Global **Malaria** Programme

Global report on insecticide resistance in malaria vectors: 2010–2016

World Health Organization

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ABBREVIATIONS AND ACRONYMS

Ace-1R	insensitive acetylcholinesterase
CDC	US Centers for Disease Control and Prevention
DDT	dichlorodiphenyltrichloroethane
GPIRM	Global plan for insecticide resistance management in malaria vectors
GST	glutathione-S-transferase
IQR	interquartile range
IRM	insecticide resistance management
IRS	indoor residual spraying
ITN	insecticide-treated mosquito net
kdr	knockdown resistance
LLIN	long-lasting insecticidal net
NMCP	national malaria control programme
PBO	piperonyl butoxide
PMI	US President's Malaria Initiative
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

2010–2016 AT A GLANCE

Resistance monitoring

Monitoring for vector resistance to insecticides commonly used in malaria control was not conducted or was not reported for all malaria endemic countries between 2010 and 2016. For those that did monitor, this was usually not done every year. Limited information was available on resistance intensity and resistance mechanisms.

- Number of countries (of 91 endemic) that reported any monitoring data: 79
  - for resistance frequency/status:[1] 72
  - for resistance intensity:[1] 10
  - for resistance mechanisms:[2] 32

Resistance status

Pyrethroid resistance was common and widespread in major malaria vectors across the five WHO regions that had ongoing malaria transmission in 2016.

- Number of countries (of 72 monitored between 2010 and 2016) for which resistance was confirmed in at least one vector to at least one pyrethroid insecticide of those that conducted monitoring: 56 (77%).
- Number of sites (of 2145 monitored between 2010 and 2016) at which pyrethroid resistance was confirmed in at least one vector to at least one pyrethroid insecticide of those monitored: 1375 (64%).

Resistance to the three other insecticide classes commonly used in IRS was confirmed in major malaria vectors across the five WHO regions that had ongoing malaria transmission in 2016, with countries commonly reporting resistance to multiple classes.

- Number of countries (of 72 monitored between 2010 and 2016) for which resistance was confirmed in at least one vector species to a total of:
  - no insecticide classes:[3] 10
  - one insecticide class: 12
  - two insecticide classes: 13
  - three insecticide classes: 19
  - four insecticide classes: 18

Resistance frequency

Pyrethroid resistance frequency (as indicated by median mosquito survival in bioassays) increased between 2010 and 2016.

- Pyrethroid resistance frequency increased:
  - significantly in *An. funestus s.l.*: 32% increase, from 26% to 58%
  - moderately in *An. gambiae s.l.*: 13% increase, from 21% to 34%
  - slightly in other malaria vectors: 5% increase, from 10% to 15%

There were small overall median changes (≤5%) in resistance frequencies to organochlorines, organophosphates and carbamates between 2010 and 2016, with some examples of significant increases (e.g. DDT resistance frequency in *An. funestus* increased by 41%).

Resistance mechanisms

Metabolic and target-site resistance mechanisms were detected across multiple vector species and WHO regions between 2010 and 2016. However, insufficient testing and reporting precluded comprehensive analyses for this report.

- Number of countries (of 23 monitored between 2010 and 2016) in which metabolic mechanisms were detected for at least one major malaria vector: 22
- Number of countries (of 30 monitored between 2010 and 2016) in which target-site mechanisms were detected in at least one major malaria vector: 27

1 By standard intensity concentration bioassays.
2 By synergist-insecticide, biochemical or molecular assays.
3 Susceptibility or possible resistance detected only, for pyrethroids, DDT, carbamates and organophosphates

EXECUTIVE SUMMARY

Insecticide-based vector control is a cornerstone in the fight against malaria. Selection of vector-control interventions should take into account the resistance status of local mosquito vectors along with other factors associated with intervention deployment and use such as availability, cost and cost -effectiveness as well as population acceptance or compliance. Strategic insecticide resistance monitoring is therefore essential to inform evidence-based vector control.

In 2012, WHO released the *Global plan for insecticide resistance management in malaria vectors* (GPIRM) (WHO, 2012). Among other priorities, GPIRM highlighted the need for strengthened resistance monitoring and better management of data, including the establishment of a global database. This database was initiated in 2014 by the WHO Global Malaria Programme; as of October 2017 it comprised almost 30 000 records, including results from bioassays to measure phenotypic resistance, and biochemical and molecular tests for resistance mechanisms. Data have been provided for 83 countries across all WHO regions, with 68% of reports for mosquitoes having been collected from 2010 onwards.

The current report presents an overview of standard information on insecticide resistance from the WHO database for malaria vectors collated for 79 countries between 2010 and 2016. The aim is to provide the baseline for subsequent status updates, and to identify any temporal trends in resistance. An online mapping tool called *Malaria threats map* was developed in 2017 to allow further interactive exploration of available data (WHO, 2017b).

Sparse information is currently available for certain vector species, insecticides and geographical areas, with relatively few data on resistance intensity and mechanisms. Major data gaps restrict our understanding of the extent of resistance, particularly outside Africa. Strengthened resistance monitoring or data reporting are clearly required. Despite this limitation, it is apparent that, across all WHO regions with ongoing malaria transmission, malaria vectors have developed resistance to the four main classes of insecticides commonly used in adult malaria vector control (WHO, 2017d). Pyrethroid (and dichlorodiphenyltrichloroethane, DDT) resistance was confirmed in malaria vectors from at least half of the sites tested worldwide, and was most common in countries of the WHO African and Eastern Mediterranean regions. Resistance to two other insecticide classes (organophosphates and carbamates) was most common in the WHO regions of South-East Asia, Western Pacific and Eastern Mediterranean, and was relatively uncommon in the WHO African Region and Region of the Americas.

Trends analyses indicated that the frequency of pyrethroid resistance in malaria vectors increased globally between 2010 and 2016. The increase was greatest in *Anopheles funestus s.l.* (32%), was moderate in *An. gambiae s.l.* (13%) and was relatively small in other malaria vectors (5%). There was limited evidence of an increase in resistance to the three other insecticide classes commonly used in IRS, with a few exceptions such as a significant increase in frequency of resistance to DDT of *An. funestus s.l.* (41%). Although the true impact of insecticide resistance on the effectiveness of insecticidal vector control is not yet known, this highlights a potential challenge to control and elimination, particularly in Africa where the malaria burden remains highest.

Insecticide resistance monitoring and management strategies require development and implementation. These should aim to maintain the effectiveness of current malaria vector-control interventions and to integrate proven new tools once available. The ultimate objective is to prevent and reduce malaria burden, while pursuing the targets set in the *Global technical strategy for malaria 2016–2030* (WHO, 2015) of reducing global malaria incidence and mortality rates by at least 90% by the year 2030, eliminating malaria from at least 35 countries, and preventing its re-establishment in malaria free countries.

1. INTRODUCTION

Vector control is a key preventive strategy for malaria. Effective malaria vector control relies heavily on two core insecticidal interventions: deployment of insecticide-treated mosquito nets (ITNs) – mainly long-lasting insecticidal nets (LLINs) treated with a synthetic pyrethroid – and indoor residual spraying (IRS) of insecticides. Significant reductions in malaria morbidity and mortality since 2000 have mainly been due to the widespread implementation of these two insecticidal interventions.

Resistance to the four insecticide classes commonly used in these interventions has emerged in malaria vector populations throughout the world. Of particular concern is pyrethroid resistance, because this insecticide class is used in all WHO-recommended LLINs and is also used for IRS in many countries. Although it is still unclear to what extent insecticide resistance impacts on the effectiveness of current malaria vector-control tools, the emergence and spread of resistance is clearly a major threat to the significant gains made against malaria in recent years.

The WHO *Global plan for insecticide resistance management in malaria vectors* (GPIRM) was launched in 2012 to provide a comprehensive approach to addressing this biological threat to malaria control and elimination (WHO, 2012). Among other actions, GPIRM identified a key need to establish a database to track the status of insecticide resistance in malaria vectors. In 2014, the WHO Global Malaria Programme started a global insecticide resistance database to consolidate the data reported by Member States and their development partners, and data extracted from scientific publications.

This report summarizes available data on insecticide resistance in malaria vectors and the key outcomes from analyses of these data. The scope is limited to data for malaria vectors collected between 2010 and 2016, with a focus on outcomes from standard assessments, in line with WHO's *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes, 2nd edition* (WHO, 2016b). More recent data can be accessed through the online *Malaria threats map* (WHO, 2017b), which allows the interactive exploration of available information from the WHO insecticide resistance database and from other databases maintained by the Global Malaria Programme.

This report summarizes recent data on malaria vector insecticide resistance. The aim is to encourage further insecticide resistance testing to continue building an evidence-base on which to provide informed communications, advocacy and policy development for malaria control and elimination. The report may also prove useful to programmes and their development partners in devising or refining strategies, although more in-depth situation analyses at national and subnational levels will clearly be required. The intention of the first edition of this report is to provide a baseline for regular updates on the global situation of malaria vector resistance to insecticides used in public health.

In preparation for subsequent editions, further detailed analyses are ongoing. These will address strategic questions related to malaria vector resistance monitoring and management.

2. BACKGROUND

Insecticide resistance is the ability of insects to survive exposure to a standard dose of insecticide, owing to physiological or behavioural adaptation (WHO, 2016b). Resistance in malaria vectors can be considered in three different ways (WHO, 2012):

- *phenotypic resistance* – the basic expression of the genetic cause or causes of resistance, as shown by a vector's ability to resist and survive the effects of an insecticide;
- *resistance mechanisms* – the underlying genes that confer the inherited trait of resistance; and
- *resistance impacting on intervention effectiveness* – the epidemiological evidence upon which physiological resistance is established as the cause of increasing malaria transmission.

A brief overview of each of these resistance outcomes and how they are measured is provided below.

2.1 Types of resistance and their measurement

Phenotypic resistance

Insecticide resistance monitoring for malaria vectors focuses on assessing the ability of *Anopheles* populations to withstand exposure to an insecticide. Monitoring should be conducted regularly and in representative sites, to assess the situation and adapt vector-control strategies, as appropriate. Control programmes should perform insecticide susceptibility tests on all local major malaria vector species at least once per year, using the insecticides already in use and those planned for use in malaria vector control.

The standard approach to measuring phenotypic resistance is to use bioassays that test the vector's ability to survive exposure to a given insecticide under set conditions. The two main methods used are the insecticide susceptibility test from WHO and the bottle bioassay from the United States (US) Centers for Disease Control and Prevention (CDC). Both methods are well established and have been widely used for a number of years. In brief, a given number of mosquitoes are exposed to a standardized concentration of insecticide (known as the discriminating concentration or diagnostic dose) for a set exposure time. Results for both methods are expressed as percentage mortality (or incapacitation) of the test mosquitoes. This outcome is used as an indicator of resistance status, with test populations classified as susceptible (≥98% mortality), possible resistance (90–97% mortality) or confirmed resistance (<90% mortality). The results are also used to calculate a resistance frequency, indicated by the proportion of the test population that survives insecticide exposure (100% minus % mortality or incapacitation) (Table 2.1).

Outcomes from the WHO susceptibility tests and CDC bottle bioassays are not directly comparable because they use different protocols and end-points. The WHO test protocol records the proportion of dead mosquitoes after 24 hours, whereas the CDC assay registers the proportion incapacitated (i.e. knocked down) after the given diagnostic time. However, resistance status outcomes have been aligned for the two datasets for the purpose of the analyses conducted in this report. Further details on the procedures are included in the WHO test procedures document (WHO, 2016b).

Bioassay methods to determine resistance status and frequency can also be used to determine resistance intensity. The percentage mortality of test mosquitoes is measured following exposure to 1×, 5× or 10× the discriminating concentration. Mosquito mortality (or incapacitation) outcomes for each concentration can indicate low, moderate (or moderate-to-high) and high resistance intensity. Data on resistance intensity are increasingly being collected, in line with the recent revisions to the WHO test procedures document (WHO, 2016b). In addition, some research chose to calculate a resistance ratio by exposing test mosquitoes to a range of insecticide concentrations and calculating the concentration required to kill (or incapacitate) 50% or 95% of those tested with the ratio determined in relation to a reference susceptible strain. However, this measure is not usually assessed in routine monitoring and is not included in this report.

TABLE 2.1.
Overview of common phenotypic resistance indicators, methods, measures and outcomes

INDICATOR	METHODS	MEASURES	OUTCOMES
Resistance status	WHO susceptibility test with discriminating concentration	% mortality of test mosquitoes (adjusted[a])	• Confirmed resistance • Possible resistance • Susceptibility
	CDC bottle bioassay with diagnostic concentration	% incapacitation of test mosquitoes	• Confirmed resistance • Possible resistance • Susceptibility
Resistance frequency[b]	WHO susceptibility test with discriminating concentration	100% minus % mortality of test mosquitoes (adjusted[a])	• % alive
	CDC bottle bioassay with diagnostic concentration	100% minus % incapacitation of test mosquitoes (adjusted[a])	• % not incapacitated.
Resistance intensity	WHO susceptibility test with intensity concentrations	% mortality of test mosquitoes (adjusted[a]), in relation to % mortality for other concentrations tested	• High intensity • Moderate intensity • Low intensity • Could not be reliably assessed
	CDC bottle bioassay with intensity concentrations	% incapacitation of test mosquitoes (adjusted[a]), in relation to % incapacitation for other concentrations tested	• High intensity • Moderate intensity • Low intensity • Could not be reliably assessed

CDC, US Centers for Disease Control and Prevention; WHO, World Health Organization
[a] Using Abbott's formula as required (Abbott, 1925).
[b] This refers to phenotypic resistance only and is different to resistance gene frequency (see Table 2.2)

Cross-resistance has also been observed, in which resistance to one insecticide confers resistance to another, even if the insect has not been exposed to the second insecticide. To gain a deeper knowledge of resistance mechanisms and cross-resistance, mosquito populations that have been identified as resistant to insecticides can be subjected to further testing (see Section 2.2). This may include further processing of those mosquitoes that survived bioassays, or further assessments of different individuals from a single mosquito population.

Resistance mechanisms

Insecticide resistance mechanisms are the means by which insects overcome exposure to an insecticide. These mechanisms reduce susceptibility of the insect to the lethal effects of the chemical (or chemicals), and thereby allow their survival. Various types of resistance have been observed to date, and can be broadly categorized as metabolic, target-site, cuticular (reduced penetration) or behavioural resistance (WHO, 2012). These mechanisms can confer resistance to one or more classes of insecticides; where a mechanism confers resistance to more than one class of insecticide this is termed "cross-resistance".

In some malaria vector populations more than one resistance mechanism has evolved. This response may have been triggered by exposure to one or more insecticide classes, rendering the vector population resistant to these insecticide classes as well as to other classes they have not been exposed to. Mechanisms underpinning resistance can be tested for using bioassays, biochemical assays and molecular techniques. These tests are used to ascertain the presence or absence or frequency of resistance alleles, determine whether metabolic enzymes are upregulated, or provide an indication of the likely involvement of mechanisms in conferring observed phenotypic resistance (Table 2.2).

Metabolic resistance

Metabolic resistance is a common mechanism type that confers resistance in malaria vectors. It occurs when internal enzymes in mosquitoes break down or sequester insecticide molecules before they can have a toxic effect. Insect strains that have developed higher amounts or more efficient forms of these enzymes that can metabolize insecticides may exhibit phenotypic resistance. Three families of metabolic enzymes are strongly associated with resistance in malaria vectors: monooxygenases (P450s), esterases and glutathione-S-transferases (GSTs). For instance, increased expression of multiple monooxygenases has been associated with pyrethroid resistance (Edi et al., 2014; Wondji et al., 2012), esterase-mediated resistance has been shown to reduce susceptibility of malaria vectors to both organophosphates and pyrethroids (Brogdon et al., 1999; Vulule et al., 1999) and increased expression of GSTs has been associated with dichlorodiphenyltrichloroethane (DDT) resistance (Ranson et al., 2001; Riveron et al., 2014). These enzyme systems may also have a broad spectrum of activity and be capable of detoxifying a range of insecticides (Table 2.2).

Metabolic mechanisms are commonly detected using biochemical and molecular assays with dead mosquitoes, or bioassays with live mosquitoes. In bioassays, mosquitoes from a resistant population are exposed to an insecticide only or to a synergist,[1] and then an insecticide using an adaptation of the WHO susceptibility or CDC bottle bioassay method. If a higher mortality is observed for mosquitoes exposed to the synergist and insecticide than for those exposed to the insecticide alone, this is considered a proxy for the involvement in resistance of the metabolic enzymes targeted by the specific synergist. For example, piperonyl butoxide (PBO) affects monooxygenase activity; therefore, if mosquito mortality is higher with pre-exposure to PBO than without exposure, this serves as a proxy indication of the involvement of monooxygenases in resistance. However, there may be additional effects with other forms of resistance (e.g. knockdown resistance, *kdr*); hence, this should not be considered a definitive indicator of metabolic mechanisms only. Synergists for the other important detoxification enzyme groups are available, but these compounds are still being validated for their use in assays (WHO, 2016b). Molecular and biochemical assays are more resource intensive than bioassays and tend to require more

1 A synergist is a chemical that acts by inhibiting certain metabolic enzymes (e.g. mixed-function oxidases) within the mosquito that detoxify or sequester insecticides before they can have a toxic effect on the mosquito.

developed facilities. Therefore, these assays are rarely conducted on a routine basis and data are not as readily available (WHO, 2016b).

Target-site resistance

Target-site resistance occurs when a genetic mutation has modified the protein receptor within the mosquito that an insecticide is supposed to attack, which effectively blocks or reduces the toxic effect of the insecticide. For example, the main target sites for pyrethroids and organochlorines are voltage-gated sodium channels of nerve cell membranes. A *kdr* mutation reduces sensitivity of the channels to the binding of these insecticide classes. Similarly, mutations in the gene for acetylcholinesterase (called *Ace-1R* – insensitive acetylcholinesterase) confer resistance to organophosphates and carbamates. Molecular assays are generally used to measure the frequency of *kdr* mutations, and molecular or biochemical assays are used to measure the frequency of *Ace-1R* mutations or to measure the activity of acetylcholinesterases.

Monitoring of gene frequency can add valuable information during resistance monitoring. Initially, a resistance gene will be rare in a population, introduced through random mutation, and will be hard to detect as it requires large sample sizes. However, there is evidence that if resistance genes reach high frequencies these costs may decline such that resistance is sustained in the population independently of selection pressure from insecticide exposure. Individuals with the resistance gene will have a selective advantage in the presence of the insecticide, and these genes will become increasingly common in the population while it is under selection pressure. The initial rarity of the gene means that, even if the frequency is increasing, it could take a long time to reach detectable levels. However, once detectable levels are reached, the transition – for example, from 1% to 100% – could happen within a few generations and thus be relatively quick. Resistance genes are generally thought to have an associated fitness cost;[2] that is, in the absence of the insecticide, they will no longer be favourably selected and frequencies will decrease. However, this is no longer true if the resistance gene reaches high frequencies and is sustained in the population independently of the selection pressure from the insecticide. Monitoring of resistance gene frequencies may guide mitigating measures to circumvent fixation of a resistance gene in a vector population; however, this requires advanced laboratory capacity.

Cuticular resistance

Cuticular resistance (or reduced penetration) occurs when the absorption of insecticide into a mosquito is reduced because of changes in the insect's outer cuticle, the hard outer covering layer composed of epidermis. This type of resistance can reduce the efficacy of various insecticides, and often occurs in the presence of other resistance mechanisms. Presence of this mechanism is identified by examination of physical aspects of individual mosquitoes (e.g. cuticular thickness in relation to susceptible mosquitoes); however, standard methods and reporting have not yet been established.

Behavioural resistance

Behavioural resistance is defined by a change in mosquito activity, such as avoidance of insecticide-treated surfaces or changes in feeding or resting patterns in response to the presence of insecticide. Relatively little is known about the extent and impact of this type of resistance because no standard methods to detect and report such changes have been established, and it can be difficult to measure because longitudinal observations are required.

2 This is not the case for some populations of *An. funestus* from southern Africa (M. Coetzee, University of Witwatersrand, personal communication).

TABLE 2.2.
Overview of common metabolic and target-site resistance mechanism indicators, methods, measures and outcomes

INDICATOR	METHODS	MEASURES	OUTCOMES
Metabolic resistance	WHO synergist-insecticide bioassays	% mortality of test mosquitoes (adjusted[a]) when exposed to synergist and insecticide compared with % mortality when exposed to insecticide only	• Full involvement • Partial involvement • No involvement • Could not be reliably assessed
	CDC bottle synergist-insecticide bioassays	% incapacitation of test mosquitoes (adjusted[a]) when exposed to synergist and insecticide compared with % incapacitation when exposed to insecticide only	• Full involvement • Partial involvement • No involvement • Could not be reliably assessed
	Molecular assays	Upregulation of gene expression[b]	• Present • Absent
	Biochemical assays	Enzymatic activity, in relation to susceptible mosquitoes	• Present (upregulated) • Absent (not upregulated).
Target-site resistance	Molecular assays	% allelic frequency	• Present • Absent
	Biochemical assays	Enzymatic activity, in relation to susceptible population or % allelic frequency (or both)	• Present (upregulated) • Absent (not upregulated)

CDC, US Centers for Disease Control and Prevention; WHO, World Health Organization
[a] Using Abbott's formula as required (Abbott, 1925).
[b] Molecular assays that measure allelic frequencies are also available but are not commonly used.

Resistance impacting on intervention effectiveness

Insecticide resistance in a vector population can reduce the effectiveness of insecticide-based control measures. In its extreme form, insecticide resistance (along with other factors related to the ecology and behaviour of vectors) may lead to control failure, which is the point at which an intervention has no effect on disease transmission. The most quoted example of resistance causing programme failure is in KwaZulu-Natal, South Africa. In 1996, a policy change was made to switch from DDT to pyrethroids for IRS. By 2000, the number of reported malaria cases had quadrupled. *An. funestus*, a vector that had been eliminated by DDT spraying, had reappeared because it was susceptible to DDT but resistant to pyrethroids (Hargreaves et al., 2000). The entomological data were analysed in conjunction with epidemiological data because drug resistance appeared at the same time (Barnes et al., 2005). Based on this analysis, DDT was reintroduced in 2000 for vector control and the number of malaria cases fell. More recently, a study in Sudan found that pyrethroid resistance in *An. arabiensis* may have had an impact on the effectiveness of pyrethroid IRS when used in conjunction with pyrethroid LLINs, based on observations of additional protection when switching to bendiocarb IRS (Kafy et al., 2017). This finding was similar to earlier observations with *An. gambiae* from a retrospective study in Uganda that showed that IRS was the most beneficial after switching from a DDT/pyrethroid to a carbamate insecticide formulation (Kigozi et al., 2012).

For ITNs, control failure as reported in Senegal was attributed to insecticide resistance (Trape et al., 2011). However, the lack of longitudinal resistance data made it difficult

to validate this conclusion. A WHO-coordinated five-country evaluation in areas with pyrethroid-resistant malaria vectors did not find an association between malaria disease burden and resistance frequency, and showed that LLINs provided personal protection against malaria (WHO, 2016a). This study had a number of limitations making it difficult to extrapolate the findings; it was conducted in areas where resistance was on average of moderate frequency and it provided estimates of personal but not community protection provided by LLINs. In addition, it has not been possible to compare the current relative protective effectiveness of ITNs with results from studies conducted before the emergence of resistance because of the variety of study designs and settings.

