# Status of pyrethroid resistance in *Anopheles gambiae* sensu lato

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The present study confirms the presence of pyrethroid resistance among *Anopheles gambiae* s.l mosquitos in Côte d'Ivoire and reports the observation of such resistance in two other countries in West Africa (Benin and Burkina Faso). Malaria vector populations from Cameroon (Central Africa), Senegal (West Africa) and Botswana (southern Africa) were found to be susceptible to pyrethroids. In the most resistant mosquito populations, resistance to permethrin was associated with reduced mortality, not only with respect to this compound but also towards deltamethrin. Moreover, a significant increase in knockdown time was observed in some mosquito populations before any decrease in mortality, suggesting that knockdown time could be a good indicator for the early detection of pyrethroid resistance. In view of the current extension of such resistance, there is an urgent need to set up a network in Africa to evaluate its development. It is also vital that the impact of this resistance on pyrethroid-impregnated bednets be assessed.

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## Introduction

Vector control is an important component of the WHO Global Strategy for Malaria Control, whose objective is to break the transmission of malaria parasites using indoor residual spraying or pyrethroid-impregnated materials (bednets and/or curtains). Pyrethroids are preferred for impregnation because they are highly effective and fast-acting insecticides with a strong excitorepellent effect on mosquitos.

During the Global Malaria Eradication Programme of the 1950s and 1960s, dieldrin resistance, involving a specific resistance mechanism (ϒ-aminobutyric acid (GABA) receptors), was recorded among most *Anopheles gambiae* s.l. populations in Africa. In contrast, only a few cases of DDT resistance have so far been recorded in Africa. The first, involving *A. gambiae* s.s., was observed in 1967 in Bobo Dioulasso (Burkina Faso) and attributed to the use of DDT against cotton pests (*1–3*, J. Hamon et al., unpublished data, 1968). Soon after, it was also observed among *A. arabiensis* from Senegal (*1*). DDT resistance can be due either to a specific detoxification mechanism (glutathione-*S*-transferase) or to a nerve insensitivity resulting from a modification of the target site (sodium channel). The latter, governed by the *kdr* gene, reduces both the knockdown and lethal effects of DDT. In West Africa, it induces a cross-resistance to pyrethroids, which also depends on *kdr* mutation (*4*). In Zanzibar, however, DDT resistance induced by glutathione-*S*-transferase did not cross with pyrethroids (*5*).

Since the 1970s, pyrethroids have been extensively used in urban areas (as domestic coils and aerosols) as well as for agricultural purposes in rural areas. In both cases, the selection pressure exerted on *A. gambiae* s.l. populations was not negligible: the first case of pyrethroid resistance in *A. gambiae* s.l. was recorded in Bouaké (Côte d'Ivoire) and was attributed to the domestic use of aerosols (*6*). Later, reduced susceptibility was observed in an area of Kenya where permethrin-impregnated bednets were used (*7, 8*). In the Gambia, however, no change in the pyrethroid susceptibility of *A. gambiae* s.s. occurred as a result of an impregnated bednet project (*9*).

In view of the pyrethroid resistance observed at Bouaké and the current prospects for extending the use of impregnated materials, surveys were carried out in Africa through an informal collaboration network. The objectives were first to confirm the resistance recorded in Bouaké and evaluate its spread outside that city, and to obtain more information on the susceptibility of *A. gambiae* s.l. to pyrethroids in other countries.

## Materials and methods

Mosquitos were harvested in six countries (see Fig. 1): Benin (Cotonou), Botswana (Sebina), Burkina Faso (Kou valley near Bobo Dioulasso),

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Cameroon (Yaoundé), Côte d'Ivoire (Abidjan, Bouaké, Daloa, Kafine, Katiola, Korhogo, Odienné, Tai, and Yao Koffikro) and Senegal (Dakar).

Most of the tests were carried out on nonblood-fed, 2–4-day-old females that emerged from field-collected larvae or with the $F_1$ progeny of females collected in the field. Only in Cotonou were engorged wild-caught females tested directly. All the specimens were *A. gambiae* s.s. except in Dakar and Sebina where they were *A. arabiensis* (determined where possible by polymerase chain reaction (PCR)). A susceptible reference strain of *A. gambiae* s.s from Kisumu was used as the control.

