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SHORT COMMUNICATION

Knockdown times in a simple assay determination of insecticide resistance in malaria vectors

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Background: Determining the insecticide resistance status of malaria vectors, particularly to insecticides used on mosquito nets, is important but is limited to a relatively small number of locations. We describe a simple assay that enables this information to be obtained over a much wider area.

Methods: The time to knockdown of mosquitoes in an insecticide-treated netting-covered metal frame cage were recorded. The shape of the curve of the proportion of knocked down mosquitoes provides information on resistance status.

Results: Resistant *Anopheles funestus* took significantly longer for knockdown than did susceptible *Anopheles arabiensis*.

Conclusions: This simple technique will enable a wide range of locations to be sampled, enhancing our understanding of the spread of resistance to pyrethroids.

Keywords: insecticide resistance, insecticide-treated bed nets, malaria, mosquito vectors

Introduction

Mosquito nets treated with insecticide remain the main means of malaria control worldwide. Resistance to the insecticides used on the nets is becoming an increasing problem.[1] Presently the standard tests to determine resistance status in vectors requires a laboratory and a reasonable degree of training of the person doing the testing.[2] This limits the number of locations from which data are available and makes decision making harder. New and simpler tests that may help in determining the development of resistance and that can be widely used are needed.[3] Here we describe a simple assay that can be performed without the need of a laboratory or sophisticated equipment and which may provide information on the development of resistance in mosquitoes.

Methods

An unused insecticide-treated net is wrapped around a wire frame cage (15 cm on a side) and field-collected mosquitoes are introduced. The time to knockdown is recorded. A similar cage covered with an untreated net is the control. If necessary, insects can be removed when they fall and can be stored individually by the time of knockdown. Thus, supposing there was a mixture of two species in the collection (e.g. *Anopheles gambiae* and *Anopheles coluzzii*) with a different resistance status, they could be identified post-test (and individual curves developed as a result). The cumulative total number of mosquitoes knocked down is plotted over time and the resulting curve is compared with a set of (yet to be developed) standard curves.

Results

In the tests described, mosquitoes were collected in the field using Furvela tent-traps.[4] The tests were carried out with the nets that we had available. After removal from the trap, mosquitoes were held for 1 h before being assigned to either a control or treatment cage. We did not have access to an untreated Olyset net so used a standard polyester net for this. The number knocked down per minute was recorded and plotted as a percentage of the total knocked down (Figure 1). In the case of resistant insects, the tests were stopped after 1 h. In the present experiments, none of the insects in the control cages were knocked down.

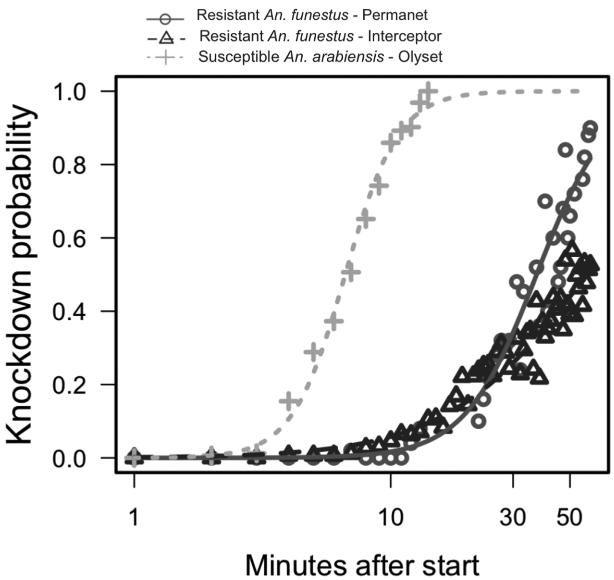

Figure 1. Knockdown times of susceptible *A. arabiensis* from Muleba, Tanzania, exposed to a new Olyset net (n=4 replicates of 25 mosquitoes per replicate) and resistant *A. funestus* from Furvela, Mozambique, exposed to used Interceptor nets, which killed susceptible *A. arabiensis* (n=13 replicates of 25 mosquitoes per replicate) or a new Permanet (n=1 replicate of 27 mosquitoes per replicate). Resistance status based on standard World Health Organization assays.

The average exposure time required to knock down 50% (KDT_{50}±95% confidence interval [CI]) and 95% (KDT_{95}±95% CI) of a resistant population of *Anopheles funestus* from Mozambique when exposed to the Interceptor net was 55.4 min (interquartile range [IQR] 53.2–57.6) and 341.3 min (IQR 296.1–386.5), respectively. The estimated KDT_{50} and KDT_{95} on exposure to a new, unused Permanet was 42.4 min (IQR 40.4–44.3) and 86.3 min (IQR 74.9–97.7), but the KDT_{50} and KDT_{95} for a susceptible population of *A. arabiensis* from Muleba, Tanzania, exposed to a new, unused Olyset net was only 6.64 min (IQR 6.15–7.14) and 13.40 min (IQR 11.51–15.29). These are the kinds of difference that might be seen between resistant and susceptible populations elsewhere. Should resistance begin to develop in a population, then the curve will tend towards the right.

Given that long-lasting insecticidal nets remain the most widely used method of vector control, testing insecticides used for indoor residual spraying (IRS) in this way is less important. The provision of pretreated netting with the insecticides used for IRS could also be produced for this purpose.

Conclusions

This simple test requires very little equipment and does not require a laboratory. This would enable a wide geographical spread of tests to be undertaken. It could easily be added to other tests to obtain greater insights into the spread and development of resistance to insecticides by vectors. The development of standard curves would allow comparisons with presently used techniques (such as the World Health Organization or Centers for Disease Control tests). If necessary, this test can be followed up using conventional techniques. Widespread use would allow decision makers to more easily determine the spread and intensity of resistance and thus should inform future policy.

Authors' contributions: JDC conceived the study. JDC and AK designed the study protocol and carried out the assays. AK carried out analysis of the data. JDC and AK drafted the manuscript, critically revised the manuscript for intellectual content and read and approved the final manuscript. JDC and AK are the guarantors of the paper.

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Competing interests: None declared.

Ethics approval: The collections conducted in Tanzania were done as a component of the Pan African Malaria Vector Research Consortium project 'Evaluation of a novel long lasting insecticidal net and indoor residual spray product, separately and together, against malaria transmitted by pyrethroid resistant mosquitoes', which received ethical clearance from the ethics review committees of the Kilimanjaro Christian Medical College (certificate 781 on 16 September 2014), the Tanzanian National Institute for Medical Research (20 August 2014) and the London School of Hygiene and Tropical Medicine (reference 6551 on 24 July 2014). The collections in Mozambique took place under the aegis of the joint Instituto Nacional de Saúde–Danish Bilharziasis Laboratory Centre for Health Research and Development project 'Turning houses into traps for mosquitoes', which obtained ethical clearance from the National Bioethics Committee of Mozambique on 2 April 2001 (ref: 056/CNBS/01).

Data availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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