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Persistence of DDT, malathion & deltamethrin resistance in *Anopheles culicifacies* after their sequential withdrawal from indoor residual spraying in Surat district, India

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***Background & objectives*: Under the national antimalaria programme DDT was introduced in early 1950s for vector control and later hexachloro cyclohexane (HCH) followed by malathion and recently synthetic pyrethroids in 1990s to manage the insecticide resistance in *Anopheles culicifacies.* Subsequent replacement led to development of multiple resistances in *An. culicifacies* in Surat district in Gujarat State. Indoor residual spray (IRS) was completely withdrawn in southern villages in Surat in 2002. This study was undertaken in these areas to study the persistence of resistance to DDT, malathion and deltamethrin after sequential withdrawal of IRS with these insecticides at different times.**

***Methods*: Susceptibility tests on *An. culicifacies* were conducted using standard WHO methods and kits. Mortality, knockdown time and lethal times were calculated for *An. culicifacies* exposed to WHO prescribed diagnostic concentrations of different insecticide impregnated papers.**

***Results*: Persistence of DDT-resistance was observed even after 30 yr of its withdrawal from IRS. Similarly, persistence of malathion resistance was also observed after 9 yr of its withdrawal from IRS, while reversal of deltamethrin-resistance was observed very fast within 2-3 yr after its withdrawal from IRS in 2002.**

***Interpretation & conclusion*: Present data indicate that the quantum of reversion of insecticide resistance in a population is relative and depends on the genetic stability of the respective resistance genes in the mosquitoes. In the present study withdrawal of pyrethroid-IRS resulted in increased susceptibility against pyrethroids alone and was independent of existence of resistance to insecticides of other groups. This study emphasizes that appropriate rotation of different insecticides; including carbamates may prevent or delay the onset of resistance.**

Key words *Anopheles culicifacies* - DDT - deltamethrin - genetic stability - indoor residual spray (IRS) - insecticide-resistance - malathion

The tropicopolitan mosquito *Anopheles culicifacies* (Diptera: Culicidae) is the most important vector of malaria in India[1]. Gujarat State being highly endemic to malaria, indoor residual spray (IRS) with DDT (dichloro-diethyl-trichloroethane, 1 g/m^2 and two rounds per year) had been carried out to control

An. culicifacies population since 1950s, which though showed spectacular success initially, in due course resulted in widespread resistance in *An. culicifacies*[2,3]. In 1958, DDT was replaced from regular spray by HCH (hexachloro cyclohexane), but was effective only for a short duration as the vector successfully developed high levels of resistance[4]. In order to curtail the malaria transmission in Gujarat, organophosphorous insecticide, malathion was introduced for IRS in 1969 (2 g/m^2 and three rounds per year)[5]. Malathion resistance in this species was first reported from Surat in 1973, but its use was continued till mid 1990s and was replaced in 1996 by deltamethrin, a synthetic pyrethroid (0.02 g/m^2 and two rounds per year)[6]. Studies in some villages in District Surat in Gujarat State indicated development of low levels of deltamethrin resistance in 2001[6]. Deltamethrin was withdrawn from IRS in these villages in Surat in 2002 as the annual parasite index (API) became <2 (personal communication 2008; District Malaria Officer, District Surat, Gujarat). The present investigation was carried out to assess the persistence of respective insecticide resistance in field populations of *An. culicifacies* after sequential withdrawal of DDT, malathion and deltamethrin and to study the resistance/susceptibility pattern in Surat.

Material & Methods

Surat district is situated on the west coast of India between 21-22°N latitude and 73-74°E longitude. In 2005, five villages, namely Serula, Munkiya, Satkashi, Pagharduwa and Nimbi Moti Pipal of Primary Health Centre (PHC) Borda, and in 2006, eight villages under two PHCs, Borda and Bhadkunja, namely Serula, Munkiya, Satkashi, Pagharduwa, Amalpada, Nani-pipal, Limbi and Anandpur were surveyed. Female *An. culicifacies* mosquitoes were collected from indoors during the morning hours (0600-0800 h) using an aspirator and flash light[7] and were used for susceptibility studies.

