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## Indication of pyrethroid resistance in the main malaria vector, *Anopheles stephensi* from Iran

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### ABSTRACT

**Objective:** To investigate insecticide resistance in target species for better insecticide resistance management in malaria control programs. **Methods:** The status of insecticide resistance to different insecticides in *Anopheles stephensi* (*An. stephensi*) including DDT 4%, lambda-cyhalothrin 0.50%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.50% was performed according to WHO standard method. **Results:** The mortality rate to lambda-cyhalothrin, permethrin, cyfluthrin, deltamethrin, etofenprox and DDT was (88.0±3.2), (92.0±2.7), (52.0±5.0), (96.0±2.2), (90.0±3.0) and (41.0±5.7) percent, respectively at diagnostic dose for one hour exposure time followed by 24 h recovery period. **Conclusions:** These results showed first indication of pyrethroid resistance in *An. stephensi* in a malarious area, from southern Iran. There is widespread, multiple resistances in the country in *An. stephensi* to organochlorine and some report of tolerance to organophosphate insecticides and recently to pyrethroids. However, results of this paper will provide a clue for monitoring and mapping of insecticide resistance in the main malaria vector for implementation of any vector control.

## 1. Introduction

Malaria is still a major endemic disease in foci located in south and southeast of Iran. According to the report of Ministry of Health and Medical Education of the country the annual malaria cases have been reported from 66 075 to 3 000 during 1995–2010, indicating the sharp decline of disease. It is unstable with two seasonal peaks mainly in spring and autumn. These areas include the provinces of Sistan & Baluchistan, Hormozgan and Kerman. In this part of the country six Anopheline mosquitoes including *Anopheles culicifacies* (*An. culicifacies*) s.l., *Anopheles stephensi* (*An. stephensi*), *Anopheles dthali* (*An. dthali*), *Anopheles fluviatilis* (*An. fluviatilis*) s.l., *Anopheles superpictus* (*An. superpictus*), and *Anopheles pulcherrimus* (*An. pulcherrimus*) are known to be the malaria vectors, while *Anopheles sacharovi* (*An. sacharovi*) and *Anopheles maculipennis* (*An. maculipennis*) s.l. are considered as

malaria vectors in northern part of the country [1–11]. There are also occasional outbreaks of malaria in northwest of Iran. The country is now launching the elimination of malaria.

Different studies have been conducted during more than 80 years on malaria and its vectors. So far 33 species from 2 subspecies (*Anopheles* and *Cellia*), siblings, genotype and type forms are recorded in the country, 18 out of them are in complexes or groups that introduced as malaria vectors. Different studies have introduced *An. stephensi* (type, intermediate and mysorensis forms), *An. superpictus* (X, Y, Z genotypes), *An. culicifacies* (sibling A,B), *An. fluviatilis* (sibling T,U), *An. maculipennis* group (*An. atroparvus*, *An. maculipennis*, *An. melanoon*, *An. messeae*, *An. persiensis*, *An. sacharovi*) and *An. dthali* as confirmed malaria vectors in the country. Also *An. pulcherrimus* is detected serologically positive to malaria parasite(s) in Baluchistan malaria foci, southeastern of the country. Figure 1 shows the distribution of *An. stephensi* in the country.

Seasonal activity of Anopheline mosquitoes varies in different areas due to environmental conditions. It shows one peak in northwest especially in summer, however, there are two peaks of activity in coastal warm and humid regions

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in the southern part of Iran with oriental epidemiological characteristics.

*Plasmodium vivax* (*P. vivax*) is dominant agent of malaria in Iran, while *Plasmodium falciparum* (*P. falciparum*) is reported and infects about 15% of patients.

