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Evaluation on current screening methods for insecticide resistance in mosquito vectors

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ABSTRACT

Mosquito-borne illnesses are a major concern for worldwide public health and insecticides persist as the principal method for reducing potential vectors and disease control. As a result of widespread insecticide use emergence of resistance in mosquitoes is a global problem. In order to overcome this issue vector control programmers should routinely check localized mosquito vectors for insecticide resistance, which is essential for the efficient management of vector-borne diseases and schedule preventive actions in accordance. The major techniques for determining mosquito vectors insecticide resistance (such as dose-mortality bioassay, WHO tube testing, and CDC bottle bioassay) are highlighted in this review along with some insights into challenges that researchers are encountering when evaluating resistance.

Figure : 00 References : 17 Table : 01
KEY WORDS : CDC bottle bioassay, Insecticide resistance, Mosquito, WHO tube test

Abbreviations

AI-Active ingredient; CDC- Centers for Disease Control and Prevention; DD-Diagnostic dose; DT-Diagnostic time; FP-Formulated products; IGR-Insect growth regulator; IR-Insecticide resistance; LC-Lethal concentration; PBO-Piperonyl butoxide; RR-Resistance ratio; VCP-Vector control programme; WHO-World Health Organization ; WHOPES-World Health Organization Pesticide Evaluation Scheme

Introduction

Mosquitoes belong to the family Culicidae (Order: Diptera) and transmit numerous diseases to people. Mosquito-borne illnesses continue to be a problem for worldwide public health, which has increased mortality and morbidity[7,8]. Despite increased efforts to manage and eradicate vector borne diseases during the past couple of decades, there has been no appreciable decline in the number of cases around the world. Their efficient therapies and vaccinations are frequently unavailable. However, being part of integrated mosquito management (IIM), preventing mosquitoes bite with utilising insecticides to reduce probable vectors remain the major ways to prevent disease[6]. Insecticide exposure to mosquitoes occurs from a variety of sources, including: public (governmental) vector control programmers, private pest management (residential, urban, industrial), homeowner implementation, and agricultural applications (by using formulated products [FP] and various active ingredients [AI]. Based on the target species, exposure to adulticide is probably more common from domestic and urban sites than through public VCP applications[3,4,12,13].

Insecticide resistance towards the majority of the WHO permitted public health insecticides is now being recorded throughout the world as a result of the extraordinary amount of insecticides that are used[9,11]. Target site alterations (knockdown resistance) and enhancements in insecticide metabolism are recognised as the main mechanisms of insecticide resistance[1]. Inappropriate insecticide application techniques, insecticide resistance (IR), and other variables may become a reason for control failures and because of limited resources it is underappreciated. Since the precise causes of mosquito control inefficiencies or failures must be recognised and remedied, it is crucial that mosquitoes are regularly and successfully screened for insecticide resistance to guide treatment decisions[5,14]. Field assessment of insecticides assay efficacy and development of resistance may have different to lab assessment. Understanding the lab field

TABLE-1 : Comparison between CDC and WHOPES Bioassay methods

	CDC protocol	WHOPES protocol
Advantage	❑ Availability of insecticides and bottles makes it very simple to test a range of insecticide concentrations without the requirement for specialist testing tools. ❑ Avoid using pre-assembled test kits and insecticide-impregnated papers to increase the range of insecticide types and concentrations that can be assessed. ❑ The process is comparatively quick and easy(no need of 24-hour holding period).	❑ Less susceptible to variation because control/treated and testing apparatus are obtained from a specific supplier. ❑ User does not need to manage liquified insecticide supplies because the filter papers are already impregnated with insecticides. ❑ Minimising the inconsistency in dealing and assessment as well as the probability of exposures as a result of spills.
Disadvantage	❑ The need that entomologists put on personal protective gear when making the glass bottles and clean all bottles completely after every use. ❑ Lack of “mosquito recovery phase” makes it unable that CDC testing correctly detect metabolic resistance.	❑ If mosquitoes settle on the tubular untreated portions at the top and bottom of the tubes, they might ignore the insecticide impregnated sheets. ❑ The user may require to make treat and stocks filter sheets if alternative insecticide doses are required in addition to those that are specified by the World Health Organization.

condition of IR testing in vector control programmes is costly. In the field study differences in weather condition, mosquito age, resting time on foliage. Insecticide application method and other unknown variables interpret the result. In this review, we provide (i) information regarding phenotype and genotype bioassay (ii) overall view of the assessing methods of insecticide resistance (iii) issues and solutions that people facing while use those methodologies.

