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Reviewing the evidence for and against selection of specific pyrethroids for programmatic purposes - Executive Summary

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Key messages:

- Compelling evidence – based on molecular, laboratory and field data - suggests that in areas where pyrethroid resistance exists, differences between adult mosquito mortalities in insecticide resistance assays are not indicative of a true or operationally relevant difference in potential performance of the specific pyrethroids currently in common use (deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin).
- As an insecticide resistance management strategy, it is not advisable to switch between the pyrethroids that are in common use given the strong evidence of cross resistance.
- It is possible that pyrethroids not in common use – e.g. bifenthrin and etofenprox – may have differential resistance profiles to the other pyrethroids, though further work would be needed to examine this possibility.
- Analyses conducted in this review shed light on the variability, and main drivers of variability, in insecticide resistance monitoring results, leading to recommendations on how to improve the usefulness of such testing to inform operational decisions.

Introduction

Pyrethroid resistance is widespread in malaria vectors. Pyrethroids are present in all WHO prequalified insecticide treated nets (ITNs) and are still used for indoor residual spraying (IRS). Where susceptibility monitoring suggests differential levels of resistance to different pyrethroids, PMI, a key vector control procurement agency, supports the choice of products containing pyrethroid active ingredients which show higher mortality in bioassay tests against the local mosquito population. However, there is some uncertainty about whether current methods for susceptibility monitoring can reliably identify differential resistance phenotypes, and if so, whether effective resistance management can be achieved by such targeted use of insecticides of the same class. This desk review aims to answer the following two main questions:

1. Should countries interpret differential mortality in discriminating dose susceptibility assays as indication of differential levels of susceptibility within the pyrethroid class? Or should these be interpreted in another way (e.g. inherent variability in mortality results; differently calibrated discriminating doses, other).

If yes:

2. Should countries with evidence of differential susceptibility in pyrethroid assays consider preferentially selecting a specific insecticide for programmatic use? i.e. does a difference in mortality in a diagnostic dose or resistance intensity bioassay imply either different control potential or potential to use multiple pyrethroids in resistance management approaches?

Evidence from the following areas were examined: i) molecular information ii) insecticide resistance patterns and testing results in laboratory colonies iii) insecticide resistance patterns and testing results in field data iv) lessons from behavioural assays.

Main questions and the sources of evidence used to address them

Molecular evidence

1. Is there molecular evidence for differential resistance among members of the pyrethroid insecticide class?
2. Are different pyrethroids equally susceptible to different resistance mechanisms?

Laboratory strains

Using discriminating dose assays on laboratory reared colonies to ask:

3. Are the discriminating doses of permethrin and deltamethrin suitable and comparable?
4. What intrinsic variability do we see in the results of dose response assays?
5. Is there evidence for divergent resistance in colonies routinely selected using a single pyrethroid?

Field populations

Using data from discriminating dose and intensity assays on field populations:

6. What are potential sources of (non-resistance associated) variability in the assay?
7. What is the evidence for the existence of divergent resistance between pyrethroids?
8. Can difference seen in molecular studies (question 1) be detected in wild mosquito populations?

Mosquito behaviour

9. Do mosquitoes, resistant or susceptible, exhibit different behavioural responses to different pyrethroids?
10. How suitable are resistance monitoring methods considering the possibility of behavioural resistance?
11. How could efficacy testing be improved to take behavioural response into account?

Findings

Molecular evidence

This component aimed to review the molecular evidence for cross- versus divergent-resistance among chemicals within the pyrethroid class in relation to their structures, and to review if different members of the pyrethroid class are equally susceptible to (a) metabolic and (b) target site resistance mechanisms.

- Pyrethroids are broadly differentiated into two groups based on their biological activity that is also commonly associated with the absence (Type I: e.g. permethrin, bifenthrin) or presence (Type II: e.g., deltamethrin, λ-cyhalothrin, α-cypermethrin) of an α-cyano group.
- Pyrethroid resistance is complex but primarily associated with target-site mutations in the VGSC gene (most commonly L995F and L995S) known as knockdown resistance (*kdr*) and increased insecticide detoxification (metabolic resistance) often caused by elevated levels of cytochrome P450s.
- For target site resistance:

  - Some *Anopheles* bioassay studies suggest slight but significant differences in the effect of the two most common *kdr* mutations (995F and S) on permethrin *vs.* deltamethrin resistance. However, neither electrophysiological studies nor data from other taxa support these findings, although for structurally divergent pyrethroids *kdr*-effects might be more variable.

