Research News

Combating malaria vectors in Africa: current directions of research

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Vector control remains an important component of malaria control, particularly in Africa where most infant deaths occur. Among the different methods, insecticide-treated bednets seem to be a suitable way to reduce morbidity and child mortality in endemic areas. To facilitate their large-scale use and to investigate alternative vector control methods, the authors propose three current directions of research that are already being explored in Africa through a collaborating network involving several African countries: (1) vector genetics, (2) insecticide resistance and (3) vector control strategies.

Probably over two billion febrile episodes resembling malaria occur annually worldwide. At the very least, 700 000–2.7 million people die from malaria each year; over 75% of them are African children [1]. This proportion is on the increase as a result of human intervention (e.g. deforestation, agriculture and urbanization), breakdown in healthcare due to socio-economic obstacles or wars, and extension of resistance to drugs and insecticides [2]. To reverse this trend, an increased number of scientists and institutions [3] (e.g. Roll Back Malaria, Multilateral Initiative on Malaria, Wellcome Trust, Programme PAL+ and Gates Malaria Programme) have recently come together to develop and/or improve selective and sustainable tools for malaria control and prevention. Vector control remains an important component of malaria prevention. In sub-Saharan Africa, house spraying was clearly efficient in controlling malaria outbreaks in Madagascar, South Africa and Burundi, but vertical organization is a key factor for their efficacy [4]. In endemic areas, insecticide-treated bednets (ITNs) seem to be a method of choice to reduce morbidity and child mortality on a long-term basis, even in areas of intermediate or intense transmission [5,6]. However, large-scale implementation and sustainable use of ITNs need to overcome several obstacles linked to human behaviours and vectors. New avenues of research have been set up in Africa by many groups of scientists belonging to international or African institutions such as: International Centre of Insect Physiology and Ecology (ICIPE), Kenya; Ifakara Health Research and Development Centre (IHRDC), Tanzania; South African Institute for Medical Research (SAIMR), South Africa; Blair Research Laboratory, Zimbabwe; Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Cameroon; Centre de Recherche Entomologique de Cotonou (CREC), Bénin; Centre Pierre Richet (CPR), Côte d'Ivoire; Centre Muraz, Burkina Faso; Ecole de Médecine de Bamako, Mali; Medical Research Center (MRC), The Gambia; Pasteur Institute in Madagascar or Institut de Recherche pour le Développement (IRD) in Senegal [3]. These research activities relate to contrasting areas of investigation including vector bionomics, insecticide resistance and vector control strategies. In Table 1, the results obtained from research open new perspectives that could contribute to national malaria control programmes in the near future.

Towards a better knowledge of malaria vectors

In Africa, the knowledge of the major vectors of malaria, whether less efficient or locally important, remains incomplete [7]. All of the vectors belong to species complexes whose members differ widely in their vectorial capacity and competence. Any vector control strategy, whether based on traditional methods (insecticides and ITNs) or new ones (e.g. introduction of genes responsible for refractoriness to *Plasmodium* infection), must take into account this heterogeneity, and requires a precise identification of the targets: species and populations [8]. The recent developments in genetics and molecular entomology, in particular PCR techniques, have allowed significant progress in identifying species, population structure and GENE FLOWS (see Glossary). Among the major vectors of malaria, we focused on *Anopheles gambiae*, *Anopheles funestus* and *Anopheles nili*.

Anopheles gambiae

Anopheles gambiae is the best-known vector of malaria. Its genome is about to be sequenced, which should bring in new prospects for the control of transmission, making it possible to identify the genes involved, for example, in the trophic behaviour of *Anopheles*, their immunity, their development, or of genes encoding for receptors of *Plasmodium* spp. or insecticide targets. These genes, whose dissemination in the natural population could be evaluated by studies of population genetics, could be the stepping-stone towards a less conventional approach to vector control.

Glossary

M and S: *Anopheles gambiae* natural populations are divided into five different incipient taxa depending on chromosomal inversions. Recent efforts have focused on the pattern of variation observed with molecular markers that are not linked to inversions in order to resolve the taxonomic status of the chromosomal forms of *An. gambiae.* Genetic differentiation observed within the ribosomal DNA cluster on chromosome X suggests that the *An. gambiae* population is subdivided into two different entities referred to as the molecular forms M and S.
Gene flow: The transfer of genetic material between (mosquito) populations.
Introgression: The transfer of genetic material between (mosquito) species (or between genetically differentiated populations) through a hybridization event.
***kdr* mutations**: Mutations on the knockdown resistance gene that encodes for the sodium channel, leading to a decrease in the sensitivity of the insect nervous system to pyrethroids and DDT. These mutations confer resistance to the knockdown and lethal effect of these compounds. For *An. gambiae*, two mutations were identified on the same amino acid located in the S6 transmembrane segment of domain II of the sodium channel sequence: a leucine to phenylalanine change which is widespread in West Africa and a leucine to serine change in East Africa.

