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Long lasting insecticidal nets use, efficacy and physical integrity in a vector resistance area after a nationwide campaign in southern Benin, West Africa

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Although effective malaria control measures such as long lasting insecticidal nets (LLINs) have been promoted, relatively little is known about their coverage dimension. However, usage varies among households, and such variation may seriously limit the potential impact of nets. Moreover, the efficacy of these measures against *Anopheles gambiae* in some departments is unknown. The objective of this study was to investigate LLINs coverage and use, and their efficacy against *A. gambiae* in Ouémé department after a mass free distribution. A post survey campaign was undertaken in January, 2012 to assess the effect of campaign six months after LLINs (Olyset net) free distribution in southern Benin. A questionnaire has been provided to heads of 1,600 households randomly selected from four districts. Despite LLINs use reaching more than 80%, coverage was low at 50.9%. A considerable damage rate (14%) of Olyset nets after only 6 months of use was observed. Bioassays revealed inefficacy of olyset toward the local population of *A. gambiae* after only 6 months of use. Free distribution of LLINs rapidly improved their coverage in communities. The rapid wear observed associated with low efficacy raises the problem of the choice of impregnated materials in a context of high vector resistance to insecticides.

Key words: Long lasting insecticidal nets (LLINs), olyset, efficacy, *Anopheles gambiae*, vector control.

INTRODUCTION

Malaria is a severe public health issue, causing roughly 216 million cases of disease and 655,000 deaths per year (World Health Organization (WHO), 2011). Most victims are children under five living in sub-Saharan Africa (WHO, 2011). Malaria 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. Long lasting insecticidal nets (LLINs) are effective tools for malaria prevention and can significantly reduce severe disease and mortality due to malaria, especially among children under five in endemic areas (Lengeler, 2004).

In the recent decade, many countries across sub-Saharan Africa are rapidly increasing insecticide-treated nets (ITNs) coverage through several strategies including, social marketing (Noor et al., 2007; Grabwosky et al., 2007), free distribution to target groups (Beer et al., 2010; Grabwosky et al., 2010), and more recently, free universal population- based distribution campaigns target the entire population at risk (Beer et al., 2010;

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Teklehaimanot et al., 2007). If the results are below what is expected, this is due to several factors. In addition to the extension of resistance to pyrethroids in malaria vectors which represents a serious obstacle to the implementation and use of LLINs in Africa (Corbel et al., 2007; Yadouleton et al., 2010), significant problems remain, endangering the sustainability goals and achievements. Indeed, in recent years, the National Malaria Control Program (NMCP) in African countries has invested heavily in the acquisition and distribution of LLINs for malaria control. However, monitoring the use of these materials is often overlooked.

The use of insecticide-treated nets (ITNs) is a major component of malaria prevention. A meta-analysis by the Cochrane group of 22 studies that used random sampling revealed that sleeping under an ITN can decrease the morbidity and the mortality due to malaria in children under five, respectively by 50 and 17% (Lengeler, 2004). In communities with ITNs coverage over 60%, a group effect occurs and people who do not use the ITNs receive similar protection in comparison with those who use them (Hawley et al., 2003; Russell et al., 2010). Mass ITN distribution campaigns target all persons at risk for malaria, particularly in high transmission settings, and have the advantage of rapidly achieved high community-level coverage which benefits everyone in the community and not just those who own and sleep under nets (Teklehaimanot et al., 2007). This strategy also has the potential to achieve equity in mosquito net ownership and use as shown by a number of studies in different settings (Noor et al., 2007; Beer et al., 2010); however, the level of achievement depends largely on specific context settings and the effectiveness of the distribution strategy. It is therefore important to assess equity in mosquito net ownership and use after each mass distribution in a new setting.

Benin is currently engaging in free mass distributions of LLINs, a type of net that is factory-treated with insecticide and designed to maintain efficacy against mosquito vectors for at least 3 years. In Ouémé department, after indoor residual spray (IRS) withdrawal in 2011, the NMCP with the support of United States Agency for International Development (USAID), distributed LLINs in July 2011 to the inhabitants of the areas of this department previously under IRS intervention in order to limit the recovery of transmission. But the free distribution of LLINs to people is just not for solving social problems created by the cessation of IRS. The big challenge is their use by the communities. That is why the first goal of this study was to determine the utilization rate of nets distributed. The nets distributed are Olyset, impregnated with permethrin. However, mosquitoes have developed a strong resistance against this product. It is for this reason that, after six months of use, we found it important to determine the effectiveness of Olyset on local populations of *Anopheles gambiae*. So, we propose to identify transactions about these nets and their physical integrity, namely the degree of wear. In addition, the resistance of malaria vectors to pyrethroids represents a potential liability for the success of vector control programs in Benin. As the *kdr* mutation that confers vector resistance to pyrethroids is involved in this resistance, it is therefore important to know the impact of this mutation on the efficacy of Olyset currently in use in Benin as part of vector control.

METHODOLOGY

Study area

The study area is located in Ouémé Department and includes four districts: Adjohoun, Dangbo, Missérété and Sèmè (Figure 1), an area of approximately 1000 km^2. The total population is 310,400. There are approximately 65,000 households with 62,890 children aged 0 to 5 years, spread in 174 villages (INSAE, 2004). In 2010, the cumulative incidence of malaria was estimated at 11.2 and 12.6%, respectively in the Departments of Plateau and Ouémé (MS, 2011). Each district includes two different settings: the plateau area, characterized by the presence of temporary mosquito breeding sites and the valley area, characterized by the presence of permanent mosquito breeding sites associated with numerous pools and swamps. The entire region is characterized by a sub-equatorial climate, with two dry seasons (August to September and December to March), and two rainy seasons (April to July and October to November). The average annual rainfall is 1,500 mm with a relative humidity of 70 ± 5% and an average monthly temperature ranging from 23 to 32°C.

Sampling

Selection of study sites

A sampling plan level was used by associating each district with a uniform ponderation, followed by a simple random sample of households. Using probability proportional size (PPS) sampling methodology, two boroughs per district were first selected. PPS was then used to select two villages per borough. Thus, the boroughs of Adjohoun and Démè, Dangbo and Kessounou, Katagon and Missérété, Agblangandan and Djrègbé were selected, respectively in the districts of Adjohoun, Dangbo, Missérété and Sèmè. The purpose of each of the 2 ponderations is to meet the requirement of representativeness of our sample.

Sample size

In view of our objectives and the aim to have a good estimation of the parameters measured by the survey, we decided to visit 96 households per village with a margin of 5% to compensate for contingencies such as non-response due to errors and other registration. Thus, 100 households were visited per village, 400 households per district and a total of 1,600 households for all the study area.

LLIN coverage, use and the degree of wear

Households were interviewed about LLINs ownership and their use as means to prevent malaria. To evaluate the coverage of Olyset distributed by the NMCP in July, 2011, in each district, we

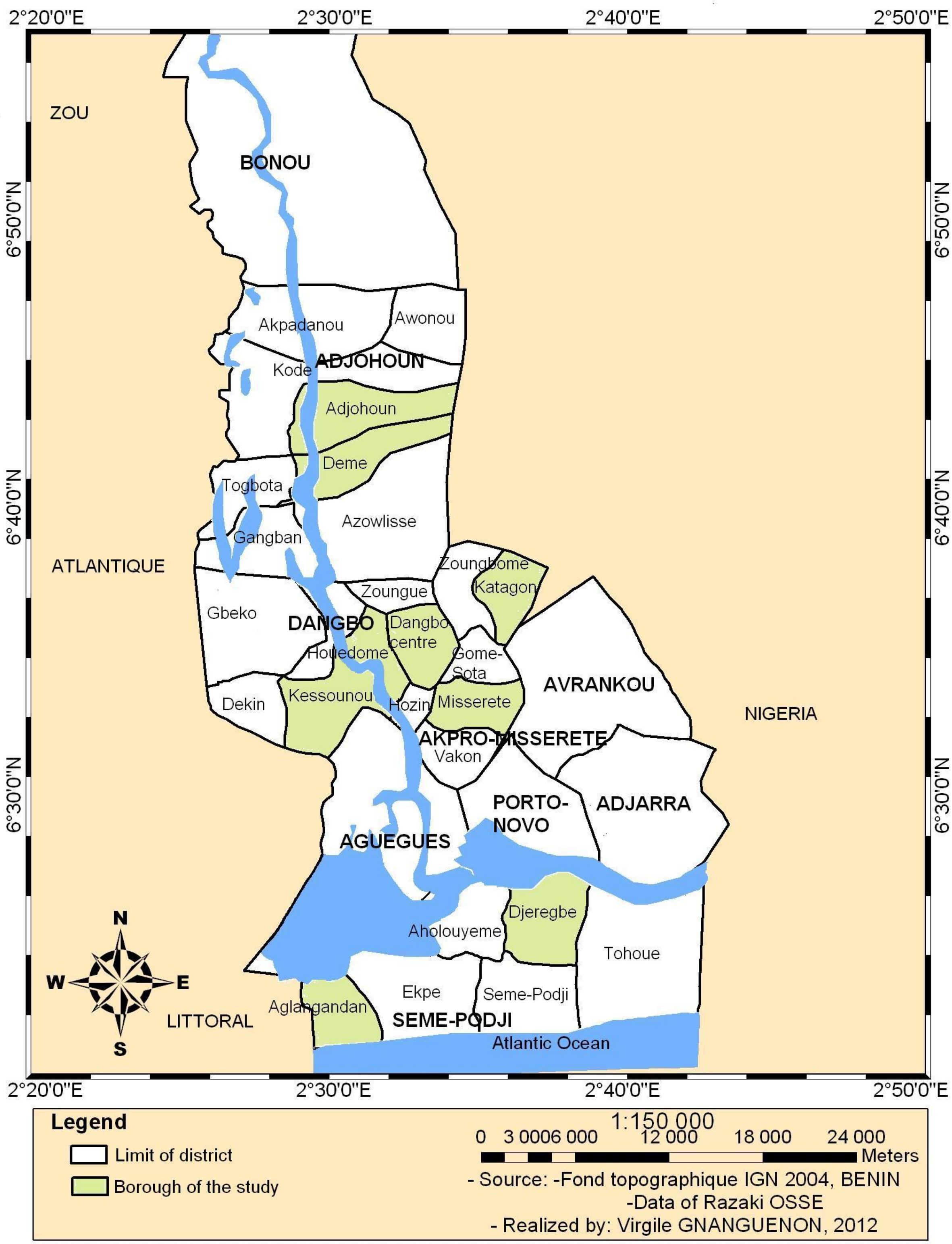

Figure 1. Map of study area - Ouémé department, Benin.

determined the number of people who declared having received Olyset NMCP 2011 and the number of people who do not receive. According to the principle of one net for two people, the coverage rate was calculated as the ratio of the number of Olyset NMCP 2011 available in households by the number of Olyset that should be present. The evaluation of utilization rate of the Olyset distributed by NMCP in 2011 required knowledge of the number of Olyset actually in use in households. People who use them are those who reported having slept under ITNs the previous night of the survey. To calculate this rate, the number of respondents reported sleeping under NMCP Olyset 2011 the previous night was divided over the total number of respondents who reported having received it. A rate of usage over 80% was considered satisfactory. In a household, any net hung or not folded meant it has been used the previous night of the survey, and is supposed to be a net in use. The physical aspect of each net was evaluated. The number and size of the holes in the nets were recorded and classified. The holes and tears observed were classified as (I) small holes size (hole that would allow a thumb to pass through), (II) middle holes size (more than a hole as in (I) but not large enough to allow a hand to pass through), (III) large holes size (hole larger than the fist).

