ENTOMOLOGIE MÉDICALE

Insecticide resistance in malaria vector mosquitoes in a gold mining town in Ghana and implications for malaria control.

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Résumé : **Résistance aux insecticides chez les moustiques vecteurs du paludisme dans une ville minière du Ghana et les implications de la lutte contre le paludisme.**

*En Afrique, les programmes de lutte contre le paludisme résident pour la plupart, dans le traitement de la maladie et la fourniture de moustiquaires traitées aux insecticides. L'impact de ces programmes est resté limité et de nouvelles approches sont préconisées, y compris des stratégies de gestion intégrée du vecteur, en partenariat avec l'industrie. Des études sur le moustique ont été menées pour AngloGold/Ashanti en vue de mettre en place un programme intégré de lutte contre le paludisme dans la ville d'extraction d'or d'Obuasi au Ghana. Les vecteurs du paludisme collectés dans les maisons ont été identifiés par espèces et par formes moléculaires au moyen de la PCR et testés pour leur résistance aux insecticides en utilisant les dosages standards de l'OMS et les dosages (*kdr*) d'insensibilité de l'emplacement de la cible moléculaire. Les espèces* An. funestus *s.s.et* An. gambiae *S et les formes M ont été identifiées. Les prélèvements d'*An. gambiae *forme S ont montré une résistance au DDT, aux pyréthrinoïdes et aux carbamates, tandis que* An. funestus *s'est montré résistant au DDT et aux carbamates. La forme M* An. gambiae *est apparue en nombre très peu élevé et leur résistance n'a pas pu être évaluée de manière fiable. Le dosage standard de la PCR pour la détection de la mutation* kdr *chez* An. gambiae *forme S a montré qu'il y a peu de corrélation avec la résistance aux pyréthrinoïdes. Le séquençage ultérieur du domaine IIS6 contenant la mutation* kdr *provenant de neuf moustiques survivants a montré que huit d'entre eux étaient homozygotes résistants et un hétérozygote. Ceci correspondait aux résultats des dosages et aux études antérieures sur le* An. gambiae *ouest africain, soulevant cependant quelques inquiétudes sur la fiabilité du dosage PCR pour la détection de la mutation* kdr. *À la suite de ces recherches, AngloGold/Ashanti ont mis en place un programme de contrôle de vecteur, en plus du traitement de prise en charge des cas, intégrant la gestion de la résistance aux insecticides en alternant différentes catégories d'insecticides en pulvérisation dans les maisons, l'approvisionnement en ITNs, le dépistage des maisons et la gestion environnementale appropriée, c'est-à-dire la gestion intégrée du vecteur.*

Anopheles gambiae
Anopheles funestus
vecteur du paludisme
lutte contre le paludisme
gestion de la résistance
Obuasi
Ghana
Afrique intertropicale

*Summary: Malaria control programmes in Africa, for the most part, address only treatment of the disease and supply of insecticide treated bed nets. The impact of these restricted programmes has been limited and new approaches are being advocated, including integrated vector management strategies and partnerships with industry. Mosquito surveys were carried out for AngloGold/Ashanti in preparation for their implementation of an integrated malaria control programme at the Obuasi gold mine in Ghana. Malaria vectors that were collected inside houses were identified to species and molecular forms by PCR, and tested for insecticide resistance using standard WHO bioassays and molecular target site insensitivity (*kdr*) assays. Species were identified as* An. funestus *s.s. and* An. gambiae *S and M forms. The* An. gambiae *S form samples showed resistance to DDT, pyrethroids and carbamates while* An. funestus *was resistant to DDT and carbamates. The* An. gambiae *M form occurred in very low numbers and could not be assessed reliably for resistance. The standard PCR assay for detection of the* kdr *mutation in* An. gambiae *S form showed little association with pyrethroid resistance. Subsequent sequencing of the IIS6 domain containing the* kdr *mutation from nine surviving mosquitoes showed that eight were homozygous resistant and one heterozygous. This correlated with the bioassay results and with previous studies on West African* An. gambiae*, but raised concerns about the reliability of the PCR assay for detection of the* kdr *mutation. As a result of these investigations AngloGold/Ashanti are implementing, in addition to treatment and case management, a vector control programme that includes insecticide resistance management by alternation of various classes of insecticides for house spraying, supply of ITNs, screening of houses and environmental management where appropriate, i.e. integrated vector management.*

Anopheles gambiae
Anopheles funestus
malaria vector
malaria control
resistance management
Obuasi
Ghana
Sub-Saharan Africa

Introduction

Insecticide resistance in African malaria vector mosquitoes is becoming an increasing problem for vector control programmes. A recent malaria epidemic in South Africa was caused by the presence of insecticide resistant *Anopheles funestus* (8, 4). The efficacy of insecticide treated bed nets has been assessed in the face of widespread pyrethroid resistance in *An. gambiae* in West Africa with varying results (1, 12). It is therefore prudent for any new initiative to assess the resistance status of the local vector mosquitoes before deciding on strategies for malaria vector control.