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Table 1. Current research activities and their contribution to national malaria control programs

Programs under way[a]	Main findings	Perspectives	Main contribution to vector control	Refs
Vector bionomics				
Genetic heterogeneity of *Anopheles gambiae* s.s.	The M and S molecular forms of *An. gambiae* are probably distinct species.	To study the spread of genes of interest for genetic control within the M and S forms.	To diversify vector control strategies.	[10]
Molecular characterization of *Anopheles nili*	Several vector species are morphologically similar.	To appreciate the role of different vector species in malaria transmission.	To target vector control better.	–[b]
Insecticide resistance				
Impact of pyrethroid resistance on the efficacy of ITNs	Preliminary results showed that ITNs preserve their full effectiveness in experimental huts.	To confirm the preliminary results at a larger scale (study in progress in Côte d'Ivoire).	To improve drug resistance management.	[16]
Distribution of the *kdr* mutation within *An. gambiae* s.s.	The *kdr* mutation is rare in the M form of *An. gambiae* and is probably a result of an introgression from the S form.	To study introgression by analysing the sequence of the gene encoding for the sodium channel.		[18]
Implementation of ITNs				
Development of LLNs	Some LLNs remain active for their average life span without any re-treatment.	To render LLNs more cost-effective by using the latest developments in bioactive fibre technology.	To improve acceptability and sustainability of ITNs.	[22]
Social and practical representations related to ITNs.	Sociocultural indicators relating to the use of ITNs are under identification.	To see how malaria control programmes could adapt their strategy for a better use of ITNs.		–[c]

[a]Abbreviations: ITNs, insecticide-treated bednets; *kdr*, knockdown resistance gene; LLNs, long-lasting bednets.
[b]P. Kengne, pers. commun.
[c]M. Akogbéto, pers. commun.

The *An. gambiae* complex is a group of more than seven species, including efficient vectors and non-vector species, such as *Anopheles quadriannulatus*, which bites cattle. *Anopheles gambiae* s.s. is made up of at least two genetic variants: the molecular forms M AND S, which are anthropophilic and are good vectors for human *Plasmodium* [9]. Using ribosomal DNA (rDNA) and microsatellite markers on some sympatric populations of Cameroon, we showed that there were no hybrids between the M and S forms, and that gene flow was restricted, suggesting a speciation in progress [10].

Anopheles funestus

Anopheles funestus is probably the best vector of malaria and belongs to a group of species with morphologically similar characteristics. Thanks to the development of molecular methods [11], recent studies in Cameroon (A. Cohuet, pers. commun.) have shown that some species of the *An. funestus* group can be collected resting indoors. Cytogenetic studies indicate strong polymorphism in this species, with >11 paracentric inversions, some of them specific to certain geographic areas [12]. Population genetics studies using isoenzyme and microsatellite markers are under way in Burkina Faso, Cameroon, Côte d'Ivoire, Madagascar and Senegal. When completed, it will be possible to assess the links between the various populations and to understand the possible speciation phenomena in progress.

Anopheles nili

Anopheles nili is also a complex species. Research on the biology, morphology and population genetics, in addition to some sequencing data from the internal transcribed spacer (ITS) 2 areas of the rDNA, show that this group comprises at least four vector species, including a new species, which are extremely close morphologically. A species-specific PCR based on the variation of the ITS2 rDNA sequence has just been developed (P. Kengne, pers. commun.), which is simple to use and helps to distinguish between the four species, and appreciate their respective role in transmission (one of them seems to be exclusively zoophilic).

Insecticide resistance

Pyrethroids are the main insecticides used in public health and are the only ones currently used for impregnation of mosquito bednets. However, pyrethroid resistance is already present in several major vectors of malaria in Africa [13]. To work out more effective strategies for managing resistance and to anticipate any possible operational failures, several areas of research are explored simultaneously. These works contribute to the drawing up, for a given area, of a resistance dynamic chart accounting for the distribution of resistance and its intensity, the mechanisms involved and the factors that significantly influence its evolution.

