CellPress

Special Issue: Vectors

Review

Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control

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Malaria control is reliant on insecticides to control the mosquito vector. As efforts to control the disease have intensified, so has the selection pressure on mosquitoes to develop resistance to these insecticides. The distribution and strength of this resistance has increased dramatically in recent years and now threatens the success of control programs. This review provides an update on the current status of resistance to the major insecticide classes in African malaria vectors, considers the evidence that this resistance is already compromising malaria control efforts, and looks to the future to highlight some of the new insecticide-based tools under development and the challenges in ensuring they are most effectively deployed to manage resistance.

A Limited Toolbox for Malaria Vector Control

Increased coverage of those at risk from malaria with **long-lasting insecticidal nets (LLINs)** (see Glossary), and to a lesser extent **indoor residual spraying (IRS)** with insecticides, together with improvements in case management have had an enormous impact, halving malaria deaths and decreasing disease incidence by over a third since the beginning of the century [1,2]. These interventions are reliant on a small number of insecticides, with just the pyrethroids available for LLINs, and therefore the emergence and spread of insecticide resistance in African malaria vectors is a critical threat to malaria control. Although increases in levels of pyrethroid resistance in *Anopheles* mosquitoes are widely documented, opinion is divided on the current and future impact of this resistance on efforts to reduce or eliminate malaria transmission. This review outlines the challenges in defining the impact of resistance, assesses the strength of the current evidence, and considers the future prospects for malaria control if resistance eventually renders the pyrethroid insecticides obsolete.

A Rapidly Changing Landscape

The distribution of pyrethroid resistance in African malaria vectors was described in a 2011 article in this journal [3]. At this time, pyrethroid-resistant populations of *Anopheles gambiae* were

Trends

Resistance to pyrethroid insecticides, the only class available to treat bed nets, is now ubiquitous in African malaria vectors and resistance to other insecticide classes used for adult mosquito control is increasing.

Critical knowledge gaps impede estimates of the impact of this resistance on malaria transmission but multiple observational studies suggest a rapidly worsening situation.

New approaches to tackle pyrethroid resistance are urgently needed; this will require new products, a more rapid and robust approach to their field evaluation, and acceleration of access to these products where the malaria control challenges posed by resistance are greatest.

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prevalent in western and central Africa but were rarer in southern and eastern countries of the continent. Pyrethroid resistance is now widely spread across the continent with *An. gambiae* in Kenya, much of Tanzania, Zambia, and Zimbabwe resistant to this insecticide class. Data remain scarce for much of central Africa, although reports are emerging of pyrethroid resistance across the Democratic Republic of Congo [4]. In summary, although *An. gambiae* populations fully susceptible to pyrethroids are still present in 2015 [e.g., in parts of Angola, Madagascar, and Mozambique (http://www.africairs.net/wp-content/uploads/2014/11/Multi-Country-Profile-of-Insecticide-Resistance-on-Malaria-Vectors.pdf)] they are becoming increasingly outnumbered by resistant populations (Figure 1A).

Data on resistance in *Anopheles funestus* remain limited (Figure 1B) but, whereas previously pyrethroid resistance in this species was thought to be restricted to southern Africa, it has now been detected in Uganda [5], Kenya [5], Benin [6], and Cameroon [7]. As with *An. gambiae*, fully susceptible populations of *An. funestus* have been reported in some areas of Mozambique (Figure 2D) but resistance is very prevalent in other areas of the country [8]. Furthermore, the trend is similar to that observed in *An. gambiae*, with susceptible populations becoming the exception rather than the norm.

Several countries have established **longitudinal monitoring** in **sentinel sites** enabling temporal changes in the prevalence of resistance to be detected. Examples from Malawi, Mozambique, Tanzania, and Uganda are shown in Figure 2. Changes in the methods and timing of mosquito collections, which may in turn alter the relative proportion of morphologically identical sibling species used in the bioassays, will undoubtedly influence the results and make estimates about the rate of spread of resistance difficult to infer from bioassay data alone. Furthermore, resistance can be remarkably focal [9,10]. Nevertheless, three of the sites shown in Figure 2 exemplify the pattern across large parts of Africa in which 10 years ago control programs were targeting vector populations that had full susceptibility to pyrethroids but are now facing the challenge of over half of the potential malaria vectors having developed resistance to this insecticide class.