- For metabolic resistance:

  The level of a pyrethroid's vulnerability to metabolic attack (by P450 resistance markers) is an indicator of how likely metabolic resistance is to arise against the pyrethroid.

  - Permethrin and deltamethrin are the pyrethroids most vulnerable to metabolic attack by the P450 resistance markers examined.
  - Bifenthrin, ʎ-cyhalothrin and α-cypermethrin are less vulnerable.
  - Etofenprox was strongly metabolised by key P450s.
- At least in the absence of concurrent *kdr* resistance, there may be differences in how different pyrethroids perform against metabolically resistant strains: metabolic resistance may result in a more important decline in deltamethrin and permethrin toxicity than in bifenthrin, ʎ-cyhalothrin, α-cypermethrin, etofenprox and transfluthrin toxicity (based on molecular data and on *in vivo* data for transfluthrin).
- P450-structure-activity relationship (P450-SAR) models give a proxy estimate of insecticide vulnerability to metabolic attack; linking this to quantitative toxicity data in the field may help to understand the contribution of P450s to resistance.

Evidence from laboratory strains

This component aimed to examine data from discriminating dose assays on laboratory reared colonies to consider: a) comparability of discriminating doses across different pyrethroids, b) intrinsic variability in discriminating dose testing and c) whether evidence exists for divergent resistance in selected colonies.

a) Comparability of discriminating doses across different pyrethroids.

- Publicly available data from the 1998 WHO multi-centre trial, and the associated methodology used to calculate the current discriminating doses (DD) for deltamethrin and permethrin were reviewed. Most centres within the study appear to have diverged from the agreed protocol in terms of sample size and replicates tested. The concentrations tested resulted in poor dose

response curves for individual strains and the data do not appear robust. The link between the reported data and final concentrations is therefore unclear.

- Based on the publicly available data, the current DDs for deltamethrin (0.05%) and permethrin (0.75%) appear to be too low (i.e we calculated the DD for deltamethrin to be 0.1% and a DD of 1.46% for permethrin).
- Given the rationale for establishing the DDs for permethrin and deltamethrin is unclear, and the data used to calculate them was not sufficient or consistent, it is unlikely that they are comparable. This is a challenge when trying to draw reliable conclusions about relative efficacy of, or resistance to, the two pyrethroids from data collected using these concentrations.

b) Intrinsic variability in discriminating dose testing.

- In general, following exposure of characterised lab strains in WHO tube bioassays under controlled conditions the level of variability in mortality among test replicates exposed to a single compound was greater in moderately resistant strains. An experiment where PBO was added to the bottle alongside permethrin increased the level of mortality and supported the observation that greater variability is observed where mortality is intermediate. Further investigation is required to establish the inherent variability in PBO synergism assays, relative to DD bioassays.
- Variation in resistance levels within strains of the same species make it difficult to conclude if there are species differences in mortality based on this data set.
- In CDC bottle assays against permethrin, a higher level of variability in mortality data was observed in resistant strains compared to susceptible strains in this data set. When comparing resistant strains, both level of mortality and variability in mortality is greater in the CDC bottle bioassay compared to the WHO tube test in response to their respective diagnostic doses, but susceptible strain mortalities were comparable.

c) Whether evidence exists for divergent resistance in selected colonies.

- In general, following exposure of characterised lab strains in WHO tube bioassays under controlled conditions, intra-strain mortality to permethrin, deltamethrin and α-cypermethrin was similar. However, in intermediately resistant strains some divergence in mortality rates to different pyrethroids was observed.
- The laboratory strains tested in this data set have been selected with deltamethrin for up to 6 years. Despite this, trends in mortality over time do not suggest divergence between deltamethrin and the other pyrethroids.