Potential for reduced efficacy of an intervention due to resistance is governed by the type and frequency of resistance mechanisms, and by the extent and intensity of resistance to the insecticides used in interventions. Although target-site resistance is widespread, particularly through *kdr* mutations, there has so far been no clear association with operational effectiveness of insecticidal interventions.[3] Metabolic resistance alone – particularly through monooxygenases – may be sufficient to lead to control failure; this was likely the case in South Africa with *An. funestus* (Hargreaves et al., 2000). Vectors with several mechanism types (e.g. target-site and metabolic) are more likely to exhibit resistance that can affect control. The occurrence of *kdr* and metabolic resistance genes has been reported in multiple pyrethroid-resistant *An. gambiae* populations; for example, in Benin, Ghana and Zambia (Hardstone, Leichter & Scott, 2009; Ranson & Lissenden, 2016; Thomsen et al., 2014).

Despite the lack of conclusive confirmation of the impact of resistance on the effectiveness of insecticide-based malaria vector control, it is considered inevitable that resistance will diminish the effectiveness of vector control. Development of new insecticides and other vector-control methods is therefore strongly encouraged, to provide options for insecticide resistance management (IRM). Meanwhile, programmes should develop monitoring and management plans for insecticide resistance that incorporate the latest WHO-recommended vector-control interventions, and should implement these proactively (WHO, 2017a; c).

2.2 Management of insecticide resistance

To minimize and mitigate the risk of insecticide resistance affecting malaria prevention and control efforts, a pragmatic approach must be taken that leverages appropriate tools on the basis of available evidence. Up-to-date monitoring information is therefore required to feed into the decision-making processes and adjust plans as required. Research and development is also needed to develop new interventions, such as those that use new insecticide classes or reduce reliance on insecticides.

Insecticidal interventions must be used carefully to ensure that effectiveness is preserved for as long as possible, to maintain the optimal impact of investments in vector control. Approaches have been developed largely based on experience with agricultural pest management. These approaches aim to limit or delay the emergence of resistance by removing selection pressure or by killing resistant mosquitoes, such as by exposing them to multiple insecticides. These form an important part of IRM, and include use of mixtures of insecticides, mosaic spraying, rotations of insecticides and deployment of multiple interventions in combination. Each approach is explained in

3 Recent evaluations have identified a strong association between both *kdr* mutations and predicted mean resistance to DDT, with a somewhat weaker association with deltamethrin, permethrin and lambda-cyhalothrin resistance, indicating that *kdr* allelic frequencies may be predictive of phenotypic resistance for these insecticides (C. Moyes and P. Hancock, University of Oxford, personal communication).

more detail below. IRM should be guided by insecticide resistance data (see Table 2.1 and Table 2.2), to tailor the strategy to the locality.

For public health vector control, there is still little evidence and no consensus on the best resistance management approaches to apply in a given situation. A 2013 review of experimental and modelling studies on insecticide, pesticide and drug resistance concluded that mixtures generally lead to the slowest evolution of resistance (REX Consortium, 2013). However, more recently, an exploration of overlaps between agriculture and public health found that – owing to caveats and case specificity – there is only weak evidence for one insecticide resistance management approach being better than another; hence, more research is needed to test interventions in the field (Sternberg & Thomas, 2017). There is a need to improve understanding of the biological mechanisms that are likely to favour different approaches in different situations (Huijben & Paaijmans, 2017; South & Hastings, 2018).

Resistance management approaches

Sequences

Sequences are the usual approach to addressing insecticide resistance. They involve first using an intervention with a single insecticide until that intervention has reduced effectiveness and stops providing sufficient protection against malaria, then switching to an intervention with an insecticide of a different mode of action (e.g. one to which there is a lower frequency or intensity of resistance). There may be an option to eventually switch back to the original insecticide if the frequency of resistance to that insecticide declines enough.

Mixtures

Mixtures are formulations that combine two or more insecticides with different modes of action. The theory is that the presence of resistance in a population should be rare, such that any individual that survives exposure to one insecticide is highly likely to be killed by the other insecticide or insecticides. Recent modelling work suggests that mixtures will be beneficial to resistance management only if at least one of the insecticides is highly effective at killing susceptible genotypes (South & Hastings, 2018). Ideally, all insecticides in a mixture should have a similar residual life and remain bioavailable over time; however, in practice this can be difficult to achieve. There are currently no WHO-recommended IRS mixture formulations, although some are under development and evaluation. An LLIN product that contains a pyrethroid and a pyrrole compound[4] has received the status of a WHO interim recommendation after completion of WHO Pesticide Evaluation Scheme (WHOPES) Phase I and II trials (WHO, 2017d) and has been requested to generate data on epidemiological impact to allow assessment of public health value (WHO, 2017g), and an LLIN with a pyrethroid and a juvenile hormone mimic[5] is under development.

Mosaics

Mosaics involve the use of insecticides of different classes in neighbouring geographical areas. The optimal spatial scale (size of areas) for mosaics has yet to be determined, but trials in Mexico provide some evidence that this is an effective method for managing resistance (IRAC, 2011). However, mosaics can be operationally challenging to implement.

4 A pyrrole is a broad-spectrum insecticide that acts on the stomach and through contact.
5 A juvenile hormone mimic can inhibit development of adult characteristics or can interrupt reproductive maturation in adult insects.

Rotations

Five insecticide classes with three modes of action are now available for use in IRS against adult malaria vectors (WHO, 2017d). Rotations involve switching between insecticides with different modes of action at pre-set time intervals, irrespective of resistance frequencies. The theory is that resistance frequencies will decline (or at least will not increase) during the period of non-use of insecticides of a specific mode of action. This approach has had some success in slowing the evolution of resistance in agriculture. However, although this approach is currently considered best practice for resistance management where IRS is used (IRAC, 2011), there is only weak evidence of its impact on resistance (Sternberg & Thomas, 2017).

Combinations

Combinations expose the vector population to two classes of insecticides with differing modes of action through the co-deployment of different interventions in the same place; for example, pyrethroid LLINs combined with a nonpyrethroid IRS (WHO, 2014).

Cross-resistance

The possibility of cross-resistance needs to be considered when managing insecticide resistance through the approaches discussed above. Use of insecticides to which there is cross-resistance in local malaria vectors – whether in a sequence, mixture, mosaic, rotation or combination – is likely to lead to poorer public health outcomes and increased resistance frequencies. General patterns of cross-resistance have been established for the four insecticide classes in common use and for five resistance mechanism types (Fig. 2.1). However, the specifics of metabolic resistance are not yet fully known.

FIG. 2.1.
Cross-resistance patterns of different classes of insecticide

Size of dot indicates anticipated relative importance of the mechanism type in conferring resistance to the specified insecticide class.

	BIOCHEMICAL MECHANISM OF RESISTANCE				
	Metabolic			Target-site	
	Esterases	Mono-oxygenases	GSH S-transferases	*kdr*	Altered AChE
Pyrethroids	●	●		●	
DDT		●	●	●	
Carbamates	●	●			●
Organophosphates	●	●			●

AChE, acetylcholinesterase; DDT, dichlorodiphenyltrichloroethane; GSH, glutathione; *kdr*, knockdown resistance
Source: GPIRM (WHO, 2012)[6]

6 Work is ongoing to update this table to include insecticide classes recently available or soon to be available for malaria vector control (e.g. neonicotinoids and pyrroles).

The cost of insecticide resistance management

There are financial and operational implications to implementing insecticide resistance monitoring and management. Combinations are relatively expensive because of the need for concurrent implementation of multiple interventions. Different insecticide formulations have different prices, and prices can vary over time due to factors such as changing supply and demand. Residual efficacy also differs between formulations, and depends on the composition of the surfaces to be sprayed. This necessitates different frequencies of application, which in turn requires different quantities and implementation costs (including those related to transport, storage and disposal) to provide effective protection over an entire malaria transmission period. Less immediate factors also need to be taken into account; for example, capacity-building, operational research, and product development and evaluation. Cost–effectiveness and operational feasibility of the potential tools and approaches need to be considered, to identify the best approach for each setting. Although limited empirical data are available that demonstrate the consequences of resistance management for public health vector control, scenario modelling may be of use for identifying viable options.

Global estimates have been made for the cost of resistance management in the GPIRM (WHO, 2012) and the *Global technical strategy for malaria 2016–2030* (WHO, 2015). These costs are not insignificant, and donors and agencies are urged to commit funding to this. In the past, the options available for resistance management have been limited, but this has changed with the availability of one new insecticide classes for IRS and will be further enhanced once LLINs with insecticides other than pyrethroids have been fully evaluated. However, these new interventions are likely to have a higher cost than current tools. Cost considerations around the integration of these new tools should extend to a broader consideration of how the use of these interventions will become essential to maintain the gains made in malaria control over the last decade.

IRM does not need to be an all-or-nothing approach. Available funds can be strategically targeted to make improvements wherever possible, such as through selection of new-generation interventions for specific conditions of insecticide resistance. The potential costs of not managing insecticide resistance should also be considered. Failure to mitigate resistance now may save money in the short term, but in the midterm it could reduce available options for malaria vector control, and in the long term could raise the cost for vector control as cheaper interventions fail and more expensive options are required. Ultimately, malaria cases could increase, causing sickness and loss of lives, and leading to negative economic consequences through loss of productivity.

3. WHO INSECTICIDE RESISTANCE DATABASE

3.1 Overview

Effective IRM requires data to inform national and global malaria vector-control policy and implementation planning. The WHO global database consolidates information collected on the insecticide resistance status, intensity and mechanisms for *Anopheles* mosquitoes from countries across all six WHO regions. It includes data from annual reports to WHO by national disease programmes and their partners, and data collated from publications in international peer-reviewed scientific journals. The collation process is therefore heavily reliant on timely and appropriate data reporting by partners to national programmes, effective data management at national level, and complete and timely reporting to WHO.

Standard data forms have been used for submission to WHO of country data for insecticide susceptibility measured via standard discriminating or intensity concentration bioassays, and resistance mechanisms measured via synergist bioassays, and biochemical and molecular assays for *Anopheles spp.* collected from 2010 onwards. Before being included in the database, data are checked for accuracy and validity at WHO country, regional and headquarters levels. Data are then aligned with the standard structure, format and nomenclature used for the database, and georeferencing is undertaken using online resources. Replicates (e.g. those reported by programmes and extracted from publications) are identified using key criteria – for example, year of mosquito collection, collection site name, vector species, insecticide or mechanism tested, number of mosquitoes tested and outcome – and are removed. Aligned data are then verified annually by national malaria control programmes (NMCPs), in conjunction with the process undertaken for compilation of data for WHO's annual *World malaria report*.

As of 20 October 2017, the WHO database contained a total of 29 363 reports for *Anopheles* mosquitoes from resistance monitoring and investigations conducted between 1947 and 2017.[1]

3.2 Data selected for report

Inclusion and exclusion criteria

This report focused on insecticide resistance data for malaria vector species collected between 2010 and 2016 from the 91 countries considered malaria endemic in 2016 (WHO, 2017e). It focused on data generated using standard procedures, as outlined in the WHO test procedures document (WHO, 2016b). For discriminating and intensity concentration bioassays, records with the following conditions were excluded from summaries and further analyses: nonstandard insecticide concentrations, insecticides for which there was no established discriminating concentration, test procedures other than WHO susceptibility and CDC bottle bioassays (e.g. cone bioassays), tests with a control mortality of more than 20% and tests conducted with fewer than 10 mosquitoes.

Data from phenotypic bioassays conducted with between 10 and 99 mosquitoes were included, even though this was below the recommended minimum of 100 adult female mosquitoes (WHO, 2016b), because these data were considered informative. Data were also included where the number of mosquitoes tested was not reported.

1 Because mosquito collection duration may cross 2 calendar years, the year indicated refers to the year in which the collection began.

For seven countries, the only data available were from bioassays with fewer than 100 mosquitoes or for which the number of test mosquitoes was not reported. Low numbers of test mosquitoes are often the result of difficulties in collecting sufficient specimens from the field, or are due to challenges in rearing immature mosquitoes to adults for testing.

Application of inclusionary and exclusionary criteria reduced the number of data for analysis by 39.3%, to a total of 17 824 records. Data were excluded mainly due to the collection year being outside the range of this report (9693; 33.0% of all records) or due to nonstandard concentrations or methods (1114; 3.8%), with the remainder excluded due to low numbers of test mosquitoes or high mortality in negative controls.

Summary

Standard insecticide resistance monitoring data for 2010 to 2016 were reported to WHO by 79 of 91 malaria endemic countries. However, monitoring was not conducted annually in all countries. Data for 2010 were reported by 34 countries, for 2011 by 41 countries, for 2012 by 39 countries, for 2013 by 44 countries, for 2014 by 51 countries, for 2015 by 41 countries and for 2016 by 37 countries. The recent decline in reporting of resistance data to WHO is of concern, although it is yet to be determined whether this is a true indication of weakened monitoring or reporting (or both). In some cases there may be a significant delay in the collection of data and their correct management and reporting, particularly for data collected by research institutes that are intended for publication in scientific journals. However, as noted above, reporting of data to WHO and inclusion in the database should not preclude publication in such journals.

Of the 17 824 records included in this analysis, most (15 146; 85.0%) were from phenotypic assays, with the remainder (2678; 15.0%) being from mechanism assays (Fig. 3.1a). Of the phenotypic assays, most (14 277; 94.3%) were from discriminating concentration bioassays, with only 5.7% (869) from intensity concentration bioassays in nine countries. The numbers of mosquitoes tested in discriminating concentration bioassays varied (Table 3.1), with almost half of the tests conducted using at least 100 mosquitoes, and a mean of 96 mosquitoes used per test.

For the mechanism assays, most of the data (1877; 70.1%) were from molecular marker studies to detect the presence and frequency of *Ace-1R* and the alleles *kdr L1014S* and *kdr L1014F*. A total of 22.1% (593) were from synergist-insecticide bioassays[2] conducted in 15 countries, of which 96.3% (571) were conducted with PBO as a proxy for monooxygenase involvement.[3] The remaining 7.8% (208) of mechanism records were from biochemical studies, including assays to detect the upregulation of monooxygenases, esterases and GSTs.

Test mosquitoes originated from a total of 2732 unique collection sites between 2010 and 2016 (Fig. 3.1b). Most of the sites were located within the WHO African Region (1403; 51.4%), followed by the WHO regions of South-East Asia (548; 20.1%), Western Pacific (341; 12.5%), Eastern Mediterranean (263; 9.6%) and the Americas (168; 6.0%), and the WHO European Region (13; 0.5%) (Fig. 3.1c). The annual number of sites tested peaked in 2013 (803) and declined by almost half in 2016 (405), largely due to a significant decrease in reported data from the WHO African Region. That decrease may have been due to weakened reporting – particularly from those countries not supported by the US President's Malaria Initiative (PMI) – rather than to a true scale-back in monitoring activities. Most of the data reported (14 101; 79.1%) were for members of the *An. gambiae* complex and the *An. funestus* group, which are considered the principal malaria vectors in sub-Saharan Africa (Fig. 3.1d).

2 This includes concurrent testing with a 1× (or discriminating) concentration of the specified insecticide.
3 This is not confirmatory evidence of involvement of P450s in observed resistance. Further research is required to determine the accuracy of results from synergist-insecticide bioassays.

TABLE 3.1.
Records of discriminating concentration bioassays for 2010–2016 by number of *Anopheles* malaria vectors tested, for each WHO region

WHO region	Total number of tests	% of tests per region by the number of mosquitoes tested			
		10–49	50–99	≥100	Not reported
African	10 099	9%	22%	49%	20%
Americas	623	2%	13%	73%	11%
Eastern Mediterranean	1 534	20%	13%	47%	21%
European	15	100%	0%	0%	0%
South-East Asia	1 102	28%	30%	38%	4%
Western Pacific	904	24%	22%	54%	1%
Total number	14 277	12%	22%	49%	17%

3.3 Analytical methods

Tables 2.1 and 2.2 outline the indicators used in this report. Regional analyses were conducted for WHO malaria endemic countries – as per 2017 classification (WHO, 2017e) – based on WHO regions and African subregions (Fig. 3.2). Trends over time were evaluated using statistical model estimates for the average change in resistance frequency based on mosquito survival for tests conducted between 2010 and 2016, with evaluation across insecticide classes and by WHO regions, subregions, major vector species groupings and individual insecticides. Generalized linear mixed-effects models were fitted to all data within an insecticide class for three vector species groupings: *An. funestus s.l., An. gambiae s.l.* and other *Anopheles* malaria vectors. The country of data origin was included as a random effect to determine overall temporal trends, taking into account different starting resistance frequencies between countries, and variable sampling effort between countries and across time.

FIG. 3.2.
Map indicating malaria endemic countries by WHO region or African subregion, for 2016

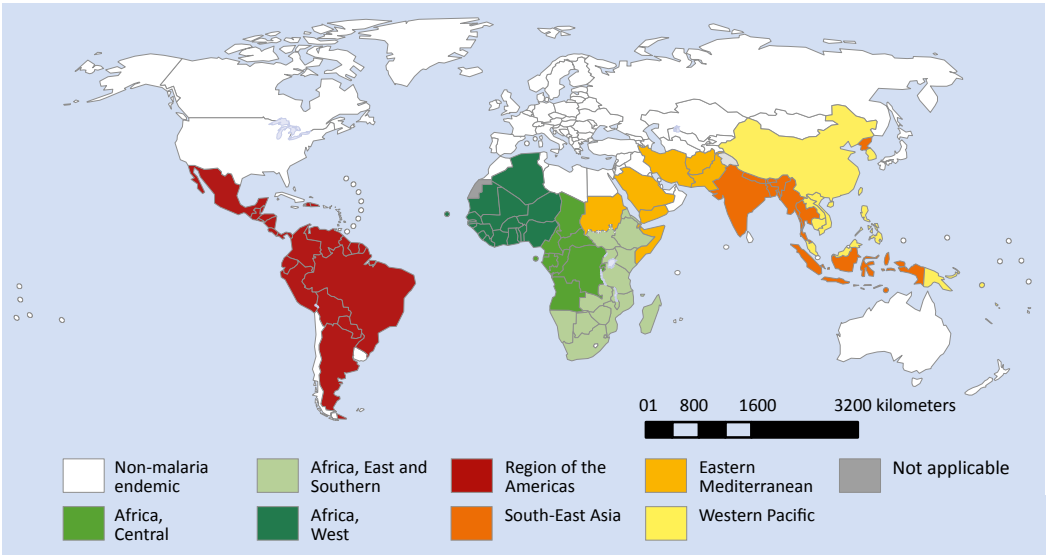
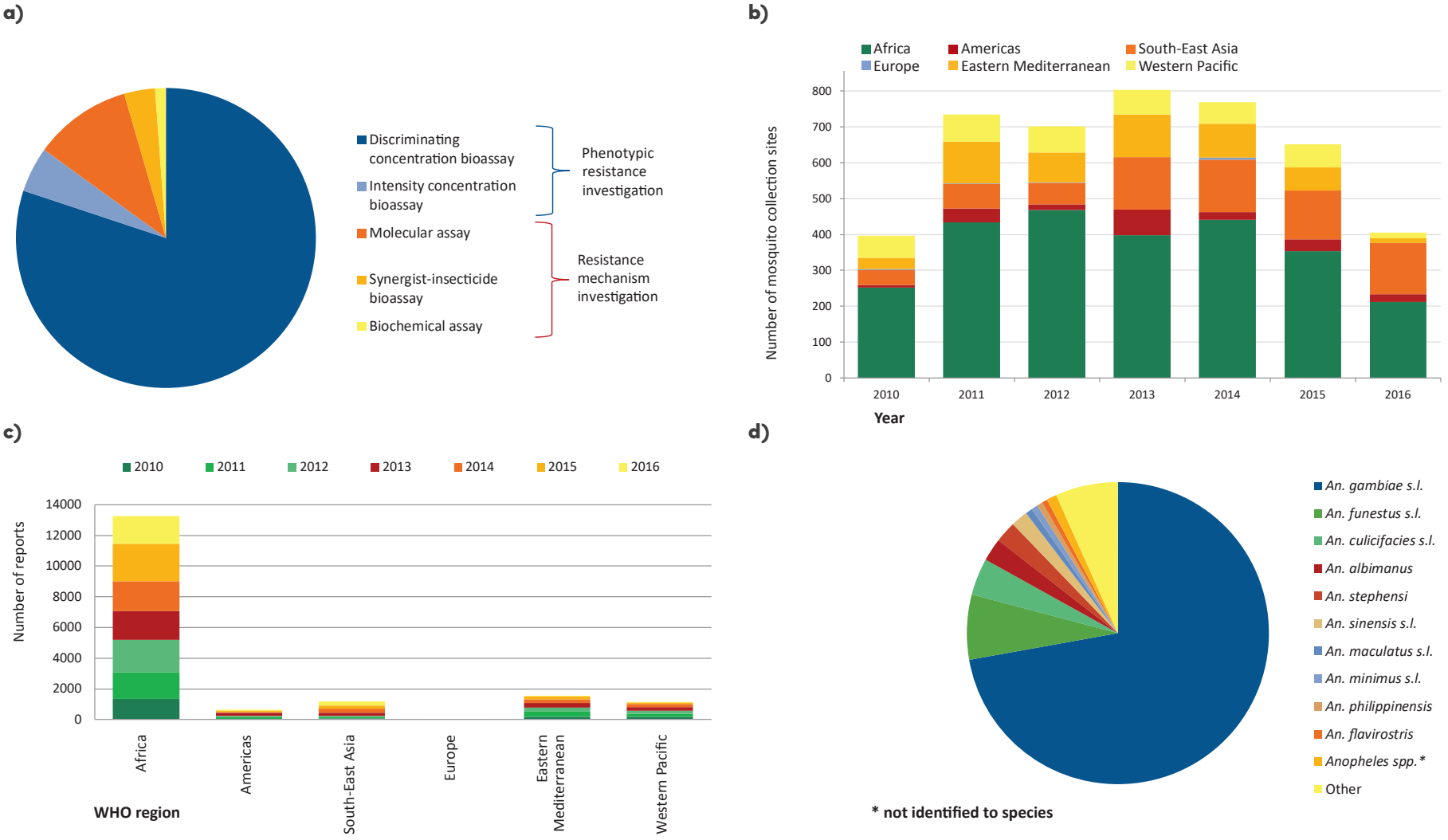


FIG. 3.1.

