Effectiveness of Permanet in Côte d'Ivoire Rural Areas and Residual Activity on a Knockdown-Resistant Strain of *Anopheles gambiae*

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ABSTRACT The effectiveness of long-lasting preimpregnated nets of Permanet type (deltamethrin, 50 mg/m^2) erected in households in rural areas of Côte d'Ivoire was tested on two laboratory strains of *Anopheles gambiae* s.s.: the Kisumu susceptible strain and the Vk per pyrethroids resistant strain with >70% kdr allelic frequency. Treated nets were distributed in households in three villages of Danané forest area in western part of Côte d'Ivoire. In each village, a net was sampled for bioassays. Three Permanets also were erected in the laboratory, serving as control samples. From May 2001 to July 2002, the effectiveness of these deltamethrin-pretreated nets was monitored using World Health Organization-cone tests on the two strains of *An. gambiae*. Mortality rates were recorded 24 h postexposure. Knockdown times for 50 and 95% mosquitoes (kdT_{50} and kdT_{95}, respectively) were estimated by means of WIN DL software. One-way analysis of variance was used to compare the knockdown times. Times to failure of nets were analyzed using Cox model. The kdT_{50} of the Kisumu susceptible strain with both laboratory samples and nets used in the field varied around 10 min. No significant difference was recorded between the kdT_{50} of the Kisumu susceptible strain with laboratory kept nets and samples of nets used in the field. The kdT_{95} values were in the same scale with the two types of nets. The kdT_{50} of the Vk per resistant strain when exposed to used nets were twofold that of the Kisumu susceptible strain at the beginning of the trial, and they increased to fivefold 15 mo later. These latter kdT_{50} significantly differed to those of the Kisumu susceptible strain tested with laboratory and field samples of nets. The kdT_{95} significantly differed from those of the Kisumu strain with laboratory kept nets and with field kept nets. Baseline bioassay mortality rates were always 99–100% with the Kisumu susceptible strain, and they did not show any significant difference between laboratory-kept nets and field-used nets during the 15-mo trial. With the Vk per resistant strain, the expected long-lasting activity was not achieved. A high decrease of mortality rates was observed from 69 to 75% in the first 3 mo to 2% at the month 15. This mortality was associated with significant differences between Vk per resistant strain tested with field-used nets compared with Kisumu susceptible strain tested with both laboratory kept-nets and field-used nets. This study emphasized the actual long-lasting effectiveness of Permanet against the *An. gambiae* Kisumu susceptible reference strain and a rapid decrease of residual activity against a strain with kdr-based resistance to pyrethroids.

KEY WORDS *Anopheles gambiae*, knockdown resistance, long-lasting impregnated nets, Côte d'Ivoire

Scaling up of insecticide-impregnated bed-nets is one of the major components of the World Health Organization (WHO) Roll Back Malaria initiative. To date, six pyrethroid insecticides are recommended for bed-net impregnation: α-cypermethrin, cyfluthrin, deltamethrin, λ-cyhalothrin, permethrin, and etofenprox (pseudopyrethroid) (Lines and Zaim 2000, WHO 2000, Zaim et al. 2000). They are advocated for vector control because of their quick knockdown and lethal effects on mosquitoes at low concentrations (Chavasse and Yap 1997).

However, a practical obstacle to sustainable implementation of this approach is the need for impregnation and regular reimpregnation of nets in the field (Chavasse et al. 1999). Net treatment requires technical knowledge and skill and available and affordable product and community participation. Inadequacy of these and other factors results in low rate of net re-impregnation and the decline of effectiveness (Kachur et al. 1999). To overcome these obstacles, various concepts have been developed: sachets and tablet pyrethroid presentations for individual treatments

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(WHO 1999), establishment of net impregnation centers at district level of primary health care (WHO 1997). The most consistent advance is some manufacturers' supplying pretreated nets, such as Moskitul (deltamethrin, 25 mg [AI]/m^2), Olyset Net (made of polyethylene fiber with 2% permethrin incorporated during manufacturer), and Permanet (deltamethrin, 50 mg [AI]/m^2) having long-lasting efficacy (4 yr). Results of field and laboratory trials have demonstrate such long-lasting efficacy of pretreated nets against mosquitoes (Nguyen et al. 1996, Njunwa et al. 1996, Carnevale et al. 1998). Considering the ecoepidemiological diversity of malaria, each tool should be trailed in different areas to precise its efficacy in various contexts, especially against pyrethroid-resistant vector strains.