Survey

The survey was conducted 6 months after the campaign in Ouémé department in January, 2012 during the dry season. It is a cross-sectional study based on quantitative and qualitative questionnaire. The quantitative questionnaire had targeted to 1,600 individuals, in particular adult male and female heads of households (family unit). The respondents' consent was sought and gained by explaining the aims of the study. The questionnaire was administered to them by trained interviewers. The questionnaire focused on the knowledge, attitudes and practices of respondents on the quality of the implementation of LLINs and their use. Individuals who could not read or write or understand French language were interviewed in the local language of the Ouémé region. Direct observations on the field allowed us to record the types of nets used by communities in the four districts, their setting, their use and their state after six months of use in order to assess the physical durability of these nets. The information gained was recorded on a form. Surveyors identified, counted and examined each type of nets that were found during the investigation. The purpose of the sociological survey is to assess the state of the nets in terms of both efficiency and utilization, and to assess the sustainability of the nets sampled.

Mosquitoes

The susceptible strain "Kisumu" of *A. gambiae* originated from Kenya and bred in the insectary of the Entomological Research Centre of Cotonou (CREC) was used for the bioassays. A local population of *A. gambiae* collected as larvae and raised to adulthood in the insectary was also used.

WHO cone bioassay

Twenty nets (10 Olyset distributed in July, 2011 and 10 Permanets which are in use for 1 to 2 years: 2009 to 2010) from the study area were randomly selected and submitted to the efficacy test (cone test) to verify the presence of insecticide residues on the fibers. Four other nets (2 new untreated nets, 1 unused Olyset, 1 unused permanet) were also tested and used as negative and positive controls. Olyset nets were treated with 2% permethrin while permanets contained 55 mg active ingredient of deltamethrin per m^2. Cone tests were performed according to the WHO protocol (WHO, 2005). Cones were placed on different sides of LLINs test: 5 to 10 young females aged 2 to 5 days of *A. gambiae* Kisumu and wild *A. gambiae* were introduced into each cone. Mosquitoes were in contact with the different sides for 3 min, then were removed using an aspirator. Once transferred in sterile cups, they were provided with sugar solution (sugar solution to 10%) and maintained at 27 ± 2°C with a relative humidity of 80 ± 10% for 24 h to assess delayed mortality. After 24 h, the mortality rates were determined for each strain of mosquitoes. Abbott (1975) was not used in this study for the correction of mortality rates in the cone test because the mortality rates in all controls was always less than 5%. Survivors and dead specimens from field bioassays were kept in different Eppendorf tubes and passed polymerase chain reaction (PCR) to find the different mechanisms involved in the resistance to insecticides.

Knock down resistance (*kdr*) diagnostic

DNA was extracted from *A. gambiae* Kisumu and wild *A. gambiae* mosquitoes (survivors and dead from the cone tests) and was used to genotype samples for the *kdr* "Leu-phe" allele, using the PCR-based method of Martinez-Torres et al. (1998).

Data processing and analysis

Data obtained from the survey were checked and recorded using Epi Info version 6 and Microsoft Office Excel 2007 for Windows. Simple descriptive statistics such as frequency and percentage of variables were computed and cross-tables were performed using R software version 2.11.1. A chi-square test for the comparison of proportions was performed to compare the proportions of each variable related to each region. Summary procedure base packages of this software were used to perform the frequency distribution of *kdr* mutations. Then the Fisher's exact test was used to compare allele frequencies of *kdr* mutations in the dead and the survivors. The same test was used to compare mortality rates observed for the different genotypes. The level of significance was 0.05.

Ethical clearance

This paper used data from the Ouémé post mosquito net free distribution campaign survey conducted on the behalf the National Malaria Control Program. Because this was part of the programmatic activity, ethical clearance was exempted. Informed consent was obtained from each participant.

RESULT

Nature of nets recorded

During our survey in the four districts, various kinds of nets were found. The majority of LLIN recorded were Olyset distributed in July, 2011 by the NMCP (59.3%: 998 Olyset from a total of 1682 registered) (Table 1) and 2% of other Olyset. LLINs like PermaNet (20.9%) and some unidentified but treated nets (17.8%) were also found. In the district of Sèmè, the distribution rate of Olyset NMCP reported is low: 15.6% against 29.6% in Adjohoun, 26.1% in Dangbo and 28.5% in Missérété district (Table 1).

Household LLIN coverage and use

Table 1 shows the coverage rate of Olyset distributed in July, 2011 in the four districts. In total, 1,996 Olyset

Table 1. Frequency of types of mosquito nets, coverage rate and use rate of Olyset NMCP mosquito nets in some districts from Ouémé department, Benin, in January, 2012.

Districts	Boroughs	Olyset NMCP (I)	Other Olyset	Permanet	Other nets	Total	Coverage rate of Olyset NMCP: People visited	Olyset NMCP which should be distributed (I × 2)	Coverage rate (%) (95% CI)	Coverage rate (%)/district	Use rate of Olyset NMCP: Olyset NMCP used	Use rate (%) (95% CI)	Use rate (%)/district
Adjohoun	Adjohoun	198	1	32	25	256	568	396	69.7 [66, 73]	70.1	184	92.9 [89, 96]	84.1
	Deme	98	7	42	26	173	276	196	71.0 [65, 76]		65	66.3 [57, 75]	
Dangbo	Dangbo Centre	96	5	59	16	176	499	192	38.5 [34, 42]	47.9	84	87.5 [81, 94]	87.4
	Kessounou	165	3	31	35	234	590	330	55.9 [52, 60]		144	87.3 [82, 92]	
Misserete	Katagon	88	0	38	21	147	405	176	43.5 [39, 48]	60.9	70	79.6 [71, 87]	69.8
	Missérété	197	0	25	20	242	531	394	74.2 [70, 77]		129	65.5 [58, 72]	
Seme	Djregbe	59	17	25	120	221	497	118	23.7 [20, 27]	29.6	55	93.2 [87, 99]	89.7
	Agblangandan	97	0	99	37	233	558	194	34.8 [31, 38]		85	87.6 [81, 94]	
	Total	998 (59.3%)	33 (2%)	351 (20.9%)	300 (17.8%)	1682	3924	1996	50.9 [49, 52]	-	816	81.8 [79, 84]	-

CI: Confidence interval.

should be distributed to 3,924 people (children and adults). Thus, 50.9% of this population has received the new Olyset meaning 1 Olyset for 1.9 person. In Adjohoun and Misséréte districts, the rate was higher, respectively, 70.1% (1 Olyset for 1.4 person) and 60.9% (1 Olyset for 1.6 person). However, the coverage rate was low in Dangbo district (47.9%: 1 Olyset for 2.1 persons) and very low in Sèmè (29.6%: 1 Olyset for 3.4 persons). But in these two latter districts, if we took into account the other nets available before the distribution campaign in July, 2011, the coverage would be improved: 1 Olyset for 1.2 person in Sèmè (86.1%) and 1 Olyset for 1.3 person in Dangbo (75.3%). Among 998 Olyset distributed, 816 were regularly used. The usage rate was 81.8% (Table 1). This rate was high in all localities visited except Misséréte where it was less than 80% (Table 1).

Households LLIN position and location

In Adjohoun, Dangbo and Sèmè districts, more than 82% of Olyset NMCP received by the populations were in use. But at Misséréte, the percentage of Olyset in use was lower (below 70%) (Table 2); 92.4% of the Olysets NMCP not in use were stored. Overall, in all 4 districts, 81.7% of Permanets and other types of Olyset were in use.

Wear on Olyset after 6 months of use

After six months of use, 14% of Olyset NMCP were found with tears and holes (Table 3). The frequency of mosquito holes varied from one district to another: 4.9% in Misséréte, 10.8% in Adjohoun, 18.6% in Sèmè and 24.5% in Dangbo (Table 3). Of the 139 Olyset found with holes, 557 holes were recorded, averageing 4 holes per Olyset. The holes of size I, II and III represent, respectively 27.1, 41.3 and 31.6% of all the holes. In Adjohoun and Misséréte districts, holes size II were the most recorded, respectively 55.2 and 75.5% (Figure 2). In both districts, there was a significant difference between the frequency of these holes and the holes of size I and III ($p < 0.05$).

In Sèmè, the frequency of holes size III (47.2%) on the Olyset NMCP was significantly higher than the holes size II (33.3%) and size I (19.5%) ($p < 0.05$). But in Dangbo, frequencies of holes size I (37.5%) and size III (34.4%) were almost the same ($p > 0.05$) and were significantly higher compared to the frequency holes size II ($p < 0.05$) (Figure 2).

Table 2. Position and place of mosquito nets in households at some districts in the department of Ouémé, Benin, in January, 2012.

Types of nets	Localities	Position of LLINs suspended		Place of LLINs unsuspended		
		N	% (CI 95%)	Suitcase	On rope	Total
Olyset NMCP	Adjohoun	245	82.8 [78, 87]	47	4	51
	Dangbo	225	86.5 [82, 90]	32	3	35
	Missérété	194	68.1 [62, 74]	86	5	91
	Sèmè	137	87.8 [82, 93]	16	3	19
Other Olyset	Adjohoun	8	100 [63, 100]	0	0	0
	Dangbo	8	100 [63, 100]	0	0	0
	Missérété	0	0	0	0	0
	Sèmè	14	82.4 [57, 96]	3	0	3
Permanet	Adjohoun	59	79.7 [69, 88]	13	2	15
	Dangbo	81	90 [82, 95]	9	0	9
	Missérété	50	79.4 [67, 89]	12	1	13
	Sèmè	107	86.3 [79, 92]	17	0	17
Other nets	Adjohoun	47	92.2 [81, 98]	2	2	4
	Dangbo	48	94.1 [84, 99]	3	0	3
	Missérété	25	61.0 [45, 76]	16	0	16
	Sèmè	125	79.6 [73, 86]	30	2	32
Total		1373	81.7 [80, 84]	286 (92.9%)	22 (07.1%)	308

N: number; CI: Confidence interval.

Effectiveness of Olyset after 6 months of use

More than 400 specimens of *A. gambiae* Kisumu (susceptible reference strain) were exposed to Olyset after 6 months of use. Most Olyset tested were effective. However, one of them (1/10) gave a mortality rate less than 80%, the threshold of bio-efficacy of ITNs on susceptible mosquito strains (Figure 3). However, for the other types of nets that were in use for more than 6 months, especially mosquito nets of type Permanet 2.0, the mortality rates of *A. gambiae* Kisumu were below 65.1% (Figure 3). With the resistant population (local *A. gambiae*), there was a remarkable decline in the effectiveness of two types of nets (Olyset NMCP and Permanet). The mortality rate recorded was between 0 and 36.4% for Olyset after 6 months of use (Figure 3), and between 0 and 11.8%, with Permanets in use for 2 years (Figure 3).

Kdr genotyping

The allele frequency of the *kdr* mutation in 154 individuals (26 dead and 128 alive) from the tested population was 90.3%. Moreover, the allele frequency of the *kdr* mutation in *A. gambiae* Kisumu was 0%. The *kdr* frequencies in dead and alive mosquitoes were, respectively 94 and 89.5%. No significant difference in allele frequency of this mutation was observed between dead mosquitoes and those alive (p = 0.5) (Table 4). As the *kdr* mutation is partially recessive, we compared the mortality rates observed in individuals with genotype RS with those observed in individuals with genotype RR. The mortality rate of mosquitoes RS genotype was 10%, and 17.7% for RR genotype. These rates were not significantly different (p = 0.5) (Table 5).

DISCUSSION

The large-scale introduction of LLINs has become a priority for malaria prevention. Access to universal coverage of LLINs and proper use of these nets are a challenge for an effective control against malaria in sub-Saharan Africa. Many African countries are already investing in the free distribution of mosquito nets. Actually, the overall coverage is below the WHO target to reach at least 80% of people at risk or suffering from malaria (WHO, 2008). According to a study by the UNDP (United Nations, 2009), the success in achieving the objective of the sixth MDGs (Objective of the Millennium Development Goals) and other global targets for malaria depends on public awareness about the value of human health, the use of treated mosquito nets and the provision of effective access to nets.

Table 3. Frequency of damaged mosquito nets in districts from the Ouémé department, Benin, in January 2012.

Localities and types of nets	Total nets	No. of damaged nets	Frequency of damaged nets (%) (95% CI)
Adjohoun			
Olyset NMCP	296	32	10.8 [7, 14]
Other Olyset	8	5	62.5 [29, 96]
Permanet	74	18	24.3 [15, 34]
Other nets	51	21	41.2 [28, 55]
Dangbo			
Olyset NMCP	261	64	24.5 [19, 30]
Other Olyset	8	4	50 [15, 85]
Permanet	90	31	34.4 [25, 44]
Other nets	51	18	35.3 [22, 48]
Misserete			
Olyset NMCP	285	14	4.9 [2, 7]
Other Olyset	0	0	-
Permanet	63	17	27 [16, 38]
Other nets	41	6	14.6 [4, 25]
Seme			
Olyset NMCP	156	29	18.6 [12, 25]
Other Olyset	17	5	29.4 [8, 51]
Permanet	124	59	47.6 [39 56]
Other nets	157	62	39.5 [32 47]

N: number CI: Confidence interval.

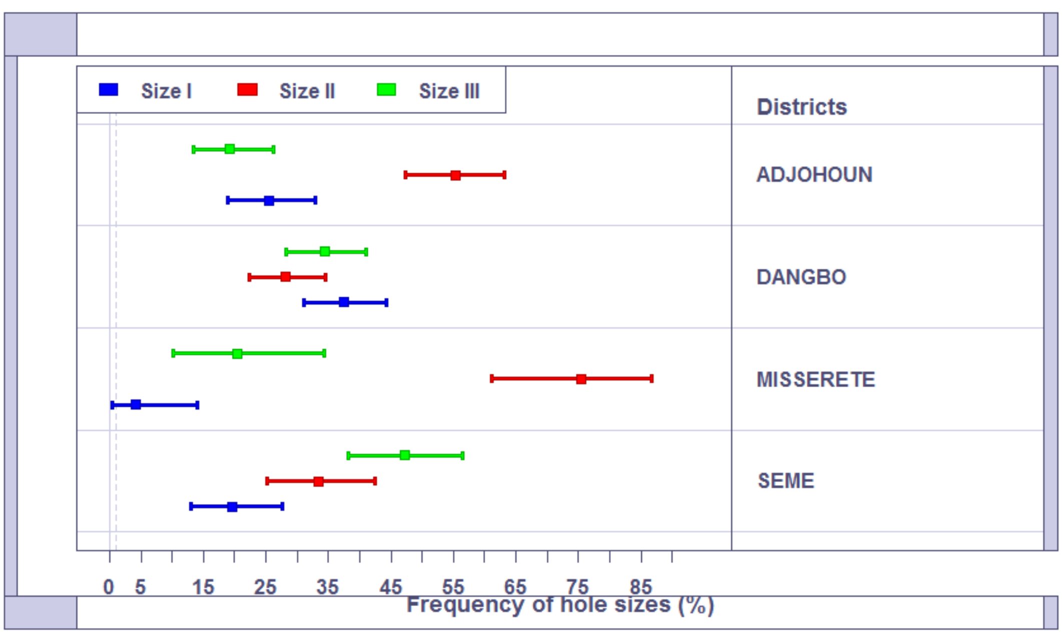

Figure 2. Frequency of hole types on the Olysets NMCP mosquito nets in some districts at the department of Ouémé, Benin, in January, 2012.