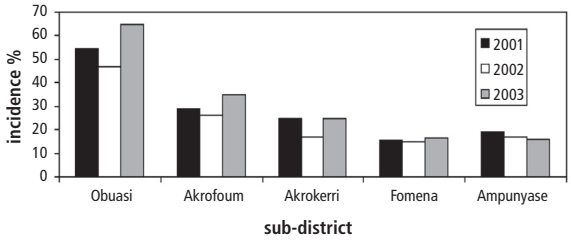
The Health Ministry of Ghana rates malaria as the number one cause of death amongst children in the country and approximately three million Ghanaians are affected by malaria annually. Malaria is not a notifiable disease but information regarding morbidity and mortality are collected at health facility level. The use of ITNs, early treatment, education and environmental sanitation are the main control measures against malaria in Ghana. In 2003 only 3.5 % of children under five and 2.2% of pregnant women slept under bed nets (Ministry of Health, Ghana, unpublished report, 2003).

Obuasi is situated in south-west Ghana in the district of Adansi-West. The 2003 unpublished Ministry of Health annual report from this district indicated that malaria was one of the major health concerns (figure 1).

Figure 1.

Malaria incidence rate (clinical cases) by sub-district, Adansi-West, Ghana, 2001-2003

Taux d'incidence du paludisme (cas cliniques) par sous-district, Adansi-West, Ghana, 2001-2003



Obuasi has a population of 173,447 people who are mostly dependent on the AngloGold/Ashanti operation for their income. Other economic activities are farming and minor trading. The crops cultivated include cocoa and citrus. During 2003 only 1000 nets were sold in the Obuasi sub-district but according to the annual report, plans are underway to strengthen seven identified bed net sales points in the district. The majority of uncomplicated (83%) and complicated (89%) malaria cases were in the age group 5 years and older. Severe anaemia was predominantly (65%) in the older age group. Data for age specific incidence rates were not available. These disease profiles are not typical of tropical Africa and have a marked impact on the workforce of the mine and the local economy in general. The malaria cases and case fatality rate at the AngloGold/Ashanti hospital for 2003 were on average over 6000 and 17 per month respectively (unpublished data). This has considerable impact on the productivity of the mine personnel.

The aim of the Anglogold/Ashanti malaria control programme at Obuasi is to implement an integrated strategy to reduce transmission of *Plasmodium falciparum*. Specific aims of the vector control programme are to implement an integrated vector management (IVM) programme, including indoor residual house spraying (IRS) with an appropriate insecticide, screening of houses, supply of insecticide treated bed nets (ITNs), selective larviciding and environmental management where appropriate. In order to do this, base line surveys of the mosquito populations were carried out.

Insecticide resistance studies of malaria vector mosquitoes in Ghana have been limited in their scope. KRISTAN *et al.* (10) carried out bioassay studies on populations of *An. gambiae* complex from south-western Ghana and showed resistance to both DDT and permethrin but did not correlate these results with presence or absence of the West African *kdr* allele. YAWSON *et al.* (15) on the other hand, at 11 localities from coastal to far northern Ghana reported on the occurrence and distribution of the *kdr* mutation in *An. gambiae* complex mosquitoes but did not carry out insecticide bioassays.

The results of baseline surveys of mosquito populations at Obuasi, Ghana, for species composition, infectivity, insecticide susceptibility and *kdr* assays are presented here.

Material and Methods

Study Site

The Obuasi area (06°15'N, 01°36'W) is characterized by its equatorial climate and hilly terrain greatly modified by mining activity and urban development. Mine personnel are to some extent housed in accommodation provided by the mine in housing clusters scattered within Obuasi town, but a fair proportion live amongst the general town and peri-urban population. Drainage in the town to remove liquid waste is via surface drains on the side of the roads. Some of the better maintained sections have well constructed concrete drains, in some instances covered with concrete slabs. In many areas the drains are damaged, creating swampy areas.

Collections

The survey was conducted during the dry season in April 2004 and again when the rains had started in June 2004. Houses were searched and resting mosquitoes were collected by hand. Samples of wild females were exposed to insecticides in the field while sub-samples were returned to the laboratory in Johannesburg for rearing of F-1 progeny and further analysis.

