Insecticide resistance of Malaria Vectors, the current update and future plan
Insecticide resistance

- Resistance is a serious threat to any vector control programme relying on insecticide
- Epidemiological impact of resistance not properly documented
- Impact may depend on insecticide and application methods:
  - IRS
  - ITN/LLIN
  - Larval Control
Areas of particular concern are SSA and India due to reports of widespread resistance and high rates of malaria transmission.
Malaria vector control relies on the efficacy of four classes of insecticides.

With the emergence and spread of insecticide resistance among malaria vectors, our global control efforts are at high risk.

**Implications for malaria control**

- High reliance of malaria vector control on pyrethroids and on the other three classes of insecticides.

- With the emergence and spread of insecticide resistance among malaria vectors, our global control efforts are at high risk.
WHO GMP 2010 meeting report

- Action must be immediate and pre-emotive
- Preserve vector susceptibility (alternative vector control)
- Prompt action (resistance management)
- Best practices for immediate use
- Monitoring
- New product
- Capacity building
Global Plan for Insecticide Resistance Management (GPRIM)

- ~140 stakeholders consulted, of whom ~50 are control programme people
- 120+ interviews conducted
- 60+ stakeholders provided detailed comments on fact-base – most of them country programme people
- GPIRM launched in May
- Funded by Bill & Melinda Gates Foundation; support from BCG
Objective Global Plan For Insecticide resistance Management

1. Define what is known, what is assumed and what remains unknown with regard to insecticide resistance among malaria vectors, its trajectory, its operational impact and options for managing the problem.

2. Estimate the potential impact of insecticide resistance on malaria burden as well as the financial cost of monitoring and managing insecticide resistance.

3. Using these elements as the foundation, define the plan for managing insecticide resistance and the way forward, including:
   - Short-term action plan with clear responsibilities
   - Ongoing research and development requirements
GPRIM Strategy

- Plan and implement IR management strategies in malaria endemic countries
- Ensure proper, timely entomological and resistance monitoring and effective data management
- Develop new, innovative vector control tools
- Fill gaps in knowledge on mechanisms of IR and the impact of current IR management approaches
- Ensure that key enabling mechanisms (advocacy, human and financial resources) are in place
Resistance mechanisms

- Typically two major mechanisms are assumed to be responsible for pyrethroid insecticide resistance:
  1. Changes in the target site that reduce the binding of insecticides (kdr resistance),
  2. Increases in the rate of insecticide metabolism that lower the amount of insecticide reaching the target site (cytochrome P450 enzyme),
  3. It is also important to consider other physiological or behavioural changes in the mosquito population that may impact on the efficacy of pyrethroid insecticides.
Definition of insecticide resistance

- The development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal population of the same species—WHO Definition.

- Resistance is a genetically inherited characteristic which increases in the vector population as a direct result of the selective effects of the insecticide.
Cross resistance

DDT  PY
Sharing the same target sites

OP  PY
Being detoxified by the same enzymes
WHO discriminating concentrations of insecticides

- The concentrations were established under standardised laboratory conditions, using known “susceptible” strains (WHO/CDS/CPC/MAL/98.12)
- Discriminating concentration for a given insecticide and a mosquito species was defined in two ways:
  1. Twice the lowest concentration that gave systematically 100% mortality after 60 minutes exposure and a holding period of 24 hrs on a susceptible strain or a susceptible population
  2. Twice the $\text{LC}_{99}$ determined by the baseline susceptible strain or a susceptible population
<table>
<thead>
<tr>
<th>Insecticide class</th>
<th>Insecticide</th>
<th>Discriminating concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organochlorine</td>
<td>DDT</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>Dieldrin</td>
<td>0.4%</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>Malathion</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>Fenitrothion</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>Pirimiphos-methyl</td>
<td>0.25%</td>
</tr>
<tr>
<td>Carbamates</td>
<td>Propoxur</td>
<td>0.1%</td>
</tr>
<tr>
<td></td>
<td>Bendiocarb</td>
<td>0.1%</td>
</tr>
<tr>
<td></td>
<td>Carbosulfan</td>
<td>0.4%</td>
</tr>
<tr>
<td>Pyrethroids</td>
<td>Permethrin</td>
<td>0.75%</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin</td>
<td>0.05%</td>
</tr>
<tr>
<td></td>
<td>Lambda-cyhalothrin</td>
<td>0.05%</td>
</tr>
<tr>
<td></td>
<td>Etofenprox</td>
<td>0.5%</td>
</tr>
</tbody>
</table>
SUPPLIES FOR MONITORING INSECTICIDE RESISTANCE IN DISEASE VECTORS

 Procedures and conditions
- Four replicates (25 mosquitoes)
- Two control replicates (25 each)
Collection

- The age, physiological status and gender of mosquitoes can influence the results of the susceptibility tests.
- Ideally, non-blood fed females aged 3-5 days post emergence.
Female mosquito of unknown age

- Wild caught females: when larval collections are not possible.
  - Physiological status (unfed/blood fed, semi-gravid, gravid) should be carefully noted.
  - Females should be provided with sugar-water until the tests are ready to be carried out.
- **Advantages:** sample size (wild genome number) and convenience.
- **Disadvantage:** age unknown => under-estimation of resistance.
Monitoring tools

- New tools for early detection of resistance:
  - Biochemical and molecular assays now available, more sanative than bioassay,
  - Biological Biochemical and molecular assays are complementary tools to detect resistance and trends
WHO definitions - 1998

- 98-100% mortality indicates susceptibility
- 80-97% mortality suggests the possibility of resistance that needs to be confirmed
- <80% mortality suggests resistance.

Where <95% mortality occurs in tests that have been conducted under optimum conditions with a sample size of >100 mosquitoes then resistance can be strongly suspected.
Interpretation of results - 2012 new guidelines

- A mortality in the range 98–100% indicates susceptibility.
- A mortality of less than 98% is suggestive of the existence of resistance and further investigation is needed.
- If the observed mortality (corrected if necessary) is between 90% and 97%, the presence of resistant genes in the vector population must be confirmed.
- If mortality is less than 90%, confirmation of the existence of resistant genes in the test population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of EACH species was tested.
- When resistance is confirmed, pre-emptive action MUST be taken to manage insecticide resistance and to ensure that the effectiveness of insecticides used for malaria vector control is preserved.
Pyrethroid Resistance In African Malaria Vectors
ANVR Atlas on Vector Resistance

Legend
Anopheles gambiae s.s.
Permethrin
- Resistant (% Mortality <90)
- Resistance to be confirmed (% Mortality 90-98)
- Susceptible (% Mortality 98-100)

Legend
Anopheles arabiensis
Permethrin
- Resistant (<90 % Mortality)
- Resistance to be confirmed (90 to 98 % M)
- Susceptible (98 to 100 % mortality)

Country limit

Kilometers
Current status of IR in Asia Pacific Region

- Except in Greater Mekong Subregion, there are no systematic and no coordinated efforts to screen IR in anophelines in this region,
- Most data if available are also out dated. In short, there is no baseline database on anopheline IR in the region,
- The principal reason is the difficulty in obtaining sufficient number of anophelines for testing using the WHO test method & the lack of anopheline colonisation skills.
Anopheles dirus s.l.
Van Bortel et al. MJ 2008, 7: 102
Bioassays: permethrin
The next steps (1)

- Capacity building and strengthening:
  - Strengthen national capacity to plan and implement routine monitoring of insecticide resistance and management as outlined in the Global Plan for Insecticide Resistance Management (GPIRM) including allocation of adequate resources to implement the proposed national plans.
  - To build country capacity on IR and IR data analysis to be able to predict the trend in the evolution of insecticide resistance and design mitigation measures,
The next steps (2)

- Information dissemination:
  - Ensure member countries of the region have adequate information on the susceptibility status of malaria vectors to all current and potential insecticides to be used by the national vector-borne disease control programmes,
  - The Asia Pacific Insecticide Resistance Network (APNVR), established in 2011 should be used as information exchange, coordination of IR
  - Establishment of Regional Reference Centre(s) on IR
Resistance Monitoring

- WHO susceptibility test remains the gold standard for early detection of insecticide resistance (following the revised Test Procedures for Monitoring Insecticide Resistance, WHO 2012),
- CDC bottle assay methods can be used as supplementary to WHO method (the level of resistance)
- Adoption of new supplementary test method e.g. biochemical tests in places where WHO test method cannot be conducted (e.g. insufficient samples)
Research

- Research on resistance detection using molecular techniques esp. on detection of kdr (knockdown resistance which cannot be detected by either bioassay or biochemical tests); elucidation of resistance mechanisms
Conclusions

- The criteria for resistance are updated in 2012 guidelines.
- The LLIN/insecticide resistance Cochrane review is currently with the editorial team.
- WHO standard test kit is still a gold standard for IR monitoring.
- The IR data on Anopheleline in the region are patchy and only conducted as research.
- More coordinated efforts on IR monitoring are needed in the region.
- Capacity building to strengthen the IR monitoring and data analysis are required.