Monitoring of insecticides resistance in malaria vector (s) in Pakistan

ABSTRACT: The development of resistance against insecticides is the major threat to any program which relies on use of chemicals. It is important to know when a vector species develop resistance in order to decide the most appropriate resistance management strategies such as interruption of spraying, change of insecticides or rotation of insecticides, or by other means. Since the eradication era during early 1960’s, Pakistan implemented vector control interventions very successfully depending on the use of chemicals, with indoor residual house spraying as the main intervention. Since 1995 Pakistan is continuously using pyrethroids while in many part of the world the resistance in malaria vectors against pyrethroids has been reported. Despite this fact there has been no systematic effort to monitor the level of resistance in malaria vectors against insecticides in the country since 1990’s due to lack of expertise and updated knowledge on vector (s). Directorate of Malaria Control (DoMC) developed this project for monitoring of insecticides resistance in malaria vector (s) to all four groups of insecticides i.e. Pyrethroids (Permethrin, 0.75%), Organochlorinated Hydrocarbon (DDT, 4%), Carbamate (Bendiocarb, 0.1%), and Organophosphate (Malathion 5%). The trials will be conducted at 8 sentinel sites according to WHO guidelines. Both unfed and F-1 generation females anophelines will be used in susceptibility tests. The exposure time will 1 hrs. with exception that in case of Deltamethrin 0.05% and Permethrin 0.75% the exposure time will be 30 minutes. The exposure tubes will be kept +90F and relative humidity 70-80%. Mortalities will be recorded after 24 hrs. of hold periods. In long term it is expected that the results from this study will generate up-graded knowledge of resistance in malaria vectors against insecticides. The final recommendations of the study will also enable the national health planners and policy makers to make better informed decisions and design more effective resistance management strategies, including switching over from one group of insecticides to new one or rotation of insecticides groups, regular operational research and capacity building program. These recommendations will also provide guidelines for judicious and rational use of residual insecticides for future vector control.
Monitoring of insecticides resistance in malaria vector (s) in Pakistan

Directorate of Malaria Control

Ministry of Health.
Islamabad
September 2009

Muhammad Mukhtar, Senior Entomologist, Directorate of Malaria Control, Ministry of Health, mukasbilumm@gmail.com
1. Title of Study:

Monitoring the resistance level of malaria vector(s) against all 4 major groups of insecticides at 8 sentinel sites established four provinces including AJK and FATA in Pakistan.

2. Investigator(s)

Principal Investigator

a). Muhammad Mukhtar  
b). Muhammad Aslam  

a). Senior Vector Control Specialist/Entomologist  
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b). Senior Advisor

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Coordinator. VCB-Health Services Academy (HAS)-Islamabad

c). Co-Researchers  
Mrs. Ghazala Nadeem  
Senior Entomologist  
NIMRT-Lahore.

2). Review Committee

The project proposal has been reviewed by a penal of following experts who are also the members of Technical Advisory Committee on Malaria (TACOM).

<table>
<thead>
<tr>
<th>Name and Designation</th>
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<tr>
<td>1 Dr. Humanyun Rathore Coordinator. VCB</td>
<td>Health Services Academy (HAS)-Islamabad</td>
<td>0333-5211478</td>
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</table>
The major TORs of TACOM are to advise Program on:

1. National priorities, policies and guidelines.
2. Programmatic strategies, activities and gaps.
3. Processes and potential source of assistance to address program gaps.
4. Research priorities, plans, ethical clearance, execution and dissemination.
5. Development of liaison and linkages with other potential partners including research institutions.
The development of resistance against insecticides is the major threat to any program around the globe which relies on use of chemicals. Appropriate monitoring of vector susceptibility/resistance to insecticides shall be an integral part of planning and evaluation of insecticide uses in malaria control programmes, and it is one of the most important tools for determination of success and failure of field application of an insecticide which provides very vital information for the selection of a new insecticide or replacement of an insecticide in use. Since the eradication era during early 1960’s, Pakistan is implementing vector control interventions very successfully depending on the use of chemicals of different groups during different periods of timing. During 1980 to 1985 there has been massive efforts made by Rathore et al., to establish the baseline for resistance in anophelines in Pakistan. However, since 1990 despite the use of insecticides on large scale there has been no systematic effort to monitor the level of resistance in malaria vectors against insecticides in the country due to lack of expertise and updated knowledge on vector (s). Directorate of Malaria Control (DoMC) developed this project for monitoring of insecticides resistance in malaria vector (s) to all four groups of insecticides i.e. Pyrethroids (Deltamethrin 0.05% and Permethrin, 0.75%), Organochlorinated Hydrocarbon (DDT, 4%), Carbamate (Bendiocarb,0.1%), and Organophosphate (Malathion 5%). The trials will be conducted at 8 sentinel sites during peak transmission periods according to WHO guidelines (2002). Both unfed and F-1 generation female anophelines will be used in susceptibility tests. The exposure time will 1 hrs. with exception that in case of Deltamethrin 0.050% and Permethrin
0.75% the exposure time will be 30 minutes. The exposure tubes will be kept 25°C and relative humidity 70-80%. Mortalities will be recorded after 24 hrs. of hold periods. In long term it is expected that the results from this study will generate upgraded and new knowledge of resistance in malaria vectors against insecticides. The final recommendations of the study will also enable the national health planners and policy makers to make better informed decisions and design more effective resistance management strategies, including switching over from one group of insecticides to new one or rotation of insecticides groups, regular operational research and capacity building program. These recommendations will also provide guidelines for judicious and rationale use of residual insecticides for future vector control.
1. Introduction

Malaria is the 2nd most prevalent and devastating disease in the country (HMIS, 2006) and has been a major cause of morbidity in Pakistan and continues to be a major threat to the health of millions who live in highly endemic areas of the country. More than 90% of disease burden in the country is shared by the 56 highly endemic districts, mostly located in Balochistan, FATA, Sindh, and NWFP. More than 40% of the reported cases from these districts are due to falciparum malaria, more dangerous form of malaria.

Malaria is a seasonal disease and major malaria transmission season in Pakistan is post monsoon (September-November) each year. However, along the coastal areas and western international bordering areas of country with Iran and Afghanistan the disease prevails throughout the year. A short transmission season during spring months (March-April) is also evident, of which most of the cases are delay expression of disease transmitted during post monsoon season or may be due to the second episode of the disease caused by relapsing vivax malaria. However there are many concrete evidences of the link between incidence of malaria and vector densities. Both transmission periods are directly linked with the malaria vector densities. After monsoon when water become stagnant and humidity level rise up, the vector...
densities starts building and attains their peaks. This shows that transmission and vector densities are inter-linked with each other.

1.2 Checklist of anophelines of Pakistan

The mosquito fauna of Pakistan is poorly studied, and there is no updated checklist of anophelines of Pakistan. First checklist of mosquitoes of Pakistan was published by Aslamkhan (1971) lists 38 species, 2 subspecies and 1 variety of anopheline mosquitoes. When species from the former East Pakistan (presently Bangladesh) are excluded, the list reduces to 22 species. Mukhtar et al., (in process) revised the mosquito fauna of Pakistan and till today recorded 132 species of mosquito from Pakistan. In 2001, Amarasinghe and Mukhtar published an up-to-date key of anophelines of Pakistan including following 24 Anopheles species:

1. *Anopheles fluviatilis*  
2. *An. subpictus*  
3. *An. sergenti*  
4. *An. culicifacies*  
5. *An. dthali*  
6. *An. turkhudi*  
7. *An. annularis*  
8. *An. pallidus*  
9. *An. moghulensis*  
10. *An. multicolor*  
11. *An. pulcherrimus*  
12. *An. stephensi*  
13. *An. superpictus*  
14. *An. splendidus*  
15. *An. maculatus*  
16. *An. Theobald*  
17. *An. willmori*  
18. *An. barianensis*  
19. *An. gigas*  
20. *An. claviger*  
21. *An. lindesayi*  
22. *An. barbirostris*  
23. *An. peditaeniatus*  
24. *An. nigerrimus*
### 1.3 Distribution of anophelines in Pakistan

<table>
<thead>
<tr>
<th>Species name</th>
<th>Punjab</th>
<th>NWFP/FATA</th>
<th>Sind</th>
<th>Balochistan</th>
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<tr>
<td>An. stephensi</td>
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<td>+</td>
<td>+</td>
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<td>An. culicifacies</td>
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Monitoring of insecticides resistance in malaria vector(s) in Pakistan

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Source: Mosquito fauna of Pakistan (Mukhtar et al., in progress)

1.3 Vectors in Pakistan

In Pakistan anophelines are chiefly zoophilic, making it difficult to interpret the role of different vectors in ecology of malaria (Reisen & Mehmood, 1980). Investigations in Punjab showed that the instability of malaria is due to overwhelming preferences of mosquitoes for bovine host (Mehmood et al., 1984, 1985; Nalin et al., 1985). Of 24 Anopheles species of Pakistan (Amerasinghe et al., 2001) only one species, An. culicifacies s.l. has been reported as confirmed malaria vector in rural areas of Pakistani Punjab (Mehmood & MecDonald, 1985; Herrel et al., 2004, Klikinburg et al., 2004). Recent studies in NWFP showed that An. stephensi can also be highly abundant in rural areas and have been documented as test positive for sporozoite antigen (Rowland et al., 1997).

1.4 Primary vectors

Currently, only An. culicifacies s.l. has been reported as primary malaria vector in Pakistan. This species has uniform distribution across the country. It has been found naturally infected with Plasmodia in some villages of central Punjab. Recently two new species An. fluviatilis and An. annularis have been identified from FATA (North and
South Waziristan) and Balochistan province, district Zhob (Mukhtar and Chandana 2006). Previously these two vector species have been considered confirmed malaria vectors in Iran and Afghanistan.

