The technical basis for coordinated action against insecticide resistance: *preserving the effectiveness of modern malaria vector control*

Global Malaria Programme

WHO HEADQUARTERS, GENEVA, 4–6 May 2010

Meeting report

World Health Organization

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Abbreviations

kdr	*Knockdown* resistance, particularly any of a series of genes involving a mutation in the target site of pyrethroids and DDT, and conferring resistance to these insecticides.
IRM	Insecticide resistance management
ITN	Insecticide-treated net
IRS	Indoor residual spraying
LD50	Lethal dose 50% (i.e. the dosage expected to kill exactly 50% of exposed insects)
LLIN	Long-lasting insecticidal net
GMAP	Global Malaria Action Plan
NMCP	National malaria control programme
RBM	Roll Back Malaria

Glossary

F1 progeny This generally means "first generation offspring"; in this context it refers to the use of adults raised from the eggs of wild-caught female mosquitoes, in order to obtain an age-standardized sample of the wild population for bioassay tests for resistance.

Insecticide combination

The use of two or more insecticide applications within a building, e.g. one insecticide on the walls and another on nets in the same household. Insecticide combinations differ from insecticide mixtures in that the same insect is likely, but not guaranteed, to come in contact with both insecticides.

Insecticide mixture

Two or more compounds are mixed within a single product or formulation so that the mosquito is guaranteed to come into contact with both at the same time.

Insecticide mosaic

The spraying of compound A in one area and compound B in another area, so that some mosquito populations are exposed to A while others are exposed to B.

Refugia A fraction of a mosquito population which is systematically removed or protected from the insecticide to which the rest of the population is exposed. Refugia are an important feature of resistance management strategies in agriculture.

Susceptibility tests (bioassays)

Bioassays in which insects from a wild population are exposed to a fixed dose of insecticide designed to reliably kill susceptible insects, so that any survivors are likely to be resistant. There are long-established WHO standard methods, and more recently-developed CDC methods.

Synergist A substance which does not itself have insecticidal properties, but which, when mixed or applied with insecticides of a particular class, considerably enhances their potency, for example by inhibiting an enzyme that normally has detoxifying activity against the insecticide.

Contents

1. Summary

Current situation – vulnerability

Modern malaria vector control is exceptionally dependent on a single class of molecule: the pyrethroids. For public health purposes, pyrethroids are probably the best insecticides ever developed. They are currently the only class of insecticide used on WHOPES-recommended nets, and are substantially less expensive and longer-lasting than almost all the alternatives for indoor residual spraying (IRS). According to the **Roll Back Malaria** Global Malaria Action Plan (RBM/GMAP), about 60% of the projected malaria control expenditure in 2010 will be used for vector control commodities and activities, mostly long-lasting insecticidal nets (LLINs) and IRS, where currently pyrethroids are the most commonly used chemical.

In the last few years, genes conferring resistance to pyrethroid insecticides have been spreading rapidly and are now widely distributed in the main African mosquito vectors of malaria. One type of resistance – *kdr* – has been spreading in *Anopheles gambiae* for several years and is now widespread in West and Central Africa. Metabolic forms of resistance have also been found in several widely scattered locations among both *An. gambiae* and *An. funestus*. These metabolic forms of resistance are more difficult to monitor and, alone or combined with kdr, may be a much greater threat. There is evidence that at least some of these genes have the potential to threaten the effectiveness of current vector control efforts. Carefully designed epidemiological trials to accurately assess the impact of different types of resistance, particularly on the efficacy of LLINs, are urgently needed.

In order to develop an appropriate and comprehensive response, the WHO Global Malaria Programme convened an informal expert consultation (Annex 1) at which participants were asked to review the current status of insecticide resistance in malaria vectors, and to identify options for a resistance management strategy that could help to preserve insecticide susceptibility, slow down the evolution of resistance, and prolong the effectiveness of current vector control interventions.

Recommended strategies to delay resistance

1. Action must be immediate and pre-emptive, not responsive

1.1 A strategy for insecticide resistance management, involving not only monitoring but also planning interventions to minimize resistance evolution, should be built into every vector control programme, and must be implemented from the outset, without waiting for evidence of resistance. Above all, such strategies must not be delayed pending indisputable proof of control failure.

1.2 Methods for delaying resistance are much more effective when resistance is rare, and much less so when it has become common (*1–3*).

2. Judicious use and operational quality

2.1 When insecticides are used for vector control, they must be used with care and deliberation – not indiscriminately. Programmes should target their vector control to reduce unnecessary insecticide selection pressure in non-transmission areas, which will also reduce unnecessary costs. Similarly, quality assurance to ensure accurate IRS application rates will reduce both waste and unnecessary selection pressure.

3. Rotations, mosaics, combinations and mixtures

3.1 ***Rotations:*** IRS programmes should avoid the practice of spraying the same insecticide (or class of insecticides) repeatedly year after year; instead, insecticides with different modes of action should be sprayed alternately, in rotation. Alternating different insecticides in space, as a mosaic, is another option.

a. Rotations and mosaics assume that the frequency of resistance will decline in the absence of selection, due to fitness cost or dilution through mixing with surrounding susceptible populations. Rotations are a common form of 'best practice' in agriculture, and are considered to have a good record in slowing down the evolution of resistance (*1*).

b. The insecticides in a rotation system will vary in cost, so programmes should focus on planning for the cycle as a whole, and consider the average annual cost over the whole cycle. Donors should consider what is needed to ensure that their own planning cycles support this approach.

3.2 ***Combination interventions*** involve using different insecticide classes applied in different forms within a house (e.g. a carbamate sprayed on the wall and a pyrethroid on an LLIN). Such combinations are likely to play an important role in resistance management in the future.

a. Combinations work on a principle which is different from that of rotations and mosaics, and similar to that of combination therapy with drugs: that if an organism resistant to A appears, and is not killed by intervention A, it will be immediately killed by B.

b. Preliminary studies of combinations have given very promising results, and further investigation, both in small-scale studies and in evaluation of operational-scale trial interventions, is urgently needed.

3.3 ***Mixtures*** are products in which two insecticides of different classes (different modes of action) are co-formulated so that both have a similar rate of decay. Mixtures should be investigated urgently, since they may be the best way to ensure that insects which survive exposure to one insecticide will be killed by the other. If so, they would probably be one of the most effective resistance management approaches (*2*). Currently, there are only a few mixture products in agricultural use, and none in public health (*1*), but manufacturers should be encouraged to develop such products. In order to do so, it will be necessary to address some critical formulation and regulatory issues. The former must be addressed by manufacturers during product development, but the latter must be addressed by the public health community.

4. Monitoring

4.1. A major intensification of resistance monitoring is urgently needed. Vector control decisions, targeting and insecticide selection must become contingent on local data.

4.2 All agencies and programmes funding or implementing large-scale vector control operations should take responsibility for the production and reporting of adequate resistance monitoring data in their target areas.