There are different methods including biological, chemical and environmental management for malaria vector control. Chemical control had been the main method for combating against adult stage of malaria vector since eradication era. Insecticide application for adult mosquito control started with organochlorines (DDT, dieldrin and BHC) during the 1960's, followed by organophosphates (malathion and pirimiphos-methyl) for 2 decades from 1966 and continued with the carbamate, propoxur during 1977–1990, and then with pyrethroids (lambdacyhalothrin/deltamethrin). Temephos, chlorpyrifos-methyl and pirimiphos-methyl was used for larviciding from 1970 to 1992. Resistance of *An. stephensi* to DDT, dieldrin and malathion was reported for the first time in 1957, 1960 and 1976, respectively. Resistance of *An. stephensi*, especially its adult stage against DDT, dieldrin and malathion have been widely reported in Middle east and Indian subcontinent regions<sup>[12]</sup>.

Malaria control in the country is now based on use of deltamethrin (5% WP) as an adulticide as 25 mg/m<sup>2</sup> for indoor residual spraying (IRS) and *Bacillus thuringiensis* as a larvicide. Recently Long Lasting Impregnated Net of Olyset is recommended for southern part. Due to simultaneous use of pyrethroids either in IRS and LLINs against target mosquitoes, and the lack of no rotation strategy with other classes of insecticides, it is expected to evolve resistance gene of tolerant and resistance in the opulation of Anopheline vectors. Therefore the aim of this study was to determine the current status of susceptibility to insecticides in *An. stephensi*, the main malaria vecotr of coastal southern part of Iran.

## 2. Materials and methods

### 2.1. Study area

This study was carried out in Chabahar sea port (25°25' N, 60°45' E) Sistan and Baluchistan province, Iran. The larvae were collected from breeding places and then maintained in the insectary. All the tests were carried out on this species during spring 2011.

### 2.2. Mosquito sampling, rearing and identification

Collected larvae were reared under insectary conditions at 25–29 °C, 12:12 (light:dark) h photo-period and 50%–70% relative humidity, and were fed with 10% aqueous sucrose solution.

The insectary is using for International Diploma Course of Malaria Programe and Planning which designated as WHO collborating centre. The field strain was reared and

F1 progeny of 2–3-day-old sugar fed females were used for the tests. Mosquitoes were identified morphologically in the field by use of a standardized key for medically important anophelines of Iran.

### 2.3. Adult susceptibility test

Tests on adults were carried out according to the method of WHO<sup>[12]</sup>. At each test at least 100 mosquitoes representing 4–5 individual replicates of 20–25 adults were tested. To reduce variability in the replicates, sugar fed females were used. The exposure tubes were held in a vertical position during the tests. The exposure time for each insecticide was 1 h. The mortality rate was scored after a 24 h recovery period. Insecticide exposure took place in a room with a temperature of (27±2) °C and holding tubes were held in a room condition of (27±2) °C and relative humidity of 55%–60%. Error bars for each mortality were calculated based on statistical method at  $\alpha = 5\%$

### 2.4. Insecticides impregnated papers

The following insecticides impregnated papers were supplied by WHO: DDT 4%, lambdacyhalothrin 0.05%, deltamethrin 0.05%, permethrin 0.75%, cyfluthrin 0.15% and etofenprox 0.5%. For the control of organochlorine insecticides and pyrethroids insecticides the, mineral oil, and silicon oil impregnated papers were used, respectively.

### 2.5. Statistical analysis

The bioassay result was corrected using the Abbott formula when the control mortality was between 5% and 20%<sup>[13]</sup>. In some circumstance we plotted regression line based for calculation of LT<sub>50</sub> and its 95% confidence intervals.

### 2.6. Criteria for susceptibility

The bioassay results were summarized in three resistance classes as defined by WHO<sup>[12]</sup>: (1) susceptible when mortality was 98% or higher, (2) possible resistant so called tolerant, when mortality was between 97% and 80%, and (3) resistant when the mortality was lower than 80%.