The data in Figures 1 and 2, and indeed the vast majority of data currently being collected on insecticide resistance in mosquitoes, indicate the response of the mosquito population to a diagnostic dose of insecticide, but this dose bears little relationship to the field dose of

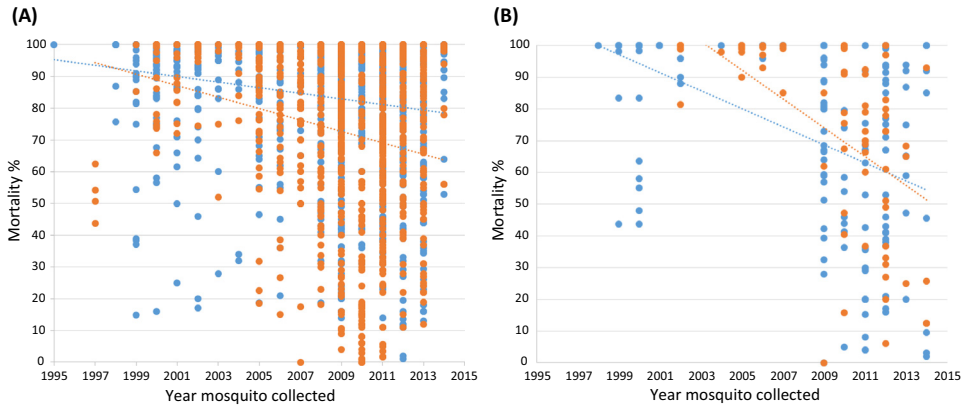

Figure 1. Changes in Pyrethroid Mortality in Major African Malaria Vectors Over Time. Percentage mortality of (A) *Anopheles gambiae* sensu lato (s.l.) and (B) *Anopheles funestus* s.l mosquitoes exposed to 0.05% deltamethrin (blue) or 0.75% permethrin (orange) in World Health Organization (WHO) susceptibility bioassays. Data from 1995 to 2015 were extracted from IR Mapper [21] in August 2015 and supplemented with a literature search for 2014 and 2015 data. Each dot represents a data point extracted from IR Mapper or from the literature search and the dotted lines show trend lines for the mortality rates for each insecticide.

Glossary

***Anopheles gambiae* sensu lato (*An. gambiae* s.l.):** nomenclature used to characterize species of the *Anopheles gambiae* complex, a group of eight distinct sibling mosquito species that are morphologically identical but exhibit different behavioral traits.

Exophilic: term used to describe mosquitoes that generally reside/rest outdoors after taking a blood meal.

Experimental hut: standardized structures that act as a proxy for local houses. Used in the evaluation of vector control tools that target indoor-biting mosquitoes. Modifications to these structures, such as closed eaves and window exit traps, allow standardized collection of entomological end points such as mosquito mortality and deterrency.

Indoor residual spraying (IRS): a vector control intervention that targets indoor-biting mosquitoes. Long-lasting insecticides are applied to wall surfaces inside houses via spraying and susceptible mosquitoes are killed when they come into contact with these surfaces.

Longitudinal monitoring: field study of mosquitoes over time. Information such as mosquito species, abundance, distribution, and susceptibility to insecticide are studied and collated.

Long-lasting insecticidal nets (LLINs): mosquito nets with pyrethroid insecticides incorporated into their fibers. Susceptible mosquitoes are killed on contact with the net's surface. Effective for approximately 3 years.

Randomized controlled trial: a study used to evaluate a specific intervention, drug, or strategy (e.g., mosquito net, malaria prophylaxis). Individuals or groups are assigned at random to one of the interventions under study, with one study arm receiving a control (or placebo) intervention.

Sentinel sites: key geographical locations where extensive information is collected and collated to help inform control programs and policy decisions affecting larger geographical areas.

Slide positivity rate: an alternative measure for malaria incidence. Defined as the number of laboratory-confirmed (using light microscopy) malaria cases per 100 suspected cases examined.

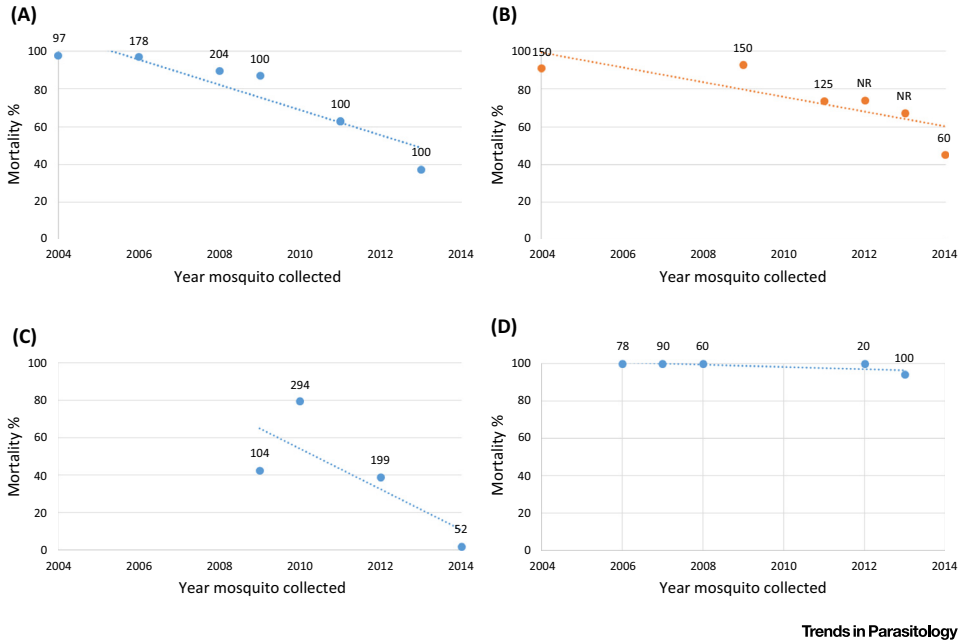

Figure 2. Changes in Mosquito Mortality Over Time. Percentage mortality of *Anopheles gambiae* sensu lato (s.l) from (A) Tororo, Uganda and (B) Arumeru, Tanzania and of *Anopheles funestus* s.l from (C) Chikwawa, Malawi and (D) Mocuba, Mozambique. Mosquitoes were exposed to 0.05% deltamethrin (blue) or 0.75% permethrin (orange) in World Health Organization (WHO) susceptibility bioassays. Data labels show number of mosquitoes exposed per assay. Data were extracted from the IR Mapper [21] August 2015 database. NR, not recorded. When multiple data points existed for a given locality for a particular year the bioassay with the largest sample size was selected or a random number generator was used to select the result.

insecticide. Alternative measures of resistance in which the intensity or strength of the resistance is measured have been described [11] and are being adopted in a small number of field studies [12–14]. Again, longitudinal monitoring of resistance intensity can provide an important insight on the rapid changes occurring in malaria vectors. For example, the exposure time required to kill 50% of the *An. gambiae* population in an area in southwestern Burkina Faso was found to increase tenfold over a single year [13].

Perhaps of more direct relevance in decision making are assays that measure the response of local vectors to locally implemented vector control tools. The simplest example of this is the cone bioassay in which mosquitoes are exposed to a bed net or a sprayed wall for a fixed exposure time and then mortality recorded 1 h and 24 h after exposure [15]. Results from cone bioassays paint an alarming picture, with very low kill rates being observed even after exposure to new nets or freshly sprayed surfaces in several settings [8,11,13,16–18].

Increasing Reports of Resistance to Other Insecticide Classes

Most ***An. gambiae* sensu lato (s.l.)** populations remain susceptible to carbamates (Figure 3A) and organophosphates (Figure 3B), although reports of resistance to these two classes [which share the same mode of action (MoA)] are increasing and may be expected to rise further in areas where IRS programs are replacing pyrethroids with these insecticide classes in response to pyrethroid resistance (see below). Of particular concern are populations that show resistance to all four classes of insecticide available for malaria control, this has been reported in several countries including Côte d'Ivoire [19] and Mali [10].