4.3 All insecticide procurement choices should be informed by adequate resistance monitoring data. Just as some donors require adequate environmental impact assessments when pesticides are used, there should be similar requirements for resistance monitoring information to support the choice of insecticide product.

4.4 A clear reporting system, with defined responsibilities and adequate funding support, needs to be created within countries, among implementation partners, and at the regional level. National programmes should coordinate national-level monitoring, and all data should be reported to them as soon as they are collected (e.g. they should not be held back for later publication).

4.5 the WHO guidelines on monitoring insecticide resistance need to be updated urgently, and a sub-group of experts from this consultation has been asked to undertake this task.

5. New products

5.1 New vector control products are needed with the utmost urgency. Above all, new classes of insecticides are needed, especially products suitable for use on nets. It is also essential to prolong the life of the currently available insecticides, which will require new formulations designed for resistance management.

5.2 In order to invest in the development of such new products, manufacturers need to be confident that the market will be adequately stable, and that there will be effective competition favouring products that are more effective at delaying resistance evolution.

5.3 One public-private partnership to support market development of new products already exists, but other institutional interventions may also be needed.

5.4 While there are some products under development for IRS, prospects for new insecticides for LLINs are far less promising. Special efforts may be needed, because the insecticide represents only a very small fraction of the value of an LLIN, but a much larger proportion with other vector control products.

6. Cost implications

6.1 All alternatives to the existing methods involve an increase in short-term costs compared to current practice. To maintain the effectiveness of vector control, a substantial increase in the short-term cost of vector control must be anticipated, as well as the costs for development and introduction of entirely new products. Ultimately, these investments will almost certainly be cost-saving, if the effectiveness of pyrethroids on LLINs is preserved.

6.2 A study should be commissioned to estimate the likely global costs of vector control commodities until the year 2030, using the same methods as those in the RBM/GMAP, but taking into account the adoption of resistance management methods (including rotations and combinations) and likely price trends. The aim is to give manufacturers assurance as to the long-term stability of the market, and thus the incentive to develop the products that are urgently needed.

2. Introduction

Current malaria vector control is heavily dependent on one class of insecticides, the pyrethroids. Accumulating evidence suggests that genes for resistance to pyrethroid insecticides have been spreading rapidly and are now widespread in African malaria vector mosquitoes. There is evidence that at least some of these genes have the potential to threaten the effectiveness of current malaria vector control interventions (*5*).

In order to develop an appropriate and comprehensive public health response to this situation, a working paper summarizing the evidence was prepared, and an informal expert consultation was convened with the following tasks:

- to review the working paper on the current status of insecticide resistance in malaria vectors;
- to review current knowledge of resistance management in theory and in practice;
- to consider and make recommendations on the general options for a resistance management strategy, i.e. the potential tactical and strategic actions that could *(i)* preserve insecticide susceptibility and slow down the evolution of resistance, and *(ii)* preserve the effectiveness of malaria vector control interventions in places where a high frequency of resistance genes has already evolved;
- to consider which elements of the resistance management strategy should await clear evidence of control failure, and which should be implemented immediately without waiting for such evidence.

3. Current situation

Recent reductions in the global burden of malaria morbidity and mortality (*4*) are due primarily to massive scaling-up of malaria vector control using LLINs and IRS. According to the RBM/GMAP, about 60% of projected global malaria control expenditure in 2010 will be used for vector control commodities and activities.

In Africa, LLINs and IRS are now being deployed on an unprecedented scale, exposing African vectors to selection pressure for insecticide resistance that is more intense and consistent than ever before. Unfortunately, since the commercialization of the pyrethroids in the 1970s and 1980s, there has been little public investment in insecticides for public health purposes. Hence, most of this pressure comes from just one class of molecule, the pyrethroid insecticides, which are the only class of insecticides currently used on treated nets and the class most commonly used for IRS. Nets are an easy-to-deliver, effective and robust form of vector control, and without them, it is unlikely that the goal of universal vector control coverage can be achieved and sustained in the most difficult-to-reach communities. It can therefore be argued that modern malaria control is even more dependent on pyrethroid insecticides than it is on artemisinin-based medicines: the threat from insecticide resistance in the vectors is at least as great as that from drug resistance in the parasite.

Genes conferring various degrees of resistance to most of the currently available insecticides are already circulating in target vector populations, especially in Africa. According to the working paper prepared for the meeting (*5*), which includes two useful maps (Figures 1a and 1b), pyrethroid resistance has now been reported in malaria vectors in 27 countries in sub-Saharan Africa.

Mechanisms of two kinds are responsible for the majority of cases of resistance that have been studied in *Anopheles* species: changes in the target site (such as the *kdr* mutations described above), and increases in the rates of insecticide metabolism. Both of these types of mechanism have been found in *An. gambiae*; metabolic forms of resistance have been found in several widely-scattered locations in *An. funestus*. It seems likely that other resistance mechanisms remain to be identified (*5*).

Resistance to a given insecticide usually confers resistance to the other insecticides in the same class, and may also confer cross-resistance to one or more other classes of insecticide. For example, pyrethroid insecticides and the organochlorine insecticide DDT are structurally very different, but both attack the same target site on the insect nerve membrane, and mutations in this target site, known as *kdr* mutations, are now the most common and widespread resistance mechanism in Africa. Thus resistance to DDT is sometimes observed in the same areas as pyrethroid resistance. Similarly, organophosphate and carbamate insecticides share another target site, and a mutation in this site conferring cross-resistance to both classes is being increasingly reported in *An. gambiae* s.l. in West Africa and there have been several reports of carbamate resistance in *An. funestus*. Multiple mechanisms may also be present in a single insect. It is clear from ongoing longitudinal studies that the geographical distribution of resistant alleles is spreading very rapidly.

Although substantial progress has been made in understanding the genetic causes and biochemical mechanisms of insecticide resistance, less is known about the epidemiological impact of resistance on current malaria control activities. This is partly because resistance cannot be randomly allocated to some communities and withheld from others, so that the effect of resistance cannot be fully separated from other factors which also influence the efficacy of vector control. A small number of published studies, and several anecdotal reports, have indicated that pyrethroid resistance can have a negative impact on insecticide-based malaria control. One well-known example in IRS occurred in South Africa where a clear case of control failure followed the appearance of both insecticide resistance in the vector and drug resistance in the parasite. On the other hand, the spread of *kdr* genes in *An. gambiae* s.l. through West Africa has not been followed by conspicuous control failure, and trials in West Africa have shown that the introduction of pyrethroid-treated nets still has worthwhile epidemiological impact, despite a high frequency of *kdr* genes. Given the state of routine epidemiological surveillance in most of the region, a partial and/or patchy decline in the effectiveness of routine vector control could occur without being detected. Thus, the picture is incomplete.

