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Impact of Olyset® Nets on malaria transmission in India

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Abstract

Background & objectives: Efficacy of Olyset nets, long-lasting insecticidal nets were tested from August 2003 to August 2006 against *Anopheles culicifacies*, the vector which transmits 60% of all malaria cases in rural India.

Methods: Three villages in District Gautam Budh Nagar (Uttar Pradesh), India were selected for the trial and Olyset nets were distributed in one village, in another village untreated nets were distributed and the third village was kept as control where nets were not used. Entomological, and epidemiological data were collected using standard methods.

Results: The use of Olyset nets reduced the indoor resting density of *An. culicifacies* and also reduced mosquito entry into the structures where Olyset nets were used. No mosquitoes were caught landing on the Olyset nets. There was a reduction in the parity rate of *An. culicifacies* in the Olyset net village as compared with untreated net and no net villages. The impact of Olyset nets was observed on malaria incidence and only one case of *Plasmodium falciparum* malaria was reported in the Olyset net village but these continued to be found in the village with untreated nets and the control.

Conclusion: Results of the present study confirmed that Olyset nets are highly effective in reducing the indoor resting density of mosquitoes, man-vector contact and malaria incidence.

Key words *Anopheles culicifacies* – human-vector contact – malaria transmission – Olyset nets – parity rate – sporozoite rate.

Introduction

Mosquito nets treated with pyrethroid insecticides have been shown to cause a decline in malaria morbidity and mortality in several trials carried out in different countries[1–4] . However, periodic re-impregnation, low treatment rates and impact of repeated washing have been problems affecting the application of insecticide-treated nets in longer duration. To overcome these problems, long-lasting insecticidal nets (LLIN) have been developed. LLINs should offer a practical solution to these problems provided that they live up to specifications. The Olyset net, approved by the WHO Pesticide Evaluation Scheme, is one such LLIN, which is made of very durable polyethylene fibre with a broad (4 mm) mesh and with permethrin incorporated into it if the surface layer of insecticide is washed off. It is intended that more insecticide will diffuse to the surface, especially at high ambient temperature or if the net is deliberately heated. Olyset nets evaluated in other countries showed encouraging results[5–15]. However, Lindblade *et al*[16] and Gimnig *et al*[17] recently reported poor performance of Olyset nets in laboratory as well as in field conditions against *Anopheles gambiae*. Results of a Phase II trial carried out in India showed

that Olyset nets are wash resistant and effective for several weeks in providing the desired level of mortality in *Anopheles culicifacies* Giles and *An. fluviatilis* (Diptera: Culicidae)[18,19] and against *An. stephensi* and *Aedes aegypti*[20]. In view of this, a village-scale trial was undertaken to evaluate the efficacy of Olyset nets against *An. culicifacies,* a principal vector of rural malaria, and on malaria incidence.

Material & Methods

Olyset nets made of polyethylene fibre of 150 denier thickness 156/m^2 mesh 4 × 4 mm hole size and incorporating 1000 mg a.i./m^2 permethrin (2% w/w) and untreated polyethylene nets of about 100 denier supplied by M/s. Sumitomo Chemicals India Pvt. Ltd., Mumbai, India were used in the present study.

Description of study sites: Preliminary surveys were carried out in District Gautham Budh Nagar, Uttar Pradesh, India and entomological and epidemiological data were collected to select the study villages. Three villages namely Khandera, Beel Akbarpur and Anandpur in Dadri Primary Health Centre were selected for the present evaluation as they had more or less similar ecotopography, socioeconomic background and vector prevalence. The distance between the three villages was >6 km. Agriculture is the major occupation in these villages and the land is irrigated through the main upper Ganga canal and its tributaries. Demographic data such as population structure, professions and literacy, mosquito species composition, etc. were also collected. Baseline data of mosquito density and malaria incidence were collected from August 2003 to July 2004.

Criteria of distribution: Olyset nets were distributed in Khandera village (Pop. 2000 living in 210 houses). Beel Akbarpur village situated about 10 km away from Khandera village was selected for distribution of untreated nets (Pop. 1800 living in 174 houses). Another village namely Anandpur (Pop. 2000 living in 193 houses) situated at a distance of ~ 6 km from both the aforementioned villages was taken as control village. Bednets were distributed on 1 August 2004 and other vector control operations were suspended during the trial period. Active surveillance was strengthened to avoid a malaria outbreak. One net was given to each individual of more than 10 years of age and the nets were shared with children aged below ten years if required. Pre-informed consent from the inhabitants was also obtained for inclusion in the study. Information, education and communication (IEC) activities were carried out to create awareness in the inhabitants about the usefulness of LLINs and instructions were given to the inhabitants on use, preservation, washing of the nets, etc. Compliance in using nets was monitored monthly. For this a volunteer in the two villages was deployed to collect data on usage pattern by the inhabitants and a vigilance team collected data fortnightly. Data on number of washes of nets performed by the inhabitants were also collected.

Entomological evaluation: Hand collections of indoor resting mosquitoes were done fortnightly in all the study villages using suction tube and flashlight in the early morning hours. Four houses were selected randomly (one in the north, south, east and west of the village) for sampling indoor resting mosquitoes. Collections were done for 15 min in each selected room and density per man-hour of collection were calculated. Data were pooled for each month and subjected to statistical analysis. Field collected mosquitoes were brought to the laboratory for species identification and further processing.

Mosquitoes landing on bednets were collected fortnightly in all the study villages and on baits in without net village. Informed consent was obtained from the human volunteers participating in the study as baits. Human volunteers were asked to sleep inside the bednets and mosquito collections were done from dusk-to-dawn (1800 to 0600 hrs). Altogether four houses in each village were selected randomly in the east, west, north and south and exit traps were fixed

to the windows. Two insect collectors were deployed in each room. One was assigned to collect the mosquitoes resting on the walls and the other to collect the mosquitoes landing on the net. The total of the mosquitoes collected from the walls, exit traps and off the bednet were considered to represent as total number entering the house. The number of mosquitoes that landed on the bednets was especially noted. Insect collectors were rotated every four hours.

An. culicifacies mosquitoes collected by all the methods were dissected and their parity or nulliparity assessed from the tracheolar skeins and, gut and gland infections with *Plasmodium* were recorded[21].

Epidemiological evaluation: Mass blood surveys were conducted in the selected villages in April 2004 prior to the distribution of nets, and in October 2004, and May and October 2005, and August 2006 after distribution of nets. Every fourth house was surveyed and blood smears of all family members were collected regardless of occurrence of fever. In addition, active surveillance for malara fever was also carried out fortnightly in all the study villages. All fever cases were identified and blood smears were collected, stained with JSB stain and examined under the microscope. Presumptive treatment was given to all the fever cases, while radical treatment was given only to malaria positive cases according to National Vector Borne Disease Control Programme, Govt. of India drug schedule in both control and experimental villages. Data were pooled together for before and after distribution periods.

Results

Altogether 1203 Olyset nets and 1289 untreated nets were distributed. Usage of nets by the inhabitants in study villages is shown in Fig. 1. It was found that >90% of the inhabitants were regularly using nets during the main malaria transmission season (August to November), and >80% in the months of March and April when the *Culex* mosquito densities are generally high. In this area, in the winter season (December to February), the density of *Anopheles* mosquitoes is generally very low due to low temperatures and people generally do not use nets. About 3.5% of Olyset nets and 2% of untreated nets were lost over a period of two years. The inhabitants using Olyset nets reported no adverse effect such as itching, nausea, vomiting, etc.

Impact on adult mosquito densities: As shown in Table 1 the number of all mosquitoes and of *An. culicifacies* collected resting indoors per man hour did not differ significantly between the villages before the intervention. After the intervention the numbers caught in the Olyset village were much less than in the control village. There were some indications that the untreated nets were also reducing the number of mosquitoes resting in the rooms.

