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Evidence of increasing L1014F *kdr* mutation frequency in *Anopheles gambiae* s.l. pyrethroid resistant following a nationwide distribution of LLINs by the Beninese National Malaria Control Programme

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PEER REVIEW

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Comments

The authors have clearly shown a widespread *kdr* frequency in the transect south-north Benin which have increased even after the distribution of LLINs. The effect of selection pressure after a large scale distribution of LLINs has confirmed previous reports by Czeher *et al.* (2008) on an increase Leu-Phe *kdr* mutation in Niger following a countrywide LLINs implementation. The study is important in its field.
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ABSTRACT

Objective: To determine the susceptibility status to pyrethroid in *Anopheles gambiae* s.l. (*An. gambiae*), the distribution of *kdr* "Leu-Phe" mutation in malaria vectors in Benin and to compare the current frequency of *kdr* "Leu-Phe" mutation to the previous frequency after long-lasting insecticide treated nets implementation.
Methods: Larvae and pupae of *An. gambiae* s.l. mosquitoes were collected from the breeding sites in Littoral, Zou, Borgou and Alibori provinces. CDC susceptibility tests were conducted on unfed females mosquitoes aged 2-5 d old. *An. gambiae* mosquitoes were identified to species using PCR techniques. Molecular assays were also carried out to identify *kdr* mutations in individual mosquitoes.
Results: The results showed that *An. gambiae* Malanville and Suru-lere populations were resistant to deltamethrin. Regarding *An. gambiae* Parakou and Bohicon populations, they were resistant to permethrin. PCR revealed 100% of mosquitoes tested were *An. gambiae* s.s. The L1014F *kdr* mutation was found in *An. gambiae* s.s. Malanville and Parakou at various allelic frequencies. The increase of *kdr* allelic frequency was positively correlated with CDC bioassays data.
Conclusions: Pyrethroid resistance is widespread in malaria vector in Benin and *kdr* mutation is the main resistance mechanism involved. More attention may be paid for the future success of malaria control programmes based on LLINs with pyrethroids in the country.

KEYWORDS
Resistance, Insecticide, Vectors, CDC bioassay, Leu-Phe *kdr* mutation

1. Introduction

There were an estimated 216 million episodes of malaria in 2010, with 149 million to 274 million cases. Approximately 81%, or 174 million cases, were in the African Region, with the Southeast Asian Region accounting for another 13%[1]. There were also an estimated 655 000 malaria deaths in 2010, of which 91% were in the African Region. Approximately 86% of malaria deaths globally were of children under 5 years of age[1]. So, malaria remains one of the most critical public health challenges for Africa despite intense national and international efforts[2].

The intensive use of insecticide in the malaria control activities means that widespread mosquito insecticide resistance could have a devastating effect on the planned upscaling of the vector control activities[3].

In many African countries, malaria mosquitoes were already resistant to pyrethroids. For instance, pyrethroid

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resistance in *Anopheles gambiae* s.s. (*An. gambiae*) has already been described in Benin[4-6].

Malaria vector resistance to insecticides in Benin is conferred by two main mechanisms: (1) alterations at site of action in the sodium channel, *viz.* the *kdr* mutations and (2) an increase of detoxification and/or metabolism through high levels of multi-function oxidases, non-specific esterases and glutathione S-transferases[4-7].

The Beninese National Malaria Control Programme has been implemented. Large-scale and free long-lasting insecticidal nets (LLINs) (OlysetNet) were distributed since July 2011 throughout the country to increase coverage of LLINs. Information on susceptibility to pyrethroid insecticides used in public health in Benin and the underlying mechanisms being investigated are crucial. This will properly inform control programs of the most suitable insecticides to use and facilitate the design of appropriate resistance management strategies.

The aim of this study is to determine the susceptibility status, pyrethroid resistance levels in *An. gambiae* Malanville, Parakou, Bohicon and Suru-lere populations to permethrin and deltamethrin using CDC (Center for Disease Control and Prevention) bottle bioassays, to evaluate the presence and extent of the distribution of the *kdr* mutation within and among these *An. gambiae* s.l. populations and to compare the current frequency of *kdr* "Leu-phe" mutation to the previous frequency in malaria vectors in the south-north transect Benin after LLINs implementation.

2. Materials and methods

2.1. Study area

The study was carried out in some localities, following a south-north transect. Four contrasting localities of Benin were selected for mosquito collection on the basis of variation in agricultural production, use of insecticides and/or ecological settings (Figure 1). The localities are as follows. Bohicon is located in the middle part of the country where the farmers used significant amounts of pyrethroids and organophosphates for cotton protection or to control agricultural pests. Parakou, an urban vegetable growing area located in the north of Benin. Malanville is a rice growing area located near the Niger River. Suru-lere is an urban locality in Cotonou district located in southern Benin.

The choice of the study sites took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. The southern zone (Cotonou) is characterized by a tropical coastal Guinean climate with two rainy seasons (April-July and September-November). The mean annual rainfall is over 1 500 mm. The middle part of the country (Bohicon) is characterized by a Sudano-Guinean climate with an average rainfall of 1 000 mm per year. The northern zone (Parakou and Malanville) is characterized by a Sudanian climate with only one rainy season per year (May to October) and one dry season (November-April). The temperature ranges from 22 to 33 °C with the mean annual rainfall of 1 300 mm.

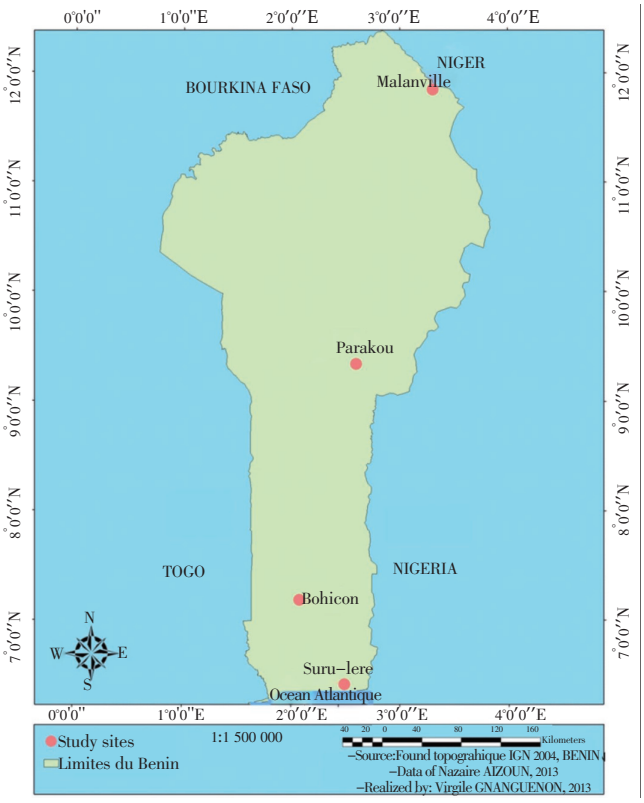

Figure 1. Map of the study area.

2.2. Sample collection

From March 2012 to November 2012, larvae and pupae of *An. gambiae* mosquito were collected several times per year, *i.e.* at the beginning and the end of rainy season from a wide range of breeding sites (puddles, shallow wells, gutters and rice fields) in Malanville, Parakou, Bohicon districts and in Suru-lere locality of Cotonou district. All larvae were brought back to laboratory of Centre de Recherche Entomologique de Cotonou for rearing. Emerging adult female mosquitoes were used for insecticide susceptibility tests. A susceptible strain of *An. gambiae* Kisumu was used as reference strain for bioassays.

2.3. Insecticide susceptibility tests

Females *An. gambiae* aged 2 to 5 d old were exposed to CDC diagnostic dosage of various insecticides according to the CDC protocol[8]. The following insecticides were tested: permethrin (21.5 μg per bottle) and deltamethrin (12.5 μg per bottle). Mosquitoes were exposed for 2 h to insecticide-treated bottles and monitored at different time intervals (15, 30, 35, 40, 45, 60, 75, 90, 105 and 120 min). This allowed us to