Comparative evaluation of insecticide resistance assessing methodologies

Resistance to different insecticides can be identified and studied at several levels, from molecular identification of the genes generating resistance and associated biochemical products to the function that gene products serve in counteracting the harmful impacts of insecticides. Metabolic resistance, cuticular resistance, target-site resistance, and behavioural resistance are all known mosquito insecticide-resistance mechanisms[10]. The first 3 can be identified employing phenotypic bioassays and genetic tests, but behavioural resistance is a little more challenging to identify in a controlled laboratory environment and is outside the purview of this review.

Phenotypic resistance bioassay

The majority of insecticide tolerance assessment is carried out using standardised bioassays to assess phenotypic resistance (when resistance found without genetical changes). The assays measure the percentage of death in localized mosquito species exposed to a specified diagnostic dose (using mosquito variants that are susceptible to it) in a regulated laboratory setting. To examine a mosquito’s susceptibility to adulticides and larvicides, phenotypic assays can be carried out on adult mosquito and an aquatic immature mosquito respectively[10].

1. Larvicide susceptibility testing

Insecticides, which kill or control larvae form of mosquitoes before their development into adult form are called larvicides. To estimate a larvicide's diagnostic concentration it is required to check mosquito's susceptibility to different kinds of larvicides. Following these methods are used to test larval susceptibility-

1.1. WHO larvicide testing

The WHO recommended larvicide testing and resistance ratio (RR) test can both be used to determine the larval mosquitoes' susceptibility to the larvicides. In both studies, third or fourth instar larvae are treated in cups of water with a specific insecticide concentration, and death is reported after 24 hours of uninterrupted exposure[17]. The WHO larvicide experiment evaluates the death rates of larvae to a diagnostic concentration, that is twofold the lethal concentration ($LC_{99.9}$), which kills 99.9% of a susceptible mosquito population of the similar species[15]. The WHO larvicide testing is simpler to carry out because it only involves evaluating a few concentrations and does not necessitate simultaneous assessing of a susceptible mosquito population, that is not always accessible in study sites around the world.

1.2. Dose-mortality bioassay (resistance ratio test)

Mosquitoes of a recognized susceptible (control) colony must be tested concurrently for the RR experiment. To establish lethal concentration (LC) which kills 50% (LC_{50}) or 95% (LC_{95}) of mosquitoes, this bioassay exposes insects to a range of insecticide concentrations. The present resistance level and variations in resistance over time are determined by dividing the LC_{50} of the tested field mosquito strain by LC_{50} of susceptible mosquito strain (LC_{50} field population/LC_{50} susceptible population)[17]. Since numerous doses are employed, these kinds of bioassays can improve the precision when susceptibility observations in field species are interpreted. They can also assist in identifying potential resistance pathways.

1.3. Insect growth regulators (IGRs) test

IGRs on treated larvae have a delayed effect. These juvenile hormone equivalents prevent pupae development from instar larvae and subsequently into adults while chitin synthesis inhibitors impede cuticle construction and influence immature phases and all instars of the mosquito. Mortality in IGR testing is measured in immature stages at every two or three days until adult emergence. Dead and moribund larvae and pupae, and also adult mosquitoes partially attached to the pupal case, are regarded as "affected" when calculating the IE percent for each dose. The empty pupal skins can also be used to count the number of adults who have properly emerged. Both a susceptible mosquito population and a wild or field species go through the same process[15].

2. Adulticide susceptibility assay

The WHO tube test and the CDC bottle assay are typically used in phenotypic susceptibility testing for adult mosquito population[2,15].

2.1. Centers for Disease Control and Prevention bioassay

The development of bottle bioassays by the Centers for Disease Control and Prevention allowed for the evaluation of IR in any insect species, includes mosquitoes. In this experiment, up to 25 mosquitoes (in every 4 replicated bottles) are exposed to insecticide doses and mortality of mature female mosquitoes is assessed across a 2-hour period at various intervals. The interior part of every glass bottle comprising remnant of a technical grade active ingredients or formulated products which may contain substances to increase effectiveness (acetone usually used for stock solution formation). The same population's control mosquitoes are exposed to previously-acetone-coated bottles. In treating bottles, mosquitoes are more likely to be resistant to an insecticide or to be gaining resistance to it if it takes longer for it to kill mosquitoes[16].

2.2. World Health Organization pesticide evaluation scheme

The World Health Organization Pesticide Evaluation Scheme (WHOPES) is the most extensively used assay and offers recommendations for items like field/laboratory testing of mosquito nets and geographical repellents. The WHO outlines a cone bioassay in which bed nets or other treated textiles are exposed to mosquitoes for three minutes underneath a plastic cone. After exposure, mosquitoes are moved to clean cages where their mortality is measured after one and twenty-four hours. In another test, mosquitoes are introduced in plastic tubes coated with papers that have been treated with an insecticide and a control substance, according to the WHO. Only one WHO-accredited lab produces papers with fixed doses, however other labs with the necessary supplies and equipment are also able to produce papers. A total of six replicate tubes each contain 20-25 mosquitoes in this experiment. Mosquitoes are moved to clean cages after a 60 minutes exposure time, and then fatality rates are measured after 1-hour and 24 hours[16].

Similar to the CDC bottle assay, mosquitoes that are unable to fly but are perhaps still moving are regarded as dead. In order to ensure consistency between tests, the WHO has centralised facilities for the manufacture and distribution of resistance testing equipment (such

as insecticide impregnated papers for tube tests) by University of Malaysia, Vector Control Research Unit.

2.3. Resistance determination by use of discriminating concentrations

Prior to doing the WHO tube test and CDC bottle bioassay, diagnostic concentrations and diagnostic periods for every AI or FP are established by assessing baseline mosquito species that show susceptibility to the insecticide. Non-resistant vectors are subjected to varying amounts of an insecticide ingredient in order to establish baseline data on sensitivity in order to evaluate resistance in the mosquito vectors. This technique can be used to calculate the concentration that corresponds to 99.9% fatality (the $LC_{99.9}$ value). The diagnostic or discriminating concentration is typically defined as being twice this concentration.

The diagnostic dose (DD) and diagnostic time (DT) may differ between various populations and species of mosquitoes. In order to test mosquitoes population (*Anopheles* and *Aedes* but species not specified) in bioassays, the WHO and CDC list beginning point DTs and DDs for various classes of insecticides, including cyfluthrin, cypermethrin, bendiocarb, DDT, permethrin, deltamethrin, malathion, fenitrothion, lambda-cyhalothrin, pirimiphos-methyl but emphasises that it would be necessary to establish DTs and DDs for mosquitoes from various geographical areas.

2.4. Synergist insecticide bioassay

The synergist-insecticide bioassay examines the impact of a synergist pre-exposure on the emergence of insecticide resistance. It is a prompt reaction to exposure test. Although a synergist is not an insecticide, some mosquito detoxifying enzymes can detect it as a substrate. This bioassay is employed to determine if metabolic resistance pathways contribute to the development of resistance phenotypes. Four bioassay exposures are included in this experiment: solvent control, insecticide only, PBO only, and insecticides after PBO insecticide. After a 24-hour recovery period, the mortality rate of mosquitoes is recorded[16].

Current issues and suggestions

There are certain effective ways for evaluating resistance in mosquitoes against insecticide in lab reared and field collected species. But environmental parameters are fluctuated over time which affects the insecticide resistance assessing in different conditions. So further development of the insecticide resistance assessment process and comprehension of the shortcomings of the current approaches are still required. Here is brief discussion of some concerns below:

1. For diagnostic dose and diagnostic time evaluation, a control (susceptible) mosquito generation is required to increase standardization. For each respective species, a baseline susceptible generation should be thoroughly described using molecular methods to evaluate the various resistance mechanisms. To establish worldwide uniform DTs and DDs for all FPs and AIs, the similar population (for every species) must be used. The evaluation of field population groups should therefore be done using such species-specific DTs and DDs. The information would be utilised for resistance evaluation, but it would require a lot of work at first[14].
2. Concerns about standardised sources of technical grade AIs (from where to get them, how to handle stock mixing, and how long they should be stored) are also frequent. To regulate the integrity of the insecticides utilized in bioassays, a recognised commercial supplier (such as Chem Service or Sigma Aldrich) for technical grade AIs must be chosen. Insecticide stock mixes utilized during bottle bioassays should be handled according to approved conventional protocols and for storage conditions (i.e., refrigerated in amber coloured containers), storage time (i.e., 1 season), and bottle impregnation and cleaning procedures (to reduce human mistake) should be standardised[14].
3. It is critical to understand to assess of IR in field under a variety of biological and environmental condition. Susceptibility test results are expected to fluctuate over time and location due to the considerable impact of ecological parameters on resistance phenotypic and the reality that parameters like humidity, temperature, and availability of food can change on a daily basis. Additionally, it has been demonstrated that resistance varies with mosquito age, with mature larvae or adults often being more vulnerable than young ones. In field conditions, the tarsi of resting mosquitoes may contact foliage that has been treated with insecticides and sometimes flying mosquitoes affected by ULV spray (not assessed via CDC bottle bioassay or WHOPES). Due to differences in contact time and the bioavailability of the active components, the amount of insecticides that mosquitoes consume in laboratory bioassays and in the field will differ[10].
4. For various FPs and AIs, a significant range in diagnostic time and diagnostic dose is seen. Additionally, if a mosquito population is labelled resistant, the VCP should determine the intensity of the resistance; in other words, in follow-up analysis, the VCP can utilise around 5-10 times diagnostic dose to further determine the intensity of the IR. In cases when IR is regionally widespread, subsequent testing of increased resistance genes and enzymes

should be taken into consideration in order to determine the underlying causes of IR[14].

5. Interpreting the findings and data analysis is a crucial aspect for VCPs. It should be emphasised that both the WHOPES tube test and the CDC bottle bioassay count any debilitated (cannot stand or fly or but they may still active) or dead mosquitoes that are present during the exposure period. Instead of using endpoint analysis of the percentage killed, the results can be processed in a more subtle way because of the kinetic nature of CDC bottle bioassay. In order to examine a semi-quantitative assessment for the CDC bottle bioassay, time-mortality curves of resistant or susceptible populations can be compared. Data on time-mortality and dose-mortality that can be applied for RR calculations can be analysed. The bioassay data on 95% confidence intervals can be used to distinguish between significance in RR estimates. In comparison to both the WHO assay one-point reading format and the CDC bottle bioassay diagnostic time, the dose mortality assay is more reliable and quantifiable[14].
6. Field mosquito groups and their developmental stages are frequently brought up in the assessment of insecticide resistance. Based on the objective, either adult mosquitoes (of various ages) from the field can be captured and placed into assay bottles, or eggs/ larvae can also be collected, raised to adulthood, and mosquitoes of a comparable age can be included in bioassays. Similarly aged mosquitoes should generally be utilised in CDC bottle bioassays because variations in IR rely on the physiological and chronological age of the mosquito[14].
7. Examining the variations between FPs and AIs is a crucial problem. VCPs frequently inquire as to whether they may still employ an FP that uses an AI after resistance assessment based on that AI. A mosquito population's susceptibility or resistance to an active ingredient does not mean necessarily that a formulated product will be effective in the field. Synergists and other substances that boost effectiveness are frequently used in formulated products. So, an FP can hide the emergence of resistance to an AI during CDC bottle bioassay. Given this restriction, it is acceptable to employ formulated products in CDC bottle assays with addition to AI assays, provided that either FPs or AIs are consistently used in standard and unknowable mosquito groups for comparison[14].

Conclusion

Standardization of technique, results evaluation, and knowledge of the practical uses of various forms of susceptibility and resistance monitoring in mosquito vectors are required since assessing resistance to insecticides should be a standard procedure throughout all vector control programmes. The ideal specification for tracking insecticide resistance in mosquitoes has been the CDC bottle assay and other techniques. These assessments are designed to track technical resistance so that outcomes can be contrasted across space and time. The ecological circumstances, however, can have a significant impact on a mosquito's response to insecticides. Susceptibility assay results are anticipated to vary across time and geography due to the significant impact that environmental factors have on resistance phenotypic and the fact that parameters like humidity, temperature, and availability of food can change daily and seasonal. Better grasp of these methods will be helpful in assessing and mitigating resistance. Addressing the problems caused by insecticide resistance may be the preliminary step toward eradicating the burden of mosquito-borne diseases. Vector control programmes at large scales ought to take into account offering services to smaller vector control programmes in their region that need to evaluate insecticide resistance, as well as offering pre-treated bottles to programmes looking to do the same. Local mosquito monitoring agencies can employ such insecticide susceptibility tests with ease.

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