Keeping track of the spread of insecticide resistance is a challenge for malaria control programs, with many lacking the resources or expertise to conduct regular monitoring and/or the

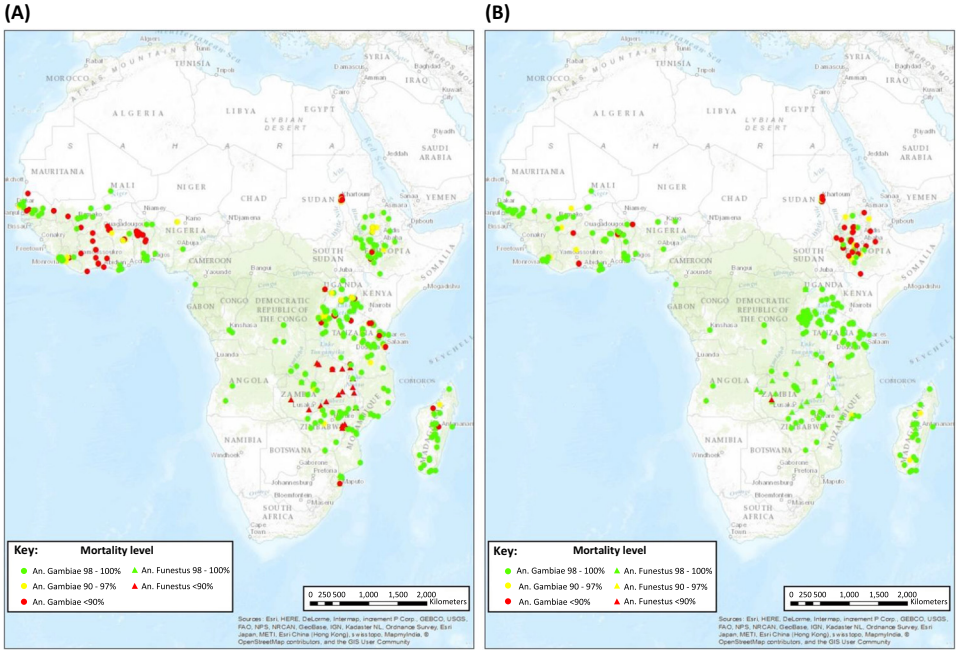

Figure 3. The Distribution of Carbamate (A) and Organophosphate (B) Resistance in Africa. Maps were created from data extracted from the IR Mapper [21] August 2015 database, using data collected between 2011 and 2015 from World Health Organization (WHO) susceptibility and Centers for Disease Control and Prevention (CDC) bottle bioassays. Data were omitted if the insecticide dose tested was not recommended by the WHO or CDC [44] or if the concentration was not recorded.

databases to store and access this data [20]. Two global databases have been established (IR Mapper [21] and VectorBase [22]), with the WHO also planning to establish their own global database [20]. These databases face difficulties in ensuring the timely and comprehensive inclusion of quality-assured data from all sources but an even greater obstacle for countries is using the available resistance data to make informed decisions on malaria control. With just one chemical class available for LLINs and two MoAs for IRS, limited budgets, and widespread resistance already circulating, preserving the susceptibility of malaria vectors is probably beyond the reach of most control programs. As a result, decisions on changes in insecticide use are generally precluded by evidence of control failure with existing tools. A greater understanding of the link between resistance bioassay results and the protective efficacy of LLINs or IRS is important to facilitate this decision making and also to predict the future prospects for malaria control in an era of widespread resistance.

What Is the Impact of Insecticide Resistance on Malaria Transmission?

Measuring the public health impact of insecticide resistance is critical for assessing the changing dynamics of malaria transmission across Africa and mobilizing resources to tackle resistance. There are major challenges in quantifying this relationship (Box 1) and even if it were possible to design and implement appropriate **randomized controlled trials** to assess the impact of resistance, these would be unlikely to yield answers in the time frame necessary for action. Below we review some of the alternative approaches to assessing the impact of pyrethroid resistance.

Experimental hut data could provide information on the impact of resistance on both the personal protection (via blood-feeding inhibition) and the community protection (by increased mosquito mortality) afforded by insecticides, if comparable studies were conducted in areas

Box 1. Challenges in Assessing the Impact of Resistance on Malaria Transmission

Extrapolating from bioassay data indicating that insecticides are killing a smaller proportion of mosquitoes to estimates of the impact of this resistance on malaria control tools is not straightforward. Some of the potential challenges and confounding factors include the following.