1.5 Secondary vectors

*An. stephensi* has been reported as secondary vector with little more importance in the urban areas in Pakistan. Recently it has been concluded that *An. stephensi* may be a more important vector than previously believed *An. culicifacies* in northern Punjab and North West Frontier Province (Rowland et al., 1997).

1.6 Suspected vectors

Apart from the above two species there are also some suspected malaria vectors in Pakistan like *An. pulcherrimus*, *An. maculates*, and *An. superpictus*. Mostly these are considered the suspected vectors in mountainous and foothill areas of NWFP and in Punjab (Mukhtar and Chandana 2006, Rowland et al., 1997).

1.7 Vector control interventions in Pakistan and resistance issues

Reduction of malaria burden in the country is both a national and provincial priority. Government of Pakistan is committed to combat malaria and achieve its national targets, which are in line with the Global Roll Back Malaria (RBM) initiative and MDGs. Since the commencement of global Malaria eradication program by WHO in 1960’s, Pakistan is implementing vector control very successfully depending on the use of chemicals, with indoor residual house spraying as the main intervention. During 1961 DDT was the main insecticide used for malaria
vector control in Pakistan. During early 1970's resistance in *Anopheles culicifacies* and *An. stephensi*, against D.D.T (Organo-chlorinated hydro-carbans) was confirmed in some districts of Pakistan (Rathore et al., 1980, WHO, 1997). Therefore in 1972 BHC of same group was introduced. Similarly, Malathion and Sumithion (Organophosphate OP) group were also introduced during 1979 and DDT was abandoned due to vector resistance. However BHC use was continued up to 1983. From early 1980’s to mid 1990’s only Malathion was used for malaria vector control. High frequency of malathion-resistance was reported in *An. stephensi* in some districts of Punjab, and lower frequencies were noted in the NWFP and the Sindh province during mid 1980’s (Rathore et al., 1980 and 1983). *An. culicifacies*, had also been reported resistant to malathion in certain parts of Pakistan during late 1980’s (Rathore et al., 1980, 1983). Since 1996 deltamethrin (Pyrethroids) is being used for residual house spraying.

1.8 Rational of the study

Since late 1980’s in Pakistan vector component has been neglected to a remarkable extent and vector resistance tests could not be again conducted in country due to human and logistic contrains. All vector control interventions such as indoor residual spraying, larviciding and fogging etc were carried out without any technical assistance and guidelines. The efforts for monitoring and evaluation of vector control interventions and their impact of disease transmission had gradually been declined.

Even at present the withdrawal of external support and less supports from Government side, there is very weak entomological surveillance system in the country. Pakistan is continuously using pyrethoroids for last 12 years while in many part of the world the resistance in malaria vectors against pyrethroids has been reported. However in Pakistan there is no systematic effort to monitor the level of resistance in
malaria vectors against insecticides in the country for last two decades. Very old knowledge of vector fauna and their bionomics further compounded the situation in the country. All these factors have made it extremely difficult for Malaria Control Program to assess the impact of vector control interventions. Keeping in view the rising trend of malaria and very old knowledge of resistance in malaria vectors against insecticides the Directorate of Malaria Control (DoMC) developed the proposal for with the overall objective to up-grade the information on resistance level in malaria vector (s) against all 4 groups of insecticides at selected 8 sentinel sites.

1.9 Specific Objectives

- To establish the baseline susceptibility of the different vector species in the area.
- To monitor the possible changes in the susceptibility/resistance level in vector population being controlled by insecticides for future vector control strategy.
- To assess the vectors susceptibility to potential alternative insecticides.
- To identify the mechanisms of resistance and cross resistance pattern
- To assess/updating and mapping the resistance level of major malaria vectors i.e. *An. culicifacies* and *An. stephensi* in country.

The secondary objectives will be;

- To establish net work of sentinel sites for monitoring of insecticides resistance in vector.
- To monitor the population densities and trend of vectors in Pakistan.
- To enhance the capacity and competence of district level malaria staff especially dealing with vector control interventions.
Monitoring of insecticides resistance in malaria vector(s) in Pakistan

- To analyze data from all sentinel sites and report writing.
- To scale up the resistance monitoring strategy in other parts of the country in light of recommendations of present proposed pilot project.
- Sharing of data with GFATM

1.9 Outcome of the study

In long term it is expected that the results from this study will generate up-graded knowledge of resistance in malaria vectors against insecticides. The final recommendations of the study will enable the national health planners and policy makers to:

1. Make better informed decisions and design more effective resistance management strategies

2. Switching over from one group of insecticides to another one or rotation of insecticides groups

3. Continuation of regular operational research and

4. Capacity enhancement on regular intervals.

This will provide necessary informations and guidelines for judicious and rational use of residual insecticides for future vector control operations in the country. Such changes are essential to make a significant contribution to ameliorate malaria in Pakistan.
2. Materials and Methodology

More details on project and its questioners can be obtained from Muhammad Mukhtar. Senior Vector Control specialist & Incharge R&D Wing of Directorate of Malaria Control. Ministry of Health.

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