Evidence from field populations

This component aimed to examine data from discriminating dose and intensity assays on field populations to consider: a) potential sources of (non-resistance associated) variability and trends in tests, b) evidence for existence of divergent resistance between pyrethroids, c) whether differences between pyrethroids identified by molecular studies can be detected in wild mosquito populations.

a. Potential sources of variability and trends in pyrethroid resistance tests

This sub-component conducted a review of the procedures involved in insecticide resistance monitoring in malaria vectors, sampling, and performing bioassays to identify sources of variability and bias in the data collected

- Trends in diagnostic dose bioassay data from 2002-2017 show that the insecticides tested have changed over time but testing of either defunct insecticides or those with very well-established resistance persists. Diagnostic dose assays were designed to identify the emergence of resistance and are poor tools for quantitative analysis of resistance levels where resistance is established. More consideration of the purpose of the testing and operational significance is required to use the available resources to most effectively monitor for resistance to current products and inform deployment decisions.
- Resistance intensity assays provide improved resolution of resistance level but for comparisons among insecticides, suffer from the same problem as the diagnostic doses on which they depend – an apparent lack of parity across insecticides as described above (*Evidence from laboratory strains (a)* point 3). The most comparable methods for comparing different insecticides are dose-response assays (which are not dependent on existence or accuracy of diagnostic doses) and vary either insecticide concentration or exposure time. However, large numbers of mosquitoes are required and an increase in direct testing of insecticidal products as part of monitoring programmes may provide more efficient operationally relevant information to aid decision making.
- With more insecticides or products to test, larger numbers of mosquitoes are required, whilst utilising collection methods that yield representative population samples. Larval collections often provide the best option for intensive sampling, but might yield many closely related individuals, biasing results. However, we provide results that show that with pragmatic, but carefully performed sampling average relatedness is low, supporting the statistical validity of large larval collections.
- All bioassays are vulnerable to very strong effects of humidity and temperature, in addition to other environmental effects more easily standardized by the user. Indeed, data show that moderate changes in conditions can affect mortality enough to change a classification of a cohort

of mosquitoes from susceptible to resistant or *vice versa*. In addition to avoiding testing in uncontrolled conditions where possible, improved reporting of sampling, rearing and testing conditions is crucial to allow consideration of possible biases when interpreting and using data.

b. Evidence for diverging resistance to pyrethroids

This sub-component performed an analysis of discriminating dose and resistance intensity bioassay data collected over years during insecticide resistance monitoring, and comparison with results from a systematic review of experimental hut trial data comparing different pyrethroid-treated ITNs, to look for evidence of differential resistance to different pyrethroids.

- The resistance intensity bioassay represents a substantial improvement over the discriminating dose bioassay in areas with moderate and high insecticide resistance. However, it is prone to substantial sampling and measurement error so results from individual data sets should not be overly interpreted and should be analysed together in a robust statistical framework to understand long-term trends.
- Evidence from discriminating dose bioassays, resistance intensity bioassays and experimental hut trials all indicate, on average, slightly higher mortality to Type II than Type I pyrethroids in wild mosquito populations. Since the discriminating dose for permethrin and deltamethrin may induce slightly different levels of mortality (*Evidence from laboratory strains (a)* point 3), so it is not clear whether these differences in mortality reflect true differences in resistance. Nevertheless, true difference of this level is unlikely to have a substantial public health impact, especially when other intrinsic differences between ITNs, such as the surface bioavailability of insecticide, are considered.
- There is no evidence of divergence of mortality over time induced by Type I and II pyrethroids in field mosquito populations, i.e. the average difference between pyrethroids has remained consistent over time rather than increasing as might be expected if substantial differences in phenotypic resistance were selected for a prolonged period.
- The variability in discriminating dose and intensity assay mortality is high. This variability is predominantly at a local geographical scale indicating that if there were a difference between Type I and II pyrethroids it will be beneath the size of the region of deployment for ITNs or IRS.

c. Investigating whether differences between pyrethroids identified by molecular studies can be detected in wild mosquito populations

This sub-component used correlation analyses to identify which pairs of pyrethroids are most similar or divergent in terms of the resistance found to them within *An. gambiae* s.l. populations, comparing

field resistance monitoring data and molecular evidence, and a review of studies investigating resistance to bifenthrin compared to other pyrethroids.