Insecticide Susceptibility Tests

The standard WHO susceptibility tests were conducted. Adults from the April collections were exposed for one hour to 4% DDT, 0.05% deltamethrin, 0.1% bendiocarb and 5% malathion. The June collections were exposed for one hour to 0.05% lambda-cyhalothrin, 0.15% cyfluthrin, 0.15% etofenprox, 0.1% propoxur, and for two hours to 1% fenitrothion. Dead and alive mosquitoes were stored separately on silica gel for molecular analysis and sporozoite infectivity tests.

Species Identification

The *Anopheles gambiae* complex was identified initially by morphology (6), to species level by PCR assay (13) and to M and S molecular forms by the PCR method of FAVIA *et al.* (5). The *An. funestus* group were identified using the method of KOEKEMOER *et al.* (9).

Molecular assay for knockdown resistance (*kdr*)

The mutation responsible for knockdown resistance to pyrethroids in West Africa was described by MARTINEZ-TORRES *et al.* (11) and this methodology was modified here for assaying members of the *An. gambiae* complex. Two

independent PCR runs were set up for each sample: the first contained the primers Agd2 + Agd4 to identify the susceptible allele and the second contained primers Agd1 + Agd3 identifying the resistant allele.

Sequence analysis of the IIS6 domain

The 293 bp fragment of the IIS6 domain containing the *kdr* mutation was amplified from 9 mosquitoes using primers Agd1 and Agd2 (11). This fragment was sent to Inqaba Biotechnical industries, South Africa, for sequencing.

ELISA for sporozoite assay for *P. falciparum* infectivity

The infectivity rates of female mosquitoes were tested using the enzyme-linked immunosorbent assay (ELISA) (14).

Results

Mosquito surveys carried out in April and June 2004 showed the presence of three anopheline species resting inside houses: *An. gambiae* ("S" form predominated with very few "M" form identified), *An. funestus* and a small number of *An. pharoensis*. Both the former species were found infected with *P. falciparum* but not the latter, based on ELISA (table I).

Resistance, as determined by the standard WHO susceptibility tests, to three classes of insecticides (pyrethroids – 46.4% mortality, organochlorines – 30.8% and carbamates – 45.3%) was demonstrated in *An. gambiae* (table II), with 100% susceptibility shown only to organophosphates.

In *An. funestus* resistance to two classes (organochlorines – 60.9% mortality and carbamates – 71.4%) was shown, with full susceptibility to pyrethroids and organophosphates (table III).

Tableau I.

Species composition and *Plasmodium falciparum* infectivity rates in malaria vector species at Obuasi.
Composition des espèces et taux d'infestation du Plasmodium falciparum *chez les espèces de vecteur du paludisme à Obuasi.*

date	species	number identified	number tested for *P. falciparum* (% positive)	
April 2004	*An. gambiae* 'S' form	111	92	(4.35)
	An. funestus	152	221	(1.81)
	An. pharoensis	12	12	(0)
June 2004	*An. gambiae* 'S' form	175	175	(5.5)
	An. funestus	13	-	
	An. pharoensis	**0**	-	

Tableau II.

Insecticide susceptibility tests of *An. gambiae* S form from Obuasi.
*Tests de sensibilité aux insecticides d'*An. gambiae *forme S à Obuasi*

insecticide	number tested	% 24-hr mortality
Deltamethrin	54	75.9
Lambda-cyhalothrin	15	40
Cyfluthrin	27	12.5
Etofenprox	21	57.1
DDT	26	30.8
Bendiocarb	39	56.4
Propoxur	38	34.2
Fenitrothion	89	100
Malathion	40	100

Tableau III.

Insecticide susceptibility tests of *An. funestus* from Obuasi, Ghana.
*Tests de sensibilité aux insecticides d'*An. funestus *à Obuasi, Ghana.*

insecticide	number tested	% 24-hr mortality
Deltamethrin	53	100
Cyfluthrin	13	100
DDT	23	60.9
Bendiocarb	56	71.4
Malathion	45	100

Tableau IV.

***Kdr* gene involved in insecticide resistance in *An. gambiae* S form from Obuasi.**
Gène Kdr *impliqué dans la résistance aux insecticides chez* An. gambiae *forme S à Obuasi.*

insecticide	*Kdr*	% 24-hr mortality (n)
Deltamethrin	S.S	69.2 (13)
	RS	90.7 (43)
	RR	66.7 (6)
DDT	S.S	75.0 (8)
	RS	50.0 (4)

Tableau V.

***Kdr* mutation in Deltamethrin survivors determined by sequencing and the MARTINEZ-TORRES PCR assay (11).**
Mutation du Kdr *chez les survivants au deltamethrin déterminé par le séquençage et les dosages de la PCR* MARTINEZ-TORRES *(11).*

Kdr Martinez-Torres	mortality/survival (n)	*Kdr* Sequencing (n)
S.S	survived (2)	RR (2)
RS	survived (4)	RR (4)
RR	survived (2)	RR (1) RS (1)
RR	died (1)	RR (1)

Molecular assays for the knockdown resistance mutation (*kdr*) showed no correlation with the bioassay data (table IV) with both survivors and susceptibles being found positive for *kdr* in each of the three classes RR, SS and RS.