Overview of reported data for the WHO insecticide resistance database considered in this report

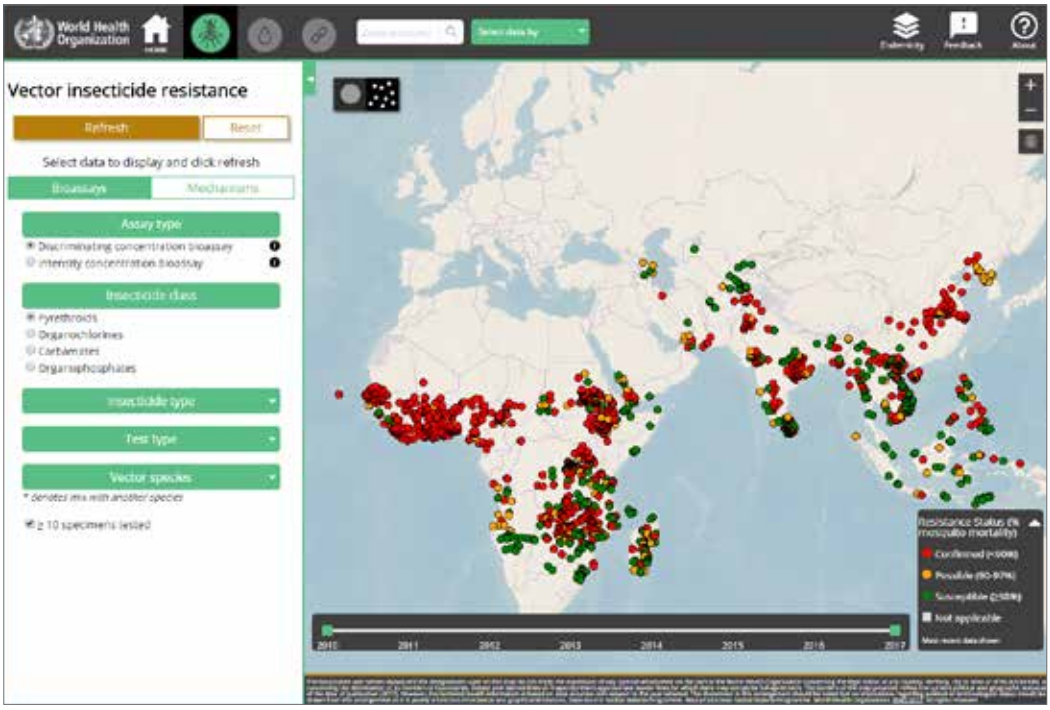
Includes a) total by investigation and assay type, b) number of collection sites by year and WHO region, c) number of reports by WHO region and year, and d) total by vector species.



3.4 Online mapping tool

In October 2017, WHO released a mapping tool that includes *Anopheles* vector insecticide resistance, *Plasmodium falciparum* gene deletions, and *P. falciparum* and *P. vivax* antimalarial drug efficacy and drug resistance marker data, as collated in Global Malaria Programme databases. This interactive mapping tool is updated periodically and thus provides a visual overview of recent data. For the vector-resistance component, there is the option to filter data by geographical area, year, assay type, insecticide or mechanism, and vector species. Data included in this report in addition to more recent insecticide resistance data can be accessed through the *Malaria threats map* (Fig. 3.3) (WHO, 2017b).[4]

FIG. 3.3.
Screenshot of *Malaria threats map* showing vector insecticide resistance data for pyrethroids for 2010–2017



Data source: WHO (2017e)

4 Other online resources are also available that map malaria vector insecticide resistance data, including IR Mapper (www.irmapper.com; accessed 18 December 2017) and PopBio (Insecticide Resistance) (https://www.vectorbase.org/popbio/map/?view=ir; accessed 18 December 2017). There is some overlap between the *Malaria threats map* and these sources in aim, scope, data sources and visualizations.

4. GLOBAL STATUS OF MALARIA VECTOR INSECTICIDE RESISTANCE

To identify major spatial and temporal trends, further evaluations were undertaken of phenotypic resistance investigations with discriminating concentration and intensity concentration bioassays, and resistance mechanism investigations with synergist–insecticide bioassays, molecular assays and biochemical assays. The data available were not randomly or uniformly distributed over time and space; sampling bias may therefore have influenced some of the trends observed.

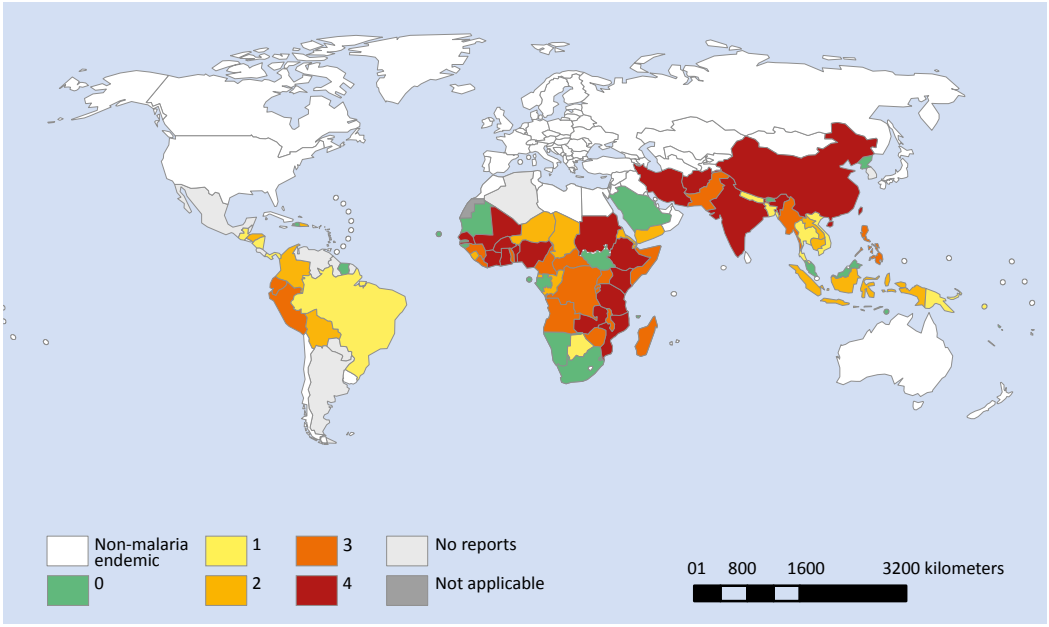
4.1 Phenotypic resistance

Discriminating concentration bioassays

Insecticide resistance was found to be widespread in *Anopheles* malaria vectors from across malaria endemic countries, with only 10 of the 79 malaria endemic countries that reported data to WHO indicating vector susceptibility to all four insecticide classes or no resistance mechanisms detected (or both)[1] (WHO, 2017e).

Between 2010 and 2016, data derived from discriminating concentration bioassays was reported by 76 malaria endemic countries,[2] of which 61 countries had detected resistance in at least one major malaria vector to at least one class of insecticide used for adult malaria vector control. In 50 of these countries, resistance was detected to two or more insecticide classes. A total of 18 countries from four WHO regions detected resistance to all four classes between 2010 and 2016, of which 12 are in the WHO African Region (Fig. 4.1); in some instances, resistance to all four classes was detected in individual vector test populations.

FIG. 4.1.
Map showing number of insecticide classes to which resistance in malaria vectors was reported, by country, for the period 2010–2016



1 Comoros, Guatemala, Haiti, Malaysia, Namibia, Saudi Arabia, Solomon Islands, Swaziland, Tajikistan and Timor-Leste.
2 Considered malaria endemic as of 2016 (WHO, 2017e).

Prevalence of confirmed resistance as a proportion of the sites tested for which resistance was confirmed varied widely among WHO regions and among insecticide classes. Malaria vector resistance to the four insecticide classes was detected in all WHO regions except the WHO European Region, where monitoring was limited (Fig. 4.2). Pyrethroid resistance prevalence was very high in the WHO African Region (78.2% of sites tested) and Eastern Mediterranean Region (69.8%); it was also high in the South-East Asia Region (38.3%) and Western Pacific Region (50.9%), but was lower in the Region of the Americas (20.0%). Organochlorine resistance (predominantly for DDT) was of similar prevalence across all WHO regions (62.4–69.8%) except the Region of the Americas (9.4%), where testing was limited. Prevalence of carbamate resistance ranged between 22.2% and 53.7%. Organophosphate resistance prevalence varied widely across regions, and (excluding the WHO European Region due to the limited monitoring) was lowest in the WHO African Region (14.1%) and highest in the WHO Western Pacific Region (64.6%)

The frequency of resistance, as indicated by percentage of mosquitoes in discriminating concentration bioassays that were alive (for WHO susceptibility tests) or not incapacitated (for CDC bottle bioassays) varied widely between the WHO regions. This was the case for pyrethroids (ranging from a median of 0% in the WHO Region of the Americas and European Region to 35% in the WHO African Region) and organochlorines (ranging from a median of 0% in the WHO Region of the Americas to 60% in the South-East Asia Region) (Fig. 4.3a and 4.3b). There was also high variability in resistance frequencies within WHO regions, as indicated by wide interquartile ranges (IQRs). In general, the distribution of resistance frequencies was similar for pyrethroids and organochlorines within each WHO region, with the exception of South-East Asia, where frequencies of DDT resistance were significantly higher for DDT (median of 60%) than for pyrethroids (median of 5%). This regional trend was probably driven by the large dataset from India, which included many reports of high frequency of DDT resistance. Median pyrethroid and DDT resistance frequencies were 0% in the WHO Region of the Americas, although relatively few sites were monitored there compared with other WHO regions (excluding the WHO European Region).Widespread resistance to pyrethroids may be linked to selective pressure exerted by the massive scale-up in pyrethroid-treated ITNs and LLINs since 2000, especially in sub-Saharan Africa (Cibulskis et al., 2016). DDT resistance may also be a driver of cross-resistance to pyrethroids, with extensive prolonged use of DDT historically, particularly in the WHO African and Eastern Mediterranean regions.

Organophosphate resistance frequencies varied widely for the WHO regions of Eastern Mediterranean, South-East Asia and Western Pacific, with median frequencies of 7%, 18% and 25%, respectively (Fig. 4.3c). Resistance frequencies were generally very low in the WHO African Region and Region of the Americas (medians of 0%), indicating that resistance to organophosphates may have developed more recently, or that selection pressure is relatively low. However, resistance was widespread in certain countries of west Africa including Côte d'Ivoire, Ghana and Nigeria, with pockets of resistance in other subregions, including in Angola, Ethiopia, Madagascar and Mozambique. This heterogeneity underscores the importance of resistance monitoring to ascertain the situation in local vectors. Carbamate resistance frequencies were relatively low in the WHO African Region, Region of the Americas and Eastern Mediterranean Region (medians of 0–1%), and were higher in the South-East Asia (8%) and Western Pacific (16%) regions (Fig. 4.3d).

Further evaluations are required to ascertain the factors influencing the emergence, frequency and intensity of phenotypic resistance, although this is beyond the scope of this current report.

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FIG. 4.2.

Reported insecticide resistance status as a proportion of sites for which monitoring was conducted, 2010–2016, by WHO region

Status was based on mosquito mortality where <90% = confirmed resistance, 90–97% = possible resistance, and ≥98% = susceptibility. Where multiple insecticide classes or types, mosquito species or time points were tested at an individual site, the highest resistance status was considered. Numbers above bars indicate the total number of sites for which data were reported (n).

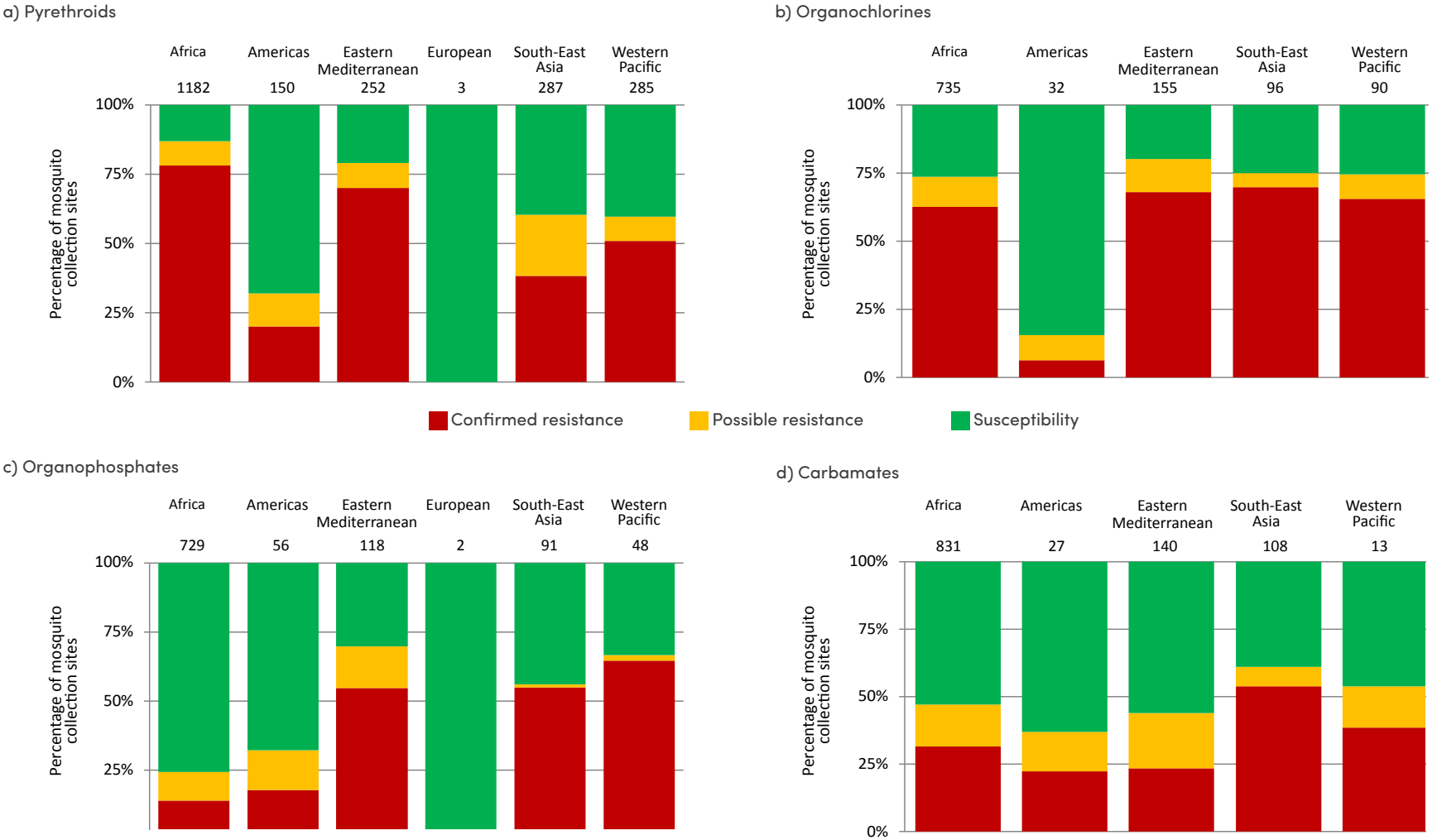
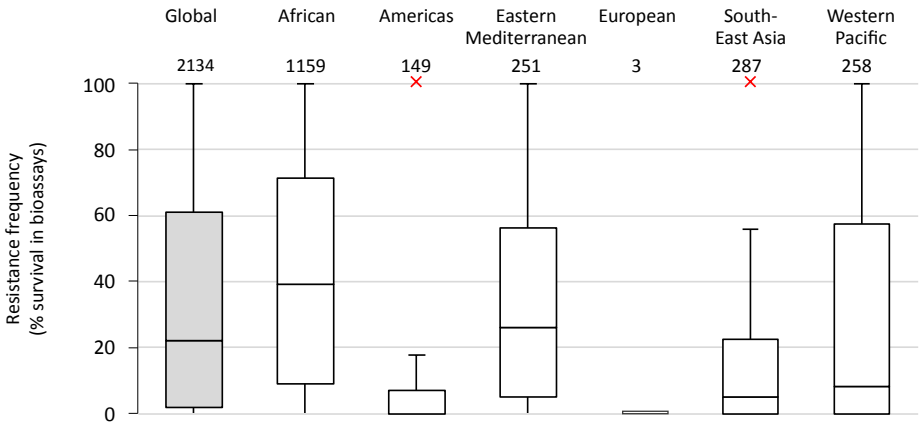


FIG. 4.3.

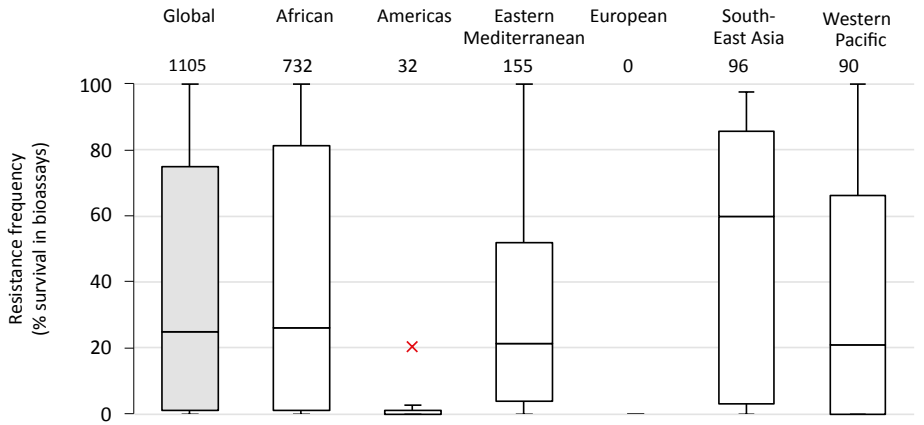
Resistance frequency (%) as measured in discriminating concentration bioassays (100% minus adjusted mosquito mortality) for most recent data available for each site for 2010–2016 (n=2354 total), using minimum for any insecticide within the class, globally and by WHO region

Boxes show the first and third quartile and whiskers show 1.5× interquartile range (IQR) above third quartile and 1.5× IQR below first quartile. Maximum outliers (red crosses) are shown if outside this range. Horizontal lines in boxes show the median. Numbers above bars indicate the total number of bioassays for which data were reported (n).

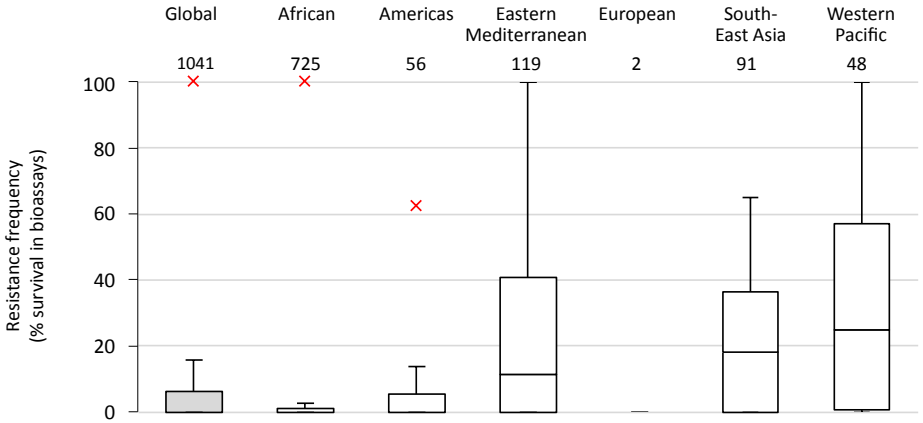
a) Pyrethroids



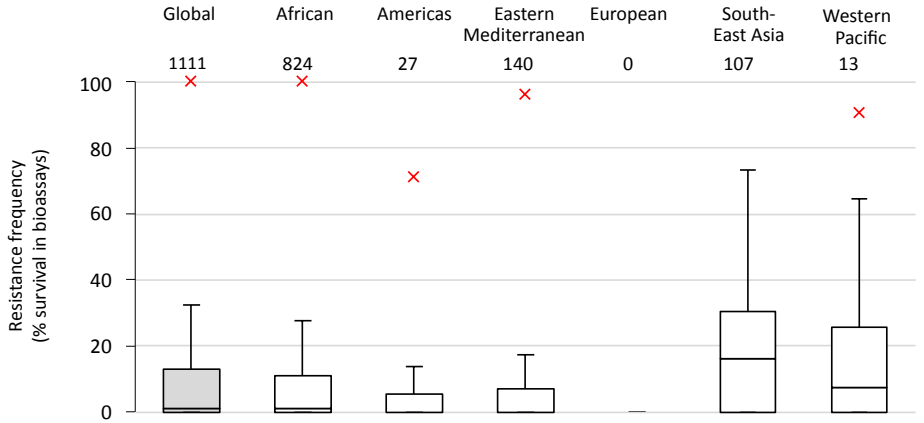
b) Organochlorines



c) Organophosphates



d) Carbamates



Changes in resistance frequency over time

There was an overall increase between 2010 and 2016 in the proportion of test mosquitoes surviving (or not incapacitated) in standard discriminating concentration bioassays with pyrethroids. Median resistance frequency from statistical model estimates rose from 16% in 2010 to 26% in 2016. Although this broad analysis grouped together all pyrethroid insecticides and vectors, the high number of bioassays considered (7844) means that this is probably a reliable indicator of a global increase in pyrethroid resistance in malaria vectors since 2010. Increases were apparent in the WHO African, Eastern Mediterranean and South-East Asia regions, and were of a similar magnitude for these three regions (11–14% rise), even though initial median resistance frequencies differed significantly between the regions in 2010 (25%, 9% and 1%, respectively). This may indicate a steady overall increase in pyrethroid resistance frequency, irrespective of the pre-existing frequency. When examining data for the three African subregions, an increase from 2010 was most notable in west Africa (22% rise, from 13% to 35%), and was similar for the two other WHO subregions of central Africa (14% rise, from 16% to 30%) and east and southern Africa (11% rise, from 42% to 53%).

There was no clear indication of an overall global increase in resistance frequency to the other three insecticide classes commonly used in malaria vector control. However, increased resistance since 2010 was evident for the WHO South-East Asia Region for organochlorines (predominantly DDT) (12% rise, from 17% to 29%), carbamates (9% rise, from 0% to 9%) and organophosphates (11% rise, from 1% to 12%). There was also some evidence of an increase in organochlorine resistance frequency in the WHO Eastern Mediterranean Region since 2010 (9% rise, from 24% to 33%). The only increase in resistance frequency for nonpyrethroid insecticides identified for any African subregion was an increase in carbamate resistance in central Africa (6% rise, from 6% to 12%).

Some of the observed regional or subregional trends may be accounted for by a preponderance of data for a particular country, with comparatively large datasets available for India (52% of reports for the WHO South-East Asia Region), Sudan (52% of reports for the WHO Eastern Mediterranean Region) and Nicaragua (37% of reports for the WHO Region of the Americas). For the WHO European Region, data were included for only one country (Tajikistan).

Trends analyses were conducted to determine whether there were any significant changes between 2010 and 2016 in malaria vector resistance to specific insecticides, and in specific vector groups (Fig. 4.4 and Fig. 4.5). A global increase in resistance frequency was observed for all pyrethroid insecticides tested. Increases were greatest for etofenprox (44% rise, from 7% to 51%), alphacypermethrin (40% rise, from 10% to 50%) and cyfluthrin (28% rise, from 4% to 32%). The increase was less pronounced for the other pyrethroids although these also had a higher initial resistance frequency in 2010: deltamethrin (14% rise, from 20% to 34%), permethrin (5% rise, from 40% to 45%) and lambda-cyhalothrin (3% rise, from 33% to 36%). This indicates that increasing resistance is an issue for all pyrethroids, and that reductions in susceptibility are most marked for those insecticides for which susceptibility was highest in 2010. Further evaluations will be undertaken to identify whether there are differences in resistance frequency and trends over time between insecticides of the pyrethroid class, in order to guide requirements for insecticide resistance monitoring.

For carbamates, the global increase in survival in discriminating concentration bioassays was much greater for propoxur (15% rise, from 9% to 24%) than for bendiocarb (2% rise, from 7% to 9%). For the organophosphates, there was an evident increase in resistance to malathion (5% rise, from 3% to 8%) and fenitrothion (3% rise, from 12% to 15%). For pirimiphos-methyl there was no clear evidence of an increase in resistance frequency (with only 19% probability of a rise).

