Status and trends of insecticide resistance in malaria vectors

GMP Entomology and Vector Control and Imperial College London

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Background

• **Good news:** Major progress in malaria prevention & control this century, mainly due to insecticidal vector control

• **Bad news:** Insecticide resistance in malaria vectors threatens these gains

• **Potential threat:** Increased morbidity and mortality from malaria

• **Response:** WHO *Global plan for insecticide resistance management in malaria vectors* (2012)
Key resources

Global plan for insecticide resistance management in malaria vectors (2012)
http://www.who.int/malaria/publications/atoz/gpirm/

Test procedures for insecticide resistance monitoring in malaria vector mosquitoes (Second edition) (2016)
http://www.who.int/malaria/publications/atoz/9789241511575/

Malaria Threats Map
http://www.who.int/malaria/maps/threats

http://www.who.int/malaria/publications/atoz/9789241512138/
Insecticide resistance monitoring

• Should be conducted annually (minimum)
• **Step 1:** Phenotypic monitoring with discriminating concentration bioassays using either:
  ➢ WHO susceptibility tests OR CDC bottle bioassays

• **Step 2:** If resistance confirmed -> further investigations
  ➢ Measure resistance intensity
  ➢ Identify resistance mechanisms, such as via:
    ▪ Synergist-insecticide bioassays
    ▪ Other molecular or biochemical assays

Images: Sven Torfinn/WHO
Insecticide resistance monitoring: procedures

**To determine phenotypic resistance frequency**

- Susceptibility test* with discriminating concentration (1x)
  - ≥ 98% mortality: Susceptible
  - 90–97% mortality: Possible resistance
  - < 90% mortality: Confirmed resistance
  - Repeat test
  - < 98% mortality: Confirmed resistance

**Resistance monitoring outcomes are shown in bold**
- a) WHO insecticide susceptibility test or US Centers for Disease Control and Prevention (CDC) bottle bioassay following standard procedures and using defined dose/concentration with adjustment of mortality outcomes if necessary
- b) Conducted using untested mosquitoes of the same population
- c) Can be conducted using progeny of surviving mosquitoes from bioassays (F1 reared under laboratory conditions)
- d) Can be conducted using mosquitoes tested in bioassays
- e) Test for known resistance mechanisms only
- f) Refers to mechanism of the broad group(s) related to the specific synergist used in the bioassay (e.g., P450 mono oxygenases for PBO)
- g) Implies the involvement of other mechanisms in conferring resistance
- h) Can be reliably assessed only where adjusted mortality for insecticide-only exposure is <90%
- i) Higher considered to be where difference is ≥10%

**To determine resistance intensity**

- Susceptibility test* with intensity concentration (5x)
  - ≥ 98% mortality: Low intensity resistance
  - < 98% mortality: Moderate to high intensity resistance

- Susceptibility test* with intensity concentration (10x)
  - ≥ 98% mortality: Moderate intensity resistance
  - < 98% mortality: High intensity resistance

**To determine resistance mechanism(s)**

- Synergist-insecticide bioassay* comparing insecticide versus synergist-insecticide exposures*
  - Insecticide-synergist mortality not higher* than for insecticide-only
    - Metabolic mechanism* not involved
  - Insecticide-synergist <98% mortality but higher than for insecticide-only
    - Metabolic mechanism* partially involved
  - Insecticide-synergist ≥98% mortality and higher than for insecticide-only
    - Metabolic mechanism* fully involved

- Molecular* or biochemical* assays
  - Outcome and interpretation depend on test used
    - Assessment of resistance allele(s)
      - 0% allelic frequency
      - >0% allelic frequency
    - Other process and outcome
      - Mechanism not detected
      - Mechanism detected
      - Not detected

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**FIG. 3.1**

Overview of process and outcomes for insecticide resistance monitoring in malaria vector mosquitoes. Includes measures of: a) phenotypic resistance frequency via discriminating concentration bioassays, b) resistance intensity via intensity concentration bioassays, and c) resistance mechanisms via synergist-insecticide bioassays, molecular and biochemical assays.
Global report on insecticide resistance in malaria vectors

• **Scope:** Summarize *Anopheles* malaria vector insecticide resistance data from WHO database, for standard monitoring procedures for 2010-2016

• **Aim:** To provide status and baseline for subsequent updates, and to identify any temporal trends in resistance

• **Audience:** National programmes and partners involved in malaria vector control planning and implementation
WHO insecticide resistance database

a) Total data by investigation and assay type

- Discriminating concentration bioassay
- Intensity concentration bioassay
- Molecular assay
- Synergist-insecticide bioassay
- Biochemical assay

b) Total number of collection sites by year and WHO region

- Africa
- Americas
- Eastern Mediterranean
- South-East Asia
- Western Pacific

Data origin (majority): Discriminating concentration bioassays in Africa for An. gambiae s.l. and An. funestus

c) Total data by vector species

- An. gambiae s.l.
- An. funestus s.l.
- An. culicifacies s.l.
- An. albimanus
- An. stephensi
- An. sinensis s.l.
- An. maculatus s.l.
- An. minimus s.l.
- An. philippinensis
- An. flavrostris
- Anopheles spp.*
- Other

