoumba	3504	169	4.82	10.30	0.50	357.63	3.98	[3.16-5.01]	<0.0001
	R^{+} - Itassoumba	237	14	5,91	0.39	0.02	16.62	0.18	[0.1-0.32]	<0.0001

zones compared to 9.71 infected bites/h/6months to 16.62 infected bites/h/24 months in the R + - Itassoumba zones (Table 2). On the other hand, the situation is opposite in the zones R + + Itassoumba. In these areas, the EIR was significantly higher (p <0.0001) than in areas of

Table 3. Parity rates observed in *An. gambiae* in low and high resistant areas 2 years after the establishment of LLINs in Plateau department.

Periods	Areas	N tested	Parous	Parity rate	OR	IC-95% (OR)	p
6 months (July 2011-December 2011)	R^{+++}	304	241	79.27	1.00		-
	R^{+} + Itassoumba	698	576	82.52	0.81	[0.58-1.14]	0.2495
	R^{+} - Itassoumba	48	37	77.08	1.14	[0.55-2.36]	0.7059
12 months (July 2011-June 2012)	R^{+++}	509	396	77.80	1	-	-
	R^{+} + Itassoumba	1296	930	71.76	1.38	[1.08-1.76]	0.0091
	R^{+} - Itassoumba	160	128	80.00	0.88	[0.56-1.36]	0.5842
18 months (July 2011-December 2012)	R^{+++}	649	514	79.20	1.00	-	-
	R^{+} + Itassoumba	2430	1822	74.98	1.27	[1.03-1.57]	0.0263
	R^{+} - Itassoumba	261	217	83.14	0.77	[0.53-1.12]	0.1969
24 months (July 2011-June 2013)	R^{+++}	758	611	80.606	1.00	-	-
	R^{+} + Itassoumba	3361	2536	75.45	1.35	[1.11-1.65]	0.0024
	R^{+} - Itassoumba	328	269	82.01	0.91	[0.65-1.27]	0.6138

high resistance R +++ throughout the study period

Impact of LLINs on the longevity of *An. gambiae* in areas of low and high insecticide resistance

A parous mosquito is an epidemiologically dangerous mosquito. Thus, the parity rate, which is a secondary parameter of transmission, makes it possible to have an idea of the longevity of Anopheles in the environment.

The results that we obtained showed a variation in the parity rates according to the zones of resistance and the study periods (Table 3). The parity rate is the same (p> 0.05) in the zone of low resistance without Itassoumba (R + - Itassoumba) as in the zone of strong resistance (R +++). On the other hand, when we consider the low resistance zones with Itassoumba, we noted a significant difference between the R +++ zones and the R + + Itassoumba zones (p <0.05) 12, 18 and 24 months after the establishment of LLINs (Table 3).

DISCUSSION

LLINs have had a large impact on Anopheles vectors and on malaria transmission in areas of low resistance without Itassoumba (R+ - Itassoumba) since its implementation compared to areas of high resistance. Despite the presence of mosquito nets in areas of high resistance (R +++), there has been an increase in the indicators of malaria transmission in the Plateau department. The distribution of Long-Lasting Insecticide-treated nets (LLINs) to the entire population implemented by the National Malaria Control Program (NMCP) in the Plateau department in 2011 achieved coverage universal. This campaign covered 85.5% of households in the Plateau department who reported receiving LLINs in 2011 according to the results of the evaluation carried out for this campaign.

The study showed that the Anopheles aggressiveness expressed in this work in terms of bites of *An. gambiae* per human per night (Hbr) was high in areas of high resistance compared to areas of low resistance R + - Itassoumba throughout the study period. The high value of Hbr noted in areas of low resistance with Itassoumba (R + + Itassoumba) compared to areas of high resistance throughout the study period is explained by the fact that in Itassoumba, a village R +, fish and vegetable production activities is practiced (Sovi et al., 2013a; Sovi et al., 2013b).

Indeed, the development and maintenance of fish ponds and market gardens serve as breeding grounds for spawning and feeding Anopheles throughout the year. These results confirm the work of Protopopoff et al. (2009) who showed that, the density of Anopheles is higher in localities located near watercourses, rivers and marshes compared to those distant from them. Similarly, Klinkenberg et al. (2008) conducted studies in Ghana and Yadouléton et al. (2010) in Benin they showed that, vegetable crops are places of high development of mosquito larvae. The dynamics of Anopheles populations is therefore related to the functioning and dynamics of larval breeding sites. Infectivity rates were similar (p> 0.05) in both zone categories (R +++, R + - Itassoumba) during the study period (24 months). These results confirm the work of Ossè et al. (2012), who showed that there is no relationship between pyrethroid resistance and *P. falciparum* infectivity. On the other hand, the transmission of *P. falciparum* was higher in the zone of high resistance (R +++) compared with that of low resistance R + - Itassoumba. The question of the entomological impact of resistant mosquitoes on the effectiveness of LLINs in field conditions is therefore of concern. These results are similar to those of Trape et al.

(2001) and Ossè et al. (2013) who also showed an increase in the level of transmission in regions under universal coverage of MIILDs in resistance zone of vectors to pyrethroids.

In addition, the work of Asidi et al. (2012) revealed a loss of efficacy of these nets in the zone of resistance compared to a zone of susceptibility. The work of Gnanguenon et al. (2013) showed that resistant mosquitoes can resist the excito-repellent effect of LLINs, and penetrate impregnated mosquito nets to feed on humans. However, our results are contrary to those of Henry et al. (2005) and Dabire et al. (2006), who showed that LLINs continue to protect regardless of vector resistance to pyrethroids. Indeed, these authors have shown the reduction of the density of vectors and the transmission of malaria in homes with LLINs, in zones of high resistance of *An. gambiae* to pyrethroids.

Moreover, no significant difference was recorded between the infectivity of the populations of *An. gambiae* with *P. falciparum* in the R +++ and R + + Itassoumba zones 18 months after the establishment of the LLINs and despite the high Anopheles density in the Itassoumba locality. However, 24 months after the implementation of the intervention, the level of transmission is significantly higher ($p < 0.0001$) in R + + Itassoumba areas than that of R +++. This situation could be explained by the strong anopheles aggressiveness observed in the locality.

The absence of a zone of clear sensitivity of the vectors towards the pyrethroids is a limiting factor in the realization of this study. In addition, the spatial heterogeneity of EIR observed in this study shows the importance of local conditions on the intensity of malaria transmission, which is an environmental problem.

Conclusion

This study has not shown evidence of an impact of resistance on the efficacy of Long-Lasting Insecticidal Nets. Indeed, after two years of evaluation, the biting rate and the entomological inoculation rate increased in areas of low resistance compared to areas of high resistance.

However, the high EIR observed in areas of low resistance is perhaps due to the aggressive density which is particularly high in Itassoumba. The lack of evidence of the impact of resistance on the efficacy of LLINs in our study area was shown by sporozoite indices and similar parturition rates in R + and R +++.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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