Results of hourly night landing collections on human volunteers sleeping with or without net protection and entry rate in rooms are presented in Table 2. The entry of *An. culicifacies* was substantially reduced in the Olyset net village in comparison to the untreated net village and without net village. No mosquitoes were caught landing on the Olyset net suggesting that it has either air borne action or strong irritant action so that mosquitoes only remain in contact with the net for a very short time. Surprisingly, about 47.4% of *An. culicifacies* and 44.5% of all mosquitoes were found dead after 24 h observation that were caught inside the rooms with Olyset net.

After net distribution there was a significantly lower parous rate in *An. culicifacies* in most surveys in the Olyset net (mean parity 22.2%) village in comparison to the untreated net (mean 47.7%) and control villages (mean 73.1%) (Table 3). There was a statistically significant difference when the parity rates of all the villages were compared ($p < 0.0001$). Observations among those that were caught inside the rooms with Olyset nets for sporozoites revealed zero from the Olyset and untreated net villages and 1/447 from

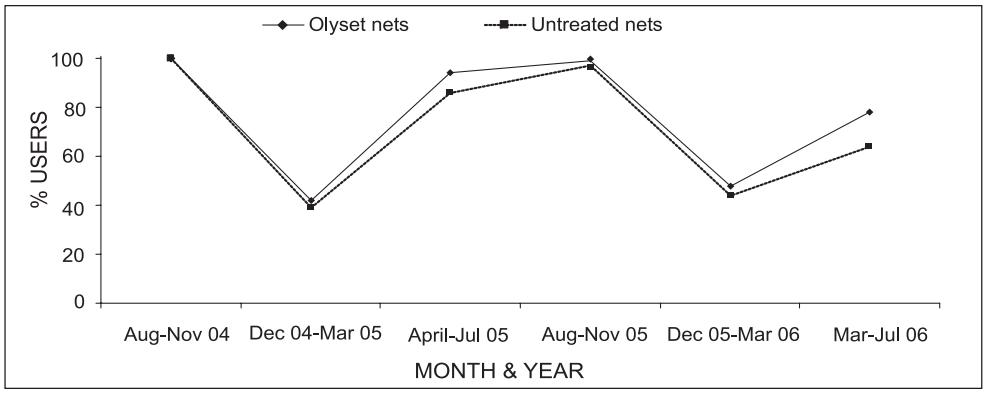

Fig. 1: Usage pattern of nets in study villages

Table 1. Number of indoor resting mosquitoes caught per man hour in the villages with Olyset or untreated nets or without nets

Period	Olyset net	Untreated net	Without net
	Anopheles culicifacies		
Pre-distribution			
Aug03–Jan 04	310 (12)[a]	489 (12)[a]	424 (12)[a]
Feb–Jul 04	464 (12)[a]	324 (12)[a]	382 (12)[a]
Monthly mean	64.5 ± 31.9[a]	67.7 ± 44.8[a]	67.16 ± 38.6[a]
95% CI for mean	41.71 – 87.29	44.9 – 90.5	44.38 – 89.95
Post-distribution			
Aug–Dec 04	124 (10)[a]	296 (10)[b]	640 (10)[b]
Jan–Jun 05	107 (12)[a]	211 (12)[b]	257 (12)[b]
Jul–Dec 05	113 (12)[a]	299 (12)[b]	358 (12)[b]
Jan–Jul 06	106 (14)[a]	245 (14)[b]	287 (14)[b]
Monthly mean	18.75 ± 12[a]	43.8 ± 26.7[b]	64.25 ± 46.7[b]
95% CI for mean	5.78–31.72	30.83–56.76	51.28–77.22
	All mosquitoes*		
Pre-distribution			
Aug 03–Jan 04	3062 (12)[a]	3097 (12)[a]	2574 (12)[a]
Feb–Jul 04	2962 (12)[a]	2940 (12)[a]	3014 (12)[a]
Monthly mean	502 ± 238[a]	503 ± 241[a]	465.6 ± 177[a]
95% CI for mean	372.4–631.6	373.5–632.7	336.1–595.3
Post-distribution			
Aug–Dec 04	536 (10)[a]	1520 (10)[b]	2437 (10)[b]
Jan–Jun 05	1110 (12)[a]	2506 (12)[b]	3035 (12)[b]
Jul–Dec 05	1029 (12)[a]	2042 (12)[b]	2678 (12)[b]
Jan–Jul 06	1291 (14)[a]	2229 (14)[b]	3026 (14)[b]
Monthly mean	165.25 ± 98.2[a]	345.7 ± 223[b]	465.6 ± 296[b]
95% CI for mean	75.15–255.4	255.6–435.8	375.6–555.8