Insecticide susceptibility test: Susceptibility to different insecticides was determined following standard WHO method and kit (from University Sans Malaysia- *www.usm.my*) by exposing the mosquitoes to the insecticide impregnated papers of the specified WHO diagnostic concentration and time of exposure[8]. Mosquitoes were exposed to DDT (4%), malathion (5%), bendiocarb (0.1%), propoxur (0.1%), deltamethrin (0.05%), cyfluthrin (0.15%), permethrin (0.75%) and λ-cyhalothrin (0.05%) impregnated papers for 1 h while to fenitrothion (1%) for 2 h[8] and to α-cypermethrin (0.1%) for 1 h. A minimum of 3-4 replicates of 15-20 mosquitoes were used. Mortality was corrected using Abbott's formula wherever required[9]. The mortality data in insecticide susceptibility tests were subjected to χ^2 analysis to determine the significance of the observed variations in mortalities before and after the withdrawal of insecticide sprays.

Knockdown and lethal time: The toxicity indices KD_{50} and KD_{90} and LT_{50} and LT_{90} were determined using standard methods of exposure[8] and using log-probit regression analysis[10]. Exposures were made for one hour for all insecticides except for DDT (8 h).

Results

The susceptibility status of *An. culicifacies* to different insecticides in villages of district Surat (Table I) shows that resistance to DDT was persistent in 1985 (9% mortality), even after complete withdrawal of regular DDT-IRS for about 15 yr[11]. Later, in our studies in 1987 and 1992, the populations were still resistant to DDT (≤16% mortality). These results indicated the stability of DDT-resistance in *An. culicifacies* population even after 22 yr of suspension of DDT spray[12,13]. Further, in 2001 (after >30 yr of withdrawal), the mortalities against DDT remained <10 per cent and there was no significant difference in mortalities reported among the years 1985, 1987, 1992 and 2001.

Table I. Susceptibility of *An. culicifacies* to diagnostic dose of different insecticides

Insecticide, %	Per cent M ± SE (n)					Present study	
	1985[11]	April 1987[12]	Nov 1987[12]	1992[13]	2001[6]	2005	2006
DDT 4	9 ± 4.74 (140)	16 ± 9.2 (60)	0 (48)	6 ± 4.65 (100)	9 ± 4.04 (192)	40 ± 11.82 (66)	20 ± 6.05 (168)
Malathion 5	12 ± 9 (50)	44 ± 10.68 (83)	17 ± 6.14 (144)	17 ± 9.5 (60)	(-)	68 ± 7.59 (145)	57 ± 6.54 (220)
Deltamethrin 0.05	100 (220)	100 (40)	100 (22)	(-)	66 ± 5.17 (322)	98 ± 3.48 (62)	99 ± 1.41 (190)

% M ± SE: Mean ± standard error; Per cent mortality ± SE at 95% CL; n = No. exposed; (-) Not done
Superscript numerals denote reference numbers

However, in 2005 and 2006, there was an increase in mortality in *An. culicifacies* against DDT up to 40 per cent and the observed differences in mortalities were statistically significant for the comparisons among the studies of 2001, 2005 and 2006 ($P<0.001$).

Malathion was introduced for IRS during 1969-1970 to control DDT and HCH-resistant *An. culicifacies*. Our studies in 1985, 1987 and 1992 registered ≤44 per cent mortality against malathion (Table I) and there was no significant difference in mortality against malathion among the years 1985, 1987 and 1992. However, mortality increased to ≤68 per cent in 2006. These mortality ranges were significant from the mortalities observed in 1992 ($P<0.001$). Our present survey indicated that even 8-9 yr after the withdrawal of malathion-IRS, *An. culicifacies* population was found resistant though an increase in mortality (from 42% in 1992 to 68% in 2005) was reported.

Regular deltamethrin-IRS was introduced in the study areas in 1996. *An. culicifacies* was completely susceptible to deltamethrin in 1985 and 1987 prior to the introduction of deltamethrin-IRS (Table I). However, in 2001, the species registered 66 per cent mortality to deltamethrin indicating development of deltamethrin-resistance[6]. Deltamethrin-IRS was discontinued from 2002 owing to lower incidence of malaria as API was <2. However, studies in 2005 and 2006 revealed that the species registered ≥98 per cent mortality. The χ^2 analysis showed that the observed mortality to deltamethrin in 2001 was significantly different compared to mortalities in the preceding years, 1985 and 1987 ($P<0.001$), and to the mortalities observed in 2005 and 2006 ($P<0.001$), *i.e.*, after the withdrawal of deltamethrin-IRS in 2002. The per cent mortality in the species against other pyrethroids, namely cyfluthrin, permethrin, λ-cyhalothrin and α-cypermethrin during the years 2005 and 2006 was in the range of 96-100 per cent indicating increased susceptibility of *An. culicifacies* to other pyrethroids. The per cent mortality against other organophosphate (fenitrothion) and carbamate (bendiocarb and propoxur) insecticides were >85 per cent indicating susceptibility to these classes.

The results showed a decrease in the KD_{50} and KD_{90} values against deltamethrin in 2005 and 2006 compared to 2001 indicating gradual increase in the susceptibility of the mosquito population to deltamethrin (Table II). Further, in case of other pyrethroids also decrease in knockdown time was observed from 2005 to 2006. The LT_{50} and LT_{90} in *An. culicifacies* to other insecticides, namely DDT, malathion, fenitrothion, bendiocarb and

Table II. Knockdown time (min) against different pyrethroids in *An. culicifacies* during the years 2001, 2005 and 2006

Insecticide, %	2001			2005			2006		
	KD_{50} (95% FL)	KD_{90} (95% FL)	χ^2 value (DF)	KD_{50} (95% FL)	KD_{90} (95% FL)	χ^2 value (DF)	KD_{50} (95% FL)	KD_{90} (95% FL)	χ^2 value (DF)
Deltamethrin 0.05	83.75 (79.40-88.84)	246.40 (216.84-286.96)	14.22 (22)	31.37 (31.37-67.93)	67.93 (60.83-78.24)	8.1 (10)	32.35 (30.95-33.72)	48.52 (45.96-51.81)	23.92 (10)
Cyfluthrin 0.15	(-)	(-)	(-)	33.10 (31.38-34.81)	57.25 (53.11-62.86)	16.26 (10)	29.80 (29.15-30.45)	49.97 (48.51-51.61)	4.20 (10)
Permethrin 0.75	(-)	(-)	(-)	28.30 (27.41-29.16)	44.66 (42.98-46.62)	7.12 (10)	23.62 (21.29-25.78)	39.93 (36.16-45.42)	61.08 (10)
λ-Cyhalothrin 0.05	(-)	(-)	(-)	(-)	(-)	(-)	40.29 (39.12-41.50)	67.45 (63.91-71.86)	9.43 (10)
α-Cypermethrin 0.1	(-)	(-)	(-)	(-)	(-)	(-)	23.74 (21.54-25.80)	40.75 (37.06-46.02)	41.06 (10)

KD_{50} and KD_{90}, Time required for knockdown of 50% and 90% of exposed mosquitoes
95% FL, Fudicial limit at 95% confidence level; χ^2, Chi-square for the heterogeneity of response; DF, Degree of freedom (K-2); (-), not done

Table III. Lethal time (min) against different insecticides in *An. culicifacies* during the years 2005 and 2006

Insecticide, %	2005			2006		
	LT_{50} (95% FL)	LT_{90} (95% FL)	χ^2value (DF)	LT_{50} (95% FL)	LT_{90} (95% FL)	χ^2value (DF)
DDT 4	155.08 (119.23-224.47)	755.60 (453.36-1668.64)	27.85 (14)	277.72 (139.88-4929.13)	785.52 (266.95-75008.29)	1.59 (10)
Malathion 5	51.45 (48.93-54.57)	100.92 (89.85-117.37)	9.58 (10)	87.25 (80.52-96.80)	168.21 (143.88-206.42)	4.30 (10)
Fenitrothion 1	38.40 (36.93-39.82)	50.08 (47.81-53.14)	8.63 (10)	67.32 (64.01-70.54)	101.8 (95.68-110.02)	29.65 (10)
Bendiocarb 0.1	24.42 (22.47-26.29)	48.53 (44.18-54.60)	18.16 (10)	35.67 (34.48-36.87)	57.08 (54.28-60.58)	11.32 (10)
Propoxur 0.1	21.46 (18.54-24.04)	34.35 (30.40-40.85)	42.70 (10)	28.68 (27.43-29.91)	53.38 (50.28-57.23)	11.1 (10)

LT_{50} and LT_{90}, Lethal time in minutes to kill 50% and 90% of exposed mosquitoes
95% FL**,** Fudicial limit at 95% confidence level; χ^2, Chi-square for the heterogeneity of response; DF, Degree of freedom (K-2)

propoxur are given in Table III. The lethal times of different insecticides also confirmed the results of the susceptibility tests against *An. culicifacies*.

Discussion

In the present study, stability in DDT and malathion resistance was observed even after long-term withdrawal of IRS with these insecticides, whereas reversal of resistance was observed in case of deltamethrin withdrawal. DDT was introduced in 1950s in India for IRS and was continued up to 1970. First report of DDT-resistance appeared in 1958[14] and later widespread resistance was reported[3]. The DDT resistance in *An. culicifacies* was stable and even after >30 yr of its withdrawal remained high indicating increased genetic stability of the resistance in the population. In our earlier studies in 1985, 1987, 1992 and 2001 per cent mortality against DDT in *An. culicifacies* ranged between 0 and 16. After the total withdrawal of insecticide-IRS in 2002, the susceptibility to DDT fluctuated between 40 and 20 per cent during 2005 and 2006. This indicates that DDT resistance was remarkably stable for more than 4 decades. Similarly, resistance to malathion was also stable. Though was there an increase in the susceptibility of *An. culicifacies* to DDT and malathion after their withdrawal from the regular spray, the population was still resistant to these insecticides. In contrast, reversal of resistance to deltamethrin was observed after its withdrawal for a short span.

The relatively low levels of reversion against DDT and malathion could be due to increased genetic stability of DDT and malathion resistance in the populations. Observations made on DDT-resistance in *An. albimanus* during a large scale field trial in Mexico also indicated increased genetic stability of DDT-resistance contemplated to be due to counterbalancing of negative selection against DDT-resistance genes by other genetic changes that might have removed the negative fitness costs of the resistance genes[15]. The development of insecticide resistance is an evolutionary phenomenon. However, withdrawal of selection pressure from populations which could not have resulted in developing homozygous resistance may promote a faster rate of reversion to susceptibility. This could be due to interbreeding of resistant individuals with heterozygotes or homozygous susceptibles promoting gradual increase in the number of susceptible individuals in the population. This sustenance or reversion of insecticide resistance is further dependent on the nature and stability of the respective resistance genes involved[16]. Further, the reversion of resistance depends on intrinsic fitness ratios of homozygotes and heterozygotes and frequency of resistance gene[17]. Cases of reversion of insecticide resistance owing to its withdrawal of selection pressure were earlier reported in organophosphate resistant strain of *Culex pipiens pallens*[18], pyrethroid resistance in *Blattella germanica*[19], *Bacillus thuringiensis* resistance in *Plutella xylostella*[20] and pyriproxyfen resistance in *Bemisia tabaci*[21].

The observed specific reversal of pyrethroid resistance alone in DDT-malathion-pyrethroid-resistant *An. culicifacies* populations in the study area has shown absence of cross-resistance among these insecticides. It is also evident that DDT-resistance in *An. culicifacies*

did not promote deltamethrin-resistance as was observed in *An. albimanus*[15]. In *An. albimanus* the pyrethroid resistance is conferred by *kdr* that also shows cross-resistance to DDT. Indian scenario depicts prevalence of DDT-resistance co-existing with susceptibility to synthetic pyrethroids. The quick reversion of deltamethrin-resistance to susceptibility could be due to the contemplated nature of the recessive resistance gene. This further indicates that deltamethrin and synthetic pyrethroids excel other groups of insecticides in vector control as their useful life can be enhanced if used judiciously for vector control. Present data indicate that the quantum of reversion of insecticide resistance in a population is relative and depends on the genetic stability of the respective resistance in the mosquitoes. *An. culicifacies* in India being a multi-resistant species and in view of dearth of alternative effective insecticide molecules in immediate future, the option for its control is resistance management. This can be practiced by appropriate rotation of different groups of insecticides including carbamates to prevent or delay the onset of development of resistance.

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References

1. Sharma VP. Fighting malaria in India. *Curr Sci* 1998; *75* : 1127-40.
2. Singh J. Activities of the Malaria Institute of India during World War II. *Indian J Malariol* 1962; *16* : 504-14.
3. Raghavan NGS, Wattal BL, Bhatnagar VN, Choudhury DS, Joshi GC, Krishnan KS. Present status of susceptibility of arthropods in public health importance to insecticides in India. *Bull Indian Soc Malar Commun Dis* 1967; *4* : 209-45.
4. Patel TB, Rao TR, Halgeri AV, Deobhankar RB. A preliminary note on a probable case of dieldrin resistance in *Anopheles culicifacies* in Thana district, Bombay state. *Indian J Malariol* 1958; *12* : 367-70.
5. Rajagopal R. Malathion resistance in *Anopheles culicifacies* in Gujarat. *Indian J Med Res* 1977; *66* : 27-8.
6. Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *An. culicifacies* in Surat district, Gujarat, west India. *Curr Sci* 2002; *82* : 547-50.
7. World Health Organization. *Manual on practical entomology in malaria,* Part II. Geneva: WHO; 1975. p. 191.
8. World Health Organization. *Test procedures for insecticide resistance monitoring in malaria vectors, bioefficacy and persistence of insecticides on treated surfaces*. 1998. Geneva: WHO/CDC/CPC/MAL/1998.12.
9. Abbott WS. A method for computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18 :* 265-7.
10. Finney DJ. *Probit analysis*. Cambridge, England: Cambridge University Press; 1971. p. 333.
11. Das M, Srivastava SP, Khamre JS, Deshpande LB. Susceptibility of DDT, dieldrin and malathion resistant *Anopheles culicifacies* population to deltamethrin. *J Am Mosq Control Assoc* 1986; *2* : 553-5.
12. Raghavendra K, Vasantha K, Subbarao SK, Pillai MKK, Sharma VP. Resistance in *Anopheles culicifacies* sibling species B and C to malathion in Andhra Pradesh and Gujarat states, India. *J Am Mosq Control Assoc* 1991; *7* : 255-9.
13. Raghavendra K, Subbarao SK, Pillai MKK, Sharma VP. Biochemical mechanisms of malathion-resistance in Indian *Anopheles culicifacies* (Diptera: Culicidae) sibling species A, B and C: Micro plate assays and synergistic studies. *Ann Entomol Soc Am* 1998; *91* : 834-9.
14. Rahman J, Roy ML, Singh K. Development of increased tolerance to DDT in *Anopheles culicifacies* Giles, in the Panch Mahal district of Bombay state (India). *Indian J Malariol* 1959; *13* : 125-30.
15. Rodriguez AD, Penilla RP, Rodriguez MH, Hemingway J. Insecticide resistance management in a multi-resistant malaria vector scenario: a Mexican trial shows sustainability. *Public Health J* 2006; *18* : 24-9.
16. Georghiou GP, Taylor CE. Factors influencing the evolution of resistance. In: *Pesticide resistance: strategies and tactics for management*. Washington DC: National Academy Press; 1986. p. 157-69.
17. May RM, Dobson AP. Population dynamics and the rate of evolution of pesticide resistance. In: *Pesticide resistance: strategies and tactics for management*. Washington DC: National Academy Press; 1986. p.170-93.
18. Wang J, Lu S, Chen R, Wang L. Relative fitness of three organophosphate-resistant strains of *Culex pipiens pallens* (Diptera: Culicidae). *J Med Entomol* 1998; *35* : 716-9.
19. Cochran DG. Decline of pyrethroid resistance in the absence of selection pressure in a population of German cockroaches (Dictyoptera: Blattellidae). *J Econ Entomol* 1993; *86* : 1639-44.
20. Tabashnik EB, Finson N, Groeters FR, Moar WJ, Johnson MW, Luo K, *et al*. Reversal of resistance to *Bacillus thuringiensis* in *Plutella xylostella*. *Proc Natl Acad Sci USA* 1994; *99* : 4120-4.
21. Wilson M, Moshitzky P, Einat L, Ghanim M, Rami Horowitz A, Morin S. Reversal of resistance to pyriproxyfen in the Q biotype of *Bemesia tabaci* (Hemiptera: Aleyrodidae). *Pest Management Sci* 2007; *8* : 761-8.

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