- Resistance is a variable trait. The phenotype is influenced by, for example, the age and physiological status of the mosquito [45], the rearing conditions of the larvae before the assay, and the temperature and humidity in the testing room [29]. This introduces large variability into bioassay data, making significant trends difficult to detect.
- Bioassays do not capture the lifetime impact of insecticide exposure. Routine surveillance typically assesses resistance on the basis of 24-h mortality responses. However, if resistant mosquitoes have reduced fitness after surviving exposure to LLINs, the impact of resistance on parasite transmission may be reduced.
- LLINs provide a physical and chemical barrier to mosquitoes. Intact LLINs may still provide a high degree of personal protection even when most mosquitoes are resistant. Yet, once holes appear or insecticide efficacy declines with net age, resistant mosquitoes will have a greater competitive advantage. Studies should evaluate the performance of LLINs that have been in use for a minimum of 1 year under field conditions.
- The interactions between mosquito behavior and insecticide resistance are very poorly understood. Does resistance impact the mosquitoes' ability to detect a blood meal? Are resistant mosquitoes less likely to avoid an insecticide-treated surface? Resistance should be evaluated for its impact on all of the key behavioral traits that influence vectorial capacity [46,47].
- The role of multiple vectors is overlooked. Most studies look at resistance in the species that is easiest to collect (typically *Anopheles gambiae* s.l.) and ignore the role of other major or minor vectors.
- The perfect study to assess the epidemiological impact of resistance is probably impossible to implement. For example: resistance is a constantly evolving trait and cannot be randomized; for ethical reasons it is not practical to withhold an intervention from one study arm to assess the level of protection added by insecticides; and the lack of longitudinal data on resistance generally precludes robust assessments of the changing impact of interventions as resistance emerges.

differing in their resistance phenotype. A recent systematic review of experimental hut studies evaluating LLIN performance demonstrated a small but significant impact of pyrethroid resistance on these entomological indicators [23], but most studies included in this review were conducted before the most potent pyrethroid-resistance mechanisms were widely established and hence may underestimate the current situation across Africa. Mathematical models of malaria transmission can be used to translate entomological outcomes from experimental hut trials into estimates of the number of additional malaria cases due to resistance. A study employing this approach found a positive correlation between insecticide susceptibility status and protection against blood feeding by LLINs [24] and concluded that LLINs would avert up to 40% fewer episodes of malaria in the most resistant areas compared with areas with a fully susceptible population. More recently, modeling of the outcomes of a study in which susceptible or resistant mosquitoes were released into experimental huts containing holed LLINs concluded that the impact of LLINs on reducing malaria transmission was dependent on the level of resistance in the population [25]. A similar conclusion resulted from an earlier study measuring entomological indicators in households using LLINs in areas of Benin where the vectors were either susceptible or resistant to pyrethroids. Sleeping under a LLIN was no more protective than sleeping under an untreated net, regardless of its physical condition, in areas with high pyrethroid resistance [26].

A further indicator that resistance may be compromising the efficacy of control tools is provided by studies reporting the collection of sporozoite-infected mosquitoes either resting on walls newly treated with IRS or inside LLINs [27]. An extension of this approach is to test for association between insecticide resistance markers and *Plasmodium* infection in wild-caught mosquitoes. If resistance is enabling mosquitoes to survive repeated insecticide exposure, the prevalence of sporozoites would be expected to be higher in mosquitoes containing insecticide-resistant alleles; the development of further molecular markers of resistance will facilitate studies of this nature [28].

Under operational settings the impact of insecticide resistance on malaria transmission will be influenced by a large number of factors including those unrelated to the vector itself (e.g., the

efficacy of case management approaches, drug resistance). Ongoing observational studies are attempting to assess the impact of resistance by comparing malaria transmission across areas where vector populations differ in susceptibility to insecticides [29]. However, the challenges of this approach are manifold, particularly due to the confounding effect of differing transmission intensity and vector ecology across sites.