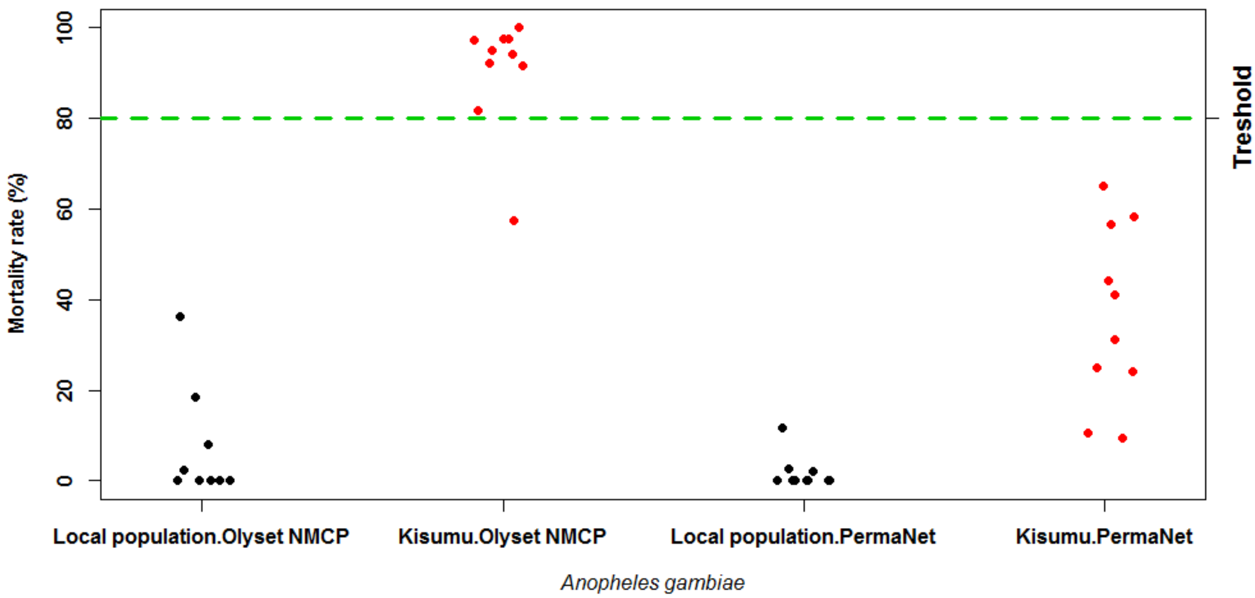

Figure 3. Mortality rate of *Anopheles gambiae* Kisumu (susceptible strain) and local population (resistant strain) observed 24 hours after a 3 min- exposure to Olyset NMCP and Permanet nets withdrawn from the study areas.

Table 4. Allele frequencies of the *kdr* mutation observed in *A. gambiae* after exposure to Olyset mosquito nets in the department of Ouémé, Benin, in January 2012.

Status of mosquitoes	*Kdr* mutation					
	Total	RR	RS	SS	Frequency (%)	p
Kisumu (susceptible strain)	25	0	0	25	0	
Survivors	129	102	27	0	89.5	0.5
Dead	25	22	3	0	94.0	
Total	154	124	30	0	90.1	

Table 5. Mortality rate of *A. gambiae* exposed to Olyset mosquito nets according to genotypes for the kdr mutation in the department of Ouémé, Benin, in January, 2012.

Genotype	N tested	N dead	Mortality rate (%)	p
RR	124	22	17.7	0.5
RS	30	3	10.0	

This survey showed that the Olyset LLIN average coverage in the department of Ouémé in 2011 is generally good with a rate of 50.9% (one Olyset for 1.9 person), which corresponds to the expectations of the NMCP. But, the Olyset coverage rate was especially low in Dangbo (1 Olyset for 2.1 persons) and very low in Sèmè (1 Olyset for 3.4 persons). In 2001, before the national campaign distribution of LLINs in southern Benin, only 4.3% of homes owned an ITN and 2.4% of children under 5 were sleeping under insecticide treated nets (Kinde-Gazard et al., 2004). The significant increase in coverage after the distribution of LLINs campaigns is quite logical and has been previously recognized (Skarbinski et al., 2007; Thwing et al., 2008; Matovu et al., 2009). However, the coverage rate obtained is lower than those observed in households in Kenya (68%), Niger (70%) and Mali (81%) after the national campaign distribution (Cervinskas et al., 2008; Thwing et al., 2008; Hightower et al., 2010).

The use of Olyset in turn is better in the whole population (81.8%) except Missérété, where the Olyset use is lower than 80% (74%). This high use of LLINs observed among populations of the four districts during the dry season can be explained by the presence of permanent breeding spots and swamps in those areas, causing a permanent nuisance of Culicidae. These rates are higher than those recorded by Ahorlu et al. (1997) and Toe et al. (2009) in previous studies in West Africa during the same period (dry season). Indeed, these studies that were conducted in Ghana and Burkina Faso showed that people were less motivated to use the nets during the dry season with high temperatures at night.

The physical barrier provided by LLINs is very important. It is the first factor of personal protection against mosquito bites and we must ensure that they retain this physical integrity. Adjohoun, Dangbo and Sèmè showed that more than 10% of Olyset were torn after only 6 months of use, so there is an accelerated degradation of nets fiber. The degradation was increased in areas where lake water is permanently available (Adjohoun, Dangbo and Sèmè). The proximity with water and high anopheline densities are some of the reasons of the extensive use of nets and their regular washing. LLINs fibers were thereby weakened and this reduces their durability, besides insecticide concentration. Indeed, when children urinate or oil stains nets, these nets were systematically washed. On the other hand, in some communities, another factor contributing to accelerate the degradation of LLINs are the types of beds (the racks) and the use of burning lamps. The degradation is less pronounced in Missérété. Indeed, in this locality, nets sustained less pressure due to the lower use rate in comparison with the other districts.

In a sociological study conducted in 2005 in the framework of Pal + program, residents of Kétonou, a district of high nuisance of mosquitoes like Dangbo and Sèmè located along lake Nokoué, said that in their area, people “eat and sleep” in nets. If this statement is true, it shows the frequent contact that might exist between man and mosquito net in some environments. The results of the sociological survey at that time showed more than 95% of the population regularly used their nets. Additional nets were also available for visitors.

The percentage of holed LLINs and the size of their holes vary from one district to another. The presence of large holes constitutes a big problem because according to Hill et al. (2006), once LLINs are holed, they lose their protection. But to reinforce the effectiveness of holed LLINs and to increase then lifetime, we suggest to provide repair kits to communities to repair nets with large holes. Preliminary works showed that ITNs were effective even if they were holed. Nowadays, due to the high level of resistance of mosquitoes to insecticides, these data must be updated.