Resistance to pyrethroid insecticides in *An. gambiae*, the major malaria vector in Africa is a growing issue in several African countries (Elissa et al. 1993; Darriet et al. 1997; Chandre et al. 1999a, 1999b, 2000; Etang et al. 2003; Awolola et al. 2005; Reimer et al. 2005). This resistance is mainly due to a single point mutation in the gene coding for the sodium channel structure of the nerve, resulting in leucine amino acid change in phenylalanine (in West Africa) or in serine (in East Africa) (Martinez-Torres et al. 1998, Ranson et al. 2000). The spread of kdr mutation conferring cross-resistance to a wide range of pyrethroids and DDT is seen as a threat for malaria vector control, because dieldrin resistance was one of the major reason of failure of house-spraying campaign in the 1950s (Carnevale and Mouchet 2001).

The aim of this study was to evaluate the effectiveness of a long-lasting net, Permanet, hung in rural areas in Côte d'Ivoire, and to monitor its residual activity against an *Anopheles gambiae* s.s. pyrethroid-resistant strain carrying kdr gene at high frequency.

Materials and Methods

Permanet Distribution in Study Sites. Supply of Permanet (long-lasting nets) preimpregnated with deltamethrin at 50 mg (AI)/m^2 was supported by the PAL+ Programme/Institut de Recherche pour le Développement (IRD) (Montpellier, France). Nets are made of white multifilament polyester fabric (75 denier, 156 mesh, 12 by 13 holes/in.^{2}, 15 m^2, Vestergaard Frandsen A/S, Copenhagen, Denmark).

The bed-nets were kept into households of three villages Finneu, Bouenneu, and Danta, all near Danané town (7° 15′ N, 8° 9′ W, 365-m altitude) in western Côte d'Ivoire, West Africa. Impregnated bed-nets were not used in this area before this trial. The average of daily temperature in study sites during the trial was ≈25.4°C, the minimum and maximum being 20 and 34°C, respectively. One Permanet was hung in a hut per village, without washing during the whole trial for bioassay. Three control nets were hung in a warehouse, i.e., in lower conditions of damage at Institut Pierre Richet Bouaké (25°C and 70–80% RH).

From May 2001 to July 2002, pieces (15 by 15 cm) of Permanet were sampled each 3 mo, both from the field and laboratory nets, and they were used for bioassays with WHO cone tests (WHO 1998). For each test, a total of six pieces of nets were randomly sampled per netting; two from the top and one from each side.

***An. gambiae* Strains.** Two reference laboratory strains of *An. gambiae* were used for bioassays. The Kisumu susceptible reference strain originated from Kenya, and the Vk per resistant strain originated from Valley of Kou in Burkina Faso. The latter strain is resistant to pyrethroids and DDT, with >70% kdr allelic frequency (our unpublished data). Both Kisumu and Vk per were reared under laboratory conditions (25 ± 2°C and 70–80% RH) in separate rooms at Institut Pierre Richet Bouaké in Côte d'Ivoire.

Bioassays. Bioassays were carried out each 3 mo from fresh nets to month 15 in the laboratory, by using 3- to 5-d-old nonblood-fed females. Six WHO cones were fastened on Permanet nettings. Ten mosquitoes were introduced in each cone by using a plastic aspirator. After 3-min exposure to treated netting, mosquitoes were transferred to another WHO cone fixed to untreated netting, and the number of individuals knocked down was recorded each 10 min during 1 h. Then, mosquitoes were kept in the insectarium and supplied with 10% honey solution. Mortality rates were determined after 24-h holding period.

Ten to 20 batches of 10 mosquitoes were used for each bioassay, and five batches were exposed to untreated netting as control. Bioassays were done in the laboratory; temperature and humidity were held at 25°C and 70–80% RH, respectively.

Data Analysis. Knockdown data were analyzed using WIN DL software, version 2.0 (Giner et al. 1999), according to the Finney model (Finney 1971). The knockdown times for 50 and for 95% (kdT_{50} and kdT_{95}, respectively) tested mosquitoes were determined. These values were compared with one-way analysis of variance (ANOVA). The level of significance of each test was adjusted to account for the other tests using the sequential procedure of Bonferroni (Rice 1989).

Mortality rates were calculated and analyzed according to World Health Organization (WHO 1999) to determine whether nets were effective. Thus, nets were considered as effective when mortality was >80%. A Cox proportional hazard model was used to estimate the time of nets failure on the Kisumu susceptible strain and the Vk per resistant strain. Both the one-way ANOVA test and the Cox model were realized using SPSS software, version 11.5 (SPSS Inc. 2002).

Results

Control Assays with Untreated Nets. No knockdown effect was observed after the exposure of both Kisumu susceptible strain and the Vk per resistant strain to untreated nets. The mortality rates recorded with untreated nets was always below 2%.

Knockdown Times. With new nets, the kdT_{50} of the Kisumu susceptible strain was ≈5 min, whereas that of the Vk per resistant strain was 16.4 min, i.e., threefold

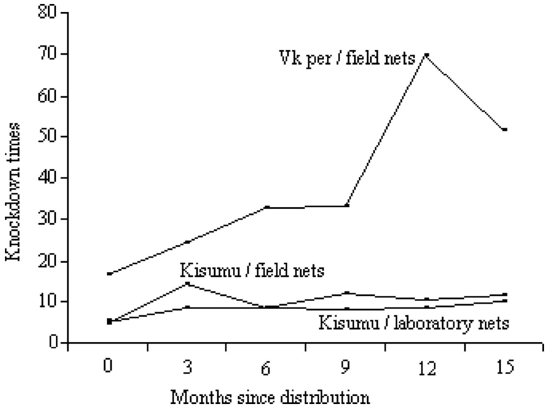

Fig. 1. kdT_{50} in minutes for 50% *An. gambiae* of the Kisumu and Vk per strains tested with laboratory kept nets and field used nets.

that with Kisumu (Fig. 1). Similarly, the kdT_{95} was ≈10 min with the Kisumu strain and 32.7 min with the Vk per strain (Fig. 2).

From the third month to the end of the trial, the kdT_{50} of the Kisumu susceptible strain was ≈10 min, i.e., approximately twofold the kdT_{50} observed with new nets (both laboratory-kept and field-used nets). No significant difference was observed between the kdT_{50} of the Kisumu susceptible strain with laboratory kept nets and with field-used nets ($P = 1$, 95% CI). The kdT_{95} values were in the same magnitude, without any significant difference between the two types of nets ($P = 1$, 95% CI).

The kdT_{50} of the Vk per resistant strain with field-used nets steadily increased to reach 50 min at month 15, i.e., fivefold that of the Kisumu susceptible strain. The kdT_{50} significantly differed from that of the Kisumu susceptible strain tested with laboratory-kept nets and with field-used nets ($P = 0.007$ and $P = 0.005$, 95% CI, respectively). As for the kdT_{50}, the kdT_{95} of the Vk per resistant strain tested with field-used nets increased and values were significantly different from those of the Kisumu strain tested with laboratory-kept nets and with field-used nets ($P = 0.046$ and $P = 0.031$, 95% CI, respectively).

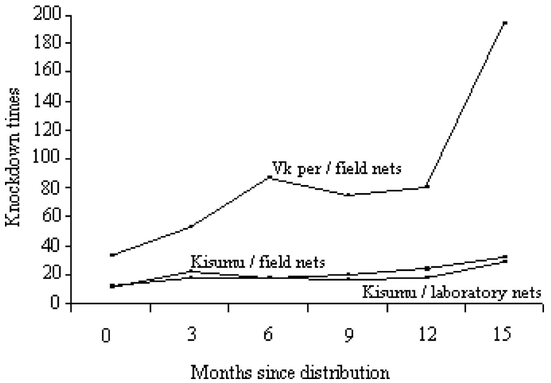

Fig. 2. kdT_{95} in minutes for 95% *An. gambiae* of the Kisumu and Vk per strains tested with laboratory-kept and field-used nets.

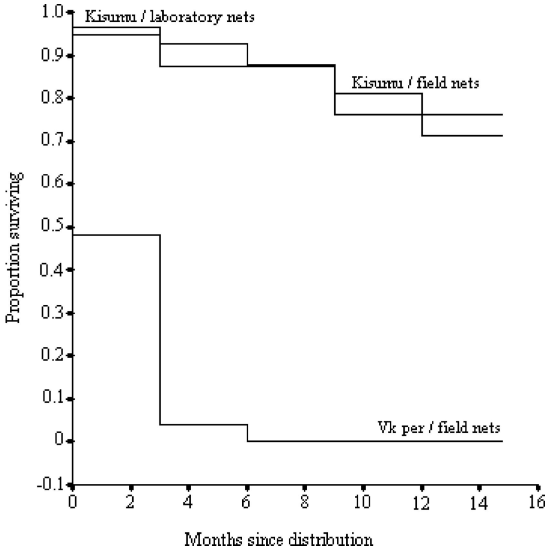

Fig. 3. Kaplan–Meier survival estimates of the proportion of nets remaining effective in killing the Kisumu susceptible strain and the Vk per resistant strain of *An. gambiae*.

Mortalities from Bioassay and Survival Analysis. The mortality rates recorded with the Kisumu susceptible strain when exposed to laboratory-kept nets and field-used nets were very high during the entire experiment (mortalities ranging from 99 to 100%). No significant difference was observed between the two types of nets during the 15 mo of study ($P = 0.51$ 95% CI). But, with the Vk per resistant strain, mortality rates were relatively high during the first 3 mo (69–75%). This mortality significantly reduced to 2% at month 15. Mortality rates of the Vk per when exposed to nets from the field differed significantly with Kisumu when exposed to laboratory-kept nets and field-used nets ($P = 0.020$ and $P = 0.022$, 95% CI, respectively). This was associated with a significant reduction of the proportion of field nets remaining effective in killing the Vk per resistant strain along the time (Fig. 3). From month 6 to the end of the trial, the expected long-lasting activity of the field-used nets in killing mosquitoes were canceled.