Resistance of *An. gambiae* s.s. to pyrethroids, first observed in the early 1990s in Côte d'Ivoire [14], is widespread in West Africa; primarily as a result of a modification in the target site (*kdr* MUTATIONS), which confers resistance to all pyrethroids and to DDT [15]. The frequency of *kdr* mutations among natural populations can reach up to 80%, particularly in areas where pyrethroids are used for agricultural purposes. The impact of this resistance on the entomological and parasitological parameters is being investigated because our results showed that impregnated mosquito bednets can preserve their full effectiveness [16] and that their use on a large scale did not affect the resistance levels of the vector populations studied. In addition, we established that no *kdr* mutation had been detected on other species of the *An. gambiae* complex and the distribution of this mutation was heterogeneous even within the *An. gambiae* s.s. spp. In the savanna areas of Côte d'Ivoire, the *kdr* mutation is only encountered in individuals with the S form, but never in those with the M form, including in places where the two forms are sympatric [17] and both submitted to a strong insecticide selection pressure. These results suggest the existence of a strong genetic barrier between the two forms, but

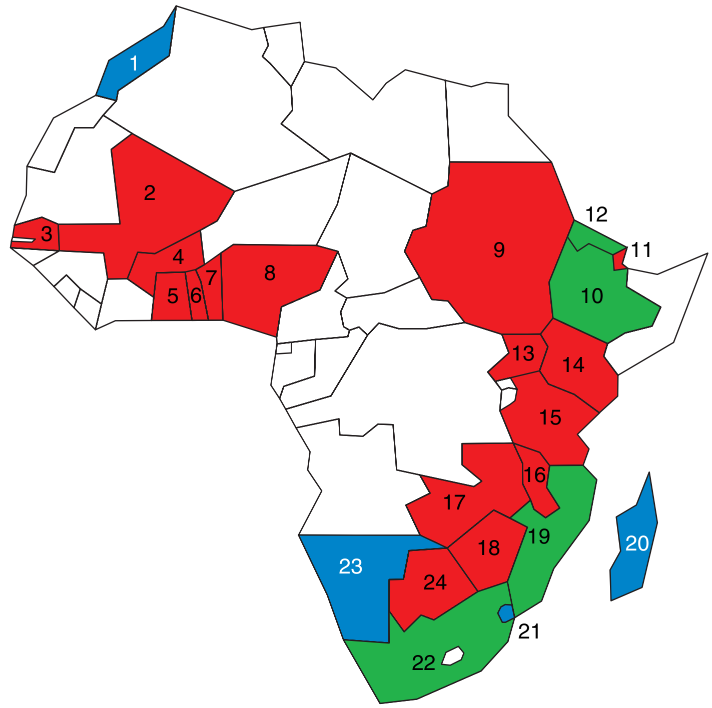

Fig. 1. African countries that are finalizing their national Roll Back Malaria strategic plan with insecticide residual spraying (blue), with insecticide-treated bednets (red) and with both integrated into the plan (green) are indicated. The countries that have not finalized their plans are in white. (Source: Roll Back Malaria, 14 March 2002.)

do not exclude a total absence of gene flows. Indeed, a resistant population of the M form of *An. gambiae,* as a result of an INTROGRESSION of the *kdr* mutation from the S taxa, was recently discovered south of Bénin [18]. It is thus essential to assess the level of such exchanges that are likely to accelerate the dissemination of resistant genes. Other investigations are under way, especially those focusing on another target site mutation, the insensitive acetylcholinesterase, a modified acetylcholinesterase which is suspected in some *An. gambiae* populations of West Africa (the development of a diagnostic test is envisaged to follow its evolution in the field). The search for metabolic-type mechanisms is also under way because their presence could lead to operational failures, such as those already observed with *An. funestus* in South Africa [19].

