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Wash resistance and residual efficacy of long-lasting polyester netting coated with α-cypermethrin (Interceptor) against malaria-transmitting mosquitoes in Assam, northeast India

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ABSTRACT

Malaria is endemic in Assam, northeast India, with low-to-moderate transmission of the causative parasites, mostly by *Anopheles minimus*. *Plasmodium falciparum* is the predominant parasite (>60%), with remaining cases being due to *P. vivax*. As an alternative intervention for malaria control, long-lasting insecticidal nets [Interceptor coated with α-cypermethrin 10% suspension concentrate (SC), 0.667% w/w, 0.2 g/m^2] underwent field evaluation for laboratory wash resistance and residual efficacy in field conditions against malaria-transmitting mosquitoes. Based on entomological observations, the Interceptor net intervention was the most effective, corresponding to the lowest mosquito vector density in experimental villages. There was virtual disappearance of *A. minimus* in Interceptor net villages in contrast to the untreated net intervention and the no-net control. Contact cone bioassay tests revealed 100% mortality in the *A. minimus* group of mosquito species in the community using the Interceptor net, which was consistent during the follow-up monitoring period (October 2006 to April 2007) in field conditions. Similar levels of mortality were observed in laboratory-washed nets compared with unwashed nets, and wash resistance was consistent even after the 20th serial wash at fortnightly intervals. Community compliance and acceptance of the Interceptor net was high, with decreased nuisance due to biting mosquitoes and other household insect pests being reported.

1. Introduction

Malaria is a major public health illness in the Indian state of Assam (24°44′ to 27°45′ N latitude; 89°41′ to 96°02′ E longitude), contributing >5% of reported cases in the country annually. Both *Plasmodium falciparum* and *P. vivax* are prevalent, but *P. falciparum* is the predominant parasite accounting for >60% of malaria cases.[1] Malaria transmission is low to moderate, maintained mostly by *Anopheles minimus*, and cases are recorded throughout the year with a seasonal peak during May–September/October corresponding to the wet season (monsoon months). *Anopheles minimus* mosquitoes have been incriminated in most districts of Assam and are solely responsible for focal disease outbreaks that are characterised by a high rise of *P. falciparum* infection and attributable deaths. Although *A. minimus* is highly susceptible to DDT (the residual insecticide used in control programmes), malaria transmission continues uninterrupted due to inadequate vector control interventions on account of a high refusal rate to spraying, difficult terrain and recurrent floods limiting access to high-risk population groups. Among

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alternative approaches, insecticide-treated nets (ITN) have been a runaway success,[2] but the sustainability of ITNs as a lasting intervention largely rests on re-impregnation coverage, which remained at <5% of the target population (State Health Directorate of Assam). The availability of a mosquito net that retains bioefficacy despite repeated washings is a landmark development offering the opportunity for an operationally feasible and cost-effective intervention.[3,4] Among a variety of long-lasting insecticidal nets (LLIN) that are subject to field evaluation in India, the objective of this study was to determine the residual efficacy of Interceptor, an α-cypermethrin-coated polyester netting (BASF AG, Ludwigshafen, Germany), against prevailing malaria vectors in the northeastern region of India and to assess wash resistance following serial washings in laboratory conditions. Here we report the research findings for the first year of observations from August 2006 to April 2007, which could help programme managers to formulate appropriate policy for effective vector control.

2. Materials and methods

2.1. Mosquito netting, study populations and net distribution

The Interceptor nets subject to field evaluation were made of 100% texturised, multifilament (36–48 filaments) polyester netting fabric with a 75 denier strength and a mesh size of 156 (12 × 13 holes/square inch). The net contains Fendozin, which binds the polymer insecticide Fendona [active ingredient α-cypermethrin 10% suspension concentrate (SC), 0.667% w/w, 0.2 g/m^2] and forms a thin, slow-release coating to the polyester fibre of the net. Fendona diffuses in a controlled manner to the surface of the coating to become bioavailable; it then repels and has a quick knockdown effect against mosquitoes and other pest insects.