- There is good evidence that resistance to deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin are strongly correlated across *An. gambiae* s.l. communities and environments. These correlations were also seen for resistance to deltamethrin, permethrin and λ-cyhalothrin (α-cypermethrin was not tested) in the *An. funestus* subgroup and in *An. arabiensis*, *An. coluzzii*, *An. funestus* and *An. gambiae*.
- Strong associations between resistance to deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin mean that switching between these compounds is not recommended.
- If individual susceptibility test results at a site show differences between deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin, it is likely that these are due to data noise and more evidence is required before a decision to switch is made.
- There is evidence that resistance to etofenprox could diverge from resistance to the more commonly used pyrethroids. The potential high vulnerability to metabolic attack by P450s shown by molecular studies might preclude an operational switch to etofenprox, but higher resistance to etofenprox, compared to the more commonly used pyrethroids, wasn't detected in field-collected *An. gambiae* s.l.
- The divergence between bifenthrin and the other pyrethroids identified by molecular studies was detected in terms of phenotypic resistance by a small number of studies of field-collected *An. sinensis* and *Ae. aegypti* populations from one site in Korea and seven in Mexico, respectively. However, more direct evidence for whether resistance is lower in targeted vector populations, compared to the other pyrethroids, is needed before a switch could be considered.

Evidence for different behavioural responses to pyrethroids

This component aimed to look for evidence of different behavioural responses of malaria vectors to different pyrethroids and how this is affected by resistance through a review of the available literature. This was used to inform a discussion of the limitations of existing WHO assays for efficacy testing and resistance monitoring and proposal of new bioassay methods to better capture behavioural endpoints.

- While the mechanisms of insecticide resistance in malaria vectors have been studied and characterised extensively at the molecular level, knowledge of behavioural changes associated with resistance is relatively poor.

- Pyrethroid-treated nets have a rapid killing effect, but some insecticide residues also have repellent properties that are detectable by some mosquitoes which respond by taking flight from the net rapidly to break contact.
- There is great variability in study design, treatments and treatment groups in the literature describing the behavioural response of mosquitoes to pyrethroid-treated nets, and therefore it is not possible to draw clear conclusions about the deterrent properties of individual pyrethroids, nor attempt to compare them.
- The overall response of pyrethroid resistant mosquitoes to an ITN may comprise alterations in multiple behaviours and any differences in responses to individual pyrethroids provide an opportunity for the efficacy of pyrethroids to diverge.
- Current resistance monitoring bioassays are unable to detect differences between responses to different insecticides or differences between susceptible and resistant mosquitoes, yet the results of these bioassays may be confounded by such changes in behaviour.
- A better understanding of behavioural responses and how they differ between susceptible and resistant mosquitoes should inform the deployment of the most effective products. This will require the development and adoption of new bioassays. Methodologies to collect more valuable data about the behavioural response of *Anopheles* mosquitoes under more relevant conditions, including large scale testing arenas, are under development.

Conclusions

- The WHO or CDC discriminating dose bioassay is a useful tool to monitor emergence of resistance to new chemistries, but is not sufficient in a situation of high pyrethroid resistance to draw conclusions about relative efficacy of or resistance to one compound or another, and a relatively higher kill by one pyrethroid is not sufficient evidence to switch to deployment of another.
- There are multiple limitations and sources of noise in the insecticide resistance data, which is collected on field populations of mosquito, which mean drawing firm conclusions about the existence of divergent resistance to different pyrethroids is not straightforward.
- Nevertheless, meta-analysis shows consistent trends between data sources that suggest a strong association between deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin, meaning that rotation between the commonly used pyrethroids is unlikely to be a successful management strategy.
- Bifenthrin may be distinct enough to warrant consideration for rotation with other pyrethroids already in use; further investigation of this compound alone and in combination with other pyrethroids is warranted.
- No evidence has been found for differential efficacy of PBO nets on the basis of which pyrethroid they include. In order to better understand the possible operational implications for recommendations on the use of PBO nets in areas of pyrethroid resistance, further investigation is needed to elucidate the biological impact of PBO on P450s, and the molecular basis for any differential efficacy of PBO in synergising different pyrethroids.
- Improvements to and standardisation of the sampling and testing procedures used to collect field insecticide resistance data are strongly recommended.
- Given the limited products available for vector control, and narrow collection of available chemistries, programmes must make deployment decisions based on the data which can realistically be collected. The current monitoring system for insecticide resistance is imperfect but could be improved to optimise use of the available resources.