Discussion

Bioassays were carried out with nets hung in the laboratory and in domestic conditions (temperature, humidity, natural deposit of dirt particles on the nets) to monitor the delay of net activity without daily use. Numerous studies have demonstrated the actual effectiveness of pretreated nets on susceptible vector populations (Nguyen et al. 1996, Njunwa et al. 1996, Faye et al. 1998).

Data reported here showed high and long-lasting effectiveness of Permanet even when used under field conditions against the Kisumu susceptible *An. gambiae* strain. No significant difference was observed in knockdown and mortality rates of the Kisumu susceptible strain in contact with laboratory-maintained nets compared with field-used nets. The two types of nets performed very well during the 15-mo trial.

Against the Vk per resistant strain, these nets were found still partially effective during the first 3 mo, and the residual activity dropped after 6 mo of use in villages.

The high knockdown effect and mortality rates recorded with the Kisumu susceptible strain are consistent with what has already been reported with deltamethrin hand-treated nets (Hougard et al. 2003). The 6-mo period of efficacy for the WHO-recommended deltamethrin dosage (25 mg [AI]/m^2) (Njau et al. 1993) was extended to 15 mo in this study with these "long-lasting" nets; this may be due to the industrialized impregnation technique and the increase of dosage at 50 mg (AI)/m^2 on Permanet. Current studies showed that Permanet in the field remained effective in killing susceptible anophelines, ≈2 yr after they were distributed (Lindblade et al. 2005). This is consistent with effectiveness (100% mortality rates) reported on Moskitul nets pretreated with deltamethrin at 25 mg (AI)/m^2 and kept in original bags for 5 yr (Carnevale et al. 1998). Olyset Net incorporated with permethrin 2%, were also found effective against the Kisumu susceptible strain of *An. gambiae* (98–100% mortality on new and clean nets, 72–92% mortality on nets used for 3 mo) (N′Guessan et al. 2001).

However, Permanet was found partially effective against the Vk per DDT/pyrethroids resistant strain of *An. gambiae.* Hand treatment of deltamethrin at 25 mg (AI)/m^2 as well as long-lasting Moskitul and Olyset Net pretreatment tested in laboratory and experimental huts in Côte d'Ivoire were found moderately effective against resistant populations of *An. gambiae* with kdr allelic frequency higher than 70% (Carnevale et al. 1998, N′Guessan et al. 2001, Hougard et al. 2003). Mortality rates reported in the latter studies (28% for Olyset Net in experimental huts, 35% for Moskitul in bioassays) are lower than that data gained with Permanet (69–75% during the first 3 mo), suggesting good activity of Permanet even against kdr-based resistant populations of *An. gambiae.* The lessening of pyrethroid exito-repellent and irritancy effects against knockdown-resistant mosquitoes allows longer contact with treated nets, resulting in high killing effect (Chandre et al. 2000). Mortality rates similar to those registered with Permanet were recorded using nets hand treated with permethrin at 500 mg (AI)/m^2 against a laboratory pyrethroid-tolerant strain of *An. gambiae* originated from Yaoundé, Cameroon with high oxidase level (Etang et al. 2004a). Nevertheless, no significant decrease of knockdown effect was observed compared with the Kisumu strain. The contrast between knockdown and mortality rates in the OCEAC laboratory strain was attributed to the involvement of metabolic detoxification in insecticide resistance that does not systematically induce the decrease of knockdown effect.

Despite the overall decrease of knockdown effect on the Vk per resistant strain, the good mortality recorded during the first months of the trial (69–75%) may confer a significant level of protection of Permanet even in areas where *An. gambiae* has developed kdr-based resistant to pyrethroids. Moreover, the threshold of insecticide-treated nets (ITNs) effectiveness established by WHO (WHO 1999) is 80% mortality rates through bioassays. However, the epidemiological impact of ITNs at community level was reported to be similar to that observed in areas with susceptible vectors (Henry et al. 2005).

The long-lasting activity obtained with Permanet against the Kisumu susceptible strain was not achieved against the Vk per strain. The dramatic decrease of Permanet efficacy after the third month may be related to some external factors such as temperature, dust, and fumes on nets hung in rural conditions. Moreover, for nets hanging in the field, dust or fumes cover the molecules of insecticide inhibiting their contact with mosquitoes and reducing their effectiveness (N′Guessan et al. 2001, Etang et al. 2004b). In a next step, we shall try to assess whether washing process could expose the sufficient amount of chemical needed to restore Permanet activity against resistant mosquitoes.

Current data highlight Permanet suitability for malaria vector control even in areas with or without vector resistance to pyrethroids, with emphasis on proper conditions of use.

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