The large amount of data submitted for species of the *An. gambiae* complex and the *An. funestus* group is likely to have been the main driver for the trends observed in the global overview. Resistance frequencies for these two species groups were compared with all other malaria vectors to determine whether there were any differences in observed trends. Increases in pyrethroid resistance frequencies were striking for *An. funestus s.l.* (32% rise, from 26% to 58%), moderate for *An. gambiae s.l.* (13% risk, from 21% to 34%) and minimal for other malaria vectors (5% rise, from 10% to 15%). There was also a significant increase in the frequency of organochlorine resistance (41% rise, from 0% to 41%) in *An. funestus s.l.*, but an apparent drop in carbamate resistance (20% decline, from 42% to 22%) and a slight rise in organophosphate resistance (2% decline, from 2% to 0%) between 2010 and 2016 (Fig. 4.4 and Fig 4.5). However, far fewer data points were available for the latter two insecticide classes; thus, results should be interpreted with caution.

Overall, there were significantly fewer records for *An. funestus s.l.* than for *An. gambiae s.l.*, because the former species group is notoriously difficult to collect and rear to adults in the insectary for susceptibility testing. Nevertheless, the clear increase in resistance to pyrethroids, DDT and possibly organophosphates could signify that this vector group presents an increasing challenge to effective malaria vector control.

Cross-resistance and multiple resistance

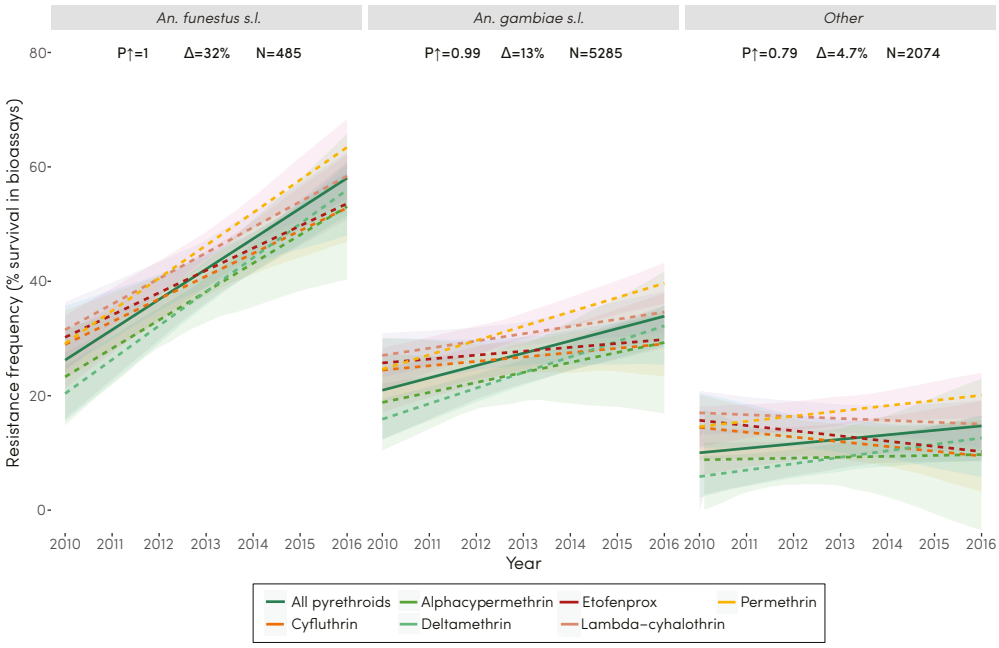
Table 4.1 summarizes reports of resistance to the different insecticide classes and detected resistance mechanisms for all populations tested across each country. A detailed examination of cross-resistance and multiple resistance in individual vector populations is beyond the scope of this report. Analyses conducted by the University of Oxford identified clear associations in resistance in *An. gambiae s.l.* between individual insecticides (i.e. deltamethrin, permethrin, lambda-cyhalothrin and DDT). For pyrethroids, this indicated that in low resource settings testing one insecticide in multiple populations is more informative that testing multiple insecticides in one population (C. Moyes and P. Hancock, University of Oxford, personal communication).

FIG. 4.4.

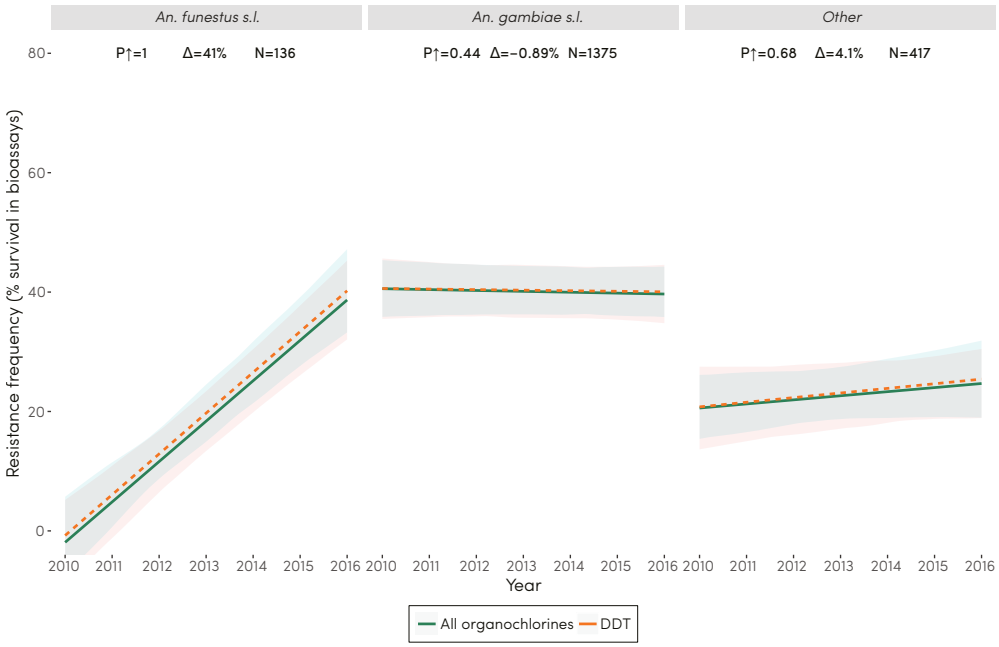
Resistance frequency in *An. funestus s.l.*, *An. gambiae s.l.* and other malaria vectors for 2010–2016, defined as the proportion of surviving mosquitoes in discriminating concentration bioassays with a) pyrethroids and b) organochlorines

Solid lines indicate best-fit estimates generated by bootstrapping methodology for insecticides of the class, and dotted lines indicate best-fit estimates for individual insecticides of the class; shaded areas indicate 95% confidence interval estimates. Values in boxes show P↑ = estimated probability of an increase in resistance frequency between 2010 and 2016, Δ = change in resistance frequency between 2010 and 2016, and n = number of assays.

a) Pyrethroids



b) Organochlorines (mostly DDT)[3]



DDT, dichlorodiphenyltrichloroethane[3]

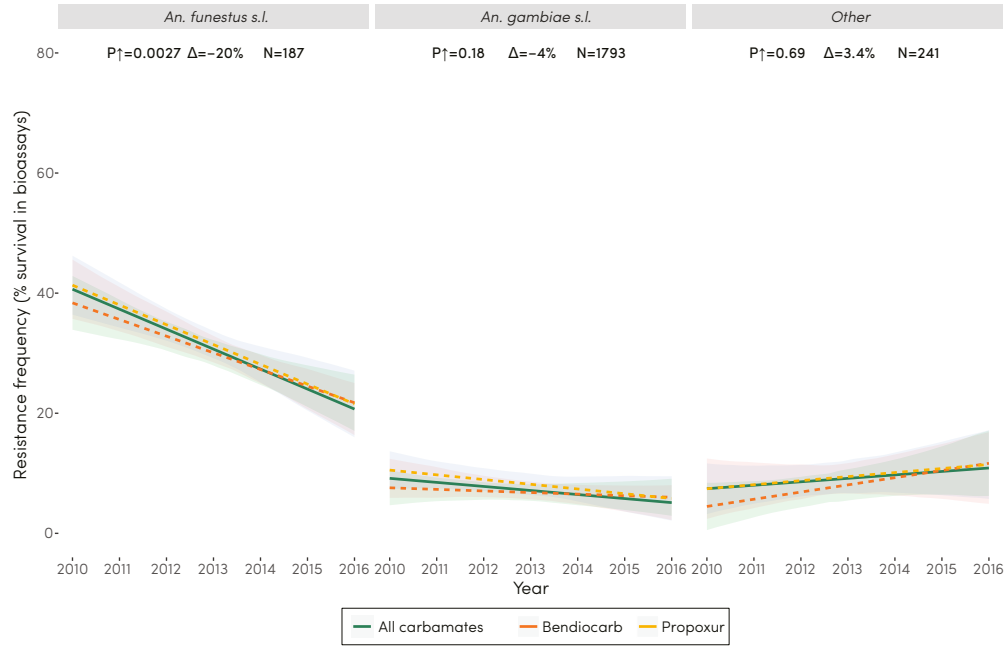
3 Includes some reports for dieldrin that are not displayed due to low numbers and wide variability.

FIG. 4.5.

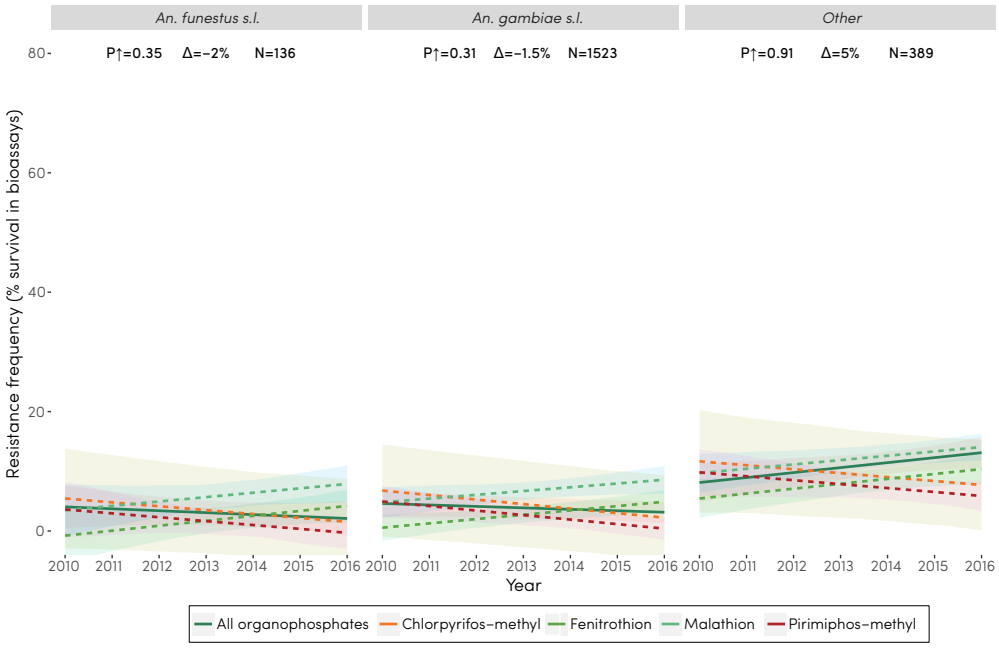
Resistance frequency in *An. funestus s.l., An. gambiae s.l.* and other malaria vectors for 2010–2016, defined as the proportion of surviving mosquitoes in discriminating concentration bioassays with a) carbamates and b) organophosphates

Solid lines indicate best-fit estimates generated by bootstrapping methodology for insecticides of the class and dotted lines indicate best-fit estimates for individual insecticides of the class; shaded areas indicate 95% confidence interval estimates. Values in boxes show P↑ = estimated probability of an increase in resistance frequency between 2010 and 2016, Δ = change in resistance frequency between 2010 and 2016, and n = number of assays.

a) Carbamates[4]



b) Organophosphates



4 Includes some reports for carbosulfan that are not displayed due to low numbers and wide variability.

TABLE 4.1

Resistance status to four insecticide classes and resistance mechanisms tested or detected (or both) for adult malaria vectors, for 2010–2016

For resistance status, outcomes are indicated based on at least one local Anopheles vector species tested by standard bioassays for which results indicated R = confirmed resistance to at least one insecticide of the class, or S = susceptibility or possible resistance for all insecticides of the class tested, or that – = no insecticide of the class was tested. For resistance mechanisms, outcomes are indicated based on reports for at least one local *Anopheles* vector species that indicated that the specific mechanism (or group) was D = detected, or ND = tested for but not detected, or that – = there were no reports of testing for the mechanism in any species in the country.

COUNTRY	RESISTANCE STATUS				RESISTANCE MECHANISMS							SPECIES[a] EXHIBITING RESISTANCE[b]
					METABOLIC			TARGET SITE				
	Pyrethroids	Organochlorines	Carbamates	Organophosphates	Monooxygenases	Esterases	GSTs	*kdr L1014*	*kdr L1014S*	*kdr* (unspecified)	*Ace-1R*	
Central Africa												
Angola	R	R	R	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Burundi	R	R	R	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Cameroon	R	R	R	S	–	–	–	D	ND	D	ND	*An. coluzzii, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
Central African Republic	R	R	S	R	D	D	D	–	–	–	–	*An. funestus s.l., An. gambiae s.l.*
Chad	R	R	S	S	ND	ND	ND	D	–	–	–	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
Congo	R	R	S	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Democratic Republic of the Congo	R	R	S	R	–	–	–	D	–	–	–	*An. gambiae s.l., An. gambiae s.s.*
Equatorial Guinea	R	R	S	S	–	–	–	–	–	–	–	*An. coluzzii*
Gabon	–	–	–	–	–	–	–	–	–	–	–	
Sao Tome and Principe	–	–	S	–	–	–	–	D	–	–	ND	*An. gambiae s.s.*
East and southern Africa												
Botswana	R	S	S	–	–	–	–	–	–	–	–	*An. gambiae s.l.*
Comoros	S	–	–	–	D	–	–	–	–	ND	–	*An. gambiae s.l.*
Eritrea	R	R	S	S	–	–	–	–	–	–	–	*An. funestus s.l., An. gambiae s.l.*

Ethiopia	R	R	R	R	–	–	–	D	ND	–	–	*An. arabiensis, An. gambiae s.l.*
Kenya	R	R	R	R	D	D	D	D	D	–	–	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. rivulorum*
Madagascar	R	R	R	S	–	–	–	–	–	–	–	*An. funestus s.l., An. gambiae s.l.*
Malawi	R	R	R	S	–	–	–	–	–	–	–	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l.*
Mayotte	–	–	–	–	–	–	–	–	–	–	–	
Mozambique	R	R	R	R	–	–	–	–	–	D	–	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. leesoni, An. maculipalpis, An. pretoriensis, An. rufipes*
Namibia	S	S	–	–	–	–	–	–	–	–	–	
Rwanda	R	R	R	S	–	–	–	–	–	D	–	*An. gambiae s.l.*
South Africa	R	R	R	S	–	–	–	–	–	–	–	*An. arabiensis, An. merus*
South Sudan	–	–	–	–	–	–	–	–	–	–	–	
Swaziland	S	S	–	–	–	–	–	–	–	–	–	
Uganda	R	R	R	S	ND	–	–	D	D	–	–	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s., An. parensis*
United Republic of Tanzania	R	R	R	R	D	–	–	D	D	–	ND	*An. arabiensis, An. gambiae s.l., An. gambiae s.s.*
Zambia	R	R	R	R	D	D	–	D	ND	–	ND	*An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s.*
Zimbabwe	R	R	R	S	–	D	–	–	–	–	–	*An. funestus s.l., An. gambiae s.l.*
West Africa												
Benin	R	R	R	R	D	D	D	D	D	D	D	*An. arabiensis, An. coluzzii, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. melas*
Burkina Faso	R	R	R	R	D	D	D	D	D	D	D	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s., An. gambiae s.s.*
Cabo Verde	–	–	–	–	–	–	–	–	–	–	–	
Côte d'Ivoire	R	R	R	R	D	D	D	D	ND	–	D	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
Gambia	–	–	–	–	–	–	–	–	–	–	–	
Ghana	R	R	R	R	–	–	–	D	–	D	D	*An. coluzzii, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s.*
Guinea	R	R	R	–	–	–	–	D	–	–	–	*An. coluzzii, An. gambiae s.s.*
Guinea-Bissau	–	–	–	–	–	–	–	D	–	–	D	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*

COUNTRY	RESISTANCE STATUS				RESISTANCE MECHANISMS							SPECIES[a] EXHIBITING RESISTANCE[b]
					METABOLIC			TARGET SITE				
	Pyrethroids	Organochlorines	Carbamates	Organophosphates	Monooxygenases	Esterases	GSTs	*kdr L1014*	*kdr L1014S*	*kdr* (unspecified)	*Ace-1R*	
Liberia	R	R	R	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Mali	R	R	R	R	D	D	D	D	ND	–	D	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
Nigeria	R	R	R	R	–	–	–	D	ND	–	ND	*An. coluzzii, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
Senegal	R	R	R	R	D	–	D	D	D	–	–	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s.*
Sierra Leone	R	R	S	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Togo	R	R	R	S	–	–	–	–	–	–	–	*An. gambiae s.l.*
Americas												
Belize	–	–	–	–	–	–	–	–	–	–	–	
Bolivia (Plurinational State of)	R	S	R	–	–	–	–	–	–	–	–	*An. darlingi*
Brazil	R	–	–	–	–	–	–	–	–	–	–	*An. darlingi*
Colombia	R	R	–	S	–	–	–	–	–	–	–	*An. albimanus, An. darlingi, An. marajoara, An. nuneztovari*
Costa Rica	–	–	–	–	–	–	–	–	–	–	–	
Dominican Republic	R	S	–	R	–	–	–	–	–	–	–	*An. albimanus*
Ecuador	R	S	R	R	–	–	–	–	–	–	–	*An. albimanus*
El Salvador	–	–	–	–	–	–	–	–	–	–	–	
French Guiana, France	–	–	–	–	–	–	–	–	–	–	–	
Guatemala	S	–	S	R	–	–	–	–	–	–	–	*An. albimanus*
Guyana	–	–	–	–	–	–	–	–	–	–	–	
Haiti	S	S	–	S	–	–	–	–	–	–	–	

Honduras	R	–	S	R	–	–	–	–	–	–	–	*An. albimanus*
Mexico	–	–	–	–	–	–	–	–	–	–	–	
Nicaragua	S	–	R	S	–	–	–	–	–	–	–	*An. pseudopunctipennis*
Panama	–	–	R	–	–	–	–	–	–	–	–	*An. albimanus*
Peru	R	–	R	R	–	D	–	–	–	–	D	*An. albimanus, An. darlingi*
Suriname	–	–	–	S	–	–	–	–	–	–	–	
Venezuela (Bolivarian Republic of)	–	–	–	–	–	–	–	–	–	–	–	
Eastern Mediterranean												
Afghanistan	R	R	R	R	–	–	–	D	D	–	–	*An. culicifacies s.l., An. hyrcanus s.l., An. pulcherrimus, An. stephensi, An. subpictus s.l., An. superpictus*
Djibouti	R	R	R	R	–	–	–	–	–	–	–	*An. gambiae s.l.*
Iran (Islamic Republic of)	R	R	R	R	D	D	D	ND	ND	–	–	*An. sacharovi, An. stephensi, An. subpictus s.l.*
Pakistan	R	R	–	R	–	–	–	–	–	–	–	*An. culicifacies s.l., An. stephensi*
Saudi Arabia	S	–	–	–	–	–	–	–	–	–	–	
Somalia	R	R	S	R	–	–	–	–	–	–	–	*An. arabiensis*
Sudan	R	R	R	R	–	–	–	–	–	–	–	*An. arabiensis*
Yemen	R	R	S	–	–	–	–	–	–	–	–	*An. arabiensis*
South-East Asia												
Bangladesh	R	–	–	–	–	–	–	–	–	–	–	*An. philippinensis, An. vagus*
Bhutan	S	–	–	–	–	–	–	–	–	–	–	
Democratic People's Republic of Korea	S	S	S	S	–	–	–	–	–	–	–	
India	R	R	R	R	D	D	ND	D	D	–	–	*An. culicifacies s.l., An. fluviatilis, An. stephensi*
Indonesia	R	S	R	S	–	–	–	–	–	–	–	*An. aconitus, An. barbirostris, An. peditaeniatus, An. vagus*
Myanmar	R	R	–	R	–	–	–	–	–	–	–	*An. aconitus, An. annularis, An. hyrcanus s.l., An. minimus s.l., An. peditaeniatus, An. philippinensis, An. sinensis s.l., An. vagus*
Nepal	–	R	S	S	–	–	–	–	–	–	–	*An. annularis, An. culicifacies s.l.*
Thailand	R	–	–	–	–	–	–	–	–	–	–	*An. barbirostris*

COUNTRY	RESISTANCE STATUS				RESISTANCE MECHANISMS							SPECIES[a] EXHIBITING RESISTANCE[b]
					METABOLIC			TARGET SITE				
	Pyrethroids	Organochlorines	Carbamates	Organophosphates	Monooxygenases	Esterases	GSTs	*kdr L1014*	*kdr L1014S*	*kdr* (unspecified)	*Ace-1R*	
Cambodia	R	R	–	–	–	–	–	–	–	–	–	*An. barbirostris, An. maculatus s.l., An. vagus*
China	R	R	R	R	–	–	–	D	–	–	D	*An. minimus s.l., An. sinensis s.l., An. vagus*
Lao People's Democratic Republic	R	R	–	–	–	–	–	–	–	–	–	*An. aconitus, An. dirus s.l., An. hyrcanus s.l., An. kochi, An. maculatus s.l., An. minimus s.l., An. neivai, An. nivipes, An. philippinensis, An. umbrosus s.l., An. vagus*
Malaysia	S	–	–	–	–	–	–	–	–	–	–	
Republic of Korea	–	–	–	–	–	–	–	D	D	–	D	*An. sinensis s.l.*
Solomon Islands	S	R	–	S	–	–	–	–	–	–	–	*An. farauti s.l.*
Vanuatu	S	–	–	–	–	–	–	–	–	–	–	
Viet Nam	R	S	–	–	–	–	–	–	–	–	–	*An. aconitus, An. annularis, An. epiroticus, An. kochi, An. maculatus s.l., An. minimus s.l., An. nivipes, An. philippinensis, An. sinensis s.l., An. vagus*

[a] Ordered alphabetically as reported; for reports of *An. gambiae s.s.* M form the species name was altered to *An. coluzzii* in line with Coetzee et al. (2013a).
[b] Either by confirmed resistance in standard bioassays or by detection of resistance mechanisms in synergist-insecticide bioassays, biochemical assays or molecular assays.

Intensity concentration bioassays

Data were available for pyrethroid intensity concentration bioassays conducted for mosquito collections in 2013–2016 from 101 sites in nine countries, of which eight were in Africa (Burkina Faso, Ethiopia, Kenya, Mali, Nigeria, Uganda, United Republic of Tanzania and Zambia) and one was in South-East Asia (India). Four pyrethroid insecticides were used: alphacypermethrin, deltamethrin, lambda-cyhalothrin and permethrin, and CDC bottle bioassays were predominantly used because WHO intensity concentration papers did not become available until late 2016. Intensity assays with organophosphates (malathion and fenitrothion) were also used at one site in Peru at which high-intensity resistance was detected.