The effects of resistance on vector control are unlikely to be simple. A resistance gene that does not cause complete and immediate control failure may nevertheless reduce the effective lifespan of an intervention. Moreover, in agricultural insects, there are cases where a series of resistance genes have evolved in succession (*6*). In Benin, experimental hut trials have suggested that the multiple resistance mechanisms found in the local *An. gambiae* s.l. population reduces the effectiveness of conventional LLINs to low levels (*7*).

Malaria vector control depends primarily on LLINs and IRS largely because these interventions are reliable and robust, i.e. they are effective in a wide range of situations, even when the circumstances are sub-optimal and coverage is imperfect. The effect of resistance may be to compromise this robustness. The authors of the working paper therefore concluded that an immediate proactive response to resistance management is essential to sustain the effectiveness of malaria vector control. Furthermore, large-scale, controlled epidemiological trials to accurately assess the impact of different types of resistance on the efficacy of LLINs and IRS are urgently needed.

Research priorities identified during the meeting are listed in Annex 2.

4. Sources of selection for insecticide resistance

In some places, *Anopheles* malaria vectors are exposed not only to public health insecticides but also to agricultural insecticides, including pyrethroids. If all the insecticide resistance in anophelines were due to agricultural chemicals, there would be little point in modifying malaria vector control practices to delay the evolution of resistance. However, the evidence as a whole suggests that public health insecticides are also important as a source of selection for resistance in vectors. There are cases where agricultural insecticides, particularly those used on cotton and rice, have clearly been responsible for resistance in malaria vectors (*8*). However, there are other cases where IRS was clearly the only source of selection for resistance (*9*). Finally, there are also cases where agricultural chemicals seem to have created a situation where a resistance gene was present and maintained at low gene frequencies in a local vector population, and subsequent anti-malaria spraying then caused that gene to be selected to high frequencies.

It was concluded that:

- Agricultural insecticides contribute to some, but not all, of the resistance problem in malaria vectors; the evolution of high gene frequencies of resistance is more commonly associated with IRS. Thus, resistance management measures to prevent resistance in malaria vectors should include, but not be restricted to, agricultural insecticides.
- As many more insecticides are currently available for agricultural use than for malaria control, it would be a valuable contribution to public health if the agricultural sector (especially large-scale applications on cotton and rice) were to use classes of insecticide that are not available for use in public health (see recommendation 7).
- It is in the interest of both the agriculture and public health sectors to work together on management of resistance (including coordinated monitoring of the insecticides used for both purposes). Further research is required to understand how the different agricultural and domestic uses of pesticides influence the appearance and spread of insecticides resistance of public health importance.

Resistance genes are rare to begin with, and this probably means that initially they have a fitness cost, i.e. there is selection against them in the absence of the insecticide. The magnitude of this fitness cost probably depends on the resistance mechanism, and is likely to be large for some mechanisms and smaller for others (*10*). Conversely, it is clear that the fitness cost is also subject to modification by the evolution of other genes. Thus, when a resistance gene has been maintained by insecticide pressure at high frequencies for some time, it can gradually lose its fitness cost as the population evolves and adjusts its genetic background to the presence of the resistance gene. When this happens, resistance in effect becomes the new "wild type", and the population is then not expected to revert to susceptibility when insecticide pressure is withdrawn (*11*).

The fitness cost of resistance is obviously helpful for efforts to delay the spread of resistance, and some common forms of resistance management, including mosaics, are theoretically effective only if there is such a cost. This, together with the general principle that fitness costs are often present while resistance is rare but may be lost when resistance becomes common, argues strongly that resistance management measures should be instituted when the resistance gene is still rare.

5. Impact and monitoring

More resistance monitoring is urgently needed. It was noted with concern that currently, many vector control interventions are delivered with no or insufficient effort at resistance monitoring. Sometimes it is suggested that resistance testing is a research activity, and not a core part of implementation.

- All implementation agencies engaged in large-scale vector control operations, including NGOs delivering large-scale LLIN programmes, should take responsibility for the production and reporting of adequate resistance monitoring data in their target areas, before and after the intervention. This should be coordinated by the national programme.

Similarly, it was noted with concern that insecticide choice decisions, including procurement decisions, are sometimes taken without considering resistance data, even when such data are available and the risk of resistance is substantial. Therefore, as an extension to currently-accepted good practice:

- Before a given procurement decision is ratified and approved by the donor, there should be a simple check that the decision has been informed by adequate resistance data, just as some donors already check that an environmental impact assessment has been carried out.

Within every NMCP, there should be a suitably qualified individual with specific responsibility for the collation and interpretation of insecticide resistance data. Any person or agency conducting any form of resistance testing within the country should be required to report the test results to the programme within three months of the test being carried out, whether or not the entire study has been completed, and prior to any scientific publication or dissemination of the data. The responsible individual should make active efforts to seek out and collect such data, and should respect the confidentiality of research data, but should report the collated data in a standard format through sub-regional networks and/or WHO.

The WHO guidelines on monitoring insecticide resistance (*12*) were last updated in 1998 and a fresh revision is urgently needed, taking into account new technology and information. A sub-group of the meeting participants was asked to undertake this task. The group recommended that a detailed, step-by-step guide to interpreting bioassays should be included in the updated monitoring guidelines.

The relative roles of bioassay and genetic tests for resistance were discussed:

- It was agreed that bioassays (standard susceptibility tests) should be the primary method by which resistance to insecticides is initially detected and identified.

Surveillance for the appearance of new cases of resistance should not rely on molecular or biochemical assays alone, because the latter cannot be relied upon to detect new and unexpected mechanisms.

- There is no simple one-to-one correspondence between resistance phenotype (survival in a standard susceptibility test) and genotype (the molecular detection of a resistance gene or mechanism in an individual insect) (*2*). Most susceptibility tests use a "discriminating dosage" that is intended to reliably kill susceptible insects, but with some insecticides it is quite common to observe 0.5% or 1% survival on the discriminating dose when wild insects are tested, even in a fully susceptible population. Conversely, the discriminating dose may kill some insects that are genetically resistant, especially heterozygotes.
- The spread of resistance is fundamentally an evolutionary process, and must ultimately be described and understood in genetic terms. Hence molecular genetic tests, and to a lesser extent biochemical tests, are also necessary. In particular, simple PCR tests such as those that have already been widely used to track the spread of *kdr* ("knock-down resistance") genes in *An. gambiae*, have now been developed into rapid through-put methods which can be combined with molecular tests for sibling species identification and the presence of *Plasmodium* infection. These are especially useful tools for monitoring the spread of resistance.

In updating the guidelines for bioassay monitoring, inclusion of the following points was recommended:

- As a minimum sample size, no less than 100 mosquitoes should be tested on each insecticide, and there should always be least two control tests.
- Bioassays should be performed at least once per year to test each insecticide that is in use or to be used within the next year. Diagnostic doses will need to be recommended for new insecticides becoming available.
- Testing should be carried out using samples from multiple sentinel sites within a country. Sites should be selected to represent different endemic areas based on ecological zones and malaria transmission intensity.
- Changes in susceptibility should be monitored longitudinally at the same site to differentiate between mosquito susceptibility evolving over time vs varying among different locations.
- Temporal and geographical changes in species distribution should be recorded for all of the major vectors in each of the sample sites. This may mean that sampling needs to be carried out more than once per year.
- Areas with high agricultural activity and use of the same insecticides as those used in vector control require increased surveillance.