Based on comparable malaria endemicity, three clusters of villages in the Sonapur Primary Health Centre area (a typical foothill malaria-endemic area) located 20 km east of Guwahati, the capital city of Assam, were selected. Each cluster was at least 6 km apart with similar population groups and comprised three villages that received Interceptor nets (population 2494), seven villages that received untreated nets, i.e. nets without insecticide (population 2199) and four villages of no-net control (population 2092) for comparative purposes. The geographic location and topographical features of the study area are detailed elsewhere.[2] The populations in the villages were predominantly tribal aborigines living in poor socioeconomic conditions. In the experimental villages, given the study protocol that had approval of the institutional scientific advisory committee (http://www.mrcindia.org), except for disease surveillance and treatment of cases, intervention based on DDT spraying operations (of which two rounds were scheduled in the high-transmission season each of 75 days duration) was withdrawn for the study period. However, for the no-net cluster of villages (i.e. without net intervention) adequate provision was made for focal spraying of DDT to check disease outbreak for which constant vigil was maintained by door-to-door disease surveillance throughout the study period.

The area receives heavy rainfall associated with the monsoons (2–3 m) annually, much of which occurs during April–September/October (wet season); for the rest of the year there is little rainfall. The mean relative humidity varies from 60% to 80% and most of the year is hot and humid (22–33 °C), except November–February (minimum 10 °C), allowing proliferation and longevity of malaria-transmitting mosquitoes. Typically, houses have two to three rooms made of bamboo with thatched roofing, which are ideal places for indoor-resting mosquitoes. To ensure full protection of the entire family, a population census was performed in July 2006 and each household was provided with a sufficient number of nets in October 2006 with the written consent of householders for willingness to use it in lieu of DDT spraying. Post-distribution evaluation was performed at regular intervals in experimental villages, i.e. Interceptor net, untreated net and no-net control areas, from October 2006 until April 2007; data for August–September 2006 (pre-intervention period) served as a baseline for comparative purposes. Necessary instructions were given to householders for compliance in using the given mosquito net, which was cross-checked periodically during community assessment surveys.

2.2. Insecticide susceptibility status

Prior to introduction of the Interceptor net as an alternative intervention to DDT residual spraying, the insecticide susceptibility status was ascertained for *A. minimus* (the predominant mosquito vector species) against DDT and α-cypermethrin (the active ingredient of the Interceptor net) using a WHO standard test kit. Field-collected, mixed age, adult females of *A. minimus* were exposed to diagnostic concentrations for 60 min and mortality was recorded after a 24-h recovery period under laboratory conditions. Data were pooled based on different replicates against each insecticide and corrected mortality was ascertained using Abbott's formula.

2.3. Mosquito sampling and abundance

Indoor day-resting catches were made by experienced insect collectors in randomly selected houses in experimental villages by the hand-catch method using a suction tube aided by torch battery light in the early morning hours (06:00 h–08:00 h) at fortnightly intervals. Collections were made for 15 min in each selected structure of mosquitoes resting indoors on walls, hanging clothes and other household articles. Field-collected mosquitoes were identified using standard keys to the species level and relative abundance was expressed as person-hour density, i.e. the number of mosquitoes collected per person-hour. In addition, dusk-to-dawn (18:00 h–05:00 h) human-bait landing catches (sleeping both indoor and outdoor) were made in August 2006 (pre-intervention period) and in January 2007 and March 2007 (post-intervention period). Pre-informed consent was obtained from the participating human baits on each count. All mosquitoes landing over the net/human volunteers were collected and pooled hourly to measure mosquito landing rate per person-night.