Outcomes from intensity assays indicated that high-intensity pyrethroid resistance was present in malaria vectors at over half of all sites tested in Africa, and was particularly prevalent in Mali and Zambia (Fig. 4.6 and Fig. 4.7). High-intensity pyrethroid resistance was found in *An. albimanus, An. arabiensis, An. funestus s.l., An. gambiae s.l.* and *An. vagus*. In India, *An. culicifacies s.l.* and *An. stephensi* were found to have at least moderate intensity resistance. Testing with 10× concentration was not conducted; hence, it could not be determined whether resistance was high intensity. Intensity assays are performed once the presence of pyrethroid resistance has been confirmed and where there is suspicion of high resistance (e.g. as indicated by prior tests such as mechanism assays); therefore, there is significant selection bias and the results should not be considered indicative of the broader situation. It is clear that further testing for resistance intensity for vectors throughout Africa and other regions is required to generate a comprehensive overview of the extent of pyrethroid resistance intensity.

Intensity testing using the same insecticide over multiple years was conducted for only 18 sites in four countries (India, Mali, Nigeria and Zambia). Of these sites, 13 were in Mali and all remained of high-intensity resistance over the test period. For the remaining five sites: at one there was an increase in intensity from moderate to high (in Zambia); at three moderate intensity remained (two in India, one in Zambia); and, at one there was a change from susceptible to low intensity resistance (in Nigeria) over the monitoring period.

A clear linkage between outcomes from resistance intensity concentration bioassays and effectiveness of insecticidal interventions has not been established. However, it is envisaged that, through increased use of resistance intensity assays, it will be possible to identify regions and areas where resistance is most intensively expressed, allowing further investigation of a potential negative correlation between resistance intensity and the effectiveness of current interventions in these high resistance areas.

FIG. 4.6.

Outcomes from intensity concentration bioassays with pyrethroids, 2014–2016

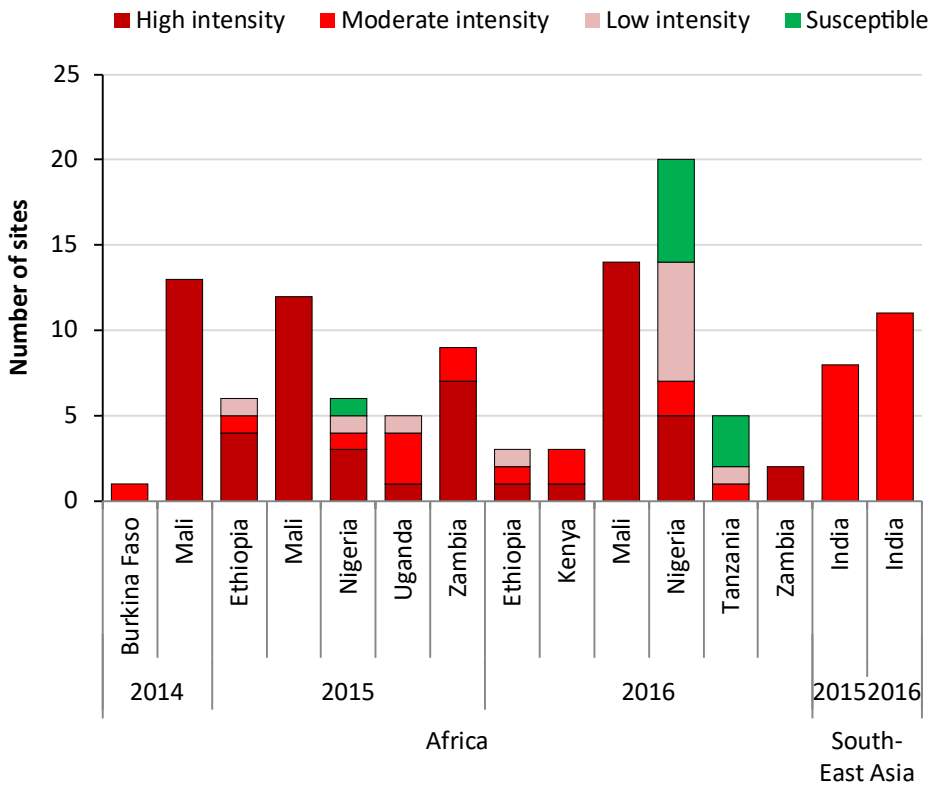
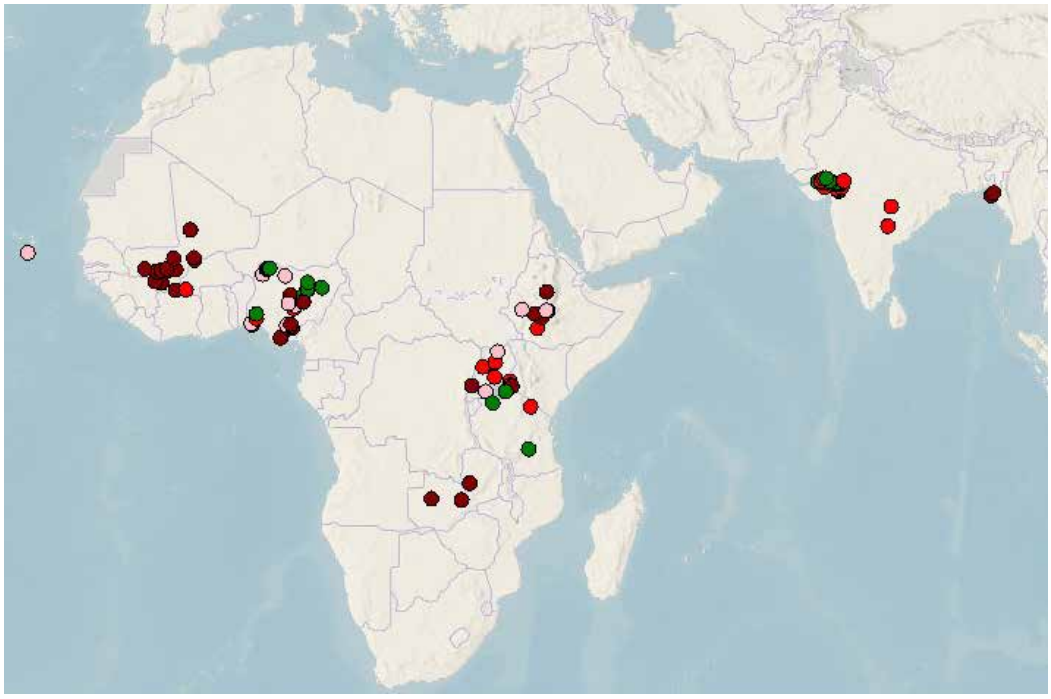


FIG. 4.7.

Pyrethroid intensity concentration bioassay data reported for the WHO African and South-East Asia regions, 2014–2016

The most recent outcome for any individual site is indicated by colour: dark red = high intensity, red = moderate intensity, pink = low intensity, and green = susceptibility.



4.2 Resistance mechanisms

Data on resistance mechanisms were available from 32 countries from all WHO regions except the WHO European Region, with the majority of data available for the WHO African Region. Detection of metabolic mechanisms is generally more difficult than for target-site mutations because the former requires the use of fresh mosquito specimens or more advanced analytical methods (for the latter, samples can be easily preserved for subsequent testing in a standard molecular laboratory). Alternatively, synergist-insecticide bioassays can be conducted using an adaptation of the WHO susceptibility test or CDC bottle bioassay, as outlined in the updated WHO test procedures document (WHO, 2016b).

As with phenotypic resistance monitoring, evaluation of resistance mechanisms is best conducted periodically for vector species at the same sites in order to identify changes over time. In reality, mechanism assessments are usually conducted as part of research projects or when specific issues (e.g. increases in malaria cases or phenotypic resistance) are identified. Significant sampling bias therefore exists, precluding the ability to conduct wide-scale temporal trend analyses.

Metabolic mechanisms

Synergist-insecticide bioassays, molecular assays and biochemical assays indicated that monooxygenase-based P450s were present throughout Africa in members of the *An. gambiae* complex (including *An. arabiensis, An. gambiae s.s.* and *An. coluzzii*) as well as the *An. funestus* group (Fig. 4.8a). This mechanism was also detected in vectors at one site each in India (*An. culicifacies s.l.*) and Iran (*An. stephensi*). Synergist-insecticide bioassays showed that this mechanism was fully involved in (or fully explained in) observed pyrethroid resistance at numerous sites across all subregions of Africa (Fig. 4.8b).

The main two other groups of metabolic resistance mechanisms were not widely tested, and there were few data for synergist-insecticide bioassays. Both esterase and GST-based mechanisms were detected in *An. gambiae s.l.* and *An. funestus s.l.* populations in Africa, with most data available for west Africa (Fig. 4.8c and Fig. 4.8d). For the vector populations in India and Iran found to harbour monooxygenase-based resistance mechanisms, the former had esterase and the latter had both esterase and GST-based metabolic mechanisms.

Target-site mutations

Molecular assays indicated that the *kdr L1014F* mutation is widespread in *An. gambiae s.l.* throughout Africa (Fig. 4.8e). Previously referred to as west Africa *kdr,* the mutation was also detected in populations from 20 sites in east and southern Africa and *An. stephensi* from Afghanistan, *An. stephensi* and *An. culicifacies s.l.* from India, and *An. sinensis s.l.* from the Republic of Korea. The allelic frequency of this mutation varied widely, with no clear temporal trend (Fig. 4.9a).

The *kdr L1014S* mutation was detected in *An. gambiae s.l.* from seven countries in Africa as well as in *An. sinensis* from China and the Republic of Korea, *An. culicfacies* from India and *An. stephensi* from Afghanistan (Fig. 4.8f). It was not detected in any of the 12 vector species tested in Iran and Papua New Guinea, despite being monitored for. The frequency of the resistance allele remained relatively low in most vector populations (Fig. 4.9b). In some cases, full details of the *kdr* mutation type were not reported (Fig. 4.8g).

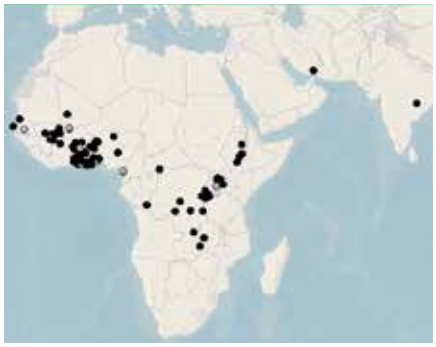
The main target sites for organophosphate and carbamate insecticides are acetylcholinesterases. Modification of *Ace-1R* leads to an insensitive form that confers resistance to these classes. *Ace-1R* has been detected throughout Africa in some species of the *An. gambiae s.l.* complex, although not in *An. melas* (Fig. 4.8h). *Ace-1R* was also detected in *An. sinensis s.l.* from 13 sites in China and the Republic of Korea, and in *An. albimanus* at one site in Peru. There were fluctuations in frequency in particular years (Fig. 4.9c), but due to the relatively small dataset this was probably influenced by the availability of data for specific areas. In particular, few data are available for 2013 onwards. With increasing use of organophosphate and carbamate formulations for IRS, monitoring should include this mechanism.

FIG. 4.8.

Maps show outcomes from resistance mechanisms tests with synergist-insecticide bioassays, molecular assays and biochemical assays for malaria vectors collected 2010–2016

Dots indicate test outcomes by site, where black = detected, grey = tested but not detected/no involvement, dark purple = full involvement, and light purple = partial involvement. Data are shown for a limited geographical area; for all data see Malaria threats map (WHO, 2017b).

a) Monooxygenases (all assays)



b) Monooxygenases (synergist-insecticide)

c) Esterases

d) GSTs

e) *kdr L1014F* mutations

f) *kdr L1014S* mutations

g) *kdr* mutations (type not specified)

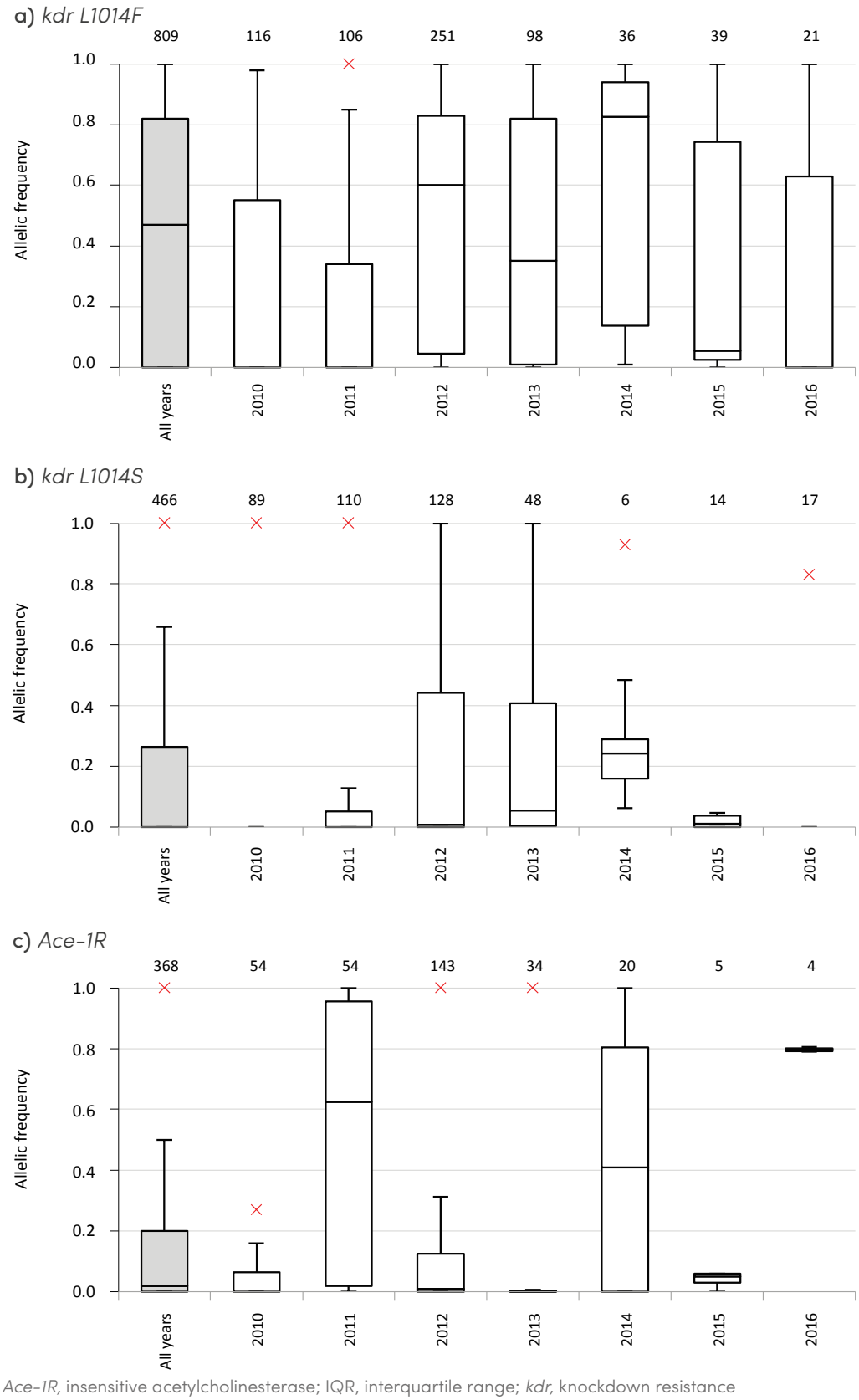
h) *Ace-1R*

Ace-1R, insensitive acetylcholinesterase; GST, glutathione-S-transferase; *kdr*, knockdown resistance

FIG. 4.9.

Frequency of target-site mutations for malaria vectors collected 2010–2016

Boxes show the first and third quartile and whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile. Maximum outliers (red crosses) are shown if outside this range. The horizontal line for each bar shows the median.



Ace-1R, insensitive acetylcholinesterase; IQR, interquartile range; *kdr*, knockdown resistance

5. REGIONAL AND SUBREGIONAL PROFILES OF INSECTICIDE RESISTANCE

Approach

Summary profiles are provided in the following pages for the period 2010–2016 (Fig. 5.1 to Fig. 5.7). Data are presented by WHO region and African subregion for each of the four insecticide classes commonly used in malaria vector control. No profile is included for the WHO European Region owing to a paucity of data.

Maps that show site-level data were generated using the WHO *Malaria threats map* (WHO, 2017b). Resistance status is displayed based on the most recent data available for each site, with priority display of the highest resistance status. Graphs show resistance frequency from the percentage of mosquito survival in discriminating concentration bioassays for the most recent data for each site, with priority display of maximum resistance frequency in the event that more than one insecticide of the class was tested. Graphs may contain data not displayed on maps if geo-reference information is not available

Summary

Resistance to pyrethroid and DDT is ubiquitous throughout all subregions of Africa, with the exception of a few countries in east and southern Africa for which data are limited (Comoros, Namibia and Swaziland) (Fig. 5.1, Fig. 5.2 and Fig. 5.3). Resistance frequencies for these two insecticide classes are particularly high in most countries of west Africa, and some countries of central, east and southern Africa (including Central African Republic, Chad, Ethiopia and Uganda). There is evidence of resistance to carbamates for many countries of the WHO African Region, particularly in west Africa. In general, there is low-frequency organophosphate resistance in central, east and southern Africa, with the exception of the Central African Republic and Ethiopia.

Relatively few or no data were available for most countries of the WHO Region of the Americas, with the exception of Colombia and Nicaragua (Fig. 5.4). Pyrethroid resistance was detected in all but two countries (Guatemala and Nicaragua) in which monitoring was undertaken. There was no DDT resistance detected at most sites tested. There was evidence of emerging resistance to carbamates, particularly in Bolivia, Ecuador and Nicaragua, and organophosphate resistance was detected in four countries.

In the WHO Eastern Mediterranean Region, pyrethroid resistance was confirmed in all countries monitored except Somalia (Fig. 5.5). DDT resistance was detected at most sites tested, and carbamate and organophosphate resistance was detected for at least one site in all countries tested except. Resistance frequency to pyrethroids, DDT and organophosphates was particularly high in malaria vectors tested in Pakistan.

For the WHO European Region, data reporting was required by Tajikistan only, although additional information was previously provided for 2010–2016 by Uzbekistan. These data for 15 sites indicated confirmed resistance in *Anopheles spp.* to DDT and carbamates in some areas, but no confirmed resistance to pyrethroids and organophosphates.

Pyrethroid resistance was detected at sites in seven of the eight countries tested in the WHO South-East Asia Region, although in Timor-Leste no resistance was detected at all 10 sites monitored (Fig. 5.6). Susceptibility to carbamates and organophosphates

was widespread in the region, with the exception of India. Data reported for Sri Lanka (which are not shown in graphs because the country has been certified as having eliminated malaria) show resistance confirmed to all four insecticide classes at sites distributed across the country.

In the WHO Western Pacific Region, pyrethroid resistance was detected in countries of mainland Asia (Cambodia, China, Lao People's Democratic Republic and Viet Nam) and in some Pacific Island countries (Philippines and Vanuatu); resistance frequencies were particularly high at some sites in China (Fig. 5.7). Carbamate resistance was monitored in China only, where it was detected at a few sites. Testing for organophosphate resistance was limited to two countries, where it was detected at many sites in China and one site in the Philippines.

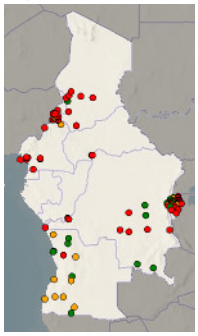
African Region – Central Africa

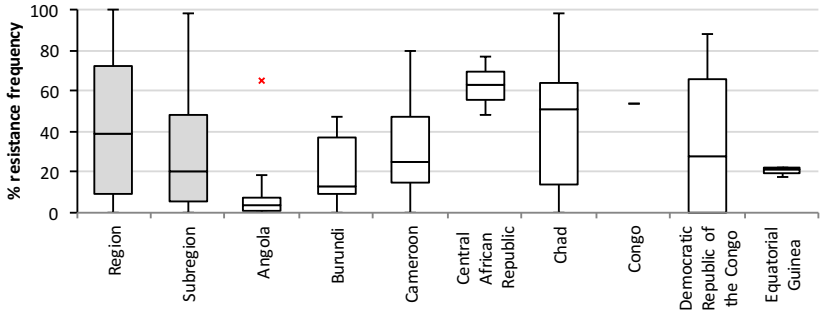
FIG. 5.1.

Resistance status for *Anopheles* malaria vectors from the Central Africa subregion of the WHO African Region for four insecticide classes, 2010–2016

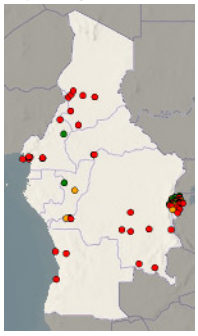
Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range. Region = WHO African Region; Subregion = central Africa.

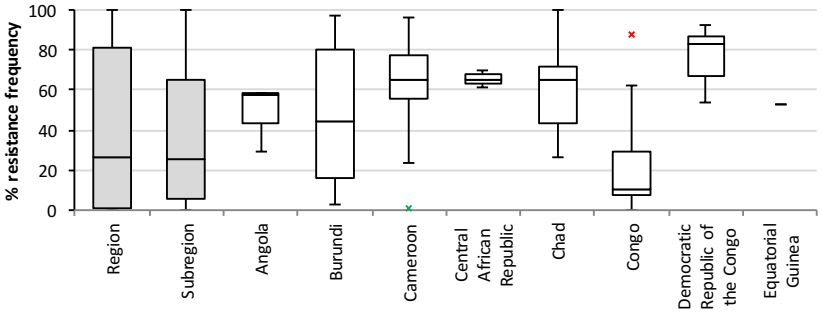
a) Pyrethroids



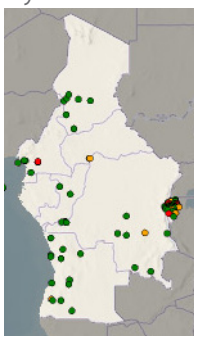


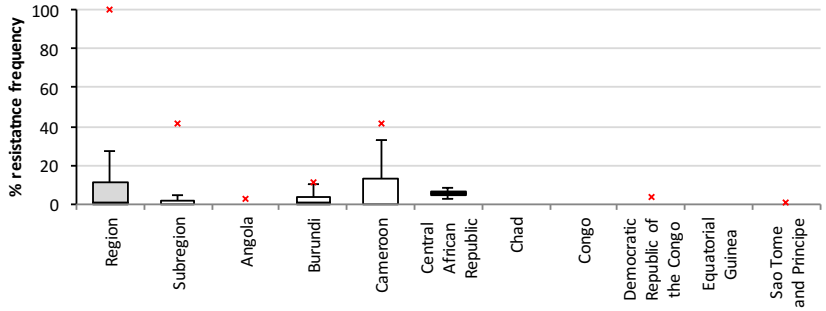
b) Organochlorines



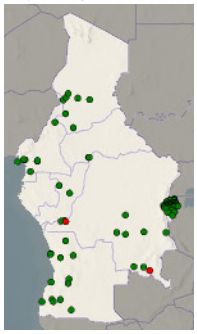


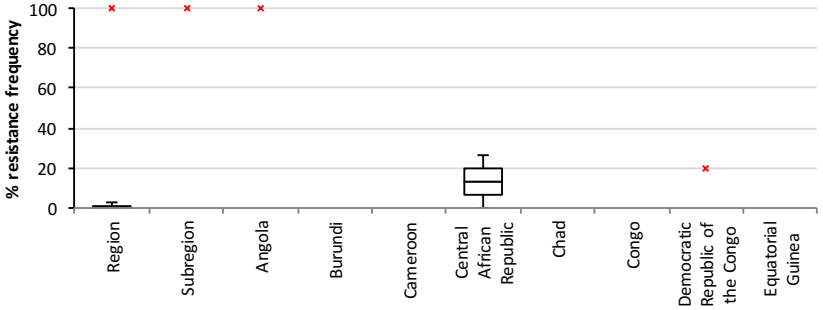
c) Carbamates





d) Organophosphates





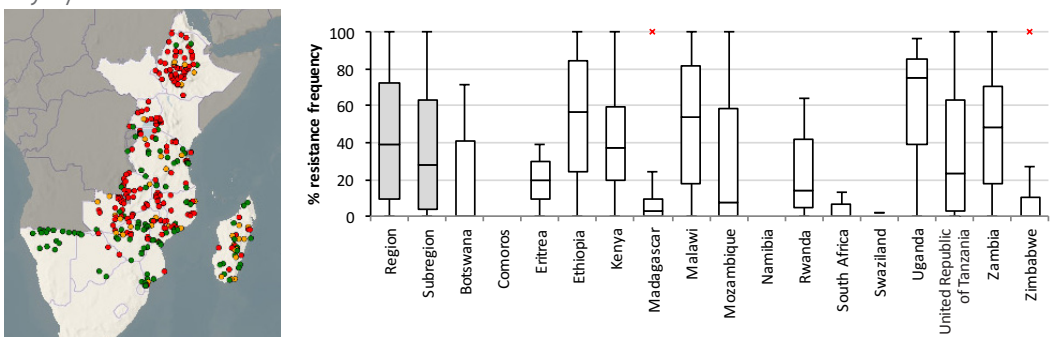
African Region – East and Southern Africa

FIG. 5.2.