## Resistance tests

WHO test kits for adult mosquitos were used (*10*). Impregnated papers were prepared using silicone oil (Dow Corning 556) with technical deltamethrin and 25/75 permethrin active ingredient samples. Papers were impregnated using 3.6 mg of 0.25% (w/v) permethrin/oil solution per $cm^2$ and 0.025% (w/v) deltamethrin/oil solution per $cm^2$ (WHO diagnostic concentration (*11*)). Some tests were carried out with 4% DDT (w/v). The exposure time was 60 min in the exposure tube in the normal vertical position. In addition to mortality after 24 h, the number of mosquitos knocked down was recorded 10, 20, 30, 40, 50, 60 and 80 min after the start of exposure. Knockdown times ($KDT_{50}$ and $KDT_{95}$) were calculated by means of a log time-probit model using software (*12*) based on that described by Finney (*13*).

## Results

### Mortality

Mortality with permethrin at the diagnostic concentration of 0.25% (w/v) (proposed by WHO (*11*)) was rarely 100%, even for the susceptible reference strain (Table 1). By contrast, 100% mortality of the susceptible strain was systematically obtained with 0.025% (w/v) deltamethrin and 4% (w/v) DDT. We therefore considered as resistant the samples with <70% mortality towards permethrin and <95% mortality towards deltamethrin.

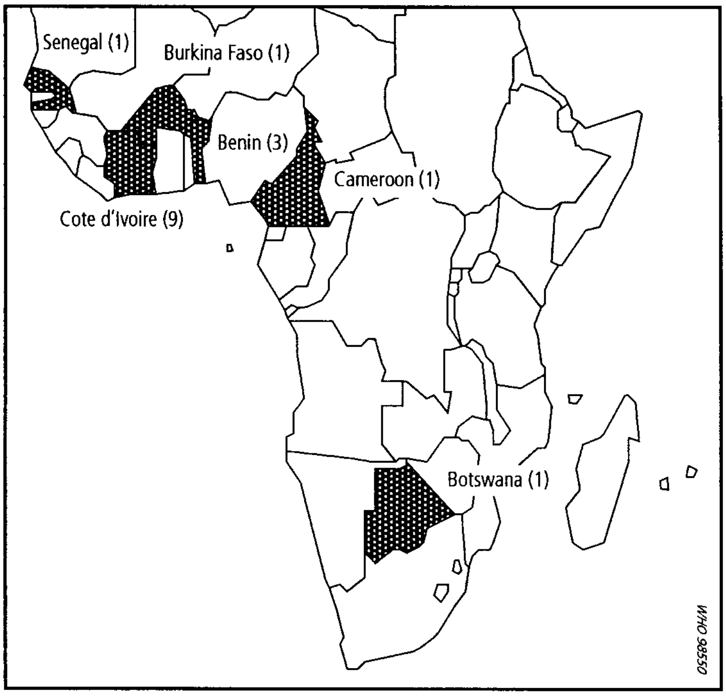
Permethrin resistance was detected in Cotonou (Benin), Kou valley (Burkina Faso) and several localities in Côte d'Ivoire (Table 1) including Bouaké, where it had been reported as early as 1993. Normal susceptibility was found in Yaoundé (Cameroon), Dakar (Senegal) and Sebina (Botswana), the latter two with *A. arabiensis.*

Deltamethrin resistance was observed in four out of the six populations tested (Cotonou, Tai, Korhogo and Yao Koffikro).

### Knockdown

In most samples, the relationship between knockdown rate and the logarithm of the exposure time was linear. In resistant samples, $KDT_{50}$ and $KDT_{95}$ (Table 2) increased markedly, with nearly a complete loss of knockdown effect for the locations with the greatest resistance (Cotonou, Korhogo and Yao Koffikro). More interesting is the 2-fold increase in $KDT_{50}$ and 2–4-fold increase in $KDT_{95}$ in some field samples considered susceptible according to their mortality records.