Longitudinal studies, with accurate records of malaria transmission and resistance levels, may provide the best opportunity to observe the impact of resistance. The most widely cited evidence for the impact of resistance comes from such a study in KwaZulu Natal, which demonstrated a correlation between the emergence of pyrethroid resistance and a spike in malaria cases that was later contained by the reintroduction of dichlorodiphenyltrichloroethane (DDT) [30]. More recently a similar conclusion was reached in Senegal, where reduction in LLIN efficacy was attributed to resistance, although the absence of longitudinal resistance data makes this conclusion difficult to validate [31]. Unfortunately, the opportunity for initiating new studies of this nature, at least for pyrethroid resistance in Africa, may have passed, unless good historical data sets already exist.

Indirect evidence that insecticide resistance is impacting malaria transmission can be obtained from retrospective analysis in countries that have changed insecticide class in IRS programs (usually in response to either reports of resistance or increases in malaria cases) and seen an improvement in control. As an example, DDT and pyrethroids were being used for IRS in Uganda despite the known presence of resistance [32]. When these insecticides were replaced with the carbamate bendiocarb, a marked improvement in **slide positivity rates** was observed [32]. Similarly, in Ghana, pyrethroid resistance triggered a switch to the use of the organophosphate insecticide Actellic (primiphos-methyl) for IRS that was associated with a noticeable impact on key indicators of malaria transmission such as the number of children with parasitologically or clinically diagnosed malaria (Figure 4) (http://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy-15/fy-2015-ghana-malaria-operational-plan.pdf?sfvrsn=3).

Despite the rapid emergence of resistance, it is important to remember that sleeping under an LLIN still provides protection from malaria, even in areas with pyrethroid resistance [33].

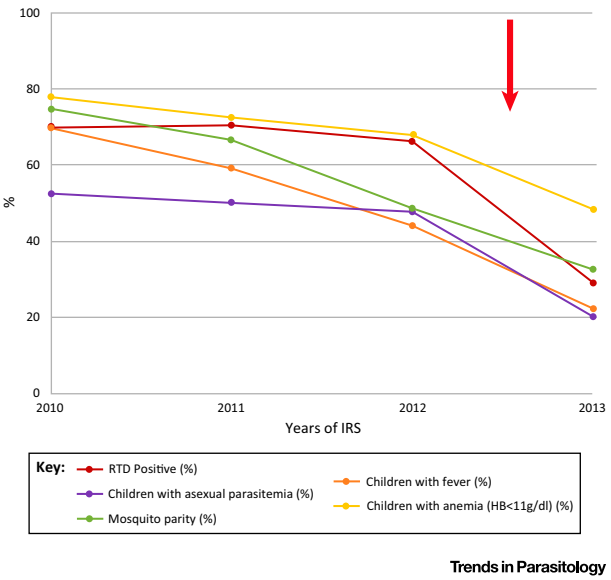

Figure 4. Improvement in Malaria Indicators in Bunkpurugu-Yunyoo, Ghana. Data were collected between 2010 and 2013 after a switch in insecticide class from pyrethroids to organophosphates for indoor residual spraying [President's Malaria Initiative (2015) *Ghana Malaria Operational Plan FY 2015* (http://www.pmi.gov/docs/default-source/default-document-library/malaria-operational-plans/fy-15/fy-2015-ghana-malaria-operational-plan.pdf?sfvrsn=3)]. Arrow indicates when spraying with the organophosphate Actellic CS was introduced. RTD, rapid diagnostic test.

However, vital questions remain to be answered about how much of the protection is due to the physical barrier of the net itself and how the longevity of the protective response (at both the personal and community level) is affected by resistance.

The Response of Control Programs to the Emergence of Resistance

In 2012, the WHO published the Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM), which provides a series of recommendations on action to take if resistance is detected [34]. The options for programs reliant on LLINs are clearly very limited given that there are currently no alternative insecticides for net impregnation and the WHO policy, adopted by the majority of countries in Africa, is to achieve universal LLIN coverage (http://www.who.int/malaria/publications/atoz/who_recommendations_universal_coverage_llins.pdf?ua=1). Essentially, the recommendation is to continue as is if the resistance mechanisms do not impact control but introduce IRS if more potent metabolic resistance mechanisms are present. However, the introduction of IRS is beyond the financial capabilities of many control programs. Furthermore, the benefits of combining the two interventions are far from clear. A meta-analysis of data from 11 countries found significantly reduced parasitemia when the two interventions were combined in areas of medium or high transmission [35], but this study did not include information on the resistance status of the vectors in the various sites. More recently, several randomized controlled trials have been conducted and, interestingly, no benefit was observed by combining LLINs with carbamate or DDT IRS in two studies with resistant vectors [36,37]. However, in a trial in Tanzania, where the more **exophilic** *Anopheles arabiensis* is an important vector, there was significant additional benefit from combining the interventions [38].