Efficacy, sustainability and acceptability

Malaria vector control in Africa mainly relies on interventions targeted on adult anopheline vectors through the use of ITNs or indoor residual spraying (IRS). Under coordination of the WHO within the Roll Back Malaria partnership [20], African Member States have prepared or are finalizing their national strategic plans for malaria control which all include ITNs and some also include IRS (Fig. 1). Based on current prices for bednets, ITNs are as cost-effective as IRS in malaria prevention [21]. Implementation of IRS relies on well-structured vector control services that did not or no longer exist in many countries, especially with the ongoing decentralization process and health structure reform. Because ITNs do not require such services, they could have a significant advantage over IRS in endemic areas. To be effective in areas with high malaria transmission, bednets must be treated by dipping in pyrethroid insecticides and re-treated at least once a year. When integrated into large control programmes, there is the danger that rates of regular re-treatment of the bednets with insecticide will drop, greatly limiting their effectiveness as a public health intervention. It is estimated that <5% of the bednets currently used in Africa are properly treated or re-treated (i.e. with correct insecticide solution and suitable time intervals, every four to six months). To overcome this problem, the development of long-lasting insecticide-treated mosquito bednets (LLNs) has been promoted using the latest developments in bioactive fibre technologies. These bednets remain active for their entire average life span (up to five years) and do not need any re-treatment [22]. Already, several LLNs are under development and will gradually replace conventionally treated bednets. In addition to mosquito bednets, long-lasting treated materials have a range of potential applications in the prevention of vector borne diseases, particularly in the development of new tools that can be used at community level, such as treated blankets, tents and plastic shelters.

To be acceptable, ITNs should also have a visible impact on nuisance from mosquitoes or other non-target arthropods, such as bedbugs [23], which is the main motivating factor for people to use ITNs. In Africa, nuisance due to mosquitoes is mostly induced by *Culex quinquefasciatus*. Because pyrethroids have limited impact on this particular mosquito, users frequently think that ITNs are not effective and do not perceive their need. More attention is now given to the behavioural response of mosquitoes to insecticides and in selecting insecticides that are most active on nuisance and vector species. Non-pyrethroid insecticides have also been tested on mosquito bednets [24] and research is currently moving towards a combined use of insecticides from different families on the same net, either separately (equivalent to mosaics) or in association (mixture). These studies take into account safety considerations and a recently proven synergism between pyrethroid and non-pyrethroid insecticides [25]. Field studies looking for the optimal and spatial distribution of pyrethroid and non-pyrethroid insecticides on bednets are being performed. These studies are based on human behaviour (to keep the non-pyrethroid insecticide out of reach of children) and host-seeking behaviour of mosquitoes when flying around the net (to favour the mosquito's contact with the most active insecticide) [26]. This combined use of insecticides (equivalent to a bi-therapy) is conceived primarily to increase acceptability of ITNs, but may also be used as a tool for the prevention and management of insecticide resistance in malaria vectors, and nuisance [27].

Until now, the participation of communities in the use of ITNs has not been up to expectation. Although they are effective, they are still used infrequently and incorrectly. In endemic areas, an average of 10–15% of households have at least one net when a coverage rate of ~80% is needed to have an impact at the community level [22]. Often, mosquito bednets were introduced in Africa by projects not taking into account the practices, tastes or expectations of the potential users. Health professionals have collected some data during knowledge, aptitudes and practices (KAP) investigations [28,29] or during epidemiological surveys. It was noted that the mosquito was perceived as a nuisance first and foremost (bites and noise) and not as a vector of a parasite, even when the link with malaria is established. The investigations by health professionals also showed that the use of mosquito bednets depended on their intrinsic characteristics (cost, size, colour, form and solidity) and that they were often regarded as objects whose functions went well beyond a simple anti-mosquito barrier. Mosquito bednets can constitute a privileged place of intimacy or protection against external aggressions (e.g. cold and crawling insects) and their use can be perceived as a mark of social or cultural status [30].

There is no doubt that the attitudes, the representations related to mosquito bednets, and the practices and experiences of communities encourage their recourse and sustainable use. It is necessary that their promotion must go through a thorough knowledge of the behaviour and expectations of the target populations, which can vary considerably from one community to another. That is why a three-year multi-local socio-anthropological study investigating the users of mosquito bednets has just been started in four countries of West Africa for the first time (M. Akogbéto, pers. commun.). The sites were selected according to their diverse epidemiological and sociocultural levels. The objective is to provide, test and evaluate sociocultural indicators relating to the reasons and modes of using mosquito bednets, in addition to their acceptability. The techniques used for data collection are based on direct observations, on focus group discussions, on interviews of householders and on structured questionnaires. One year after the beginning of the study, the results should help national malaria control programmes to adapt their approaches to the realities of the target communities and to reinforce their impact in the field of the promotion and use of ITNs.

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