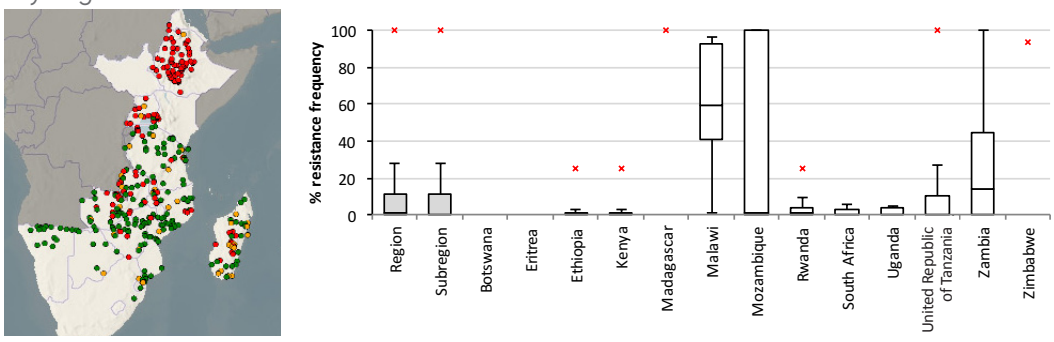
Resistance status for *Anopheles* malaria vectors from East and Southern Africa subregion of the WHO African Region for four insecticide classes, 2010–2016

Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range. Region = WHO African Region; Subregion = east and southern Africa.

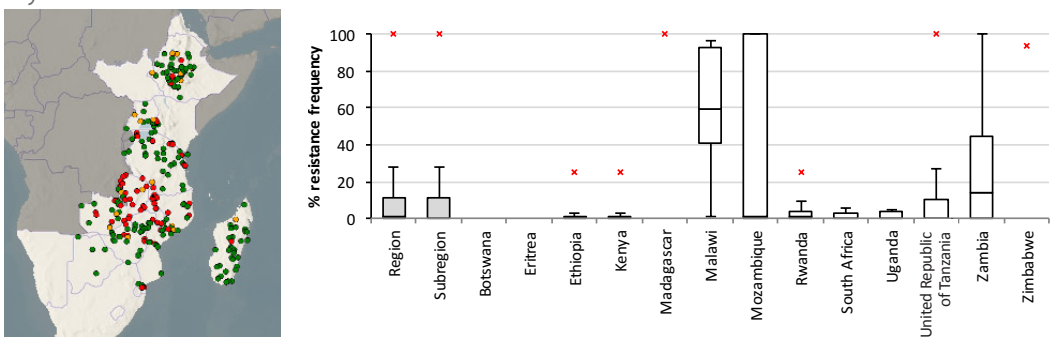
a) Pyrethroids



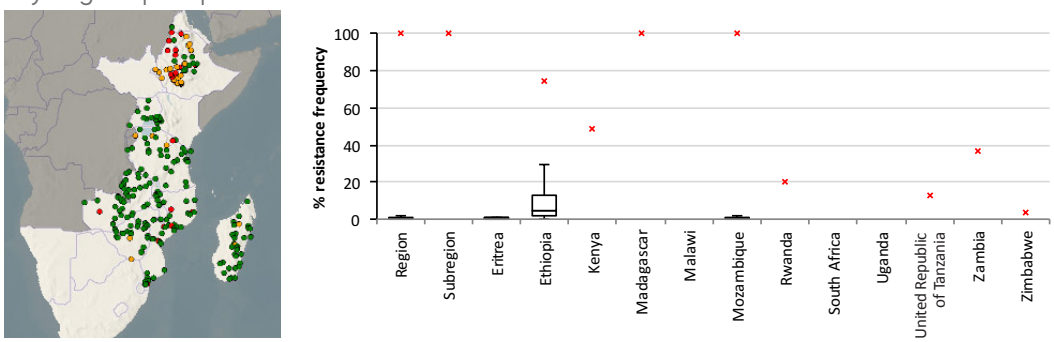
b) Organochlorines



c) Carbamates



d) Organophosphates



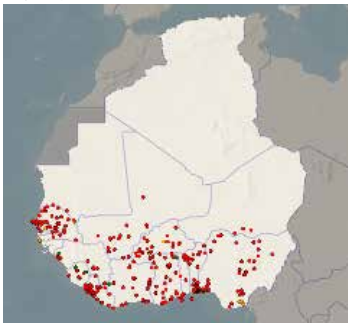
African Region – West Africa

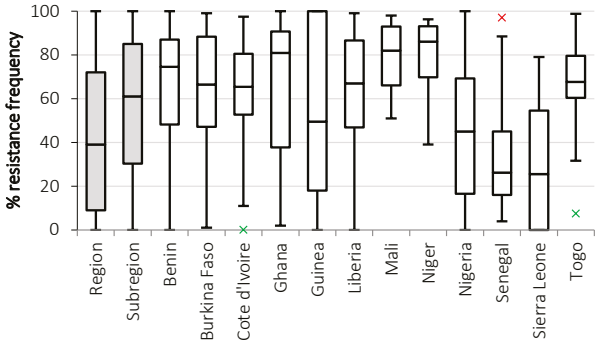
FIG. 5.3.

Resistance status for *Anopheles* malaria vectors from the West Africa subregion of the WHO African Region for four insecticide classes, 2010–2016

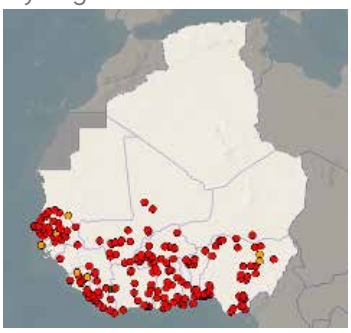
Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Dark grey shading indicates disputed areas or non-Member States. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range. Region = WHO African Region; Subregion = west Africa.

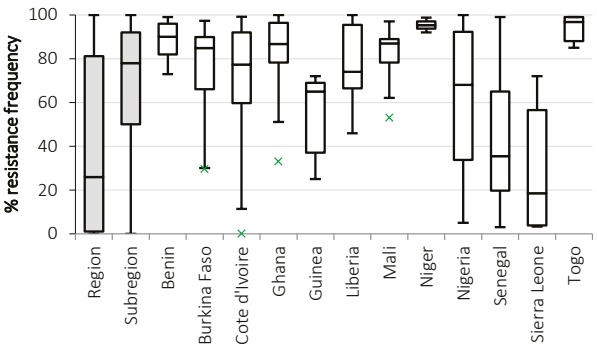
a) Pyrethroids



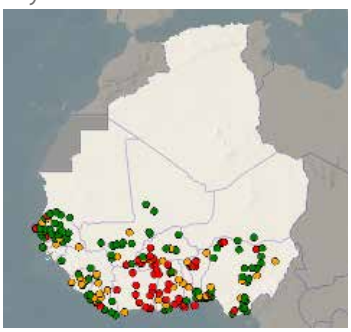


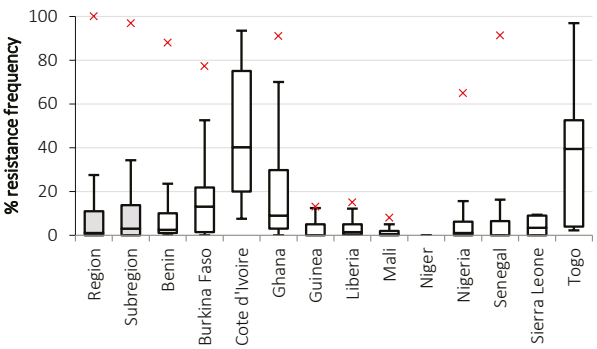
b) Organochlorines



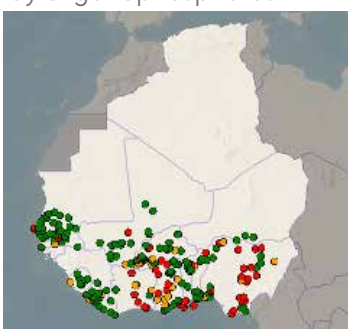


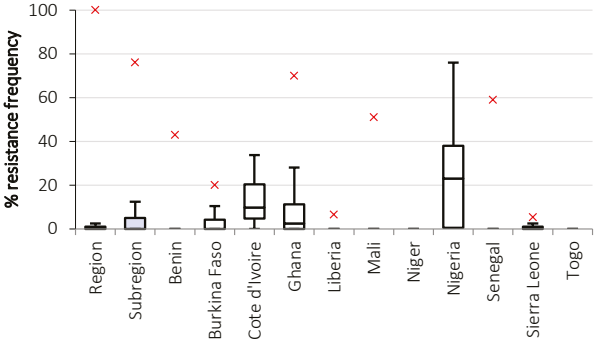
c) Carbamates





d) Organophosphates





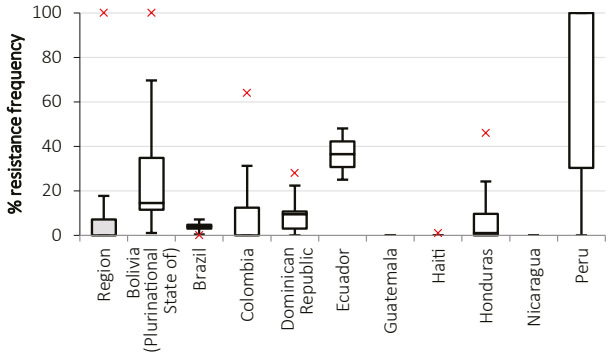
Region of the Americas

FIG. 5.4.

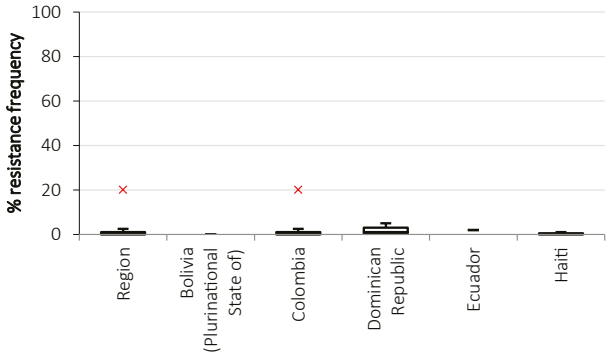
Resistance status for *Anopheles* malaria vectors from the WHO Region of the Americas for four insecticide classes, 2010–2016

Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Dark grey shading indicates disputed areas or non-Member States. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range.

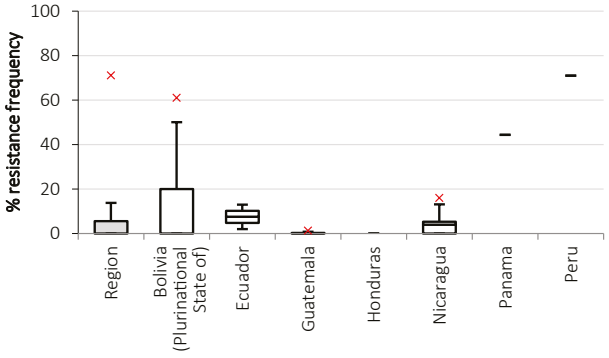
a) Pyrethroids



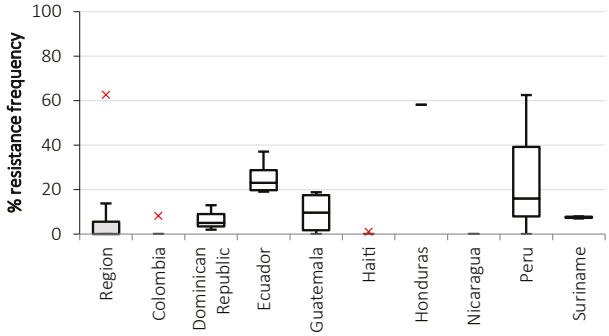
b) Organochlorines



c) Carbamates



d) Organophosphates



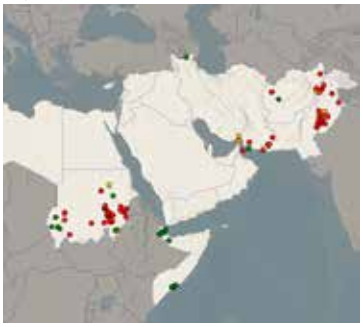
Region of the Eastern Mediterranean

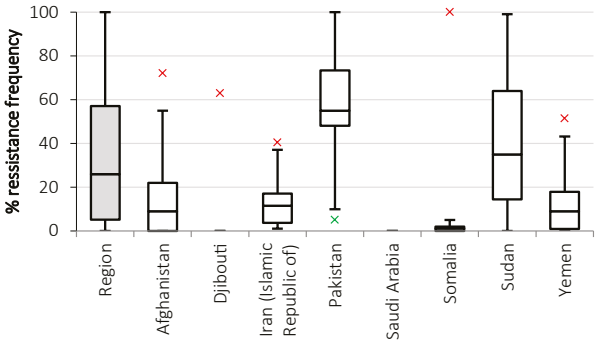
FIG. 5.5.

Resistance status for *Anopheles* malaria vectors from the WHO Eastern Mediterranean Region for four insecticide classes, 2010–2016

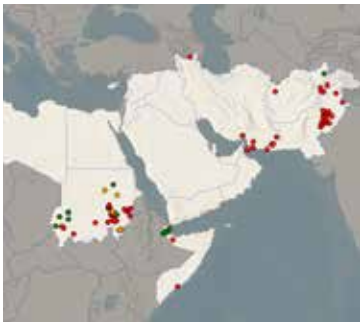
Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Dark grey shading indicates disputed areas or WHO non-Member States. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range.

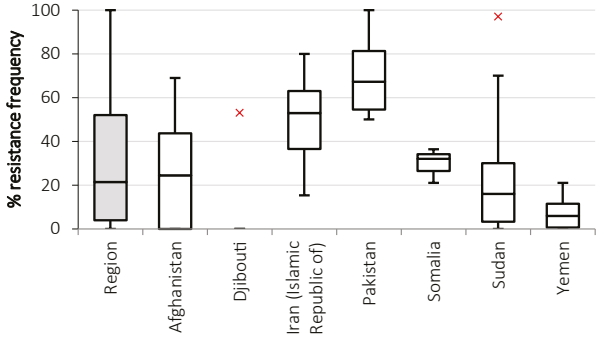
a) Pyrethroids



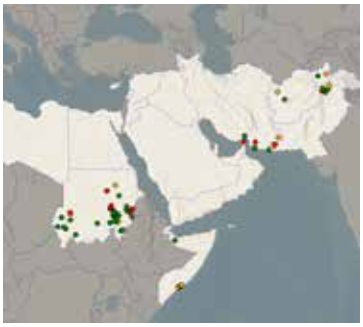


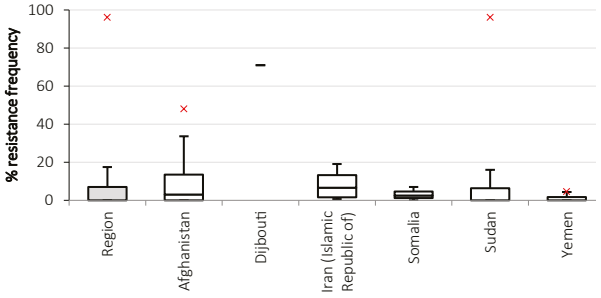
b) Organochlorines



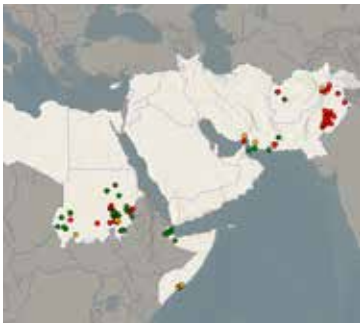


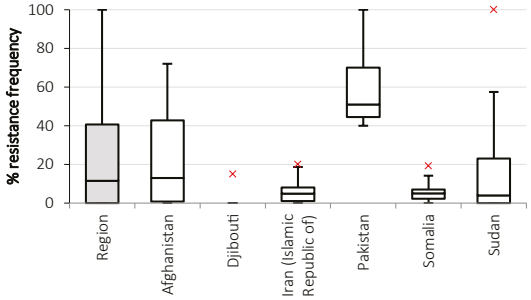
c) Carbamates





d) Organophosphates





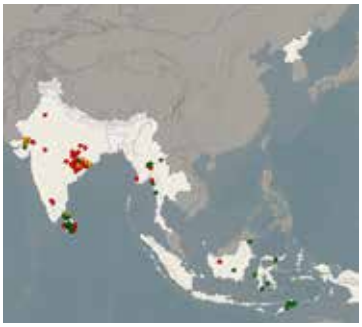
South-East Asia Region

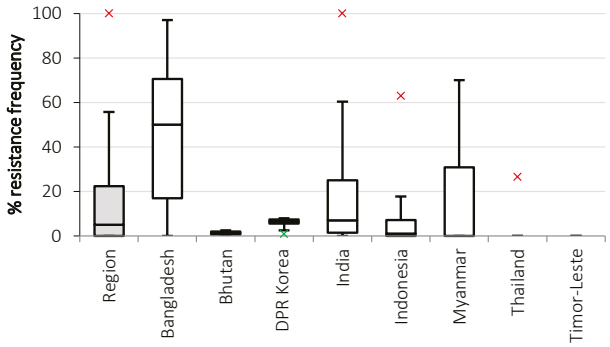
FIG. 5.6.

Resistance status for *Anopheles* malaria vectors from the WHO South-East Asia Region for four insecticide classes, 2010–2016

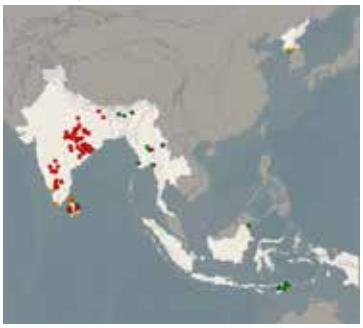
Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Dark grey shading indicates disputed areas or WHO non-Member States. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range.

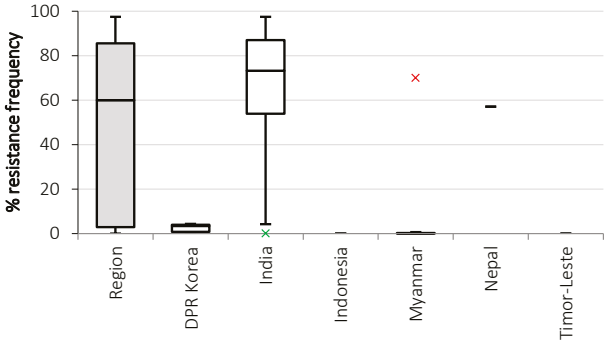
a) Pyrethroids



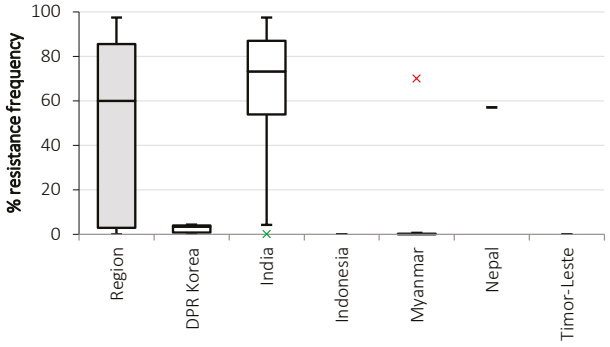


b) Organochlorines

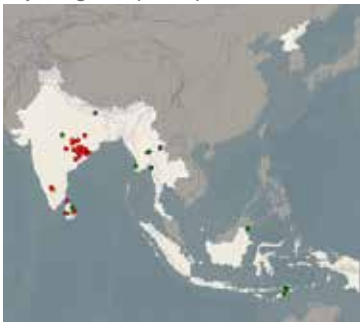


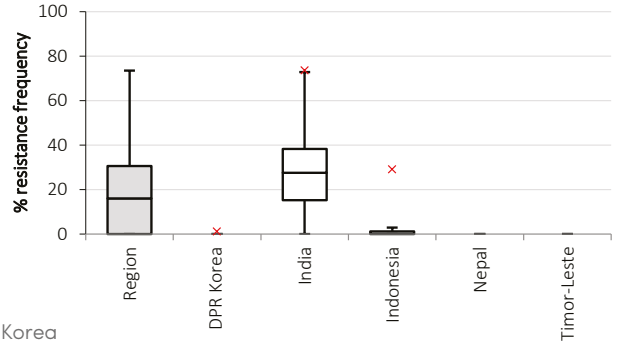


c) Carbamates



d) Organophosphates





DPR Korea: Democratic People's Republic of Korea

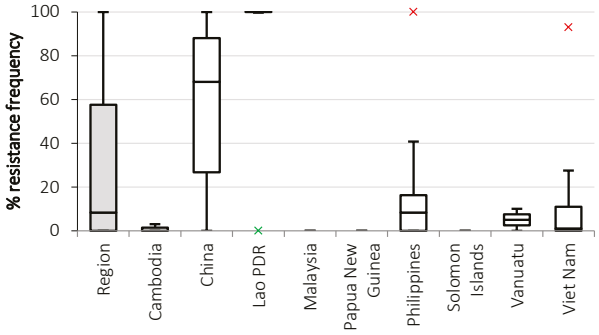
Western Pacific Region

FIG. 5.7.

