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Anopheles gambiae resistance to pyrethroid-treated nets in cotton *versus* rice areas in Mali

Moussa Fane[a,c,d], Ousmane Cissé[b], Cheick Sékou F. Traore[a,d], Philippe Sabatier[c,d,*]

[a] Malaria Research and Training Center, Faculty of Medicine, Pharmacy and Dentistry, Bamako, BP 1805, Mali
[b] CMDT, Malian Company for Development of Textile, Bamako, BP 487, Mali
[c] EPSP Team, TIMC-IMAG Lab, UMR CNRS 5525 Université Joseph Fourier, VetAgroSup, Veterinary Campus of Lyon, 1 Avenue Bourgelat, 69280, Marcy l'Etoile, France
[d] ACCIES Consortium: TIMC-IMAG, LTHE, CRC, CNRM, CERFACS, Fondation Sansouire (FS), Malaria Research and Training Center (MRTC), Institut Pasteur de Dakar (IPD), Centre de Suivi Ecologique (CSE), France

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ABSTRACT

The rise and spread of *Anopheles gambiae s.l.* (the major malaria vector sub-Saharan Africa) resistance to pyrethroids is of great concern owing to the predominant role of pyrethroid-treated nets in the WHO global strategy for malaria control. Use of pyrethroids for agricultural purposes may exert a strong selection pressure, favouring the emergence of insecticide resistance. The objective of this study was to evaluate the efficacy of alpha-cypermethrin treated nets in settings where insecticides are used against pests. This was assessed in two ways, i.e. under laboratory conditions using the WHO standard cones test technique and in experimental huts, on *Anopheles gambiae s.l.* collected in two Malian rural sites, Koumantou characterised by cotton crops and high insecticide use and Sélingué, a rice field area with low insecticide use. According to the WHO standard cones test technique, there was no difference between mosquitoes collected in the two sites; KD50 time was less than 3 min and the KD95 time below 30 min. Nevertheless, in the experimental huts with alpha-cypermethrin treated bed nets, the mosquito mortality rate was significantly lower in Koumantou (102/361, 28.2%) than in Sélingué (122/233, 52.3%) (RR: 0.65, 95%CI: 0.56–0.76) ($p < 0.001$). In addition, in Koumantou the percentage of unfed mosquitoes found in the veranda was much lower in the huts with untreated (26.0%, 33/127) than in those with treated nets (92.2%, 118/128) ($p < 0.01$) while in Sélingué there was no difference between huts with treated and untreated bed nets. Alpha-cypermethrin treated bed nets had a significant effect on mortality and repelling behaviour of *Anopheles gambiae s.l.* though in Koumantou treated bed nets were less efficacious, possibly due to the intense use of pesticide for agriculture.

Contents

* Corresponding author. Tel.: +33 632 630616.
E-mail address: Philippe.Sabatier@imag.fr (P. Sabatier).

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1. Introduction

Malaria is a leading cause of morbidity and mortality in Mali. In 2008, the Ministry of Health (MOH) reported more than 1.3 million clinical malaria cases, accounting for 37% of all outpatient visits, 42% for children under five (USAID, 2009). Thirty seven percent of all reported deaths were due to malaria, more than half of them among children under five. The National Malaria Control Program (NMCP) strategy is based on effective case management and the use of insecticide-treated nets (ITNs) targeted at vulnerable groups, i.e. children under five and pregnant women. ITN coverage has increased substantially after national distribution campaigns of free or highly-subsidized ITN. More recently, in December 2007, the Malian Ministry of Health (MOH) distributed for free 2.3 million long-lasting insecticide-treated nets (LLINs), attaining an estimated coverage of 82% in 2008 (World Health Organization, 2010).

Pyrethroids are the only insecticides currently recommended by the World Health Organization (WHO) for treatment of bed nets owing to their strong insecticidal activity at low concentrations and their low mammalian toxicity. Pyrethroid-treated nets are effective in reducing malaria morbidity and mortality and may also provide community protection through mass impact on the vector population, when coverage is high (Ilboudo-Sanogo et al., 2001; Binka et al., 1996; Nevill et al., 1996). Nevertheless, the emergence and spread of pyrethroid resistance is of concern. This is often due to mutations in the knockdown resistance (kdr) gene (Hemingway et al., 2004; Diabate et al., 2004). Both resistance to permethrin and decreased sensitivity to deltamethrin by *A. gambiae s.l.*, the main malaria vector sub-Saharan Africa, have already been observed in several African countries (Diabate et al., 2004; Chandre et al., 1999). In Bamako, Mali, the prevalence of the kdr mutation increased from 2.8% in 1987 to 62.1% in 2000 (Fanello et al., 2003). A similar trend was observed in other Malian sites (Tripet et al., 2007).