*All mosquitoes include *An. annularis, An. subpictus, Cx. quinquefasciatus, An. stephensi, Culex* sp. and *Anopheles* sp. Figures in perentheses are number of collections. Monthly data was subjected to statistical analysis and mean density is represented for a month. Data in columns sharing the same superscript letter do not differ significantly.

Table 2. Mean number of mosquitoes caught per night resting on walls and in exit traps, and landing on nets/ baits in Olyset or untreated nets or without net villages (10 night collections from August 2004 to May 2005)

Village	Mean No. entering the room	Mean No. landing on net/bait	Percent dead
	Anopheles culicifacies		
Olyset net	13.9 ± 6.4[a]	0[a]	47.4[a]
Untreated net	47.4 ± 26.7[b]	16.1 ± 8.7[b]	1.6[b]
Without net	64.8 ± 30.7[b]	31.7 ± 10.3[b]	0.9[b]
	All mosquitoes*		
Olyset net	73.1±37.5[a]	0[a]	44.5[a]
Untreated net	275.3 ± 84.9[a]	115 ± 9.52[b]	1.3[b]
Without net	384.6 ± 122.6[c]	148 ± 50.5[b]	0.2[b]

*All mosquitoes include *An. annularis, An. subpictus, Cx. quinquefasciatus, An. stephensi, Culex* sp. and *Anopheles* sp. Data in the same column sharing the same superscript letter do not differ significantly.

Table 3. Parity rate of *Anopheles culicifacies* in villages with Olyset net or untreated net or without net (August 2004 to July 2006)

	Olyset net	Untreated net	Without net
No. dissected	333	509	694
No. parous	74	243	508
Parity rate	22.2	47.7	73.1

the control village. No conclusion can be drawn from such small samples.

Impact on malaria transmission: Data are shown in Table 4 on numbers of households surveyed for fever cases, numbers of slides taken from such cases and numbers of these found positive for any *Plasmodium* and for *P. falciparum*. Before intervention the incidence of fever associated with parasitaemia for either parasites was slightly higher in the village which later received Olyset nets than the other two villages and this difference was statistically significant. However, soon after introduction of Olyset nets, such cases ceased to be found in the village which received these nets but cases (including *P. falciparum*) continued in the other two villages. Malaria incidence in between the three villages differed significantly ($p < 0.0001$).

Results of the mass blood surveys (regardless of occurrence of fever) carried out in these villages are shown in Table 5. *Plasmodium* infections were found in all three villages before intervention and they did not differ significantly in terms of malaria cases ($p > 0.05$). After intervention no infections were found in the Olyset village and prevalence of infection with *P. falciparum* was consistently less in the untreated net village than the control. Malaria cases differ significantly in all the three villages ($p < 0.0001$)

Discussion

The enhanced malaria control programme launched in 1996 in India with the financial support from the World Bank did not provide the desired impact on the increasing malaria trend in the country and the incidence of recorded cases has fluctuated from 1.5 to 2 million for the past decade. The strategy of malaria control in India consists of indoor residual spray in areas showing an annual parasite incidence per 1000 people >2 in rural areas. Conventional insecticides, antilarval methods and space spraying are used to attempt to control urban malaria. During recent years conventionally treated nets were introduced into the programme with varying degrees of success. One of the major obstacles was lack of infrastructure for timely re-impregnation of nets resulting in inadequate protection.