## 3. Results

The mortality of *An. stephensi* to lambdacyhalothrin, permethrin, cyfluthrin, deltamethrin, etofenprox and DDT was (88.0±3.2), (92.0±2.7), (52.0±5.0), (96.0±2.2), (90.0±3.0) and (41.0±5.7) percent, respectively (Table 1). Based on WHO criteria, it should be concluded that, this species is exhibiting resistance to DDT and some pyrethroids. Table 2 shows the LT<sub>50</sub> of *An. stephensi* to different insecticides.

**Table 1**

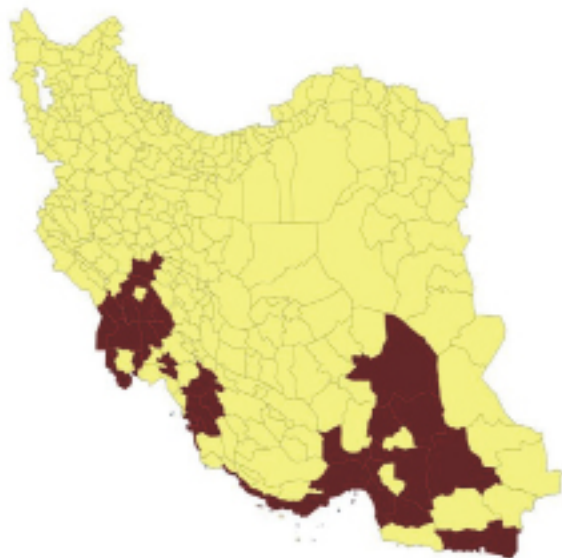
Mortality of *An. stephensi* to different insecticides at diagnostic dose for one hour exposure time and 24 h recovery period, southeastern Iran, 2011.

Insecticide	No. tested	No. Dead	Mortality (%)	Abbotts correction $\pm$ EB
Lambdacyhalothrin 0.05%	103	91	88.4	88.0 $\pm$ 3.2
Permethrin 0.75%	99	92	92.3	92.0 $\pm$ 2.7
Cyfluthrin 0.15%	100	55	55.0	52.0 $\pm$ 5.0
Deltamethrin 0.05%	77	74	96.0	96.0 $\pm$ 2.2
Etofenprox 0.50%	100	91	91.0	90.0 $\pm$ 3.0
DDT 4.00%	75	34	45.0	41.0 $\pm$ 5.7
Control	179	13	7.0	–

**Table 2**

Probit regression line parameters of different insecticides against *An. stephensi*, southeastern Iran, 2011.

Insecticides	A	B $\pm$ SE	LT <sub>50</sub> , 95% CI	$\chi^2(df)$	P	Y=aX +b
Lambdacyhalothrin	-3.281	1.305 $\pm$ 0.326	72.992 327.110 1101.824	11.215(3)	<0.05	Y = -3.281 + 1.305 X
Permethrin	-4.112	1.577 $\pm$ 0.152	314.375 404.947 507.061	6.093(4)	>0.05	Y = -4.112 + 1.577 X
Cyfluthrin	-4.682	1.863 $\pm$ 0.480	– 326.099 –	6.173(2)	<0.05	Y = -4.682 + 1.863 X
Deltamethrin	-3.529	1.468 $\pm$ 0.161	183.455 253.752 331.758	5.302(3)	>0.05	Y = -3.529 + 1.468 X
Etofenprox	-2.763	1.214 $\pm$ 0.157	120.613 188.678 263.165	7.780(3)	>0.05	Y = -2.763 + 1.214 X
DDT	-2.567	0.708 $\pm$ 0.144	2 126.921 4 208.983 16 430.847	5.613(3)	>0.05	Y = -2.567 + 0.708 X

**Figure 1.** Distribution of *An. stephensi* in Iran.

#### 4. Discussion

*An. stephensi* is found in Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Myanmar (Burma), Nepal, Oman, Pakistan, Saudi Arabia and Thailand. Previous investigations have shown that it is the most prevalent anopheline species in the malarious area of southern Iran<sup>[1]</sup>. This species is considered to be endophagous and endophilic. It is the main vector responsible for transmission of malaria to human in Persian Gulf area. Sporozoite rates of samples from the southern parts of Iran were reported to be between 0.2% and 1.8%. *An. stephensi* is one of the main malaria vectors in Hormozgan province<sup>[9]</sup>.