Fig. 1. **Locations of countries surveyed. The numbers of samples taken are given in parentheses.**




## Discussion

According to our results, the permethrin concentration of 0.25% (w/v) proposed by WHO is clearly too low for *A. gambiae* s.l. In the Gambia, for example, a mortality of only 91.5% was recorded in a population considered susceptible (*9*) and it would be better to use a higher concentration of the insecticide, either 0.5% (w/v) or 1% (w/v). For deltamethrin, however, the concentration of 0.025% (w/v) appears appropriate. The correct diagnostic concentration for all pyrethroids should be determined for each mosquito species of medical importance and the shelf-life during which impregnated papers retain their full efficacy should also be investigated.

In addition to mortality, knockdown time is a good indicator for the early detection of reduced susceptibility; it is very easy to measure and no equipment other than the WHO test kit is necessary. Knockdown time has long been accepted as an indicator of susceptibility (*6, 14, 15*). Thus the measurement of knockdown time could systematically be included in monitoring programmes for mosquito insecticide resistance, since this time provides initial information on the possible involvement of the *kdr* gene. The *kdr* gene can now be

Table 1: **Percentage mortality in field samples of *Anopheles gambiae* s.l. 24 hours after a 1-hour exposure to insecticide-impregnated papers in WHO test tubes**

| Country/locality | Permethrin[a] (0.25% w/v) | Deltamethrin[a] (0.25% w/v) | DDT[a] (4% w/v) | Resistance status[b] |
|---|---|---|---|---|
| **Botswana** | | | | |
| Sebina | 86.3 (121) [c] | – | 99.6 (188) | S |
| **Cameroon** | | | | |
| Yaoundé | 85.2 (135) | 100 (40) | 90.0 (80) | S |
| **Senegal** | | | | |
| Dakar | 76.2 (52) | – | – | S |
| **Burkina Faso** | | | | |
| Kou valley | 29.2 (113) | 95.2 (186) | 67.3 (243) | R |
| **Côte d'Ivoire** | | | | |
| Abidjan | 75.4 (57) | – | 75.8 | S* |
| Bouaké | 48.0 (102) | 98.4 (60) | – | R |
| Odienné | 61.9 (110) | – | – | R |
| Kafine | 54.5 (99) | – | – | R |
| Daloa | 50.0 (104) | – | – | R |
| Katiola | 69.2 (104) | – | – | R |
| Tai | 26.7 (60) | 36.2 (58) | – | R |
| Korhogo | 7.3 (55) | 30.6 (62) | 7.1 (84) | R |
| Yao Koffikro | 10.0 (120) | 28.4 (117) | 17.6 (102) | R |
| **Benin** | | | | |
| Cotonou 1 | 38.9 (54) | – | – | R |
| Cotonou 2 | 20.2 (376) | 18.7 (124) | 11.5 (128) | R |
| **Susceptible reference strain** | | | | |
| **Kisumu** | **86.8 (320)** | **100 (100)** | **100 (300)** | **S** |

[a] WHO diagnostic concentration.

[b] S = susceptible; R = resistant; S* = pyrethroid-susceptible with reduced susceptibility to DDT 4% (w/v).

[c] Figures in parentheses are the numbers of mosquitos.

detected from a single specimen of *A. gambiae* s.s. using PCR (*4*).

The increase in knockdown time was more marked with permethrin than with deltamethrin. In mildly resistant populations, such as those in Kou valley, knockdown time increased 4–5-fold with permethrin but only 1.5–2-fold with deltamethrin. In highly resistant populations displaying a complete loss of knockdown effect for permethrin, the $KDT_{50}$ and $KDT_{95}$ for deltamethrin were increased by only 3.5-fold and 6–8-fold, respectively. Permethrin is thus a better indicator for the early detection of *kdr*-based pyrethroid resistance. The greatly reduced mortality with deltamethrin in populations displaying a very high permethrin resistance suggests that the *kdr* gene confers cross-resistance to various pyrethroids.