In the present study with Olyset nets, long-lasting insecticidal nets significant reduction was observed in the indoor resting density of vector mosquitoes and no mosquitoes were caught landing on these nets. Earlier workers have reported high efficacy of Olyset nets in reducing man-vector contact and entry of mosquitoes into rooms[5,12,15]. In Cambodia, >70% decrease in indoor biting density of the main malar-

Table 4 Number of blood slides collected from feverish patients, number of slides positive for *Plasmodium* and for *P. falciparum*

	Olyset net	Untreated net	Without net
Pre-distribution (Aug 03 to Jul 04)			
No. of house holds surveyed	3235	3650	3970
No. of blood slides colletcted	238[a]	241[b]	210[ab]
No. positive for *Plasmodium*	79 (33%)[a]	53 (22%)[b]	55 (26%)[ab]
No. positive for *P. vivax*	36	15	19
No. positive for *P. falciparum*	43	38	36
Post-distribution (Aug 04 to Jul 06)			
No. of households surveyed	5026	6214	6750
No. of blood slides colletcted	214	209	303
No. positive for *Plasmodium*	3	18	67
No. positive for *P. vivax*	2	14	33
No. positive for *P. falciparum*	1	4	34

Data in columns sharing the same superscript letter do not differ significantly.

Table 5. Results of mass blood surveys regardless of fever in the study villages

	Olyset net	Untreated net	Without net
Pre-distribution (April 04)			
No. of houses surveyed	52	48	51
No. of blood slides colletcted	240[a]	220[a]	240[a]
No. positive for *Plasmodium*	8 (3.3%)[a]	8 (3.6%)[a]	7 (2.9%)[a]
No. positive for *P. vivax*	8	7	7
No. positive for *P. falciparum*	0	1	0
*Post-distribution**			
No. of houses surveyed	209	194	205
No. of blood slides colletcted	715	703	692
No. positive for *Plasmodium*	0	12	39
No. positive for *P. vivax*	0	8	27
No. positive for *P. falciparum*	0	4	12

* Total of four surveys carried out in October 2004, May and October 2005, and August 2006; Data in columns sharing the same superscript letter do not differ significantly.

ia vectors was reported[15]. Similarly, it was also reported that Olyset nets performed better than nets conventionally treated with permethrin in reducing man-vector contact as they significantly reduced entry and blood feeding rates[12]. The longevity and vectorial potential of the vector species was also reduced by the use of Olyset nets.

There were several reports that mosquito nets treated with or incorporating permethrin showed high efficacy in reducing the morbidity and mortality in children[2-4] . Faye *et al*[5] reported >50% reduction in malaria cases in Olyset net using areas in Senegal. They also reported a reduction of 8% in the parity rate of the vector mosquito and 90% decrease in indoor resting mosquitoes. In the present study also malaria incidence was reduced in the Olyset net village in comparison to untreated net and control villages. In particular, only one *P. falciparum* case was detected in the Olyset net village.

However, two recent studies[16,17] reported poor performance of the Olyset nets in the laboratory as well as in the field against *An. gambiae*. They reported reduced mortality after repeated washings to <5% and restoration of biological activity of Olyset nets only after heating to 60°C for 4 h. However, their method of bioassay was criticised by Maxwell *et al*[9]. Apart from the present study, other research carried out in India on the efficacy of Olyset nets against three mosquito species also gave encouraging results[18–20]. Persistence of good performance of Olyset nets in India after washing may be connected with high summer temperatures causing diffusion of permethrin to the surface of the net fibres. In the present study Olyset nets were monitored for only 24 months after distribution. More studies such as those of Maxwell *et al*[9] for a longer duration are required to investigate the long-lasting efficacy of the Olyset nets in reducing the human-vector contact and malaria incidence.

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