In Iran, insecticide resistance monitoring of *An. stephensi* has been carried out regularly, and its resistance to DDT, dieldrin and malathion was first reported in 1957, 1960 and 1976, respectively. Recent studies have shown that *An. stephensi* from Bandar Abbas region is resistant to DDT and dieldrin<sup>[14–15]</sup>. Studies in other countries showed that

*An. stephensi* is resistant to DDT in Afghanistan, India, Pakistan, Iraq, Oman, United Arab Emirates and Saudi Arabia. In Iran, after appearance of malathion resistance in *An. stephensi*, propoxur was substituted in 1978 and it was used for 13 years. In recent years, pyrethroids have been used for residual spraying in malaria control programmes. From 1990 till 2009, pyrethroid resistance has been monitored in Iran showing that almost all tested *An. stephensi* were susceptible<sup>[17–20]</sup>. The results of new study conducted in Jiroft district, Kerman province, showed that this species is tolerant to DDT and dieldrin with mortality rates of 91.3% and 90% respectively; but susceptible to pyrethroid insecticides. *An. stephensi* larvae in Hormozgan province showed susceptibility to malathion, chlorpyrifos and temephos, but resistance to fenitrothion (73% mortality in WHO susceptibility tests)<sup>[21–22]</sup>. In Kahnooj district, south of Kerman province, it found to be resistant to DDT and dieldrin and susceptible to malathion, fenitrothion, propoxur, lambda-cyhalothrin, permethrin, cyfluthrin, deltamethrin and etofenprox. That study showed larvae of *An. stephensi*, exhibited 100% mortality for temephos and malathion, but (44.00±4.32)% for discriminative dose of fenitrothion<sup>[17]</sup>. In a study it was shown that *An. stephensi* is susceptible to all types of insecticides in Kazerun in 1999 (Unpublished data). However different strains of Bandar Abbas, Iranshahr and Kazerun revealed resistant to DDT, dieldrin and fipronil and susceptible to lambda-cyhalothrin, permethrin, cyfluthrin, and deltamethrin<sup>[15]</sup>. A new report from Bashagard area, which is located in malarious area of Iran, revealed that diagnostic dose of insecticides exhibited resistance of *An. stephensi* to DDT and tolerance in *An. stephensi* to deltamethrin and bendiocarb. That study also reported (85.0±1.8)% mortality of *An. stephensi* larvae against diagnostic dose of fenthion<sup>[10]</sup>.

Because *An. stephensi* is the main malaria vector in the country and its rearing is easy, it used for different biological assays such as larvicides, irritability tests, olfaction studies, bioassay tests for bednets and indoor residual spraying, biological tests for plant extraction and repellents<sup>[23–34]</sup>.

Biochemical and molecular assays is recommended for understanding the mechanisms of pyrethroid resistance in this vector.

The waste use of pyrethroids for malaria vector control has increased in the past decade. They are used for insecticide treated net and indoor residual spraying. The major malaria vectors of the world have developed resistance to pyrethroids and the resistance genes are spreading rapidly throughout world<sup>[35]</sup>. There are several reports on mechanism of pyrethroid resistance in malaria vectors. However there are some control strategy for combating against resistance such as rotation, mixture, using biological control and Integrated Vector Management (IVM). The control strategy of the country is moving into the malaria elimination era, so that it is vital that the implications of insecticide resistance are fully understood. Monitoring and mapping of

insecticide resistance in the country is an essential tool for implementation of malaria elimination.

### Conflict of interest statement

The authors declare that there is no conflict of interest.

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