However, sequencing of the *kdr* fragment showed good correlation with the bioassay data (table V).

Discussion

The difference in species composition between the two collections in April and June 2004 was marked. The dry season vector populations were largely excluded from the town, probably due to the highly polluted state of the surface water. However, large populations, particularly of *An. funestus*, could be found just outside the urban area. The predominance of this species in the dry season is because it is dependent on permanent breeding sites such as swamps and ponds. *Anopheles gambiae*, dependent on temporary sunlit pools of rain water, was understandably in the minority. In June the picture was very different with *An. gambiae* predominating and breeding in large numbers in rain puddles, wheel ruts, etc. Such breeding sites were common in many parts of the town and mine housing complexes, resulting in generalized mosquito populations particularly in the low-lying parts of the town.

In the two communities that were sampled to obtain mosquitoes for the susceptibility tests, the use of mosquito coils was much more common during the rains than in the dry season. This was because there were many more mosquitoes, making the expense worthwhile. In fact, this reduced the populations to a level that made it difficult to obtain meaningful samples in June, which was not the case in the dry season, April.

The bioassay mortality results for *An. gambiae* S form are markedly lower than those reported in KRISTAN *et al.* (10). Twenty-four hour mortality for deltamethrin at Obuasi was 75.9% while it was >97% in the KRISTAN study and on DDT was 30.8% at Obuasi compared with >94% in the KRISTAN study. Resistance levels in mosquito populations may vary with locality and usage of insecticides in agriculture and the home, but it is probable that these levels also vary with season as mosquito populations vary in numbers with the increasing or diminishing availability of larval breeding habitats.

The marked lack of *An. funestus* in the rainy season was unexpected considering its utilization of permanent breeding sites, but could be explained by the swamps being filled to

overflowing by rainfall and thus washing out mosquito larvae from their preferred habitat. We have no data to support this speculation but cannot otherwise explain the decrease in numbers of *An. funestus* in the rainy season.

Most studies to date show strong correlation between the *kdr* mutation and bioassay insecticide resistance in *An. gambiae* in West Africa (1, 2, 3). CHANDRE *et al.* (1) in Cote-d'Ivoire showed not only positive correlation of the *kdr* mutation with the resistance phenotype but also demonstrated that the trait was incompletely recessive. They suggested that the continued efficacy of insecticide treated bed nets against resistant mosquitoes was due to either the continued killing effect of the nets because of prolonged contact with pyrethroids during blood seeking, and/or relatively few *kdr* females able to take blood meals after prolonged contact with the nets. These studies have led to the assumption that all *An. gambiae* populations in West Africa will display similar correlations of resistance phenotype to the *kdr* allele. YAWSON *et al.* (15), carrying out *kdr* analysis on *An. gambiae* from Ghana, unfortunately did not correlate their results with bioassay data but only with the molecular M and S forms.

We carried out the MARTINEZ-TORRES PCR assay (11) in two steps in order to maximize accuracy of the assay. Results showed that *An. gambiae* S form was both resistant to pyrethroids/DDT and had the *kdr* mutation, but that these two conditions were not correlated (table IV). Some mosquitoes that survived on either deltamethrin or DDT were found to be homozygous susceptible for the *kdr* allele, while some individuals homozygous for resistant *kdr* were found to be susceptible to deltamethrin. Heterozygotes on deltamethrin gave a high mortality supporting incomplete recessiveness (1), while the DDT exposures did not. Sequencing of the *kdr* fragment of the IIS6 domain from nine individuals that survived exposure to pyrethroids showed close correlation between the mutation and the resistance phenotype, contrary to data from the MARTINEZ-TORRES PCR assay (table V). These results highlight a problem with the standard PCR assay and either this assay must be modified to produce more reliable results or a new technique is needed.

The infection rates with *P. falciparum* of both *An. gambiae* and *An. funestus* are typical of those found in West Africa (7) with *An. gambiae* being a better vector than *An. funestus*.

Conclusion

The results presented here have been used in planning the malaria control programme for the Anglogold/Ashanti mine in Obuasi. An initial indoor residual spray round with an organophosphate is being implemented in early 2006. Integrated vector management (IVM) through supply of ITNs, screening of houses, larviciding and environmental management forms the basis of the vector control programme. Monitoring and surveillance of the mosquito populations will be carried out and decisions on rotation of insecticides and further IVM interventions will be made based on future results.

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