- Interpretation of results should take into account the sampling technique. For example, indoor catches may be biased towards insects that have already encountered insecticide and survived, i.e. are more likely to be resistant. Care must also be taken with speciation when the vector population consists of a mixture of different sibling species (or molecular forms), since the resistance gene frequency is likely to vary between species, and the species composition may vary between sampling methods.
- In some cases, older mosquitoes may be less phenotypically resistant. For example, when resistance is caused by a detoxifying enzyme, the activity of this enzyme may decline gradually with age. For this reason, susceptibility tests are often performed with the F1 progeny of wild-caught mosquitoes, so that age-standardized insects can be tested. However tests on wild-caught female vectors and F1 progeny can both provide useful information when used in bioassays, and both options should be considered based on the resources available. A table listing the advantages and disadvantages of using each is provided in Annex 3-A.
- If differences in susceptibility are observed, and the test insects are likely to include a mixture of sibling species, the survivors and the dead insects of the bioassay should be speciated, together with the insects in the control tubes, in order to identify in which species signs of resistance are present.
- Clear mechanisms are needed for the process of reporting and collating insecticide resistance testing data, and for the process of interpretation and ensuring that the data is used to inform planning decisions. A note on the IRBase database and its advantages is in Annex 3-B.

6. Insecticide resistance management for malaria vector control programmes

General principles for delaying the spread of resistance

The group considered the general lessons that can be drawn from the extensive modelling studies of the 1970s and 1980s (*2,13–15*) and from practical experience with other insects including agricultural pests (*1*), on the question of what can be done to delay the evolution of resistance:

- Resistance management *must* be done early while resistance is rare, as doing it later is much less effective. This point is consistently implied by the modelling studies, and by practical experience in agriculture and public health.
- The first principle is judicious use of pesticides and good pesticide management. This includes not exposing vector populations to insecticidal pressure when the human population is not at significant risk, and the intervention therefore has little or no public health benefit.
- Resistance management must become a primary consideration in the choice between alternative vector control methods. Presumably, some forms of vector control select more strongly for resistance than others, depending on the situation. Thus, the choice of alternative interventions for a vector control programme should consider not only maximizing the expected epidemiological benefits, but also minimizing the expected resistance costs. The aim must be to balance the former against the latter. Consider, for example, the choice between IRS and LLINs. It is obviously important to know which of these two selects more strongly for resistance, and whether this varies according to circumstances and vector behaviour. So far, however, it appears that this question has not been studied, and methods for doing so at the village scale need to be developed.
- Although co-formulated mixtures of insecticides have rarely been used in public health, and there are only a few precedents for their use in agricultural resistance management programmes, modelling studies indicate that in principle, a mixture formulation (see glossary) would be preferable to either combinations or monotherapies. The underlying principle here is exactly the same as that underpinning the use of drug combinations in malaria treatment: that an individual organism which is resistant to one insecticide will be killed by the other. This principle therefore assumes that the insecticides come from different classes (see recommendation 3) but have similar rates of decay over time.

- The use of combination interventions (see glossary), with one class of insecticide on the walls and another class of insecticide on nets, could be deployed immediately. The value of this approach should be investigated urgently. Theory suggests that this approach may well be preferable, in terms of resistance management if not cost, to the application of rotations and mosaics. It is of course important that the combination should never involve the same insecticide on wall and net, and as far as possible the two insecticides used in combination interventions should themselves be rotated.
- Rotation/mosaics (see Glossary) of insecticides, insecticide mixtures or insecticide combinations should always be considered as best practice, in part to promote diversity and innovation and development of more products. Rotations are particularly effective if there is a large fitness cost associated with resistance, or if the local environment means there is a large influx of susceptible vectors from refugia.
- Rotation/mosaics should always be among insecticides of different classes and different modes of action (see IRAC classifications). Information on insecticide classes should be disseminated to the programme managers.
- In the agricultural context, farmers sometimes deliberately create "refugia" habitats near to treated crops; these are areas, such as a patch of natural vegetation, where populations of the target pest insect can exist without being exposed to the insecticide. Then, even if there are a few surviving resistant insects when the crop is treated, the contribution of these survivors to the next generation will always be greatly diluted by the larger reservoir of mostly susceptible insects in the refugia. There may be useful opportunities to use this principle with vector species that are relatively zoophagic and/or exophilic, and those that exist in significant populations away from people; it is less easy to envisage how refugia could be deliberately created with vector species that are thoroughly anthropophagic and endophilic, and that are only found in association with human settlements.
- Testing the efficacy and effectiveness of non-conventional mixtures (such as insect growth regulators or fungi) should be encouraged; such products are needed to promote diversity and new products for delivery through both IRS and nets.
- Synergists (in mixtures or combinations) should be investigated and rigorously tested. However, agricultural experience is not encouraging, and does not suggest that synergists are likely to be a decisive and general answer to the resistance problem. Although the concept is attractive, there are issues with public health applications of some currently-available synergists, relating to their ability to knock out enzymes in humans as well as insects.

- Almost all of these options involve an increase in short-term costs compared to current practice. In order to maintain the effectiveness of vector control, a substantial increase in the short-term cost of vector control must be anticipated, including the need to develop and introduce entirely new products. Ultimately, these investments will almost certainly prove to be cost-saving, if the effectiveness of pyrethroids on LLINs is preserved. It is therefore essential that programmes stratify and target their interventions and deploy their interventions as efficiently as possible.
- In agriculture, farmers in some countries are persuaded to adopt a rotation strategy by encouraging them to consider the average annual cost, rather than the cost of insecticide for each year. Thus, a rotation scheme where the unit cost is US$ 1 in year 1, US$ 5 in year 2 and US$ 3 in year 3, is presented as a scheme costing US$ 3 per year over 3 years.
- A study should be commissioned to estimate and project the likely global cost of vector control commodities until the year 2030, using the basic assumptions of the existing RBM GMAP, but adding reasonable assumptions about the adoption of resistance management methods (including rotations and combinations) and about likely trends in prices for old and new products. The aim is to explore the proposition that although the cost of vector control in the future will be higher than previously estimated, it will still represent excellent public health value for money and will still be affordable. In this way, the large chemical companies (which are the only institutions with the capacity to identify completely new insecticidal chemicals) can assess the expected size and (more importantly) the long-term stability of the market, which should provide the incentive to produce the new products that are badly needed.

Practical implications for specific vector control interventions

- The vector control community should be reminded of the importance of integrated vector management in its widest sense, stressing the importance of resistance prevention and management.