2.4. Residual efficacy and wash resistance

Cone bioassay tests were performed on Interceptor nets in use by the communities under field conditions (monthly intervals) as well as in the laboratory (fortnightly) to determine persistence and bioavailability of the insecticide on washed and unwashed nets, keeping the untreated net as a control. In the laboratory, washing and subsequent washing exercises were done using commonly available branded detergent (wheel powder) that included a teaspoon full (~5 g) of detergent powder dissolved in 5 l of water followed by rinsing thoroughly in plain water two to three times and drying in open broad daylight for at least 48 h. Bioassay tests were performed just before the next wash, for which a mixture of 10 adult blood-fed, field-collected mosquitoes of *A. minimus* group, which included *A. minimus*, *A. varuna* and *A. aconitus*, were exposed using standard WHO test cones for 3 min and data for different replicates (three or more) were pooled. The number of mosquitoes knocked down following 3 min exposure and mortality after the 24-h recovery period were recorded. In addition, ring-net bioassay tests were conducted using similar procedures in which 11 mosquitoes were exposed and the time required for knockdown of the 1st, 6th and 11th mosquito was recorded, quantifying the persistence of insecticide on the net fibre.

2.5. Social acceptability and community perceptions

Cross-sectional community-based surveys were conducted among Interceptor net users for assessment of perceptions, adverse events and collateral benefits, if any, on two different occasions, first during December 2006 to January 2007 and subsequently in March 2007 using a structured questionnaire.

2.6. Statistical analyses

Data collected were subjected to statistical analyses by univariate *t*-test and one-way ANOVA using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. Susceptibility status of Anopheles minimus vector populations

Field-collected adults of *A. minimus* were ascertained to be fully susceptible to standard diagnostic concentrations of DDT (4%) and α-cypermethrin (0.10%); 100% mortality was observed against each insecticide for the given 60 min exposure and following the 24-h recovery period. Having confirmed the susceptibility status to α-cypermethrin, introduction of the Interceptor net-based intervention was considered appropriate to replace DDT residual spraying.

3.2. Vector density and relative abundance of indoor day-resting mosquitoes

In the pre-intervention period (August–September 2006), the baseline person-hour mosquito densities of *A. minimus* in the Interceptor net, untreated net and no-net villages ranged from 0.68 to 1.87 and were statistically comparable. Similarly, for commonly available indoor-resting anopheline mosquito species, including *A. varuna*, *A. aconitus*, *A. vagus* and *A. annularis,* the mosquito densities varied from 12.4 to 17.32. However, following the introduction of the net intervention in study villages in October 2006, there was virtual elimination of *A. minimus* in human dwellings (indoor) in Interceptor net villages for the entire follow-up study period (Figure 1). In contrast, for the untreated net and no-net control villages there was a seasonal decline during winter months and a rising density of vector populations of *A. minimus* observed during March/April, which was significantly higher than the Interceptor net intervention villages ($P = 0.031$; 95% CI 0.63–1.47). Similarly, for other commonly abundant anopheline mosquito species, namely *A. varuna*, *A. aconitus*, *A. vagus* and *A. annularis*, mosquito densities were significantly lower ($P = 0.048$; 95% CI 4.05–8.40) in Interceptor net intervention villages compared with the untreated net and no-net control villages (Supplementary Figure 1). These data were corroborated by overnight human-bait mosquito landing catches in target study villages (Table 1). In the pre-intervention period, i.e. August 2006, mosquito landing rates were statistically similar in all three categories of villages (95% CI 0.62–3.48). However, *A. minimus* mosquitoes were not recorded in the post-intervention months sleeping indoor/outdoor in Interceptor net villages, and there was a significant reduction in the mosquito landing rate compared with the pre-intervention period ($P = 0.017$; 95% CI 0.16–2.66). In contrast, vector populations of *A. minimus* and *A. baimaii* (formerly species D of *A. dirus*) were recorded landing on human bait both in untreated net and no-net control villages, and the change in mosquito landing rates between pre-intervention and post-intervention months was not significant.