Recommendations

Making deployment decisions about different pyrethroids based on resistance monitoring data

- Based on the available compelling evidence from both molecular studies of metabolic resistance and in vivo studies of resistance in mosquito populations, it is inadvisable to switch between the pyrethroids that are in common use (deltamethrin, permethrin, α-cypermethrin and λ-cyhalothrin).
- Improved evidence for whether resistance to less common pyrethroids such as bifenthrin is divergent from commonly used pyrethroids, is needed before a switch could be recommended.
- Systematic quantitative structure activity relationship (QSAR) using LC_{50} values calculated from dose-response data is are required to determine the bio-efficacy of the full range of pyrethroids including bifenthrin, etofenprox, α-cypermethrin, cyfluthrin, lambda-cyhalothrin, deltamethrin and permethrin against wild populations of malaria vectors which are resistant to deltamethrin and/or permethrin. This will allow a comprehensive understanding of the impact of resistance mechanism(s) on the bio efficacy of each molecule.
- Current tests lack the sensitivity to detect operationally significant differences in resistance to different pyrethroids at a single location. A difference found at a single time and place may be a true difference or the result of measurement error in the assay. Any decision to switch between pyrethroids needs to be based on tests conducted at multiple sites across the intervention target area. This approach also addresses the variation in vector populations across the target area.
- Ideally the vectors transmitting malaria should be tested and results should be obtained for each species biting humans within each target area. If this is not feasible, the evidence indicates that patterns of resistance within a species complex within an area are broadly consistent whereas there are key differences between *An. gambiae* s.l. and the *An. funestus* subgroup. Where both are present, it is vital that both are tested.
- Historical results from tests at a single site using different pyrethroids could be combined to provide a more robust estimate of pyrethroid resistance to inform decisions on the deployment of, for example, PBO-treated nets.

Improving the collection and use of resistance monitoring data and further investigation to better inform deployment decisions

- In order to better understand the suitability of the discriminating doses for permethrin and deltamethrin it would be valuable to compare dose response data from a range of susceptible *Anopheles* strains. Better calibrated DDs could then be established, and the relative potency of the pyrethroids confirmed. A meta-analysis of the literature to establish if such data exists is recommended, the results of which can then be compared to the current recommended diagnostic doses. If existing data is limited, a small-scale trial to confirm the LD values for permethrin and deltamethrin to several *Anopheles* species, ideally using multiple strains of each, should be conducted.
- Bioassays for insecticide resistance monitoring rely on wild collection of often limited numbers of mosquitoes and should therefore focus on the insecticide class/es of primary interest for current or future operational decision making.
- Although demanding in terms of mosquito requirements for testing, dose-response bioassays (independent of diagnostic doses) remain the preferred method for comparative assessment of insecticide resistance among insecticides. Ideally, and to facilitate comparisons across studies, they should also involve a standard fully-susceptible laboratory strain for calculation of resistance ratios.
- In addition to avoiding testing in uncontrolled conditions where possible, improved reporting of rearing and testing conditions is crucial to allow consideration of possible biases, along with more details of sampling protocols to assess likelihood of sampling populations representatively.
- If limited numbers of mosquitoes are available for testing, the use of PBO bioassays are not recommended for the following reasons. The issues of data noise are magnified because the end result is the difference between two bioassays. Further, most tests reveal a positive result if the WHO criteria for presence - an increase of more than 10% mortality - is used, so the evidence will almost always support deployment of PBO-treated nets. Finally, these tests cannot provide a more nuanced quantitative assessment of the level of P450-mediated resistance.
- In general, interpretation of PBO bioassay test results is challenging, with some authors apparently seeking a 10% change, but most either a full return to susceptibility or evidence of a statistically significant increase in mortality compared to the insecticide only exposure. In any of these cases, operational efficacy is difficult to predict confidently. A more practical and operationally relevant alternative might be an increase in testing products directly using cone tests, or where practical video cone test assays.

- In order to better understand the possible operational implications for recommendations on the use of PBO nets in areas of pyrethroid resistance, further investigation is needed to elucidate the biological impact of PBO on P450s, and the molecular basis for any differential efficacy of PBO in synergising different pyrethroids.
- Potential differences in behavioural responses to the different pyrethroids will not be captured by conventional resistance monitoring bioassays, and indeed results may be confounded by different levels of excito-repellency or avoidance behaviour. More research is needed to develop appropriate bioassays for the full range of behaviours that can influenced the efficacy of pyrethroids against resistant mosquitoes.