ITN

- A major limitation for the use of ITNs/LLINs in resistance management is the fact that all the products in current use contain only one class of insecticides: pyrethroids. Moreover, the fact that LLINs last for years (and the development of even longer-lasting versions should be encouraged) means that it is not possible to switch promptly to a different insecticide if it is seen that resistance develops quickly following a major LLIN campaign.

- Nevertheless, an insecticide-treated bednet is always better than no protection. There is consistent evidence that untreated nets in good condition provide about half the protection provided by treated nets (*16–21*). Thus, nets are expected to remain an important public health intervention, even if pyrethroids eventually become far less effective, and even if development of replacement insecticides for use on nets is slow. At present, this situation is still far off, and in one part of West Africa it was shown that LLINs still produce good epidemiological protection in the presence of high levels of *kdr* resistance (*22*).
- Replacement of old and physically worn nets (with holes and tears) is recognized as being very important, especially where resistance is present. There is recent and rapidly accumulating evidence that the physical durability of LLINs is highly variable and often shorter than previously expected. Systems to replace the nets lost to wear and tear are urgently needed. Net manufacturers should be encouraged, and given the incentive, to develop LLIN products that have greater physical durability, as it is believed that there is still much room for improvement.
- As LLINs become older, wear and tear causes them to lose insecticide and accumulate holes; there is some information about how these two processes separately affect the protection given by the net, but very little about how they interact as they proceed in parallel, such that eventually mosquitoes become able to penetrate the net, take a blood meal, and survive. Resistance adds a further dimension to this interaction, which needs to be studied urgently in small-scale as well as community-level studies.

IRS

- Because non-pyrethroid insecticides are available for IRS but not for nets, and because it is unlikely that universal vector control coverage can be achieved in Africa by IRS alone and without the use of LLINs, it has been suggested that the use of pyrethroids should perhaps be discontinued for IRS, and should be reserved for nets. This would be a radical change, because presently a very large proportion of all IRS is done using pyrethroids, largely because pyrethroids are several-fold cheaper (and more durable) than the currently available alternatives. The group discussed this suggestion at length. It was decided that such a recommendation is not currently justified, but might become justified in the future, should a range of new long-lasting IRS products become available.
- The interpretation of resistance data is best done at the sub-regional level: the trends and threats in one country cannot be fully understood without information from neighbouring countries. Data sharing is therefore essential. A designated organization for carrying out this function does not currently exist but it is

strongly recommended that support should be given to sub-regional resistance monitoring networks. A list of institutions with competence in the collection, collation and interpretation of this data would be helpful.

- "Level of resistance" is a loosely defined phrase used sometimes to refer to the observed mortality in a laboratory bioassay, and sometimes to the impact of resistance on the effectiveness of vector control. In either case, it must not be forgotten that the "level of resistance" of a population has two distinct underlying determinants: (*i*) the frequency of the resistance gene(s) in the population, and (*ii*) the strength of resistance conferred by that gene in heterozygotes and homozygotes, as measured, for example, by the ratio between the LD50 of resistant insects and that of susceptible insects.
- All vector control interventions should include, or be part of, a resistance management strategy, and the implementation of resistance management measures should not wait to be prompted by proof of control failure or the appearance of resistance. Nevertheless, it is of critical importance that the programme should monitor for, and respond to, the appearance of resistance and evidence of control failure. The following thresholds for decisions based on bioassay data are suggested:
  - *98–100% mortality:* continue with same strategy/package of insecticides
  - *80–97% mortality:* continue with the same package but carry out further, more frequent bioassays and impact assessment.
  - < *80% mortality:* switch away from the insecticide class to which resistance has been detected, and carry out further investigations to confirm the mechanism, gene frequency and distribution of resistance.

Thresholds such as this should be seen as one guiding element in the decision-making process, and must always be interpreted in the light of other relevant information, including the specific insecticide in question, the source and condition of the test insects, and other data from nearby locations.

- Resistance to one type of pyrethroid generally confers resistance to all pyrethroid insecticides. Susceptibility tests with different pyrethroids (e.g. permethrin and deltamethrin) may nevertheless produce different levels of mortality with a single mosquito population (e.g. 98% with one pyrethroid and 80% with another). Such data must be interpreted with caution: this can happen simply because of slight differences in the discriminating dosages set for each compound, and does not imply a greater or lesser degree of cross-resistance; in particular, it does not mean that switching from one pyrethroid to another will restore effectiveness. Nevertheless, there are some important gaps in understanding of the exact degree of cross-resistance between different sub-classes of pyrethroids (including non-ester pyrethroids such as etofenprox) and research to fill these gaps might help to prolong the usefulness of this uniquely important class of insecticides.

- Currently the impact of different levels of insecticide resistance on the effectiveness of the vector control programme is unclear but research is under way to assess this and the proposed thresholds may be reconsidered in light of their results.

Combined vector control interventions

- As it is not presently clear whether the selection for pyrethroid resistance tends to be more intense with LLINs or with pyrethroid-IRS (assuming that coverage and other factors are equal), this needs to be investigated urgently.
- Pyrethroids should continue to be used for IRS where the target vectors are susceptible and where LLINs are not being deployed at high levels of coverage.
- Pyrethroids should *not*, in general, be used for IRS in the presence of high LLIN/ITN coverage, since the combination of pyrethroids on the wall and on the net is expected to produce extremely intense selection pressure.

This recommendation has major implications, and was agreed by the group only after extensive discussion. At current prices, switching to an alternative IRS product would involve a substantial increase in the cost of insecticide. This has implications for the coverage that a programme with finite resources can achieve. Pyrethroids also have operational advantages. For example, most currently available alternatives have a substantially shorter duration of residual activity, so in places with perennial transmission or a long transmission season, switching may entail the need to spray more frequently (twice or even more times per year). New longer-lasting IRS formulations with non-pyrethroid ingredients are being developed, and it is hoped they will become available soon in order to relieve some of these problems.

However, the group noted that if there is high coverage with treated nets in good condition, there is less danger that the population will be left without any effective protection if there are gaps in the activity of the insecticidal deposit on the walls. Consideration was also given to the encouraging evidence from experimental hut work that, in a room with a treated net, pyrethroid-resistant insects have a strong survival advantage, but this advantage is reduced to low levels if a carbamate insecticide is also present on the wall (*23*).

- The recommendation that pyrethroids should not be used for IRS in the presence of high LLIN-coverage will often leave a very restricted list of alternative insecticides for IRS, making it difficult to combine LLINs with a rotation for IRS. If, because of some external constraints, only pyrethroids and carbamates can be used for spraying, a programme could find itself having to choose between (*i*) distributing LLINs and spraying only carbamate, or (*ii*) spraying pyrethroid and carbamate in alternate seasons, with no LLINs. It was agreed that either of these options might be chosen, depending on the circumstances. Option (*i*) has the advantage of offering a high degree of malaria control with year-round protection; moreover, since it puts a carbamate on the wall and a pyrethroid on the net, it is a combination intervention, and a good resistance management strategy in itself. The alternative (*ii*) is also good for resistance management, and may give adequate malaria control if the transmission season is not much longer than the effective life of the carbamate.

It was concluded that the policy of not spraying a pyrethroid in the presence of high LLIN coverage is justified, despite the increased costs and the operational difficulties, because it will help to preserve long-term susceptibility to pyrethroids, and in doing so will have important long-term public health benefits. On balance, these long-term benefits are considered likely to outweigh the short-term costs.

- The policy discussed above is a specific instance of a more general rationale. Most vector control programmes currently try to employ the cheapest intervention that is both feasible and effective. Any change from current practice which maintains effectiveness is therefore likely to bring an increase in costs, which, in the case of a resistance management strategy, may be substantial. The aim of such strategy is to delay the loss of insecticide susceptibility, prolonging the effective life of currently available interventions. The intended pay-off is a future public health benefit which may be large but which remains uncertain.
- Thus the more general question is: "in what conditions will the future benefits of preserving insecticide susceptibility outweigh the short-term costs of deploying more expensive interventions?" This issue is likely to recur whenever programmes are facing difficult policy decisions, since it concerns not only the fundamental justification of a resistance management strategy, but also the limits of this justification. Further analysis of this subject, which involves aspects of economics and population genetics as well as epidemiology, is urgently needed, first to create an adequate and agreed conceptual framework, and then to explore quantitative approaches.

Novel products

- There is a critical and immediate need for new insecticides suitable for public health use.
- There is a need to streamline and accelerate the process of WHOPES testing and recommendation, so that a greater choice of insecticidal interventions may become available sooner. The same applies to the process of national registration,

and in particular the requirement for fresh data from local trials as a condition for registration in each separate country. Current efforts to harmonize the national registration process should be encouraged, while recognizing that there are country-specific issues which must be accommodated in the harmonization process.

- There are prospects for a small number of new products for IRS: both new active ingredients and new formulations of established non-pyrethroid insecticides are under development. Such products are urgently needed. There is also active current interest in the development of new ways to apply insecticide to the walls of dwellings, in particular the use of insecticide-impregnated plastic sheeting (durable lining, DL). Non-pyrethroid versions of this technology are likely to be of particular interest.
- Alarmingly, the prospects for new active ingredients for use on nets are very poor. It was noted that with IRS, the active ingredient represents a relatively high proportion (perhaps 20%–50%) of total operational costs, but only a small proportion (e.g. < 5%) with LLINs. The probability that basic chemical research will find a new class of insecticidal molecule specifically suited for use on nets is highly dependent on the priority given to such research by the major chemical manufacturers. If those companies were to see good prospects for a secure and sizeable market in such a molecule or product, the probability of finding one would be greatly improved.
- It is now widely accepted that the best way to delay resistance to antimalarial drugs is to use a combination of drugs for treatment, preferably with a co-formulation of the two drugs together. For the same reasons, the development of co-formulated insecticides of different classes (mixtures) may be the most effective way to delay the evolution of insecticide resistance. There are a few precedents for such products in agriculture, but there are two obstacles to be overcome in the development of mixed insecticide products for public health. One is the need to ensure that the two insecticides decay at more or less the same rate, which is not easily achieved. The other is the issue of toxicology, and the possibility that the two active ingredients could interact in ways that are not expected from the toxicology assessments of the individual products. This problem should not be underestimated; it also needs to be re-considered, and if possible, methods to deal with it need to be developed urgently.

7. Capacity building

- A current major hindrance to implementing effective vector control and insecticide resistance monitoring is the lack of entomology expertise in most NMCPs in endemic countries.
- The recent massive expansion of malaria vector control activities has employed a fairly uniform set of interventions and delivery systems: either LLINs delivered through large-scale LLIN campaigns, or IRS using pyrethroids in most cases. Moreover, in Africa, the choice between these two interventions has often been made on the basis of programme capacity and malaria endemicity, rather than on entomological criteria. In the future, especially when a wider array of alternative products becomes available, vector control will have to become more diverse, and more contingent on local entomological data, Hence the need for greatly strengthened entomological capacity.

Basic entomological monitoring capacity, including resistance monitoring, should be established in all NMCPs or associated national technical institutions.

- All country NMCPs should train and appoint a senior entomologist and be provided with the necessary infrastructure for IR monitoring.
- Career development for public health entomologists should be improved.
- Regional training centres should be supported including follow-up mentoring.
- Subregional insecticide resistance monitoring networks should be strengthened, and new ones set up where necessary. These should provide sustained expert technical assistance to endemic countries, for example through annual planning meetings. The number of collaborating institutions with the capacity to carry out advanced insecticide resistance testing (i.e. molecular and biochemical assays and the characterization of resistance mechanisms) needs to be expanded, so that all NMCPs have access to the full range of available tests, preferably within their subregion. This will involve both current WHO Collaborating Centres and other institutions. Good laboratory facilities will be essential, and where they do not yet exist, must be established in new collaborating institutions.
- It would be helpful to create a register of institutions qualified to provide independent help and guidance on insecticide resistance testing methods. These institutions should pass these results on to agencies responsible for procurement decisions (see recommendation 4).
- NMCPs and national research institutions should collaborate on insecticide resistance monitoring.

8. Summary of conclusions and recommendations

1. Preserve vector susceptibility

The preservation of vector susceptibility is now, or will soon be, one of the most important criteria for choosing between alternative vector control methods, at least as important as simple short-term cost-effectiveness.

2. Prompt action

The introduction of a resistance management strategy should be part of the initial planning and implementation of any vector control intervention; it should not wait for evidence that resistance has appeared and it should certainly not wait for proof of control failure.

3. Best practices for immediate use

Although the evidence-base for insecticide resistance management is limited, a number of best practice measures are recommended and should be implemented immediately, while further evidence is gathered and new tools are under development. These recommendations can be summarised in a "***Do***" and "***Do not***" series.

a. General

- ***Do*** use pesticides judiciously, carefully and with discrimination.
- ***Do not*** apply pesticides indiscriminately, exposing mosquitoes to selection pressure for resistance with no epidemiological benefit to humans.

b. IRS

- ***Do*** rotate insecticides from year to year, using different insecticide classes in successive years/rounds (alternating between spray-rounds is another option).
- ***Do*** continue to use pyrethroids as one element of the rotation unless

  (i) there is high treated net coverage (see below) and/or

  (ii) significant levels of pyrethroid resistance have been detected.
- ***Do not*** use the same insecticide year after year, *especially* if resistance is known to be present.

c. Combination of IRS and LLIN

- ***Do*** prefer combination interventions where one class of insecticide is on the wall and another on the nets, and
- ***Do not*** use pyrethroids for IRS (or otherwise applied to the wall) where there is high coverage with treated nets.

4. Monitoring

Monitoring is a critical element of vector control. Just as the successful scale-up of intervention delivery systems required the determination of professional staff at all levels to overcome obstacles, so now resistance monitoring must be scaled up in the same way. In order to manage the resistance problem successfully, vector control must become less uniform and more responsive to local data.

- The 1998 WHO insecticide monitoring guidelines (*12*) must be updated. A working group was established during the meeting to undertake this work.
- Structures to ensure that resistance data are properly collected, collated and fed back into planning decisions are either weak or absent. Specific recommendations are made to strengthen those that are weak and to create those that are lacking, in particular:
    a. all agencies implementing large-scale vector control have a responsibility to ensure that adequate resistance data is collected, and
    b. donors supporting the procurement of vector control commodities should ensure that the choice of commodities has been informed by adequate resistance data.
- Bioassays are the primary tool for insecticide resistance monitoring, but they must be supplemented by molecular and biochemical assays where resistance is detected. Bioassays give information about the phenotype, molecular assays about the genotype; they are complementary and neither can be an adequate substitute for the other.
- Support is needed for subregional and regional networks that undertake resistance monitoring, including those that carry out advanced molecular techniques.

5. New products

There is a critical need for new insecticide products for malaria control.

- The processes for bringing new products to market should be streamlined and accelerated, and the processes for establishing a recommendation that a new category of product should be introduced in public health programmes must be clearly defined.

- There is a need to define criteria and systems by which products which are genuinely useful for resistance management can be distinguished, and can be given the correct degree of preference in procurement.

This need is especially urgent for products that can be used on nets, because for nets:

- no alternative to pyrethroids is available or in the pipeline
- market incentives to develop such a product are presently not clear, and
- it is unlikely that "universal coverage with effective vector control" will be feasible throughout Africa using other methods of vector control.

New products for resistance management are especially urgently needed. Standardized methods will be needed to measure and compare the value of new products for this purpose. This includes the development of mixtures (co-formulated combinations of insecticides) which would have great advantages, if the associated practical and regulatory obstacles can be overcome.

6. Capacity building

Lack of entomological capacity in control programmes is currently a major hindrance to effective insecticide resistance monitoring and malaria control.

a. All NMCPs should establish capacity for basic entomological monitoring, appoint senior entomologists or train entomologists where necessary, and establish the necessary infrastructure for basic entomological surveillance including insecticide resistance monitoring. A comprehensive package of that capacity should be well defined.

b. Funding applications for vector control must include in the budget a sufficient amount to establish the necessary capacity.

7. Insecticides in agriculture and public health

The agricultural sector should strive to use classes of insecticide that are not available for use in public health and engage with local vector control officials to develop an IRM strategy between sectors.

Annex 1. List of participants

The technical basis for coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control

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Declaration of interests

All participants at the meeting completed a Declaration of Interests. One participant declared an interest: Dr Mark Hoppé declared an interest as employee and shareholder in Syngenta Crop Protection AG.

Annex 2

Research priorities

1. Development of products which use a combination or mixture of two or more insecticide classes, including ITN and IRS.
2. Development of existing and novel synergists to be used to enhance currently available insecticides.
3. Development of bednets with high durability to prevent loss of effectiveness through damage.
4. Assessment of the potential role of interventions other than conventional IRS and LLINs in moderating insecticide selection pressure in some circumstances.
5. Development of non-pyrethroid LLIN products.
6. Studies to identify the major sources of selection for insecticide resistance in malaria-endemic countries, and the contributions of agriculture compared to scaled-up vector control.
7. Development of appropriate methods, other than simply measuring large-scale and long-term gene frequency change, for measuring the strength of selection for resistance imposed by an intervention; and use of these methods to compare the strength of selection associated with LLINs and pyrethroid-IRS in a range of situations.
8. Measurement of the operational impact of insecticide resistance on ITN and IRS outcomes both in experimental huts and in malaria control programmes.
9. Measurement of the extent of cross-resistance between different pyrethroids with greater precision.

Annex 3

A. Advantages and disadvantages of using F1 progeny testing versus wild caught females for bioassays

Vector sample	Advantage	Disadvantage
F1 progeny	Age of vectors can be kept constant between tests allowing results from different times and places to be compared. In areas with low mosquito density, can be used even if it is not possible to catch sufficient adult wild female mosquitoes.	Requires better entomological facilities, which limits where the tests can be carried out. Environmental conditions will differ from those within the insectary. Since many eggs may be derived from just a few adult females, the number of genomes sampled from the wild population is likely to be less than the number if insects tested.
Wild-caught females	Fewer facilities are required, so can be carried out in a greater number of locations. Changes in susceptibility will more closely reflect the changes in intervention efficacy seen in the field. The age distribution of the vectors should be representative of the wild vector population at a given time and location.	Age distribution of vectors will vary between samples reducing the comparability of results.

B. Database on insecticide resistance: IRbase

- IRbase is an open access database which is operational on the VectorBase web site: http://www.vectorbase.org/Help/Introduction_to_IRbase. It contains data from both published and unpublished surveys on insecticide resistance worldwide, and has a mapping facility.
- IRbase requires increased visibility and entry of data in order to maintain its utility for vector control programmes and insecticide resistance research.

Published data up to 2009 has already been entered, but there are large quantities of unpublished data which could be gathered and entered.

- IRbase is a valuable resource and therefore it is recommended that funding should be arranged for one or more curator positions. The role of these individuals would be to request data from control agencies and researchers, to enter and apply quality control to the data, to publicise IRbase in particular to control programme managers for its utility in decision making, and to continue to develop the website and database to improve the user-friendly format.

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Figures 1(a) and 1(b):

Collated data on pyrethroid resistance in African malaria vectors (1a) from susceptibility tests and (1b) presence of resistance alleles and/or biochemical mechanisms. Figures adapted from Ranson et al. (*3*).

Figure 1(a)

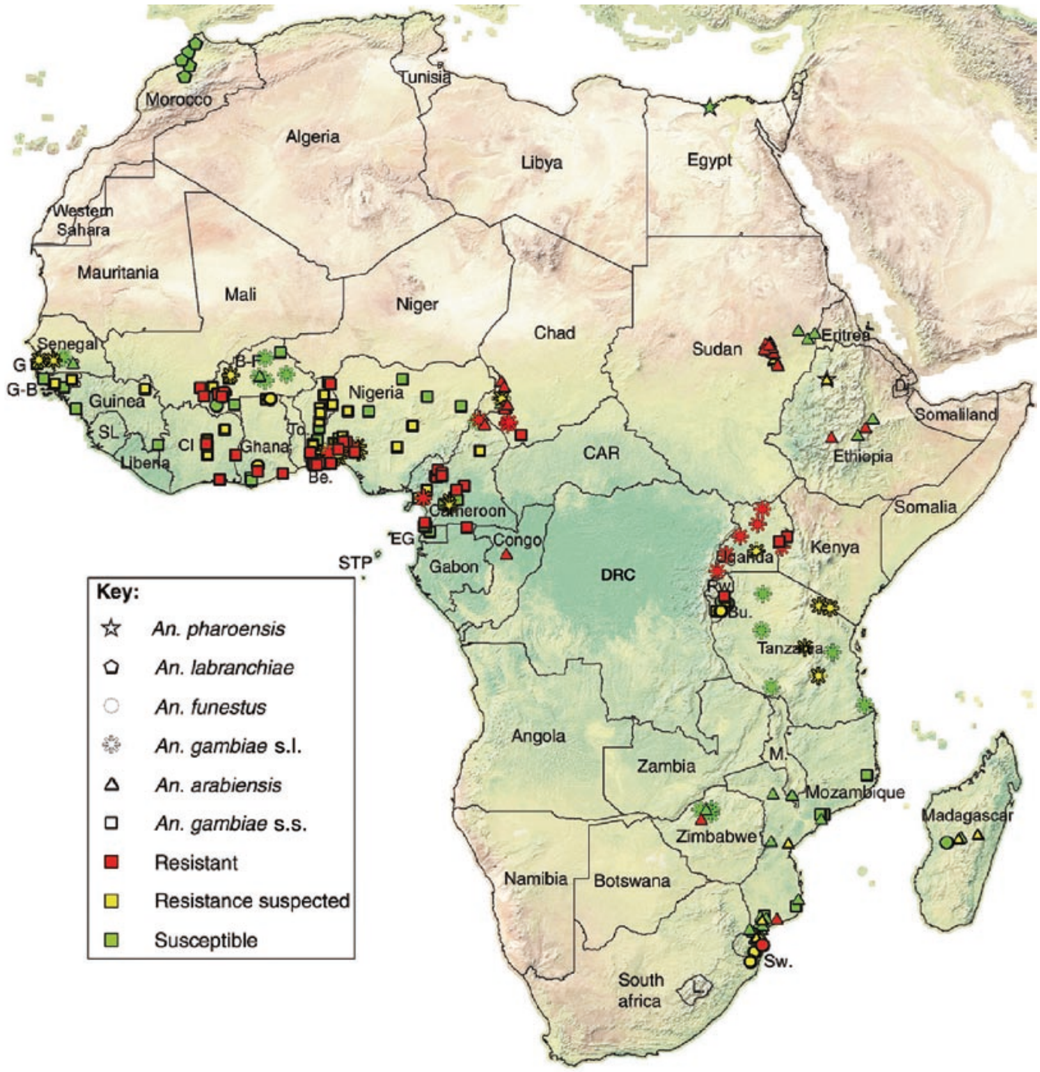
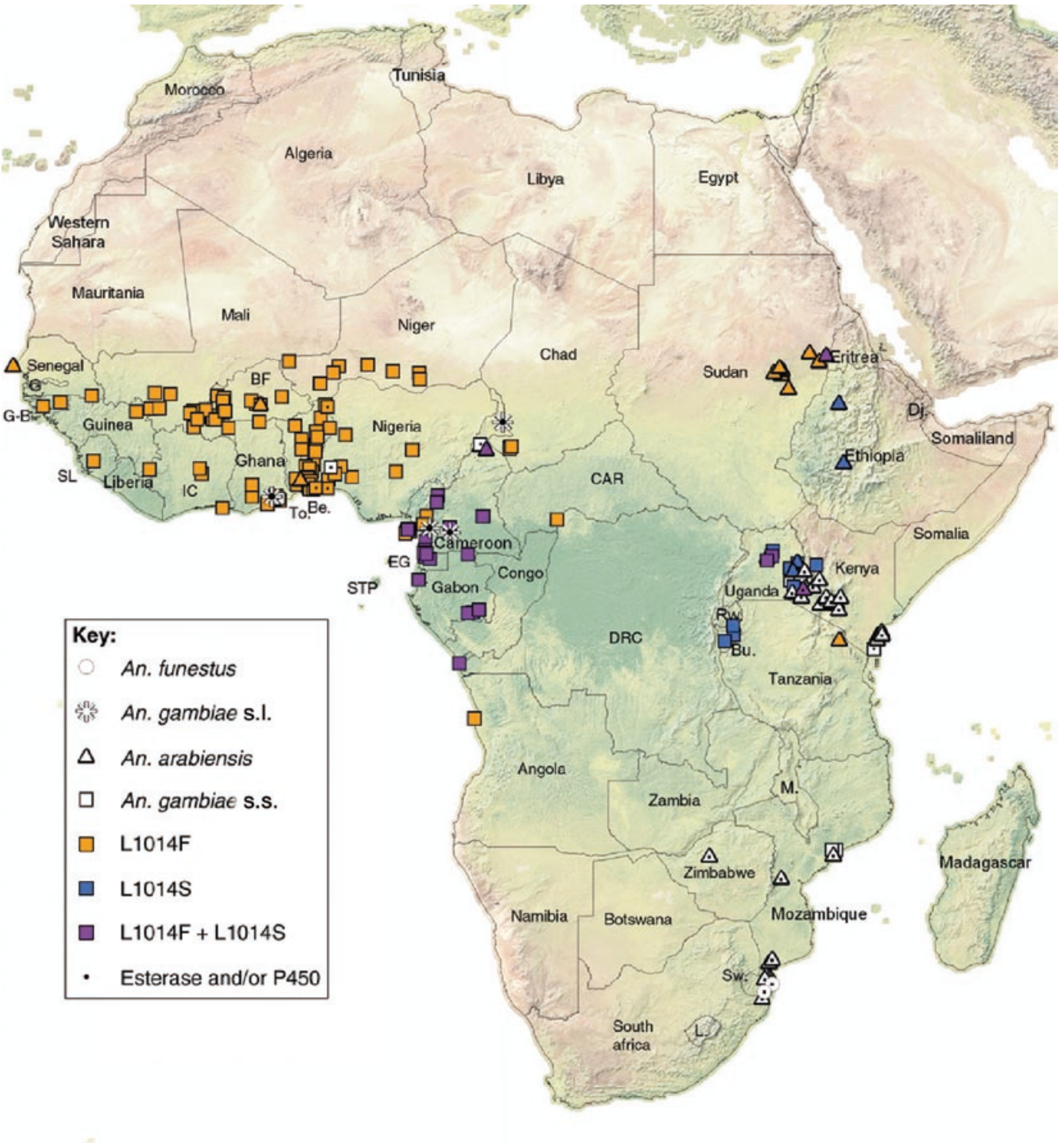


Figure 1(b)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Morocco
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Niger
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Dj.
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Somalia
Cameroon
EG
Congo
Kenya
STP
Gabon
Uganda
Rw.
DRC
Bu.
Tanzania
Angola
Zambia
M.
Zimbabwe
Madagascar
Namibia
Botswana
Mozambique
Sw.
South africa
L.
Key:
An. funestus
An. gambiae s.l.
An. arabiensis
An. gambiae s.s.
L1014F
L1014S
L1014F + L1014S
Esterase and/or P450

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