* not identified to species
### TABLE 2.1. Overview of common phenotypic resistance indicators, methods, measures and outcomes

<table>
<thead>
<tr>
<th>INDICATOR</th>
<th>METHODS</th>
<th>MEASURES</th>
<th>OUTCOMES</th>
</tr>
</thead>
</table>
| Resistance status       | WHO susceptibility test with discriminating concentration | % mortality of test mosquitoes (adjusted⁹)         | • Confirmed resistance  
                        | CDC bottle bioassay with diagnostic concentration       |                                                    | • Possible resistance  
                        |                                                               |                                                    | • Susceptibility                                             |
| Resistance frequencyᵇ   | WHO susceptibility test with discriminating concentration | 100% minus % mortality of test mosquitoes (adjusted⁹) | • % alive                                           |
|                        | CDC bottle bioassay with diagnostic concentration | 100% minus % incapacitation of test mosquitoes (adjusted⁹) | • % not incapacitated.                             |
| Resistance intensity    | WHO susceptibility test with intensity concentrations | % mortality of test mosquitoes (adjusted⁹), in relation to % mortality for other concentrations tested | • High intensity  
                        | CDC bottle bioassay with intensity concentrations       |                                                    | • Moderate intensity  
                        |                                                               |                                                    | • Low intensity                                               |
|                        |                                                               |                                                   | • Could not be reliably assessed                   |

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ª Using Abbott’s formula as required (Abbott, 1925).

ᵇ This refers to phenotypic resistance only and is different to resistance gene frequency (see Table 2.2)
2010 - 2016: Pyrethroid resistance was common and widespread. Resistance to other insecticide classes was also common.
Reported phenotypic resistance: 2010-2016

Fig. 4.1.
Map showing number of insecticide classes to which resistance in malaria vectors was reported, by country, for the period 2010–2016

 ≥ 1 class = 62 countries
 ≥ 2 classes = 50 countries

Resistance confirmed in all major vector species, and to the four commonly used insecticide classes.
There was variation in resistance frequency across all four insecticide classes, both within and between regions.
• **How?** Statistical model estimates for average resistance frequency change (mosquito survival for 2010-2016 tests)

• **What?** Across insecticide classes and by WHO regions, subregions, major vector species groupings and individual insecticides

• **Approach?** Linear mixed-effects models were fitted to all data within an insecticide class. Fixed effects:
  - 3 species groupings: *An. funestus s.l.*, *An. gambiae s.l.* and other *Anopheles* malaria vectors
  - insecticide types within a class

• Country of data origin included as a random effect to determine overall temporal trends, taking into account:
  - different starting resistance frequencies between countries
  - variable sampling effort between countries and across time
Pyrethroid resistance increased: significantly in *An. funestus s.l.*, moderately in *An. gambiae s.l.* and slightly in other vector species.

Phenotypic resistance frequency: trends 2010-2016

a) Pyrethroids

<table>
<thead>
<tr>
<th></th>
<th>An. funestus s.l.</th>
<th>An. gambiae s.l.</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P↑=1</td>
<td>P↑=0.99</td>
<td>P↑=0.79</td>
</tr>
<tr>
<td>Δ (%)</td>
<td>32%</td>
<td>13%</td>
<td>4.7%</td>
</tr>
<tr>
<td>N</td>
<td>485</td>
<td>5285</td>
<td>2074</td>
</tr>
</tbody>
</table>

Pyrethroid resistance increased: significantly in *An. funestus s.l.*, moderately in *An. gambiae s.l.* and slightly in other vector species.
Overall median changes for other insecticide classes were relatively small. Species cluster-specific changes had too few data points to be well-supported.
Phenotypic resistance: intensity

- Limited data
- Further testing needed to understand pyrethroid resistance intensity
- Further investigation needed to determine the value of intensity data for decision-making
Phenotypic resistance: intensity

Limited data
Further testing needed to understand pyrethroid resistance intensity
Further investigation needed to determine the value of intensity data for decision-making
## Resistance mechanisms: measures

### TABLE 2.2.
**Overview of common metabolic and target-site resistance mechanism indicators, methods, measures and outcomes**