In our study area, the acceptability of LLINs is good. The majority of LLINs distributed by the NMCP were found hung in households. The LLINs which were not found hung were stored or given to other family members. A similar situation was also observed in Niger (Thwing et al., 2008). The results obtained in this study show a low efficacy of Olyset against wild population of *A. gambiae*. As a matter of fact, the mortality rates observed after exposure specimens of wild *A. gambiae* to Olyset were very low, confirming the resistance of these mosquitoes to pyrethroids previously reported by several authors (Akogbéto and Yacoubou, 1999; Etang et al., 2003; Corbel et al., 2007; Yadouléton et al., 2010). These low mortality rates are due to fact that pyrethroids affect sodium channel and vectors carrying the *kdr* mutation. This target is changed and the insecticide is no longer the channel’s specific receptor and therefore cannot act. However, the dead mosquitoes were nevertheless recorded among the individuals carrying the *kdr* mutation, indicating that this is probably not the only port of the *kdr* mutation that determines the resistance of vectors (Padonou et al., 2012). It was shown that other mechanisms, including enzymatic mechanisms were involved in this resistance (Djouaka et al., 2008; Yewhalaw et al., 2011).

In population genetics, a phenotype is expressed by the combination of genotypes, environment and gene interactions. These gene interactions are important as they affect the fitness of individuals. The fact of being the bearer of the resistance allele is not the only parameter for the survival of the individual in an environment under insecticide treatment. Furthermore, it is important to note that a gene mutation also causes it to be advantageous genetic burden for the population considered. The resistance of malaria vectors to pyrethroids is a potential threat to the success of vector control using this tool. The mechanisms involved in this resistance appear to be complex and finer investigations on this subject are desirable. As part of these investigations, it is imperative to also take into account gene interactions, genetic burden is relatively difficult to quantify. If this decrease in efficacy was related to the resistance, it also is possible that the demand of LLINs in the tropics cause a decrease of rigor of the production control of these LLINs before leaving industries. In addition, some sampled nets stay long in health facilities in inadequate conditions before being used. During this long period, it is possible that the active ingredient in the insecticide content lost some of its effectiveness.

Permanets inefficacy, one to two years after their use, may be due not only to their usage time but also to some wash soaps used by recipients. In fact, the majority of Permanets removed (9/10) were washed with detergents (Kogui and Klin) since their acquisition. However, previous studies showed that some washing soaps have a negative impact on the effectiveness of insecticide-treated nets (Azondékon and Vigninou, unpublished).

The results of this study relativize the idea of the concept of "long-lasting" of nets under field conditions. Indeed, a perforation rate (14%) of Olyset after only 6 months of use was not low. One wonders if it is the material used in the manufacture of the net that is in question, or the early perforations are due to a lack of care and maintenance in households. Nets were distributed free of charge to people regularly and could be considered as a vulgar tool and lose their value. The rapid wear associated with low efficacy raises the problem of the choice of materials impregnated in a context of high vector resistance to insecticides. Each year, we propose a new formulation of insecticide solution to control resistant mosquitoes or a new type of ITN with longer duration of action. But on the field, new tools behave as much the same way as the previous tools.

Conclusion

Overall, access to universal coverage based on one Olyset for two people during the distribution of LLINs in July, 2011 was reached in the department of Ouémé. Taking into account other nets rather than Olyset, this access is exceeded. However, in Sèmè and Dangbo districts, Olyset NMCP coverage is below the level expected. It is interesting that the NMCP reviewed its strategy of distributing nets to bring all districts towards universal coverage. The results show a decrease in efficacy of Olyset towards the local mosquito populations after only 6 months of use. The concept of LLINs as defined by the designers fails to take into account current status of mosquito resistance to insecticides and the various pressures that may undergo the routine use of nets in communities. Today, LLINs are freely given to the public in many countries. This gratuity certainly improves their possession but does not guarantee effective home use. It is the awareness and involving of the social human potential that can yield results beyond our expectations and induce the perpetuation of the use of mosquito nets at the community level.

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REFERENCES

Abbott W (1975). A method of computing the effectiveness of an insecticide. J. Economic. Entomol. 18(4):265-267.

Ahorlu CK, Dunyo SK, Afari EA, Koram KA, Nkrumah FK (1997). Malaria related beliefs and behaviour in southern Ghana: implications for treatment, prevention and control. Trop. Med. Int. Health 2(5):488-499.

Akogbéto M, Yakoubou S (1999). Resistance of malaria vectors to pyrethrins used for impregnating mosquito nets in Benin, West Africa. Bull. Soc. Pathol. Exot. 92:123-130.

Azondékon R, Vigninou E. Contrôle de qualité des moustiquaires imprégnées commercialisées ou distribuées au Benin. Mémoire de fin de cycle, EPAC/UUAC, 80 p (unpublished).

Beer N, Ali AS, de Savigny D, Al-Mafazy AW, Ramsan M, Abass AK, Omari RS, Björkman A, Källander K (2010). System effectiveness of a targeted free mass distribution of long lasting insecticidal nets in Zanzibar, Tanzania. Malar. J. 9:173.

Cervinskas J, Berti P, Desrochers R, Mandy J, Kulkarni M (2008). Évaluation de la possession et de l’utilisation des moustiquaires imprégnées de l’insecticide long durée (MILDs) au Mali huit mois après la campagne intégrée de décembre 2007: Rapport Final. Ottawa: HealthBridge Canada. November 30. Disponible sur l’internet à http://www.healthbridge.ca/assets/images/pdf/Malaria/Final%20Mali%20Report%20nov%2030.pdf (http://www.healthbridge.ca/reports_e.cfm#Malaria)

Corbel V, N'Guessan R, Brengues C, Chandre F, Djogbenou L, Martin T, Akogbéto M, Hougard J, Rowland M (2007). Multiple insecticide resistance mechanisms in *Anopheles gambiae* and *Culex quinquefasciatus* from Benin, West Africa. Acta. Trop. 101(3):207-216.

Djouaka RF, Bakare AA, Coulibaly ON, Akogbeto MC, Ranson H, Hemingway, Strode C (2008). Expression of cytochrome P450s, CYP6P3 and CYP6M2 are significantly elevated in multiple pyrethroid resistant populations of *Anopheles gambiae* s.s. from Southern Benin and Nigeria. BCM. Genomics., 9:538doi:101186/1471-2164-9-538.