The resistance of mosquitoes to different pyrethroids underlines the importance of assessing vectors sensitivity to all insecticides belonging to the same family. The objective of this study was to evaluate the efficacy of alpha-cypermethrin treated nets, an insecticide not used in Mali for this purpose, on the control of malaria vectors in two areas characterized by different pattern of exposure to agricultural pesticides.

2. Material and methods

2.1. Study sites

The study was conducted in two sites in the region of Sikasso (Mali) differing by agricultural production, use of insecticides, and/or ecological settings (Fig. 1). The first site is located in the cotton crop growing area of Koumantou, where the Malian Company for Textile Development (CMDT) has a cotton factory. The climate is typical Sahelian (Sudano-Sahel), with a rainy season between May and November (annual rainfall 900–1000 mm) and a dry season between December and May. Extensive cotton crop growing (15,582 ha) is combined with widespread application of organophosphates and pyrethroids. The prevalence of the resistant kdr allele has been rising since 1996 to currently exceed 80%. To minimize the risk of resistance, CMDT uses a combination of insecticides (pyrethroid and organophosphore/carbamate) that are changed every 3 years.

The second site is located in a large flooded area of the hydro-agro-electrical dam of Sélingué, where rice is grown twice a year (more than 30,000 ha), i.e. (i) rainfall cultivation between July and November, (ii) post-rainfall cultivation between February and June. Malaria transmission is perennial with two peaks, one at the end of the rains and the other during the post-rainfall rice culture (Fondjo, 1996). *A. gambiae s.l.* is the major malaria vector. Use of pyrethroids is not as intense as in Koumantou and the prevalence of the resistant kdr allele is low (Fanello et al., 2003).

2.2. Insecticide-treated bed nets

Rectangular 100% polyester, white bed nets (12 m^2) were dipped into an insecticide solution (6 ml of alpha-cypermethrin (Fendona 6SC®, BASF) diluted in 480 ml of water (40 mg/m^2) and let dry before use. Control bed nets were untreated and protected against contamination. On each net, six holes (4 × 4 cm each) were purposely done, 2 on each width, 1 on each length, to simulate operational condition, measure insecticide efficacy and minimize the physical barrier of the net.

2.3. Mosquitoes

For the tests, wild *Anopheles gambiae* captured both in Koumantou (S-Koumantou) and Sélingué (S-Sélingué) where employed. A laboratory strain of *A. gambiae*, Kisumu strain (S-Kisumu), 100% susceptible to pyrethroids, imported from Centre Muraz of Bobo-Dioulasso, Burkina-Faso, was used as control strain for the cone test. For the laboratory study, F1 generation adults from the wild and laboratory strains were used. For the field study, mosquitoes were captured by collectors using flash lights and aspirators between 5.00 am and 7.00 am inside nets, huts and exit traps. Living mosquitoes captured in experimental huts were kept in separate cups corresponding to each collection's location: under bed net, around bed net or under veranda. A cotton wool swab soaked with 5% glucose solution was placed on the top of the cup covered by a piece of mosquito net.

2.4. Cone tests

The bioassays using WHO plastic cones (Koffi et al., 1998) were carried out at the Malaria Research and Training Center's field entomology laboratory in Sélingué. To perform one complete cones test, 4 pieces of 25 × 25 cm of both treated and untreated mosquito nets were used. For each mosquito strain, 5 unfed 2–5 days old mosquitoes were exposed to each bed net's piece for 3 min. The test was repeated 20 times, 160 times in total (80 for alpha-cypermethrin treated pieces and 80 for untreated pieces). After exposure, mosquitoes were removed from cones, grouped together in cardboard tumblers and fed by cotton soaked with 5% glucose solution. To evaluate KD50% and KD95%, the number of female knocked-down by insecticide was recorded immediately and thereafter every 10 min up to 1 h; the mosquito mortality rates were estimated at 24 h after exposure. The room for the tests located in Sélingué was air-conditioned, with glass windows and a adequate