3.3. Residual bioefficacy and wash resistance

For nets in use by the communities in field conditions, contact cone bioassay tests revealed 100% mortality

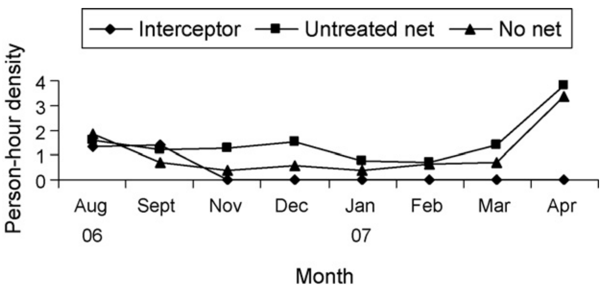

Figure 1. Relative abundance of *Anopheles minimus* in experimental villages of the Sonapur Primary Health Centre, Kamrup district, Assam, India, during August–September 2006 (pre-intervention) and November 2006 to April 2007 (post-intervention) study periods.

Table 1
Human-bait mosquito landing rates per person-night in experimental villages of the Sonapur Primary Health Centre, Kamrup district, Assam, India

Category	Mosquito species	Pre-intervention (August 2006)		Post-intervention			
				January 2007		March 2007	
		Indoor	Outdoor	Indoor	Outdoor	Indoor	Outdoor
Interceptor net	Total *Anopheles*	5.0	9.5	0.5	2.0	0	3.0
	A. minimus	2.5	3.0	0	0	0	0
	A. baimaii	0	0	0	0	0	0
Untreated net	Total *Anopheles*	4.5	12	9.0	13.0	15	14
	A. minimus	2.5	5	3.5	3.5	10	7
	A. baimaii	0	0	2.5	4.0	0	0
No net	Total *Anopheles*	2.0	8.5	9.5	7.5	7.5	11.5
	A. minimus	2.0	5.0	2.5	1.5	5.0	5.5
	A. baimaii	0	0.5	3.5	1.5	1.5	3.5

against mosquitoes exposed, which was consistent from October 2006 to April 2007 following 3 min exposure and 24-h recovery period (Supplementary Table 1). However, following 3 min exposure, a gradual reduction in knockdown mosquitoes from 70% (42/60) in October 2006 to 36% (43/120) in April 2007 was observed, which was significantly different from the baseline data ($P = 0.002$; 95% CI 15.41–39.91). In untreated net control replicates, mortality was poor and varied from 7.5% to 12.5%. Data from ring-net bioassay studies against Interceptor nets in use revealed a steady increase in knockdown time for the 1st mosquito (5.2 min to 7.1 min), 6th mosquito (8.2 min to 11.7 min) and 11th mosquito (12 min to 19.7 min) for the period from November 2006 to April 2007 (Supplementary Table 2); however, this was insignificant for all three categories of mosquitoes. Data on serial wash resistance in laboratory conditions for knockdown following 3 min exposure as well as mortality following 24-h recovery period in the Interceptor net, unwashed Interceptor net and untreated net are presented in Table 2. There was a significant reduction ($P = 0.020$) in per cent knockdown mosquitoes following 3 min exposure both in washed (from 65% to 25%) and unwashed (from 73% to 45%) Interceptor nets, however the kill effect post 24-h recovery period was 100% and was observed to be persistent even after the 20th wash of Interceptor nets, which compared well with data on unwashed Interceptor nets. Mortality in the washed untreated net control varied from 2.5% to 7.5%.

3.4. Community responses and acceptability of the Interceptor net

Based on the questionnaire-based surveys, it was observed that all community users were fully aware of the benefit of using a mosquito net as a personal protection method and compliance was 100% (425/425). In the community surveys conducted during December 2006 to January 2007 after a few months of usage, 36% of users

Table 2
Wash resistance of Interceptor nets expressed in terms of per cent knockdown of *Anopheles minimus* group of mosquito species following 3 min exposure and mortality after the 24-h recovery period in laboratory conditions following serial washing at fortnightly intervals[a]