Etang J, Manga L, Chandre F, Guillet P, Fondjo E, Mimpfoundi R, Toto JC, Fontenille D (2003). Insecticide susceptibility status of *Anopheles gambiae* s.l. (Diptera Culicidae) in the Republic of Cameroon. J. Med. Entomol. 40:491- 497.

Grabowsky M, Nobiya T, Selanikio J (2007). Sustained high coverage of insecticide-treated bednets through combined Catch-up and Keep-up strategies. Trop. Med. Int. Health 12:815-822.

Grabowsky M, Farrell N, Hawley W, Chimumbwa J, Hoyer S, Wolkon A, Selanikio J (2010). Integrating insecticide-treated bednets into a measles vaccination campaign achieves high, rapid and equitable coverage with direct and voucher-based methods. Trop. Med. Int. Health 10:1151-1160.

Hawley WA, Phillips-Howard PA, ter Kuile FO, Terlouw DJ, Vulule JM, Ombok M, Nahlen BL, Gimnig JE, Kariuki SK, Kolczak MS, Hightower AW (2003). Community-wide effects of Permethrin treated bed nets on child mortality and malaria morbidity in western Kenya. Am. J. Trop. Med. Hyg. 68(4):121-127.

Hightower A, Kiptui R, Manya A, Wolkon A, Leigh Vanden Eng J, Hamel M, Noor A, Sharif SK, Buluma R, Vulule J, Laserson K, Slutsker L, Akhwale W (2010). Bed net ownership in Kenya: the impact of 3.4 million free bed nets. Malar. J 9:183.

Hill J, Lines J, Rowland M (2006). Insecticide-treated nets. Adv. Parasitol., 61:77–128. http://dx.doi.org/10.1016/S0065-308X(05)61003-2

INSAE (2004). Fichier village Ouémé. 29 p.

Kinde-Gazard D, Gbenou D, Tohon C, Da Silva C, Nahum A, Massougbodji A (2004). Indicateurs de suivi et d’évaluation en 2001 de l’initiative « Faire reculer le paludisme» au Bénin. Bull. Soc. Pathol. Exot 97:349-352.

Lengeler C (2004). Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev. (2):CD000363.

Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Bergé JB, Devonshire AL, Guillet P, Pasteur N, Pauron D (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* s.s. Insect. Mol. Biol 7:179–184.

Matovu F, Goodman C, Wiseman V, Mwengee W (2009). How equitable is bed net ownership and utilization in Tanzania? A

practical application of the principles of horizontal and vertical equity. Malar. J. 8:109.
Ministère de la Santé (2011). Annuaire des statistiques sanitaires 2011. Direction de la Programmation et de la Prospective, Cotonou, Bénin.
Noor AM, Amin AA, Akhwale WS, Snow RW (2007). Increasing coverage and decreasing inequity in insecticide-treated bed net use among rural Kenyan children. PLoS. Med. 4:e255.
Padonou GG, Sezonlin M, Osse R, Aizoun N, Oke-Agbo F, Oussou O, Gbedjissi G, Akogbeto M (2012). Impact of three years of large scale Indoor Residual Spraying (IRS) and Insecticide Treated Nets (ITNs) interventions on insecticide resistance in *Anopheles gambiae* s.l. in Benin. Parasit. Vectors 5:72.
Russell TL, Lwetoijera DW, Maliti D, Chipwaza B, Kihonda J, Charlwood JD, Smith TA, Lengeler C, Mwanyangala MA, Nathan R, Knols BGJ, Takken W, Killeen GF (2010). Impact of promoting longer-lasting insecticide treatment of bed nets upon malaria transmission in a rural Tanzanian setting with pre-existing high coverage of untreated nets. Malar. J. 9:187.
Skarbinski J, Massaga JJ, Rowe AK, Kachur SP (2007). Distribution of free untreated bednets bundled with insecticide via an integrated child health campaign in Lindi Region, Tanzania: lessons for future campaigns. Am. J. Trop. Med. Hyg. 76 (6):1100-1106.
Teklehaimanot A, Sachs JD, Curtis C (2007). Malaria control needs mass distribution of insecticidal bednets. Lancet 369:2143-2146.
Thwing J, Hochberg N, Vanden Eng J, Issifi S, Eliades MJ, Minkoulou E, Wolkon A, Gado H, Ibrahim O, Newman RD, Lama M (2008). Insecticide-treated net ownership and usage in Niger after a nationwide integrated campaign. Trop. Med. Int. Health 13(6):827-834.
Toe LP, Skovmand O, Dabire KR, Diabate A, Diallo Y, Guiguemde TR, Doannio JM, Akogbéto M, Baldet T, Gruenais ME (2009). Decreased motivation in the use of insecticide-treated nets in a malaria endemic area in Burkina Faso. Malar. J. 8:175.
United Nations (2009). The Millennium Development Goals Report 2008. Accessed at www.un.org on August 17, 2009.
WHO (2005). Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. WHO/CDS/WHOPES/GCDPP/2005.11, 18 p.
WHO (2008). World Malaria Report 2008. Geneva, Switzerland: World Health Organization.
WHO (2011). World Malaria Report 2011. Geneva, Switzerland: World Health Organization. http://www.who.int/malaria/world_malaria_report_2011/en/ (accessed 21.05.2012).
Yadouleton AW, Padonou G, Asidi A, Moiroux N, Banganna S, Corbel V, N'guessan R, Gbenou D, Yacoubou I, Gazard K, Akogbeto MC (2010). Insecticide resistance status in *Anopheles gambiae* in southern Benin. Malar. J. 9:83.
Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, Van Bortel W, Denis L, Tessema DA, Getachew Y, Coosemans M, Duchateau L, Speybroeck N (2011). Multiple insecticide resistance: an impediment to insecticide-based malaria vector control program. PLoS One, 6(1):16066.