<table>
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</table>
| Metabolic resistance   | WHO synergist–insecticide bioassays          | % mortality of test mosquitoes (adjusted\(^a\)) when exposed to synergist and insecticide compared with % mortality when exposed to insecticide only | • Full involvement  
• Partial involvement  
• No involvement  
• Could not be reliably assessed |
|                        | CDC bottle synergist–insecticide bioassays   | % incapacitation of test mosquitoes (adjusted\(^a\)) when exposed to synergist and insecticide compared with % incapacitation when exposed to insecticide only | • Full involvement  
• Partial involvement  
• No involvement  
• Could not be reliably assessed |
| Molecular assays        | Upregulation of gene expression\(^b\)        |                                                                          | • Present  
• Absent |
| Biochemical assays      | Enzymatic activity, in relation to susceptible mosquitoes |                                                                          | • Present (upregulated)  
• Absent (not upregulated). |
| Target-site resistance  | Molecular assays                             | % allelic frequency                                                       | • Present  
• Absent |
|                        | Biochemical assays                           | Enzymatic activity, in relation to susceptible population or % allelic frequency (or both) | • Present (upregulated)  
• Absent (not upregulated) |

\(^a\) Using Abbott’s formula as required (Abbott, 1925).  
\(^b\) Molecular assays that measure allelic frequencies are also available but are not commonly used.

Global Malaria Programme
Resistance mechanisms: metabolic

- Insufficient testing/reporting precludes further analyses.

In areas where metabolic resistance mechanisms were tested for, they were often detected.
Resistance mechanisms: target-site

- Insufficient testing/reporting precludes further analyses.

In areas where target-site resistance mechanisms were tested for, they were often detected.
Considerations
Key challenges

- Availability of data (annual, representative sites)
- Quality and completeness of data
- Timely reporting
- Data sharing
- Capacity
- Funding
- Need for improved methods of surveillance
- Supply of test kits
Conclusions

• Resistance to four insecticide classes is widespread and increasing (especially to pyrethroids and in An. funestus s.l.)

• Complete extent of resistance unknown because:
  ➢ many countries do not carry out routine monitoring
  ➢ countries collecting data do not report or share data in a timely manner
  ➢ no data yet for new insecticides (e.g. neonicotinoids - IRS product PQ listed 2017)

• Impact of insecticide resistance on effectiveness of vector-control tools remains poorly-understood

• **BUT**
  ... the potential that increasing resistance may reduce the efficacy of insecticidal interventions remains concerning
Outlook

- Conclusive evidence of control failure should not be the trigger for action; pre-emptive resistance management is required
- Existing tools should be strategically deployed, as guided by a national insecticide resistance monitoring and management plan
- New tools are needed - once public health value has been validated these must be incorporated in a timely manner
- Extended monitoring required to measure vector susceptibility to those active ingredients anticipated in new tools (e.g. neonicotinoids and pyrroles)
Resistance monitoring & management plans needed. These must leverage available interventions proactively & appropriately.

FIG. 7.1.
Status of national insecticide resistance monitoring and management plans, as of October 2017

Data source: WHO (2017e)

Some progress has been made. Further effort is required.
More information: Malaria Threats Map

Malaria Threats Map

Tracking biological challenges to malaria control and elimination

VECTOR
INSECTICIDE RESISTANCE

Resistance of malaria mosquitoes to insecticides used in core prevention tools of treated bed nets and indoor residual sprays threatens vector control effectiveness

PARASITE
pfhrp2/3 GENE DELETIONS

Gene deletions among some malaria parasites cause false negative diagnostic test results, complicating case management and control

PARASITE
DRUG RESISTANCE

Resistance of malaria parasites to artemisinin – the core compound of the best available antimalarial medicines – threatens antimalarial drug efficacy

www.who.int/malaria/maps/threats
Ongoing work, through collaboration

- Build a **nonlinear statistical model** for temporal analyses and examine correlations (within and between insecticide classes; between vector species)
- Test for **relationships** between resistance indicators (frequency, intensity and mechanisms)
- Map **spatial variability** in resistance indicators to guide surveillance and control (e.g. to identify areas for potential deployment of pyrethroid-PBO nets)
- Develop **decision framework** to link epidemiology and resistance data to selection of vector control interventions
- Identify **relationships** between resistance and LLIN/IRS coverage
- Assess the **epidemiological implications** of trends in resistance
Full acknowledgements are listed in the report. In brief:

| Global report on insecticide resistance in malaria vectors: 2010-2016 | **Formulation and/or review of report:**  
• WHO Global Malaria Programme  
• Liverpool School of Tropical Medicine  
• Imperial College London  
• WHO Malaria Vector Control Technical Expert Group |
|---------------------------------|--------------------------------------------------------------------------------------------------|
| WHO insecticide resistance database | **Collection and/or validation of data:**  
• All national programmes  
• WHO regional, subregional, country and zonal offices  
• Other partners (PMI, MAP)  
• WHO Global Malaria Programme |
| Malaria Threats Map | **Design and/or implementation:**  
• WHO Global Malaria Programme  
• BlueRaster LLC  
• WHO Polio department  
• WHO ITC department |

Available on WHO website: http://www.who.int/malaria/publications/atoz/9789241514057/
Targeting mosquitoes to tackle malaria: