

quite interesting. The present study suggests that it may be possible to obtain the qualitative information on folding mechanism, folding rates and also about the stability, by modelling the more complex proteins in a similar way.

Future studies will explore the sensitivity to the potential employed and also focus on generalizing the set of potentials to accommodate more realistic potentials. Also, we have not made any study of the formation of the *specific* contacts that characterize the native state. Work in this direction is under progress.

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Reduced susceptibility to deltamethrin in *Anopheles culicifacies sensu lato*, in Ramnathapuram district, Tamil Nadu – Selection of a pyrethroid-resistant strain

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Anopheles culicifacies sensu lato, the major vector of malaria in the plain areas of rural India, has become resistant to commonly used insecticides, viz. DDT, HCH and malathion in most parts of the country. To control resistant *An. culicifacies*, synthetic pyrethroids have been sprayed during the past one decade in some areas, but so far resistance to pyrethroids has not been reported in this species. This paper reports the reduced susceptibility of *An. culicifacies* from Rameshwaram Island, Ramnathapuram district, Tamil Nadu, to deltamethrin, a synthetic pyrethroid. Knock-down bioassays revealed more than two-fold higher values of KT_{50} and KT_{90} (time for knock-down of 50% and 90% exposed mosquitoes) in *An. culicifacies* from Rameshwaram Island, than populations from other areas. Laboratory selection of the adult mosquitoes of Rameshwaram (Rmr) strain against deltamethrin for 12 generations resulted in the development of 31- and 15-fold higher resistance at LT_{50} and LT_{90} levels, respectively. The selected deltamethrin-resistant strain also showed cross-resistance to other synthetic pyrethroids, viz. lambda-cyhalothrin, cyfluthrin, permethrin and bifenthrin.

ANOPHELES culicifacies sensu lato, the major vector of malaria in most parts of the rural plain areas in India, has developed widespread resistance to DDT and HCH and also to malathion in some areas, where it has been used extensively for indoor residual spraying^{1–4}. As a result, synthetic pyrethroids have been introduced as an alternative to control double (DDT and HCH) or triple (DDT, HCH and malathion) resistant *An. culicifacies* in India, either in the form of indoor residual spray or as impregnant on mosquito nets. Synthetic pyrethroids have been reported to be highly effective for the control of *An. culicifacies* populations^{5,6}. Though past experiences have shown the development of resistance in *An. culicifacies* to different insecticides which have been used for indoor residual spray, resistance to pyrethroids in *An. culicifacies* from India has not been reported so

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far. There are however various reports on the development of pyrethroid resistance in other *anophelines* from different countries⁷⁻⁹. In West Africa cross-resistance between DDT and pyrethroids, governed by *Kdr* gene, has been reported in *An. gambiae*⁷. However, in Sichuan, China, where there has been annual treatment of millions of bed-nets with deltamethrin since 1987, no evidence of pyrethroid resistance or any difference in the knock-down time in *An. sinensis* and *An. anthropophagus* was observed¹⁰. In view of the increasing use of synthetic pyrethroids in India and widespread occurrence of DDT resistance in *An. culicifacies*, the present study was carried out to monitor the susceptibility status of *An. culicifacies* to deltamethrin, a synthetic pyrethroid, and to see the development of pyrethroid resistance through laboratory selection.

An. culicifacies sensu lato, were collected from Rameshwaram Island, Ramnathapuram district, Tamil Nadu during 1997, where moderate density of *An. culicifacies* was found even after deltamethrin spray for two consecutive years, and two areas in Haryana (pyrethroid sprayed and unsprayed). Susceptibility of *An. culicifacies* to deltamethrin was determined using knock-down bioassays in the WHO susceptibility tubes, by exposing the mosquitoes to deltamethrin papers with WHO recommended diagnostic concentration, viz. 0.025% (WHO, 1992)⁸ and 0.05% (WHO, 1999)¹¹. In addition to these field-collected populations, colonized strains of *An. culicifacies* (including Rameshwaram strain) being maintained in the insectary at Malaria Research Centre, were also tested for their susceptibility to deltamethrin to determine KT_{50} and KT_{90} (time for knock-down of 50 and 90% mosquitoes) and also the baseline data on LT_{50} and LT_{90} (time required to kill 50 and 90% exposed mosquitoes) on 0.05% deltamethrin papers¹¹.

The knock-down bioassays were carried out on 3-day-old sugar-fed female *An. culicifacies* mosquitoes collected from field (F_1 progeny) or from a mosquito colony in the laboratory. Only in Rameshwaram Island, wild caught females were tested directly. Batches of ten to fifteen mosquitoes were exposed to deltamethrin papers in the exposure tubes and the number of mosquitoes knocked-down was recorded up to 60 min at an interval of every 5 min. KT_{50} and KT_{90} were calculated by regression analysis between per cent mosquitoes knocked down and exposure time, using log-probit method¹². After 60 min, the mosquitoes (both alive or knocked down) were transferred for recovery to holding tubes for 24 h and then mortality was recorded.

Baseline data on LT_{50} and LT_{90} were determined against laboratory-colonized strains of *An. culicifacies*, following the WHO standard procedure¹³. For this, 3-day-old blood-fed female mosquitoes were exposed in batches of 15 mosquitoes per tube, to 0.05% deltamethrin papers for different time periods (0.5–32.0 min for Rameshwaram strain and 0.5–4.0 min for all other

strains) along with control. Three replicates were used for each time-period and the control. After exposure for different durations, the mosquitoes were transferred to holding tubes for 24 h and then the mortality of the exposed mosquitoes was recorded. The data on per cent mortality vs exposure time were analysed by probit regression method of Finney¹². *An. culicifacies* (Rameshwaram strain) which showed the reduced susceptibility to deltamethrin (LT_{99} = 28 min), was used for the selection of deltamethrin resistance. The selection process involved exposure of 3-day-old blood-fed female mosquitoes to 0.05% deltamethrin-treated paper in WHO susceptibility tubes, for a period equivalent to the LT_{50} value determined for the parental generation of the mosquitoes. The surviving mosquitoes after 24 h of recovery period were reared to the next generation (F_1) and the process of selection was continued up to 12 generations at LT_{50} level determined for each respective generation. After 12 generations, the LT_{50} value increased 31-fold compared to the parental generation and 75-fold higher than the LT_{50} in case of other laboratory colonized susceptible strains.

The selected deltamethrin-resistant strain of *An. culicifacies* was then used to study cross-resistance to other pyrethroids. Cross-resistance to other synthetic pyrethroids in deltamethrin-selected strain of *An. culicifacies*, was studied by comparing the LT_{50} and LT_{90} values of the laboratory-colonized deltamethrin-susceptible and deltamethrin-selected strains determined against lambda-dacyhalothrin 0.05%, cyfluthrin 0.15%, permethrin 0.75% and bifenthrin 0.1% papers supplied by WHO.

Table 1 shows the KT_{50} and KT_{90} values of *An. culicifacies* after continuous exposure up to 1 h against 0.025 and 0.05% deltamethrin and per cent mortality after 24 h holding period. The KT_{50} and KT_{90} values of *An. culicifacies* from Rameshwaram Island (field-collected and also colonized strain) were 2–3-fold higher than the KT_{50} and KT_{90} values of *An. culicifacies* from other areas. However, the per cent mortality after 24 h holding period in all *An. culicifacies* strains was 100. Though the per cent mortality after 1 h exposure and 24 h holding period in all the strains of *An. culicifacies* was 100, it may be mentioned here that WHO-recommended diagnostic concentration of deltamethrin paper and exposure period for the detection of resistance to deltamethrin in *An. culicifacies* is 0.025% and 10 min (WHO, 1992)⁸. The diagnostic concentration has been revised as 0.05% for all *Anopheles* spp.¹¹, but there is no change in the exposure period for *An. culicifacies*. Even though there was 100% mortality in all the strains of *An. culicifacies* exposed to diagnostic concentration of deltamethrin for 1 h, the increase in the knock-down time (KT_{50} and KT_{90}) of *An. culicifacies* from Rameshwaram Island, indicates the development of incipient resistance to deltamethrin in this strain of

Table 1. Susceptibility of *An. culicifacies* to deltamethrin as determined by knock-down bioassay with deltamethrin-impregnated papers in WHO susceptibility tubes

<i>An. culicifacies</i> strain	Per cent concentration deltamethrin (w/w)	KT ₅₀	KT ₉₀	Per cent mortality after 1 h exposure and 24 h holding
		(Time in minutes)		
<i>Field collected</i>				
*Rameshwaram (Tamil Nadu)	0.025	19.52	42.65	100
*Rameshwaram (Tamil Nadu)	0.05	18.63	31.58	100
*Mewat Gurgaon (Haryana)	0.05	10.02	14.83	100
Sonipat (Haryana)	0.05	8.45	12.74	100
<i>Laboratory-colonized</i>				
Burari (Delhi)	0.05	14.97	20.77	100
Haldwani (Uttar Pradesh)	0.025	14.31	21.27	100
Dehra Ghaziabad (Uttar Pradesh)	0.05	9.99	20.35	100
Ladpur-Sonipat (Haryana)	0.025	16.85	22.51	100
Rameshwaram (Tamil Nadu)	0.025	46.54	81.13	100
	0.05	22.08	50.02	100

*, Pyrethroid-sprayed areas; w/w, weight/weight.

Table 2. Baseline susceptibility of *An. culicifacies* strains from different areas to deltamethrin as determined after exposure to 0.05% deltamethrin-impregnated paper

<i>An. culicifacies</i> strain	LT ₅₀	LT ₉₀	χ^2 (df)
	(Time in minutes)		
Ladpur-Sonipat (Haryana)	< 0.5	<0.5	–
Jabalpur (Madhya Pradesh)	< 0.5	< 0.5	–
Burari (Delhi)	< 0.5	1.32	3.29 (3)
Rameshwaram (Tamil Nadu)	1.2	6.68	3.31 (3)
Rameshwaram (F-12) (laboratory-selected against deltamethrin)	37.61	99.29	11.32 (3)

LT₅₀, is the time required to kill 50% of exposed mosquitoes; LT₉₀, is the time required to kill 90% of exposed mosquitoes; χ^2 , Chi square; (df), degree of freedom.

Table 3. Comparison of toxicity (LT₅₀ and LT₉₀) of different synthetic pyrethroids against deltamethrin-susceptible and deltamethrin-selected resistant strains of *An. culicifacies*

Insecticide tested	Toxicity			
	<i>An. culicifacies</i> (Jabalpur) Deltamethrin-susceptible strain		<i>An. culicifacies</i> (Rameshwaram) Deltamethrin-selected strain	
	LT ₅₀	LT ₉₀	LT ₅₀	LT ₉₀
Deltamethrin 0.05%	< 0.5	< 0.5	38.94	97.21
Lambdacyhalothrin 0.05%	< 0.5	0.683	42.83	109.91
Permethrin 0.75%	0.76	1.825	38.24	86.25
Cyfluthrin 0.15%	< 0.5	< 0.5	39.94	127.20
Bifenthrin 0.1%	1.79	6.75	83.41	221.58

An. culicifacies. In another study with *An. gambiae* population collected from different regions in West Africa, a 2–4-fold increase in KT₅₀ and KT₉₀ values, as observed in our study, was considered as a good indicator for early detection of pyrethroid resistance⁷.

Table 2 shows the LT₅₀ and LT₉₀ values of certain laboratory-colonized *An. culicifacies* strains after expo-

sure to 0.05% deltamethrin papers. The LT₅₀ values of different colonized strains of *An. culicifacies* were less than 0.5 min, while the LT₅₀ values of *An. culicifacies* strain from Rameshwaram Islands was 1.32 min. The LT₉₀ values of different colonized strains of *An. culicifacies* ranged from less than 0.5 min to 1.2 min, while in *An. culicifacies* strain from Rameshwaram, LT₉₀ was

6.7 min, which was 7 to 13 times higher than the LT₉₀ values of other *An. culicifacies* strains. The calculated LT₉₀ value of Rameshwaram strain against deltamethrin was 28 min. These results clearly showed reduced susceptibility of *An. culicifacies* strain from Rameshwaram Island to deltamethrin. The same strain after selection with deltamethrin in the laboratory for 12 generations, showed an LT₅₀ value of 37.61 min which was 31-fold higher than the LT₅₀ values in the parental strain. These observations indicate the development of resistance to deltamethrin in Rameshwaram strain of *An. culicifacies* after selection with deltamethrin in the laboratory.

Table 3 shows the LT₅₀ and LT₉₀ values of deltamethrin-susceptible and deltamethrin-selected resistant strain of *An. culicifacies* against 5 synthetic pyrethroids. The LT₅₀ values for deltamethrin-susceptible *An. culicifacies* strain against deltamethrin 0.05%, lambda-dacyhalothrin 0.05%, cyfluthrin 0.15%, permethrin 0.75% and bifenthrin 0.1% papers were < 0.5, < 0.5, < 0.5, 0.76 and 1.79 min, respectively, while the corresponding values for the selected deltamethrin-resistant strain were 38.94, 42.83, 39.94, 38.24 and 83.4 min, respectively. These results show that deltamethrin-resistant strain had almost similar LT₅₀ values against different pyrethroids, even though it was not selected against them. This indicates the existence of one or more mechanisms of pyrethroid resistance in the selected deltamethrin-resistant strain of *An. culicifacies*, which give cross-resistance between different pyrethroids. Resistance to pyrethroid in *anophelines* has been reported due to the involvement of monooxygenases^{14,15}, and/or esterases¹⁵, and a *kdr*-type resistance, caused by a mutation in the sodium channel gene¹⁶ which also shows cross-resistance with DDT⁷. The mechanisms of pyrethroid resistance in *An. culicifacies* in the present study are however not clear. The Rameshwaram strain has also shown resistance to DDT (20% mortality on 4% DDT paper) in the parental generation. After selection with deltamethrin for 12 generations, only 3% mortality was observed against 4% DDT papers. This indicates the possibility of a *kdr*-like mechanism of pyrethroid resistance in *An. culicifacies*. An earlier report on *An. gambiae* has shown cross-resistance between pyrethroids and DDT, which is governed by the *Kdr* gene⁷. In yet another study in Zanzibar, Africa, DDT resistance

induced by glutathion-s-transferase did not cross with pyrethroids¹⁷. So far the only mechanism of DDT resistance known in *An. culicifacies*, is due to glutathion-s-transferase which has been reported from Srilanka¹⁸, but there is no report about the mechanism of DDT/pyrethroid resistance in *An. culicifacies* from India which may have a different mechanism of insecticide resistance. Further studies are required to establish the mechanism of DDT/pyrethroid resistance in *An. culicifacies*.

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