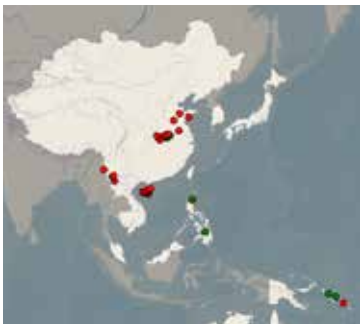
Resistance status for *Anopheles* malaria vectors from the WHO Western Pacific Region for four insecticide classes, 2010–2016

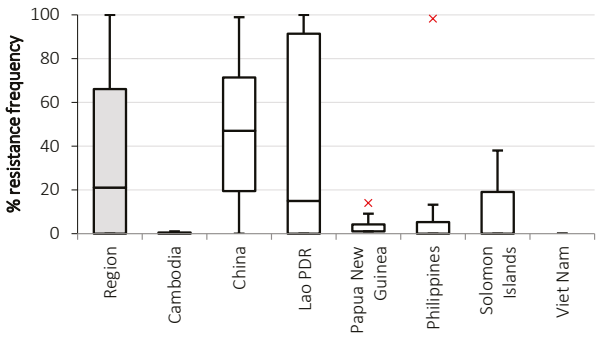
Status is indicated on the maps by colour: red = confirmed resistance, orange = possible resistance, and green = susceptibility. Light grey shading shows those countries outside of the WHO region. Dark grey shading indicates disputed areas or WHO non-Member States. Graphs show resistance frequency in discriminating concentration bioassays; the horizontal line for each bar shows the median and boxes show the first and third quartile. Whiskers show 1.5× IQR above third quartile and 1.5× IQR below first quartile; maximum (red cross) and minimum (green cross) outliers are shown if outside this range.

a) Pyrethroids

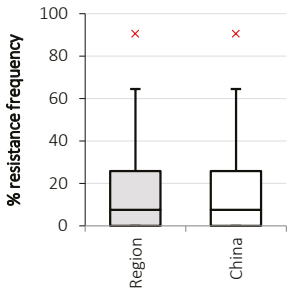


b) Organochlorines

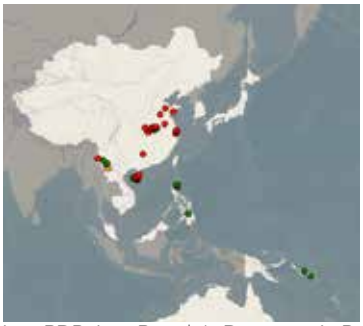


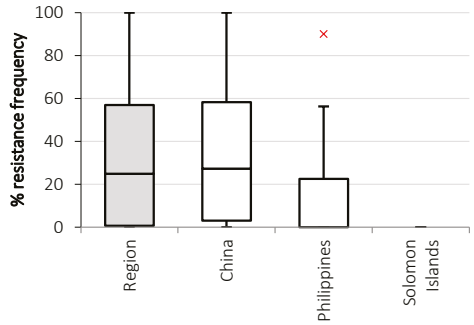


c) Carbamates



d) Organophosphates





Lao PDR: Lao People's Democratic Republic

6. CHALLENGES TO INSECTICIDE RESISTANCE MONITORING AND DATA INTERPRETATION

Monitoring of vector resistance is essential to inform the appropriate selection of insecticidal vector-control tools, with the ultimate aim of maintaining effective malaria prevention. Sufficient local capacity is required to collect, analyse and interpret resistance information to support planning and implementation. Such capacity is also needed to guide monitoring activities to ensure that data are relevant and informative (e.g. through selection of appropriate sentinel sites and consideration of the relative importance of resistance in vector species in relation to their contribution to local malaria transmission).

Funding for resistance monitoring has, in general, increased in sub-Saharan Africa since 2010.[1] Nevertheless, in many countries such monitoring is still not conducted on a routine basis because of ongoing challenges at national and subnational levels, driven largely by insufficient technical and infrastructural capacity and financial resources (Mnzava et al., 2015). The lack of data – particularly longitudinal information from established sentinel sites – limits assessment of spatial and temporal trend that could be used to better inform the development and implementation of vector-control strategies.

For the current report (and for the *Malaria threats map* application), the validity, accuracy and completeness of data are contingent on quality and timely reporting to WHO by Member States (and, in some cases, development partners such as PMI). However, it is likely that significant additional data are available that have not been reported to WHO and therefore are not considered in this report. Many control programmes rely on research institutes or other partners for support in resistance monitoring. Leveraging the capacity of partner institutions can be efficient for data generation but also relies on the coordination of activities by the NMCP and the provision of data by partners in a timely and useful manner. Data are often provided in reports rather than in a format that allows easy incorporation into the established NMCP database. In many countries, there is a clear need for improved data sharing among both national and international stakeholders operating within the country, with WHO current working to streamline data reporting requirements. This will support more appropriate use of data to inform decisions on vector-control strategies, for which the options are likely to increase as proven new tools become available for use.

Along with new vector-control tools, technologies and approaches, there is a need for improved methods of resistance surveillance. The common phenotypic monitoring procedures of WHO susceptibility tests and CDC bottle bioassays provide useful information but are subject to variability based on test conditions (mainly humidity and temperature). These tests also require a minimum of 150 adult female test mosquitoes (including controls), which can often be difficult to obtain. There have also been issues with supply of WHO susceptibility test kits that WHO are currently working to remedy. There is a need for alternative surveillance approaches that are practical and scalable at national and subnational levels, and that provide rapid information to programmes to inform decision-making. Such approaches should consider not only the tools that are currently available but also the pipeline of new tools under development. Further research and innovation in this area is encouraged.

1 Mainly with the support of PMI and the Global Fund to Fight AIDS, Tuberculosis and Malaria.

7. FUTURE OUTLOOK

The *Global technical strategy for malaria 2016–2030* (WHO, 2015) identified vector resistance to the insecticides used in core prevention tools as one of the key biological challenges to malaria control and elimination (WHO, 2016a). Resistance has now been reported in all major malaria vector species and against all classes of insecticide. Between 2010 and 2016, resistance to at least one of the four classes of insecticide recommended by WHO was reported by 61 malaria-endemic countries, with resistance to two or more classes reported by 50 of these countries (WHO, 2017e). Insecticide resistance to all four classes is widespread, and it is increasingly reported, particularly to pyrethroids and in certain vectors, such as *An. funestus s.l.*

The complete extent of resistance is, however, not fully known because many countries do not carry out routine monitoring, or because countries collect data but do not report or share those data in a timely manner. The impact of insecticide resistance on the effectiveness of vector-control tools also remains poorly understood. In general, the limited data available indicate that high frequencies of resistance can lead to failure of IRS, in turn leading to an increase in malaria incidence (Coetzee et al., 2013b). Studies in Uganda and Sudan have further indicated that protection afforded by pyrethroid IRS in areas of pyrethroid resistance is suboptimal (Kigozi et al., 2012; Kafy et al., 2017).

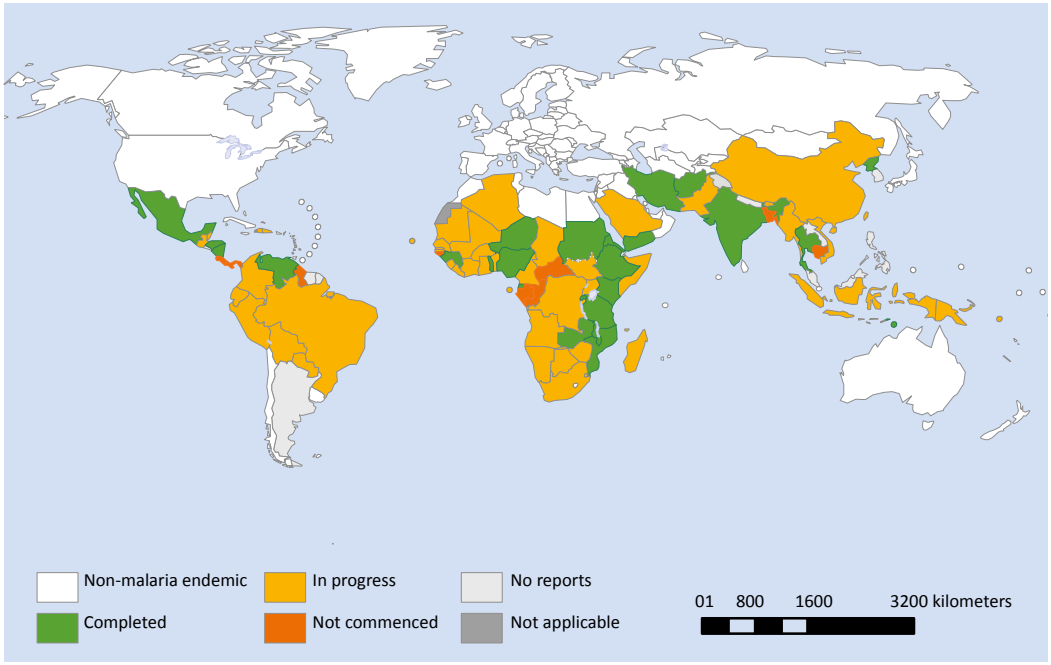
For the efficacy of LLINs, the generation of evidence is more complex, given that nets themselves provide a physical barrier and hence protection of the user. To date control failure of LLINs due to pyrethroid resistance has not be conclusively documented. A 2014 review found that, even in the presence of pyrethroid resistance, treated nets perform better than untreated nets in terms of protection against blood-feeding, and that ITNs can induce significant mosquito mortality (Strode et al., 2014). This was reiterated by a five-country study, which showed that in areas with pyrethroid resistance LLINs provided some level of person protection (WHO, 2016a). It is highly likely that the impact of pyrethroid resistance on the effectiveness of LLINs will vary from setting to setting, depending on numerous factors including the resistance profile of local vector species.

The likelihood that increasing resistance will reduce the efficacy of pyrethroid-based interventions cannot be ignored. Conclusive evidence of control failure should not be the trigger for action to apply good resistance mitigation and management practices that proactively and appropriately leverage available interventions. New tools are essential to address insecticide resistance, and once their public health value has been validated these must be incorporated into malaria control and elimination strategies in a timely manner for optimal impact. Monitoring should also extend to measuring vector susceptibility to those active ingredients anticipated in new tools (e.g. neonicotinoids and pyrroles) to ascertain their utility for disease control and resistance management. For this, standard procedures and discriminating concentrations need to be defined, with work currently ongoing to address this need.

Priority actions include establishing and implementing national insecticide resistance monitoring and management plans, in line with the WHO *Global plan for insecticide resistance management in malaria vectors* (WHO, 2012; 2017a). Some progress has been made in this regard, but further effort is required (Fig. 7.1). New vector monitoring and control tools and approaches are also urgently required.

FIG. 7.1.

Status of national insecticide resistance monitoring and management plans, as of October 2017



Data source: WHO (2017e)

8. CONCLUSION

Significant reductions in malaria burden have been achieved since 2000 but progress has now stalled (WHO, 2017). Renewed efforts are required to sustain the gains made so far, with additional efforts and financing essential to secure further declines in malaria. Vector control has been responsible for averting hundreds of millions of malaria cases, but the principal tools remain insecticide based, and their continuing effectiveness is threatened by insecticide resistance. Plans have been formulated to address this situation (WHO, 2012), but implementation has been slow and further efforts and resources are needed (WHO, 2015). Information on insecticide resistance must continue to be gathered, shared and used appropriately in local decision-making processes. National programmes require detailed information to guide their operational planning and responses to address insecticide resistance and maintain the effectiveness of malaria vector control. Tools such as the WHO database and *Malaria threats map* have been made available to assist with providing a consolidated overview of the situation. Further development of these tools is ongoing to maximize their usefulness in supporting decision-making processes and the formulation of public health policy.

REFERENCES

Abbott WS (1925). A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–7.

Barnes KI, Durrheim DN, Little F, Jackson A, Mehta U, Allen E et al. (2005). Effect of Artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. PLoS Med 2(11):e330.

Brogdon WG, McAllister JC, Corwin AM, Cordon-Rosales C (1999). Independent selection of multiple mechanisms for pyrethroid resistance in Guatemalan *Anopheles albimanus* (Diptera: Culicidae). J Econ Entomol 92(2):298–302 (http://www.ncbi.nlm.nih.gov/pubmed/10333749, accessed 18 February 2018).

Cibulskis RE, Alonso P, Aponte J, Aregawi M, Barrette A, Bergeron L et al. (2016). Malaria: Global progress 2000 – 2015 and future challenges. Infect Dis Poverty 5(61).

Coetzee M, Hunt RH, Wilkerson R, Della Torre A, Coulibaly MB, Besansky NJ (2013a). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. Zootaxa 3619:246–74 (http://www.ncbi.nlm.nih.gov/pubmed/26131476, accessed 18 February 2018).

Coetzee M, Kruger P, Hunt RH, Durrheim DN, Urbach J, Hansford CF (2013b). Malaria in South Africa: 110 years of learning to control the disease. S Afr Med J 103(10 Pt 2):770–8.

Edi CV, Djogbenou L, Jenkins AM, Regna K, Muskavitch MA, Poupardin R et al. (2014). CYP6 P450 enzymes and ACE-1 duplication produce extreme and multiple insecticide resistance in the malaria mosquito *Anopheles gambiae*. PLoS Genet 10(3):e1004236.

Hardstone MC, Leichter CA, Scott JG (2009). Multiplicative interaction between the two major mechanisms of permethrin resistance, *kdr* and cytochrome P450-monooxygenase detoxification, in mosquitoes. J Evol Biol 22(2):416–23.

Hargreaves K, Koekemoer LL, Brooke BD, Hunt RH, Mthembu J, Coetzee M (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. Med Vet Entomol 14:181–9.

Huijben S, Paaijmans KP (2017). Putting evolution in elimination: winning our ongoing battle with evolving malaria mosquitoes and parasites. Evol Appl 00:1–16

IRAC (2011). Prevention and management of insecticide resistance in vectors of public health importance (Second edition). Insecticide Resistance Action Committee (IRAC).

Kafy HT, Ismail BA, Mnzava AP, Lines J, Abdin MSE, Eltaher JS et al. (2017). Impact of insecticide resistance in *Anopheles arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation. Proc Natl Acad Sci USA 114(52):E11267–e75.

Kigozi R, Baxi SM, Gasasira A, Sserwanga A, Kakeeto S, Nasr S et al. (2012). Indoor residual spraying of insecticide and malaria morbidity in a high transmission intensity area of Uganda. PLoS One 7(8):e42857.

Mnzava AP, Knox TB, Temu EA, Trett A, Fornadel C, Hemingway J et al. (2015). Implementation of the global plan for insecticide resistance management in malaria vectors: Progress, challenges and the way forward. Malar J 14:173.

Ranson H, Lissenden N (2016). Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends Parasitol 32(3):187–96.

Ranson H, Rossiter L, Ortelli F, Jensen B, Wang X, Roth CW et al. (2001). Identification of a novel class of insect glutathione S-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. Biochem J 359:295–304.

REX Consortium (2013). Heterogeneity of selection and the evolution of resistance. Trends Ecol Evol 28(2):110–8.

Riveron JM, Ibrahim SS, Chanda E, Mzilahowa T, Cuamba N, Irving H et al. (2014). The highly polymorphic CYP6M7 cytochrome P450 gene partners with the directionally selected CYP6P9a and CYP6P9b genes to expand the pyrethroid resistance front in the malaria vector *Anopheles funestus* in Africa. BMC Genomics 15:817.

South A, Hastings IM (2018). Insecticide resistance evolution with mixtures and sequences: a model-based explanation. Malar J 17(1):80.

Sternberg ED, Thomas MB (2017). Insights from agriculture for the management of insecticide resistance in disease vectors. Evol Appl 00:1–11.

Strode C, Donegan S, Garner P, Enayati AA, Hemingway J (2014). The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: Systematic review and meta-analysis. PLoS Med 11(3):e1001619.

Thomsen EK, Strode C, Hemmings K, Hughes AJ, Chanda E, Musapa M et al. (2014). Underpinning sustainable vector control through informed insecticide resistance management. PLoS One 9(6):e99822.

Trape JF, Tall A, Diagne N, Ndiath O, Ly AB, Faye J et al. (2011). Malaria morbidity and pyrethroid resistance after the introduction of insecticide-treated bednets and artemisinin-based combination therapies: A longitudinal study. Lancet Infect.Dis. 11(12):925–32.

Vulule JM, Beach RF, Atieli FK, McAllister JC, Brogdon WG, Roberts JM et al. (1999). Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. Med Vet Entomol 13(3):239–44.

WHO (2012). Global plan for insecticide resistance management in malaria vectors. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/gpirm/, accessed April 2018).

WHO (2014). WHO guidance for countries on combining indoor residual spraying and long-lasting insecticidal nets. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/who-guidance-combining-irs-llins/, accessed April 2018).

WHO (2015). Global technical strategy for malaria 2016–2030. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/areas/global_technical_strategy/, accessed April 2018).

WHO (2016a). Implications of insecticide resistance for malaria vector control. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/insecticide-resistance-implications/, accessed April 2018).

WHO (2016b). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition). Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/9789241511575/, accessed April 2018).

WHO (2017a). Framework for a national plan for monitoring and management of malaria vectors. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/9789241512138/, accessed April 2018).

WHO (2017b). Malaria threats map: tracking biological challenges to malaria control and elimination. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/maps/threats/, accessed April 2018).

WHO (2017c). Malaria vector control policy recommendations and their applicability to product evaluation. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/atoz/vector-control-recommendations/, accessed April 2018).

WHO (2017d). WHO Pesticide Evaluation Scheme. Geneva: World Health Organization (WHO), Report of the twentieth WHOPES working group meeting (http://www.who.int/whopes/resources/who_htm_ntd_whopes_2017.04/, accessed April 2018).

WHO (2017e). World malaria report 2017. Geneva: World Health Organization (WHO) (http://www.who.int/malaria/publications/world-malaria-report-2017/, accessed April 2018).

Wondji CS, Coleman M, Kleinschmidt I, Mzilahowa T, Irving H, Ndula M et al. (2012). Impact of pyrethroid resistance on operational malaria control in Malawi. Proc Natl Acad Sci USA 109(47):19063–70.

ANNEX 1

Overview of outcomes by country for Anopheles malaria vector insecticide resistance monitoring with four insecticide classes using standard bioassays,[a] for 2010–2016. Countries are listed by WHO region. Further details are available on the *Malaria threats map* (WHO, 2017b).