For malaria control programs using IRS, it should theoretically be possible to manage insecticide resistance by careful preplanned rotation of insecticide classes with different MoAs (i.e., alternating between DDT or pyrethroids and carbamates or organophosphates). The WHO recommends that pyrethroids are not used for IRS, to reduce the selection pressure on mosquitoes to develop resistance to this class, and many programs have reduced their reliance on this insecticide class either in response to this guidance or due to the loss of control from pyrethroid-based IRS. As an example, the President's Malaria Initiative Africa Indoor Residual Spraying (PMI AIRS) program was operational in 11 countries in 2015, with all programs spraying with carbamates or organophosphates and only one including limited spraying with pyrethroids. Contrast this with the situation in 2011, when 11 of 16 active programs utilized pyrethroids. However, there are financial and logistical challenges associated with switching to an alternative insecticide class and the increase in cost of alternative chemistries is leading to reductions in the number of households protected by IRS [1,20]. Furthermore, some programs that have switched from pyrethroids to carbamates for IRS have witnessed a rapid rise of resistance to the latter class [39].

Future Prospects

New insecticides are urgently needed to counteract the rapid emergence of resistance and sustain efforts to drive malaria transmission to zero. A product development partnership, the Innovative Vector Control Consortium (IVCC), is on track to deliver its target of three new public health pesticides but these are unlikely to reach the market before 2020 (Figure 5). Meanwhile, there is hope that additional classes of insecticides will become available for the control of adult mosquitoes via the reformulation of insecticides already in use in agriculture, mostly targeted for IRS. In 2015, two agrochemical companies announced their intention to market new IRS and/or LLIN products containing previously registered agricultural insecticides (http://www.publichealth.basf.com/agr/ms/public-health/en_GB/content/public-health/our-partners/malaria_control/beating_insecticide_resistance; http://sumivector.com/news/novel-mode-of-action-indoor-residual-spray-irs-product-from-sumitomo-chemical-shows-long?

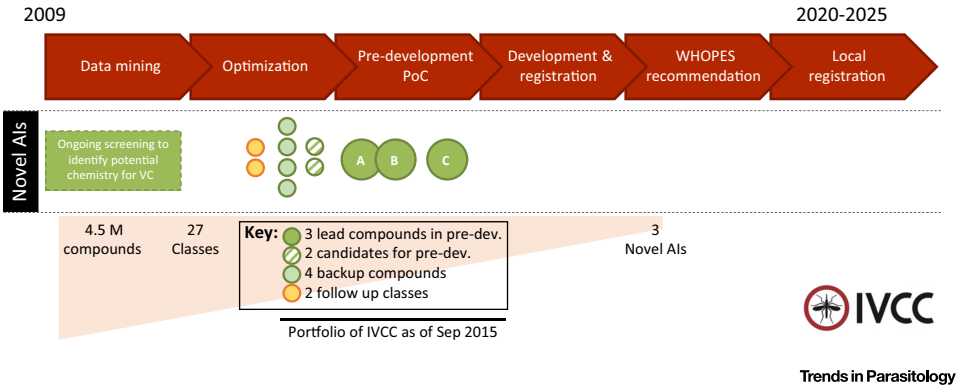

Figure 5. The Innovative Vector Control Consortium (IVCC) Pipeline of Novel Active Ingredients Currently in Development. AI, active ingredient; PoC, proof of concept; VC, vector control; WHOPES, WHO Pesticide Evaluation Scheme. A–C indicate the three lead compounds in predevelopment (pre-dev.). Image courtesy of the IVCC.

