Adaptability is key when monitoring insecticide resistance
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When no vaccine, prophylaxis or drug treatment exists, insecticides may be the only tools available for combating insect-transmitted diseases such as dengue. They remain the mainstay of most vector control programmes and are most commonly applied against adult insects through indoor residual sprays, fumigants, space sprays and treated bed nets. Insecticides are highly effective when optimally implemented, but limited local resources and operational capacity, resistance to chemicals, and the use of adulterated or poor-quality products may all combine to reduce their impact.

Bioassays provide fast and cheap ways of detecting insecticide-resistant mosquito populations. These measure the lethal effects of discriminating doses of insecticides against field-caught mosquitoes (the discriminating dose is the minimum dose required to kill susceptible insects). Data from bioassays are the key to reviewing and justifying changes in insecticide use in response to the evolution of resistance (e.g. the sequential replacement of one insecticide class with another in indoor residual spray programmes against malaria vectors in Mozambique).

The two assay methods that are in common use for monitoring resistance in mosquitoes are the WHO assay and the bottle assay but neither is suitable for all situations. For example, the WHO assay requires the purchase of all components from a centralized source. This requirement, which is unique among assay methods, removes some operator error and helps ensure that results can be compared between years and sites. However, it also increases the costs and logistical complexity of the assay and limits its use to the insecticide doses and technical compounds that are provided centrally. It cannot be used to produce locally relevant information on the efficacy and quality of insecticide formulations and local laboratories cannot alter the discriminating dose to deal, for example, with smaller, more fragile mosquito species.

In comparison to the WHO assay, some of the components of the bottle assay are more readily and cheaply available but existing protocols require the use of technical grade (pure) insecticide, which is expensive and difficult to access locally. Other stipulations of the bottle assay may be regionally problematic; acetone is used as the carrier with which to coat the bottles with insecticides but, in parts of South America, its use is restricted because of its role in the purification of cocaine. In common with the WHO assay, the recommended discriminating doses listed for the bottle assay will mask low-level resistance in some species.

Minor, peer-reviewed improvements and alterations to the existing protocols can help to make them far more robust and globally relevant. A simple exercise by a local public health laboratory in Peru showed that some of the constraints of the bottle assay could be overcome simply by replacing acetone with ethanol and by preparing the bottles with locally available insecticide formulations in place of technical grade material. With an end-point of just one hour, they found this adapted assay to be more manageable than the WHO assay (that has an end-point of 24 hours). Some innovations and adaptations will be highly specific; the Peruvian laboratory showed that pyrethroid-treated bottles could be used several times and stored for long periods under ambient conditions but this finding will be less applicable to the highly volatile organophosphates. Nonetheless, it is these local and specific adaptations that will maximize the utility of the assays. Imaginative and capable laboratories should be encouraged to rationalize and modify protocols to suit their own conditions and requirements. These modifications should be published so that they can be criticized or adopted by others.

Assay methods need to evolve and adapt. The Food and Agriculture Organization (FAO) used to recommend standardized resistance monitoring assays for agricultural pests (listed occasionally in the FAO Plant Protection Bulletin throughout the 1960s and 1970s). Some of these remain useful: emerging resistance problems have benefited from the presence of standard “off-the-shelf” monitoring tools but others have been abandoned in favour of assays that are more practical, safe, cost-effective and/or applicable to modern chemistries (e.g. the general abandonment of glass vial tests for aphids in favour of “leaf-dip” tests).

In the public health arena, however, resistance assays have remained unchallenged and unchanged. The online resources for the WHO assay and the bottle assay have not been updated since 1998 and 2002 respectively. This is largely because the approved compounds used to control adult mosquitoes are so limited. Only four insecticide classes acting on two different target sites are currently registered. Of these, only pyrethroids remain uncontroversial with regard to human and environmental toxicity. This severely limits options for management when resistance does arise. The coming years, however, promise new chemical interventions and innovations and these will demand a re-examination of the utility of existing monitoring tools. Only the most adaptable should survive.

References
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