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Central Africa							
Angola	Pyrethroids	2010–2015	18	94%	36%	100%	*An. coustani, An. gambiae s.l.*
	Organochlorines	2010	3	52%	42%	71%	*An. gambiae s.l.*
	Organophosphates	2010–2015	13	100%	98%	100%	*An. coustani, An. gambiae s.l.*
	Carbamates	2010–2015	13	99%	89%	100%	*An. coustani, An. gambiae s.l.*
Burundi	Pyrethroids	2014	6	82%	53%	100%	*An. gambiae s.l.*
	Organochlorines	2014	6	52%	3%	97%	*An. gambiae s.l.*
	Organophosphates	2014	6	100%	100%	100%	*An. gambiae s.l.*
	Carbamates	2014	6	97%	89%	100%	*An. gambiae s.l.*
Cameroon	Pyrethroids	2010–2015	46	73%	20%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.s.*
	Organochlorines	2010–2015	11	35%	4%	99%	*An. coluzzii, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Organophosphates	2010–2015	7	100%	100%	100%	*An. funestus s.l., An. gambiae s.s.*
	Carbamates	2010–2013	8	91%	59%	100%	*An. gambiae s.l., An. gambiae s.s.*
Central African Republic	Pyrethroids	2014	2	54%	23%	98%	*An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2014	2	43%	30%	59%	*An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2014	2	94%	74%	100%	*An. funestus s.l., An. gambiae s.l.*
	Carbamates	2014	2	96%	92%	100%	*An. funestus s.l., An. gambiae s.l.*
Chad	Pyrethroids	2010–2014	9	57%	2%	100%	*An. gambiae s.l.*
	Organochlorines	2011–2014	7	40%	0%	74%	*An. gambiae s.l.*
	Organophosphates	2011–2014	7	100%	100%	100%	*An. gambiae s.l.*
	Carbamates	2011–2014	7	100%	100%	100%	*An. gambiae s.l.*
Congo	Pyrethroids	2013	1	77%	46%	89%	*An. gambiae s.l.*
	Organochlorines	2013–2014	4	61%	12%	100%	*An. gambiae s.l.*
	Organophosphates	2013–2014	4	100%	100%	100%	*An. gambiae s.l.*
	Carbamates	2013–2014	4	100%	100%	100%	*An. gambiae s.l.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Democratic Republic of the Congo	Pyrethroids	2010–2016	12	81%	12%	100%	*An. gambiae s.l.*
	Organochlorines	2010–2015	9	33%	8%	100%	*An. gambiae s.l.*
	Organophosphates	2012–2015	10	99%	80%	100%	*An. gambiae s.l.*
	Carbamates	2010–2016	10	100%	96%	100%	*An. gambiae s.l.*
Equatorial Guinea	Pyrethroids	2013–2014	3	84%	78%	98%	*An. coluzzii, Anopheles spp.*
	Organochlorines	2014	1	48%	48%	48%	*An. coluzzii*
	Organophosphates	2014	1	100%	100%	100%	*An. coluzzii*
	Carbamates	2010–2014	2	100%	100%	100%	*An. coluzzii, Anopheles spp.*
Rwanda	Pyrethroids	2010–2015	46	85%	23%	100%	*An. chrysti, An. coustani, An. gambiae s.l.*
	Organochlorines	2011–2015	41	90%	51%	100%	*An. chrysti, An. coustani, An. gambiae s.l.*
	Organophosphates	2011–2015	44	100%	99%	100%	*An. chrysti, An. coustani, An. gambiae s.l.*
	Carbamates	2011–2015	43	97%	75%	100%	*An. chrysti, An. coustani, An. gambiae s.l.*
Sao Tome and Principe	Carbamates	2014–2015	7	100%	99%	100%	*An. gambiae s.s.*
East and southern Africa							
Botswana	Pyrethroids	2010–2014	7	79%	29%	100%	*An. gambiae s.l.*
	Organochlorines	2010–2014	8	99%	97%	100%	*An. gambiae s.l.*
	Carbamates	2011–2012	4	100%	100%	100%	*An. gambiae s.l.*
Comoros	Pyrethroids	2014–2015	6	100%	100%	100%	*An. gambiae s.l.*
Eritrea	Pyrethroids	2010–2014	2	86%	61%	100%	*An. gambiae s.l.*
	Organochlorines	2010–2014	2	80%	66%	91%	*An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2013–2014	2	100%	99%	100%	*An. gambiae s.l.*
	Carbamates	2013–2014	2	100%	100%	100%	*An. gambiae s.l.*
Ethiopia	Pyrethroids	2010–2016	81	48%	0%	100%	*An. arabiensis, An. gambiae s.l.*
	Organochlorines	2010–2016	69	20%	0%	93%	*An. arabiensis, An. gambiae s.l.*
	Organophosphates	2010–2016	61	92%	25%	100%	*An. arabiensis, An. gambiae s.l.*
	Carbamates	2010–2016	75	98%	67%	100%	*An. arabiensis, An. gambiae s.l.*
Kenya	Pyrethroids	2010–2016	113	71%	0%	100%	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. rivulorum*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Kenya	Organochlorines	2010–2013	14	72%	34%	100%	*An. arabiensis, An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. rivulorum*
	Organophosphates	2010–2016	46	99%	51%	100%	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. rivulorum*
	Carbamates	2010–2016	47	98%	75%	100%	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s., An. rivulorum*
Madagascar	Pyrethroids	2010–2016	50	97%	53%	100%	*An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2010–2015	43	89%	27%	100%	*An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2010–2016	41	100%	94%	100%	*An. funestus s.l., An. gambiae s.l.*
	Carbamates	2010–2016	44	98%	44%	100%	*An. funestus s.l., An. gambiae s.l.*
Malawi	Pyrethroids	2010–2015	70	58%	0%	100%	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l.*
	Organochlorines	2010–2015	17	87%	5%	100%	*An. funestus s.l., An. funestus s.s.*
	Organophosphates	2010–2015	23	100%	99%	100%	*An. funestus s.l., An. funestus s.s., An. gambiae s.l.*
	Carbamates	2010–2015	25	59%	0%	100%	*An. arabiensis, An. funestus s.l., An. funestus s.s., An. gambiae s.l.*
Mozambique	Pyrethroids	2010–2016	73	81%	0%	100%	*An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2010–2016	62	98%	50%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2011–2016	46	88%	0%	100%	*An. funestus s.l., An. gambiae s.l.*
	Carbamates	2010–2016	45	78%	0%	100%	*An. funestus s.l., An. gambiae s.l.*
Namibia	Pyrethroids	2010–2014	19	100%	100%	100%	*An. arabiensis*
	Organochlorines	2010–2014	19	100%	98%	100%	*An. arabiensis*
South Africa	Pyrethroids	2014–2015	2	94%	87%	100%	*An. arabiensis, An. merus*
	Organochlorines	2010–2015	3	93%	84%	100%	*An. arabiensis, An. merus*
	Carbamates	2014–2015	2	97%	94%	100%	*An. arabiensis, An. merus*
	Organophosphates	2015	2	100%	100%	100%	*An. arabiensis*
Swaziland	Pyrethroids	2011	1	98%	98%	98%	*An. gambiae s.s.*
	Organochlorines	2011	1	100%	100%	100%	*An. gambiae s.s.*
Uganda	Pyrethroids	2011–2016	21	53%	4%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s., An. parensis*
	Organochlorines	2011–2015	11	63%	10%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s., An. parensis*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Uganda	Organophosphates	2011–2016	13	100%	99%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s., An. parensis*
	Carbamates	2011–2016	16	97%	81%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s., An. parensis*
United Republic of Tanzania (Mainland)	Pyrethroids	2010–2016	68	79%	0%	100%	*An. arabiensis, An. gambiae s.l.*
	Organochlorines	2010–2015	50	92%	13%	100%	*An. arabiensis, An. gambiae s.l.*
	Organophosphates	2011–2016	39	99%	83%	100%	*An. arabiensis, An. gambiae s.l.*
	Carbamates	2010–2016	50	90%	20%	100%	*An. gambiae s.l.*
United Republic of Tanzania (Zanzibar)	Pyrethroids	2011–2014	16	58%	7%	98%	*An. arabiensis, An. gambiae s.l.*
	Organophosphates	2013–2014	4	100%	100%	100%	*An. gambiae s.l.*
	Carbamates	2012–2014	8	100%	100%	100%	*An. gambiae s.l.*
Zambia	Pyrethroids	2011–2016	109	60%	0%	100%	*An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Organochlorines	2010–2016	71	79%	0%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Organophosphates	2011–2016	71	100%	63%	100%	*An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Carbamates	2011–2016	87	78%	0%	100%	*An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
Zimbabwe	Pyrethroids	2011–2016	37	89%	0%	100%	*An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2011–2016	33	98%	85%	100%	*An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2012–2016	30	100%	96%	100%	*An. funestus s.l., An. gambiae s.l.*
	Carbamates	2012–2016	31	94%	6%	100%	*An. funestus s.l., An. gambiae s.l.*
West Africa							
Benin	Pyrethroids	2010–2016	70	61%	0%	100%	*An. coluzzii, An. funestus s.l., An. funestus s.s., An. gambiae s.l., An. gambiae s.s.*
	Organochlorines	2010–2015	17	15%	0%	99%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2010–2016	25	96%	57%	100%	*An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Carbamates	2010–2016	37	89%	12%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
Burkina Faso	Pyrethroids	2010–2016	42	52%	1%	100%	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
	Organochlorines	2010–2016	23	26%	3%	70%	*An. coluzzii, An. gambiae s.l.*
	Organophosphates	2010–2016	39	97%	80%	100%	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
	Carbamates	2010–2016	37	84%	23%	100%	*An. arabiensis, An. coluzzii, An. gambiae s.l., An. gambiae s.s.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Côte d'Ivoire	Pyrethroids	2010–2013	24	50%	3%	100%	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
	Organochlorines	2010–2013	18	25%	0%	100%	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
	Organophosphates	2010–2013	12	88%	66%	100%	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
	Carbamates	2010–2013	17	55%	4%	92%	*An. coluzzii, An. gambiae s.l., An. gambiae s.s.*
Ghana	Pyrethroids	2010–2016	46	50%	0%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2010–2016	36	18%	0%	67%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2010–2016	45	94%	30%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Carbamates	2010–2016	40	84%	9%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
Guinea	Pyrethroids	2012–2015	10	47%	0%	100%	*An. gambiae s.l.*
	Organochlorines	2014–2015	5	46%	28%	75%	*An. gambiae s.l.*
	Carbamates	2014–2015	5	97%	87%	100%	*An. gambiae s.l.*
Liberia	Pyrethroids	2010–2016	35	43%	1%	100%	*An. gambiae s.l.*
	Organochlorines	2013–2016	19	22%	0%	54%	*An. gambiae s.l.*
	Organophosphates	2011–2016	24	99%	94%	100%	*An. gambiae s.l.*
	Carbamates	2010–2016	26	97%	85%	100%	*An. gambiae s.l.*
Mali	Pyrethroids	2010–2016	17	43%	0%	98%	*An. gambiae s.l.*
	Organochlorines	2010–2016	18	26%	1%	76%	*An. gambiae s.l.*
	Organophosphates	2012–2016	18	99%	49%	100%	*An. gambiae s.l.*
	Carbamates	2010–2016	18	97%	66%	100%	*An. gambiae s.l.*
Niger	Pyrethroids	2013	4	32%	4%	84%	*An. coluzzii*
	Organochlorines	2013	2	5%	1%	8%	*An. coluzzii*
	Organophosphates	2013	3	100%	100%	100%	*An. coluzzii*
	Carbamates	2013	3	100%	100%	100%	*An. coluzzii*
Nigeria	Pyrethroids	2010–2016	54	79%	0%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Organochlorines	2010–2016	52	42%	0%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*
	Organophosphates	2012–2016	39	83%	24%	100%	*An. funestus s.l., An. gambiae s.l.*
	Carbamates	2010–2016	52	96%	35%	100%	*An. coluzzii, An. funestus s.l., An. gambiae s.l.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Senegal	Pyrethroids	2010–2016	50	73%	3%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Organochlorines	2010–2015	46	64%	1%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Organophosphates	2010–2016	44	99%	41%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
	Carbamates	2010–2016	46	92%	9%	100%	*An. arabiensis, An. funestus s.l., An. gambiae s.l., An. gambiae s.s.*
Sierra Leone	Pyrethroids	2010–2016	8	71%	21%	100%	*An. gambiae s.l.*
	Organochlorines	2010–2016	8	70%	28%	97%	*An. gambiae s.l.*
	Organophosphates	2010–2016	8	99%	95%	100%	*An. gambiae s.l.*
	Carbamates	2010–2016	8	96%	91%	100%	*An. gambiae s.l.*
Togo	Pyrethroids	2011–2013	5	37%	1%	93%	*An. gambiae s.l.*
	Organochlorines	2011–2013	5	6%	0%	15%	*An. gambiae s.l.*
	Organophosphates	2011–2013	2	100%	100%	100%	*An. gambiae s.l.*
	Carbamates	2011–2013	5	73%	3%	99%	*An. gambiae s.l.*
Americas							
Bolivia (Plurinational State of)	Pyrethroids	2011–2015	6	91%	59%	100%	*An. darlingi*
	Organochlorines	2011–2015	4	100%	100%	100%	*An. darlingi*
	Carbamates	2011–2015	5	87%	39%	100%	*An. darlingi*
Brazil	Pyrethroids	2011–2014	4	94%	82%	100%	*An. albitarsis, An. darlingi, Anopheles spp.*
Colombia	Pyrethroids	2011–2016	35	92%	36%	100%	*An. albimanus, An. darlingi, An. marajoara, An. nuneztovari*
	Organochlorines	2011–2015	21	98%	80%	100%	*An. albimanus, An. darlingi, An. marajoara, An. nuneztovari*
	Organophosphates	2011–2015	27	99%	92%	100%	*An. albimanus, An. darlingi, An. marajoara, An. nuneztovari*
Dominican Republic	Pyrethroids	2012–2014	10	96%	72%	100%	*An. albimanus*
	Organochlorines	2012–2014	3	98%	95%	100%	*An. albimanus*
	Organophosphates	2012–2014	3	93%	87%	98%	*An. albimanus*
Ecuador	Pyrethroids	2011–2012	2	73%	45%	100%	*An. albimanus*
	Organochlorines	2011	1	98%	98%	98%	*An. albimanus*
	Organophosphates	2011–2012	4	78%	63%	91%	*An. albimanus*
	Carbamates	2011	2	92%	87%	98%	*An. albimanus*
Guatemala	Pyrethroids	2011–2016	8	100%	100%	100%	*An. albimanus, An. darlingi, An. vestitipennis*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Guatemala	Organophosphates	2011	4	90%	81%	100%	*An. albimanus, An. vestitipennis*
	Carbamates	2011	4	100%	99%	100%	*An. albimanus, An. vestitipennis*
Haiti	Pyrethroids	2013	16	100%	99%	100%	*An. albimanus*
	Organochlorines	2013–2014	3	100%	99%	100%	*An. albimanus*
	Organophosphates	2013–2016	8	100%	99%	100%	*An. albimanus*
Honduras	Pyrethroids	2013–2016	18	94%	54%	100%	*An. albimanus*
	Organophosphates	2016	1	60%	42%	77%	*An. albimanus*
	Carbamates	2013–2016	6	100%	100%	100%	*An. albimanus*
Nicaragua	Pyrethroids	2010–2016	41	100%	98%	100%	*An. albimanus, An. pseudopunctipennis, Anopheles spp.*
	Organophosphates	2011	4	100%	100%	100%	*An. albimanus, An. pseudopunctipennis*
	Carbamates	2010–2011	8	97%	84%	100%	*An. albimanus, An. pseudopunctipennis*
Panama	Carbamates	2011	1	56%	56%	56%	*An. albimanus*
Peru	Pyrethroids	2013–2015	10	97%	93%	100%	*An. albimanus, An. darlingi*
	Organophosphates	2013–2015	3	68%	38%	100%	*An. albimanus, An. darlingi*
	Carbamates	2015	1	29%	29%	29%	*An. darlingi*
Suriname	Organophosphates	2013	2	96%	92%	100%	*An. aquasalis*
Eastern Mediterranean							
Afghanistan	Pyrethroids	2010–2016	25	89%	28%	100%	*An. culicifacies s.l., An. hyrcanus s.l., An. pulcherrimus, An. splendidus, An. stephensi, An. subpictus s.l., An. superpictus*
	Organochlorines	2010–2016	16	74%	31%	100%	*An. culicifacies s.l., An. hyrcanus s.l., An. pulcherrimus, An. stephensi, An. subpictus s.l., An. superpictus*
	Organophosphates	2014–2016	14	80%	28%	100%	*An. culicifacies s.l., An. hyrcanus s.l., An. pulcherrimus, An. stephensi, An. superpictus*
	Carbamates	2010–2016	22	91%	52%	100%	*An. culicifacies s.l., An. hyrcanus s.l., An. pulcherrimus, An. stephensi, An. superpictus*
Djibouti	Pyrethroids	2011-2016	6	77%	37%	100%	*An. gambiae s.l.*
	Organochlorines	2011-2016	6	91%	47%	100%	*An. gambiae s.l.*
	Organophosphates	2011-2016	6	96%	85%	100%	*An. gambiae s.l.*
	Carbamates	2016	1	29%	29%	29%	*An. gambiae s.l.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Iran (Islamic Republic of)	Pyrethroids	2010–2015	14	91%	48%	100%	*An. culicifacies s.l., An. d'thali, An. sacharovi, An. stephensi, An. subpictus s.l.*
	Organochlorines	2010–2016	11	62%	20%	100%	*An. culicifacies s.l., An. d'thali, An. sacharovi, An. stephensi, An. subpictus s.l.*
	Organophosphates	2010–2015	11	96%	80%	100%	*An. culicifacies s.l., An. d'thali, An. sacharovi, An. stephensi, An. subpictus s.l.*
	Carbamates	2010–2015	10	95%	81%	100%	*An. culicifacies s.l., An. d'thali, An. stephensi, An. subpictus s.l.*
Pakistan	Pyrethroids	2011–2013	20	70%	17%	100%	*An. culicifacies s.l., An. stephensi*
	Organochlorines	2011–2013	20	44%	25%	52%	*An. culicifacies s.l., An. stephensi*
	Organophosphates	2011–2013	20	58%	30%	100%	*An. culicifacies s.l., An. stephensi*
Saudi Arabia	Pyrethroids	2012	2	100%	100%	100%	*An. arabiensis*
Somalia	Pyrethroids	2010–2013	10	98%	81%	100%	*An. arabiensis, An. funestus s.l.*
	Organochlorines	2010–2012	3	72%	64%	79%	*An. arabiensis*
	Organophosphates	2010–2012	6	97%	81%	100%	*An. arabiensis, An. funestus s.l.*
	Carbamates	2010–2012	6	98%	93%	100%	*An. arabiensis, An. funestus s.l.*
Sudan	Pyrethroids	2010–2015	161	72%	1%	100%	*An. arabiensis*
	Organochlorines	2010–2015	89	76%	3%	100%	*An. arabiensis*
	Organophosphates	2010–2015	63	84%	11%	100%	*An. arabiensis*
	Carbamates	2010–2016	91	89%	4%	100%	*An. arabiensis*
Yemen	Pyrethroids	2010–2016	15	88%	49%	100%	*An. arabiensis, An. culicifacies s.l.*
	Organochlorines	2010–2016	11	93%	79%	100%	*An. arabiensis*
	Carbamates	2010–2016	11	99%	95%	100%	*An. arabiensis*
Europe							
Azerbaijan	Pyrethroids	2010	4	98%	93%	100%	*An. maculipennis, An. sacharovi*
Tajikistan	Pyrethroids	2011–2012	3	100%	100%	100%	*An. pulcherrimus, An. superpictus*
	Organophosphates	2011–2012	2	100%	100%	100%	*An. pulcherrimus, An. superpictus*
Uzbekistan	Pyrethroids	2014	6	100%	100%	100%	*An. maculipennis, An. superpictus*
	Organochlorines	2014	6	92%	49%	100%	*An. maculipennis, An. superpictus*
	Organophosphates	2014	6	100%	100%	100%	*An. maculipennis, An. superpictus*
	Carbamates	2014	6	94%	75%	100%	*An. maculipennis, An. superpictus*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
South-East Asia							
Bangladesh	Pyrethroids	2012–2014	7	63%	3%	100%	*An. annularis, An. philippinensis, An. vagus*
Bhutan	Pyrethroids	2010–2012	2	99%	97%	100%	*An. pseudowillmori*
Democratic People's Republic of Korea	Pyrethroids	2011–2016	7	98%	90%	100%	*Anopheles spp.*
	Organochlorines	2011	5	97%	96%	100%	*Anopheles spp.*
	Organophosphates	2014–2016	6	100%	100%	100%	*Anopheles spp.*
	Carbamates	2016	6	100%	99%	100%	*Anopheles spp.*
India	Pyrethroids	2010–2016	185	83%	0%	100%	*An. annularis, An. culicifacies s.l., An. fluviatilis, An. jeyporiensis, An. stephensi, An. subpictus s.l.*
	Organochlorines	2010–2016	72	42%	3%	100%	*An. annularis, An. culicifacies s.l., An. fluviatilis, An. philippinensis, An. stephensi, An. subpictus s.l.*
	Organophosphates	2010–2016	54	72%	35%	100%	*An. culicifacies s.l., An. fluviatilis, An. stephensi, An. subpictus s.l.*
	Carbamates	2013–2015	68	78%	27%	100%	*An. culicifacies s.l.*
Indonesia	Pyrethroids	2011–2016	31	94%	37%	100%	*An. aconitus, An. barbirostris, An. indefinitus, An. letifer, An. maculatus s.l., An. peditaeniatus, An. philippinensis, An. punctulatus, An. subpictus s.l., An. sundaicus s.l., An. tessellatus, An. vagus, Anopheles spp.*
	Organochlorines	2011	1	100%	100%	100%	*An. sundaicus s.l.*
	Organophosphates	2011–2014	10	100%	98%	100%	*An. barbirostris, An. farauti s.s., An. letifer, An. peditaeniatus, An. subpictus s.l., An. sundaicus s.l., An. vagus*
	Carbamates	2011–2016	26	97%	71%	100%	*An. aconitus, An. barbirostris, An. kochi, An. letifer, An. maculatus s.l., An. peditaeniatus, An. pharoensis, An. punctulatus, An. subpictus s.l., An. sundaicus s.l., An. vagus*
Myanmar	Pyrethroids	2011–2016	38	94%	22%	100%	*An. aconitus, An. annularis, An. culicifacies s.l., An. dirus s.l., An. hyrcanus s.l., An. jamesii, An. maculatus s.l., An. minimus s.l., An. peditaeniatus, An. philippinensis, An. sinensis s.l., An. sundaicus s.l., An. vagus*
	Organochlorines	2011–2015	8	95%	30%	100%	*An. aconitus, An. annularis, An. dirus s.l., An. hyrcanus s.l., An. maculatus s.l., An. minimus s.l., An. sinensis s.l., An. sundaicus s.l.*
	Organophosphates	2011–2016	10	97%	40%	100%	*An. aconitus, An. annularis, An. dirus s.l., An. hyrcanus s.l., An. maculatus s.l., An. philippinensis, An. sinensis s.l.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Nepal	Organochlorines	2014–2016	1	40%	0%	100%	*An. annularis, An. culicifacies s.l., An. fluviatilis, An. jamesii, An. peditaeniatus, An. pseudowillmori, An. splendidus, An. vagus*
	Organophosphates	2014	1	100%	100%	100%	*An. annularis, An. culicifacies s.l., An. jamesii, An. peditaeniatus, An. splendidus, An. vagus*
	Carbamates	2014	1	100%	100%	100%	*An. annularis, An. culicifacies s.l., An. fluviatilis, An. jamesii, An. vagus*
Sri Lanka	Pyrethroids	2010–2016	119	95%	24%	100%	*An. aconitus, An. annularis, An. culicifacies s.l., An. jamesii, An. maculatus s.l., An. nigerrimus, An. peditaeniatus, An. pseudojamesii, An. subpictus s.l., An. tessellatus, An. vagus, An. varuna*
	Organochlorines	2010–2014	18	74%	0%	100%	*An. annularis, An. culicifacies s.l., An. jamesii, An. subpictus s.l., An. tessellatus, An. vagus*
	Organophosphates	2010–2016	55	80%	0%	100%	*An. annularis, An. culicifacies s.l., An. jamesii, An. peditaeniatus, An. subpictus s.l., An. tessellatus, An. vagus, An. varuna*
	Carbamates	2010–2015	38	69%	0%	100%	*An. annularis, An. culicifacies s.l., An. jamesii, An. pallidus, An. peditaeniatus, An. pseudojamesii, An. subpictus s.l., An. vagus, An. varuna*
Thailand	Pyrethroids	2015–2016	7	97%	74%	100%	*An. barbirostris, An. maculatus s.l., An. minimus s.l.*
Timor-Leste	Pyrethroids	2010–2016	10	100%	100%	100%	*An. barbirostris, An. subpictus s.l., An. sundaicus s.l.*
	Organochlorines	2010–2016	9	100%	100%	100%	*An. annularis, An. barbirostris, An. subpictus s.l., An. sundaicus s.l., An. tessellatus, An. vagus*
	Organophosphates	2010–2016	10	100%	100%	100%	*An. barbirostris, An. minimus s.l., An. subpictus s.l., An. sundaicus s.l., An. vagus*
	Carbamates	2012–2016	7	100%	100%	100%	*An. barbirostris, An. subpictus s.l., An. sundaicus s.l., An. vagus*
Western Pacific							
Cambodia	Pyrethroids	2014–2016	3	91%	48%	100%	*An. barbirostris, An. dirus s.l., An. epiroticus, An. maculatus s.l., An. minimus s.l., An. philippinensis, An. vagus*
	Organochlorines	2014–2016	3	94%	44%	100%	*An. dirus s.l., An. epiroticus, An. maculatus s.l., An. minimus s.l., An. vagus*
China	Pyrethroids	2010–2016	95	45%	2%	100%	*An. jeyporiensis, An. minimus s.l., An. sinensis s.l., An. vagus, Anopheles spp.*
	Organochlorines	2010–2014	51	53%	1%	100%	*An. jeyporiensis, An. minimus s.l., An. sinensis s.l., An. vagus, Anopheles spp.*
	Organophosphates	2010–2014	42	69%	10%	100%	*An. minimus s.l., An. sinensis s.l., An. vagus*
	Carbamates	2011–2013	13	82%	10%	100%	*An. sinensis s.l., Anopheles spp.*

COUNTRY	INSECTICIDE CLASS	YEAR(S)	NUMBER OF SITES TESTED	% MOSQUITO MORTALITY[b]			MOSQUITO SPECIES TESTED[c]
				MEAN	MIN	MAX	
Lao People's Democratic Republic	Pyrethroids	2013–2015	13	43%	0%	100%	*An. aconitus, An. dirus s.l., An. hyrcanus s.l., An. kochi, An. maculatus s.l., An. minimus s.l., An. neivai, An. nivipes, An. philippinensis, An. umbrosus s.l., An. vagus*
	Organochlorines	2013–2015	11	66%	0%	100%	*An. aconitus, An. hyrcanus s.l., An. kochi, An. maculatus s.l., An. minimus s.l., An. neivai, An. nivipes, An. philippinensis, An. umbrosus s.l., An. vagus*
Malaysia	Pyrethroids	2012–2015	7	100%	100%	100%	*An. balabacensis, An. donaldi, An. letifer, An. maculatus s.l., An. sundaicus s.l.*
Papua New Guinea	Pyrethroids	2015	4	100%	100%	100%	*Anopheles spp.*
	Organochlorines	2015	4	96%	86%	99%	*Anopheles spp.*
Philippines	Pyrethroids	2011–2015	39	95%	0%	100%	*An. annularis, An. dispar, An. flavirostris, An. greeni, An. litoralis, An. ludlowae, An. maculatus s.l., An. philippinensis, An. subpictus s.l., An. tessellatus, An. vagus*
	Organochlorines	2011–2015	17	92%	2%	100%	*An. annularis, An. flavirostris, An. ludlowae, An. maculatus s.l., An. vagus*
	Organophosphates	2011–2015	4	78%	10%	100%	*An. annularis, An. flavirostris*
Solomon Islands	Pyrethroids	2013–2015	9	100%	100%	100%	*An. farauti s.l.*
	Organochlorines	2013–2015	3	87%	62%	100%	*An. farauti s.l.*
	Organophosphates	2015	2	100%	100%	100%	*An. farauti s.l.*
Vanuatu	Pyrethroids	2013	2	97%	90%	100%	*An. farauti s.l.*
Viet Nam	Pyrethroids	2010–2016	113	93%	7%	100%	*An. aconitus, An. annularis, An. dirus s.l., An. epiroticus, An. jamesii, An. jeyporiensis, An. kochi, An. maculatus s.l., An. minimus s.l., An. nimpe, An. nivipes, An. philippinensis, An. sinensis s.l., An. splendidus, An. subpictus s.l., An. tessellatus, An. vagus*
	Organochlorines	2011	1	100%	100%	100%	*An. minimus s.l.*

[a] WHO insecticide susceptibility or CDC bottle bioassays using discriminating concentrations.
[b] Adjusted as necessary using Abbott's formula (Abbott, 1925).
[c] Listed alphabetically.

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