This large-scale survey indicated that pyrethroid resistance in *A. gambiae* s.s. is already developing in large parts of West Africa corresponding to those areas where DDT resistance was previously found. This is consistent with pyrethroid resistance being inherited from DDT resistance. The level of resistance has possibly been increased by the agricultural and domestic use of pyrethroids. On the contrary, pyrethroid resistance never developed in some areas where impregnated bednets were used extensively, such as China (*14*) and the Gambia (*9*). The main use of pyrethroids is not for malaria control, however, but for agricultural purposes and, to a lesser extent, for domestic applications. These selection pressures against *Anopheles* populations are almost impossible to control. Since pyrethroid resistance mainly results from agricultural applications, it is likely that such resistance will evolve regardless of the organized use of pyrethroids in properly managed malaria control campaigns.

Even if pyrethroid resistance is a serious threat to vector control, it would be dangerous to extrapolate from these results alone before the geographical extent of the resistance and its impact on the protective effect of impregnated bednets have been determined. The risk that resistance will increase is very difficult to estimate because it is almost impossible to evaluate the overall selective pressure exerted by pyrethroids and the specific impact of malaria programmes on the build-up of pyrethroid resistance.

Insufficient attention has been paid to pyrethroid resistance in malaria vectors, either in Africa or indeed elsewhere in the world. It is therefore urgent to set up networks to monitor their levels of susceptibility or resistance. The main objectives of the networks would be to gather more information on the current situation and to follow the evolution of resistance, especially in areas where vector control operations are planned. It is also important to strengthen basic research on resistance mechanisms and operational research on the impact of resistance on the efficacy of bednets in the field.

As a result of cross-resistance due to the *kdr* gene (*16*, F. Chandre et al., unpublished data, 1999) there is an urgent need for alternatives to the currently available pyrethroids. In this regard, WHOPES (WHO Pesticide Evaluation Scheme) has a major role to play. Collaborating centres need to be revived, priority being given to those working on malaria vectors in areas where *A. gambiae* s.l. has already developed resistance to pyrethroids. Collaboration with agrochemical companies should also be stimulated to ensure that any useful existing insecticide will not be withdrawn from the market and that the search for new insecticides will be actively encouraged. ■

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Table 2: **Correspondence between mortality and knockdown time using WHO test tubes**

| Insecticide | Sample | % mortality | $KDT_{50}$ (min) | $KDT_{95}$ (min) | Resistance status[a] |
|---|---|---|---|---|---|
| **Permethrin (0.25% w/v)** | Kisumu[b] | 86.8 | 23.5 | 44.5 | S |
| | Sebina | 86.3 | 40.2 | 86.7 | S |
| | Yaoundé | 85.2 | 46.1 | 158.3 | S |
| | Dakar | 76.9 | 40.3 | 140.7 | S |
| | Kou valley | 61.6 | 80.7 | 228.2 | R |
| | Abidjan | 75.4 | 99.3 | 786.3 | S* |
| | Tai | 26.7 | 110.2 | 378.0 | R |
| | Yao Koffikro | 10 | No kd[c] | No kd | R |
| | Korhogo | 7.3 | No kd | No kd | R |
| | Cotonou 1 | 38.9 | No kd | No kd | R |
| | Cotonou 3 | 34.1 | No kd | No kd | R |
| **Deltamethrin (0.025% w/v)** | Kisumu[b] | 100 | 21.4 | 39.5 | S |
| | Yaoundé | 100 | 21.3 | 56.4 | S |
| | Kou valley | 95.2 | 31.6 | 66.3 | R |
| | Tai | 36.2 | 84.0 | 252.5 | R |
| | Yao Koffikro | 28.4 | 78.7 | 339.7 | R |
| | Korhogo | 30.6 | 76.6 | 260.0 | R |
| **DDT (4% w/v)** | Kisumu[b] | 100 | 23.6 | 38.3 | S |
| | Sebina | 99.6 | 23.3 | 46.8 | S |
| | Yaoundé | 90.0 | 60.7 | 127.4 | S |
| | Yao Koffikro | 17.6 | 118.3 | 581.7 | R |
| | Korhogo | 7.1 | No kd | No kd | R |

[a] S = susceptible; R = resistant; S* = pyrethroid-susceptible with reduced susceptibility to DDT 4% (w/v).