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In addition, net manufacturers have developed new LLINs that contain the pyrethroid synergist piperonyl butoxide (PBO) and others are developing nets containing multiple active ingredients [40,41]. Experimental hut trials suggest that these LLINs provide better protection against pyrethroid-resistant mosquitoes than conventional LLINs [41,42] but they have yet to be evaluated in large-scale field trials. Their use may help maintain the personal and community benefit of LLINs in areas with pyrethroid-resistant vectors until novel public health insecticides become available.

It is critical that future insecticide-based approaches are not dependent on a single active ingredient in the way we have been reliant on the pyrethroids since the scale up of malaria control efforts in 2000. There must not be a race to be first to the finish line in the introduction of new insecticides into the marketplace. This would provide a very short-term solution and inevitably lead to the same issues now being faced with pyrethroid resistance. This is the primary driver behind the IVCC's target to develop three novel public health insecticides, allowing for combination and rotation strategies that will optimize performance and reduce the likelihood of resistance developing. However, in addition to new insecticides a multitude of supporting activities are needed to maximize the time until resistance undermines the efficacy of any of these new chemistries. Manufacturers, donors, control programs, the WHO, and other stakeholders must work together to develop and implement resistance management strategies.

New chemistry is only part of the picture. Alternative approaches that reduce our reliance on chemical insecticides are needed, not least to tackle transmission that occurs outside the home where LLINs or IRS are not protective (http://www.who.int/malaria/publications/atoz/technical-note-control-of-residual-malaria-parasite-transmission-sep14.pdf).

Concluding Remarks

Pyrethroid resistance is ubiquitous in African malaria vectors and is rapidly increasing in strength in many regions. Relatively little is known about the fitness costs of resistance although unselected mosquito colonies can maintain their resistance in insectaries suggesting that some of the highly resistant phenotypes now selected for in the field may be stable traits [43]. Even if pyrethroid-resistant mosquitoes were less competitive than their susceptible counterparts when

Outstanding Questions

What is the most informative measure of insecticide resistance? Do current discriminating dose bioassays provide sufficient information or should they be supplemented by an additional test for 'operationally significant' resistance?

Can molecular markers of resistance inform decision making in insecticide selection and resistance management?

How does insecticide resistance influence mosquito behavior and fitness? How does this affect the impact of resistance on malaria transmission?

What is the resistance 'breakpoint' above which LLINs provide only a physical barrier against mosquito bites? Has this already been reached in some settings?

What is the economic impact of insecticide resistance? Can we put a monetary value on preserving susceptibility and, if so, who should bear this cost?

How should new public health insecticides be introduced to delay the emergence of resistance? Can modeling help predict the dynamics of insecticide resistance and develop practical strategies to minimize resistance selection?

selection pressure was removed, it is unlikely that resistance alleles would be rapidly selected against if malaria control programs ceased use of this insecticide class. This is because *Anopheles* mosquitoes are continually exposed to pyrethroids via their use in agriculture and household products (e.g., aerosol sprays, coils). Besides, with hundreds of millions of LLINs in use in Africa, and no non-pyrethroid LLIN products expected for 5 years or more, pyrethroid resistance can only be expected to increase.

Although there are many indicators that pyrethroid resistance is already compromising control, indisputable evidence is lacking. Yet given the complexities in measuring the impact of insecticide resistance (Box 1) we cannot equate lack of evidence of impact with evidence for no impact. LLINs in good physical condition undoubtedly still provide protection against malaria and the spread of resistance should not derail plans to increase access to the most effective tool to reduce malaria transmission. However, the malaria community cannot afford to be complacent about insecticide resistance. Critical knowledge gaps on the causes and consequences of insecticide resistance need to be filled (see Outstanding Questions), the development, evaluation, and implementation of new products must be accelerated, and an evidence base for how best to deploy insecticide to minimize the spread of resistance must be generated to ensure the success of future malaria control efforts.

Acknowledgments

The authors thank Dr Nick Hamon, CEO of IVCC, for helpful comments on the manuscript and Dr Christen Fornadel, USAID, for sharing information on insecticide use by the President's Malaria Initiative and permission to use the data in Figure 4. The financial support of the European Union Seventh Framework Programme FP7 (2007–2013) under grant agreement no 265660 AvecNet is gratefully acknowledged. N.L. was supported by an ISSF Grant from the Wellcome Trust.

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