[b] Susceptible reference strain.

[c] No kd = complete loss of knockdown effect (<15% of mosquitos knocked down after 1 h of exposure).


## Résumé

### Résistance d'*Anopheles gambiae* s.l. aux pyréthrinoïdes

La sensibilité d'*An. gambiae* s.l. à deux pyréthrinoïdes (perméthrine, deltaméthrine) et au DDT a été évaluée dans 4 pays d'Afrique de l'Ouest (Bénin, Burkina Faso, Côte d'Ivoire et Sénégal), au Cameroun (Afrique centrale) et au Botswana (Afrique australe). Les tests ont été réalisés sur des femelles sauvages selon le protocole OMS et en observant l'évolution du nombre de moustiques abattus au cours de l'exposition à l'insecticide (effet de choc ou de «knock down»). La résistance à la perméthrine a été confirmée à Bouaké ainsi que dans 7 des 8 autres localités testées en Côte d'Ivoire. Cette résistance a également été observée au Bénin et au Burkina Faso. Pour les populations les plus résistantes à la perméthrine, on observe une forte baisse de la mortalité avec la deltaméthrine. Les populations du Sénégal, du Cameroun et du Botswana se sont révélées sensibles. La résistance aux pyréthrinoïdes s'accompagne également d'une diminution de l'effet de choc, plus marquée avec la perméthrine qu'avec la deltaméthrine. La baisse de l'effet de choc peut même être significative avant que l'on observe une baisse de la mortalité. Elle peut donc être considérée à ce titre comme un indicateur précoce de la résistance. Le gène kdr est hérité de la résistance au DDT qui était apparue dans les années 60 suite à l'utilisation massive de ce produit contre les ravageurs du coton. Il s'est maintenu dans les populations anophéliennes sous la pression de sélection exercée par les pyréthrinoïdes agricoles et peut-être domestiques. Il est urgent, à l'heure où de nombreux pays envisagent de lancer des programmes de lutte contre le paludisme par moustiquaires imprégnées de pyréthrinoïdes, de mettre en place un réseau de surveillance de la résistance afin d'évaluer son extension actuelle et de préciser son impact sur la protection conférée par les moustiquaires imprégnées.

## Resumen

### Situación de la resistencia de *Anopheles gambiae* s.l. a los piretroides

Se ha estudiado la sensibilidad de *A. gambiae* s.l. a dos piretroides (permetrina y deltametrina) y al DDT en cuatro países de África occidental (Benin, Burkina Faso, Côte d'Ivoire y el Senegal), el Camerún (África central) y Botswana (África austral). Las pruebas se han realizado con hembras salvajes siguiendo el protocolo de la OMS y observando la evolución del número de mosquitos abatidos (kd, de «knockdown») durante la exposición al insecticida. Se confirmó la resistencia a la permetrina en Bouaké, así como en siete de las otras ocho localidades estudiadas en Côte d'Ivoire. Se observó también esa resistencia en Benin y en Burkina Faso. En las poblaciones más resistentes a la permetrina se observa una gran disminución de la mortalidad asociada al uso de deltametrina. Las poblaciones del Senegal, el Camerún y Botswana se revelaron sensibles. La resistencia a los piretroides se acompaña asimismo de una disminución del efecto kd, más pronunciada con la permetrina que con la deltametrina. La reducción del efecto kd puede ser significativa antes incluso de producirse el descenso de mortalidad, y puede considerarse por tanto en este caso como un indicador precoz de la resistencia. El gen kdr se ha propagado como consecuencia de la resistencia al DDT inducida en los años sesenta por la utilización masiva de ese producto contra los insectos que destrozaban el algodón, y se ha mantenido en las poblaciones de anofeles bajo la presión selectiva ejercida por los piretroides agrícolas y quizá domésticos. En un momento en que numerosos países prevén lanzar programas de lucha antipalúdica con mosquiteros impregnados de piretroides, urge poner en marcha una red de vigilancia de la resistencia para evaluar la magnitud del fenómeno y determinar con precisión su efecto en la protección conferida por los mosquiteros impregnados.


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