No. of serial washings (fortnightly)	Washed Interceptor net		Unwashed Interceptor net		Washed untreated net
	No. (%) knockdown post 3 min exposure	No. dead post 24-h recovery period (% mortality)	No. (%) knockdown post 3 min exposure	No. dead post 24-h recovery period (% mortality)	No. dead post 24-h recovery period (% mortality)
1	26 (65)	40 (100)	29 (73)	40 (100)	1 (2.5)
2	24 (60)	40 (100)	24 (60)	40 (100)	2 (5)
3	22 (55)	40 (100)	26 (65)	40 (100)	2 (5)
4	18 (45)	40 (100)	26 (65)	40 (100)	2 (5)
5	22 (55)	40 (100)	28 (70)	40 (100)	2 (5)
6	19 (48)	40 (100)	21 (53)	40 (100)	3 (7.5)
7	16 (40)	40 (100)	20 (50)	40 (100)	2 (5)
8	20 (50)	40 (100)	22 (55)	40 (100)	3 (7.5)
9	18 (45)	40 (100)	24 (60)	40 (100)	3 (7.5)
10	16 (40)	40 (100)	22 (55)	40 (100)	2 (5)
11	17 (43)	40 (100)	20 (50)	40 (100)	2 (5)
12	18 (45)	40 (100)	22 (55)	40 (100)	2 (5)
13	17 (43)	40 (100)	20 (50)	40 (100)	2 (5)
14	14 (35)	40 (100)	18 (45)	40 (100)	3 (7.5)
15	14 (35)	40 (100)	18 (45)	40 (100)	3 (7.5)
16	15 (38)	40 (100)	20 (50)	40 (100)	3 (7.5)
17	16 (40)	40 (100)	20 (50)	40 (100)	3 (7.5)
18	15 (38)	40 (100)	22 (55)	40 (100)	3 (7.5)
19	13 (33)	40 (100)	21 (53)	40 (100)	3 (7.5)
20	10 (25)	40 (100)	22 (55)	40 (100)	3 (7.5)

[a] Based on exposure of 10 anopheline mosquitoes per cone bioassay for a total of 40 mosquitoes.

(81/225) complained of skin-related reactions, i.e. skin irritation and itching, 8.9% (20/225) reported eye irritation and 0.4% (1/225) complained of headache; however, all these were reported to be transitory in nature lasting a few days of usage. However, during follow-up community surveys conducted in March 2007 there was no such complaining, except for 1% of users (2/200) who reported skin irritation. For both surveys combined, the majority (80%; 341/425) reported a reduction in mosquito bites and 24.5% (104/425) observed other collateral benefits such as decreased nuisance due to body lice, head lice and bedbugs. Most householders (385/425; 91%) recommended the use of a LLIN as personal guard against malaria and other pest insects.

4. Discussion

Malaria continues to be a major public health concern in the northeastern states of India. For its effective control there is an imperative need for an alternative intervention that is evidence-based and sustainable. LLINs are increasingly being accepted as a newer intervention tool for disease vector control worldwide. The National Vector Borne Disease Vector Control Programme of India has accorded due priority to the northeastern states of India for the vast population living in poverty (>36%) in high-risk remote forest fringe areas that report the most cases of malaria and malaria-attributable deaths (State Health Directorate of Assam). Among the variety of LLINs that have been considered by the WHO (http://www.who.int/whopes), Interceptor nets were granted an interim recommendation and were subject to evaluation for residual efficacy and acclaimed wash resistance against prevalent malaria-transmitting mosquitoes specific to the northeastern region of India.

From the data presented here, *A. minimus* was observed to be fully susceptible to α-cypermethrin, the active ingredient used in Interceptor nets. Interceptor nets appeared to provide adequate personal protection to users as measured by the vector abundance of *A. minimus* (Figure 1) and the mosquito landing rate on human hosts in experimental villages, which were supportive of deterrence and blood-feeding inhibition by the vector mosquito (Table 1). The Interceptor net retained adequate bioefficacy against target mosquito vector species even after the 20th serial wash in laboratory conditions as per WHO guidelines for testing of LLINs (http://www.who.int/whopes). The kill effect against *A. minimus* (the predominant vector species) was 100% and was persistent, i.e. well above the cut-off point of 80% mortality 24 h post exposure (Table 2). Data on wash resistance of these nets in laboratory conditions corresponded well with their retention of biological activity in the community when using LLINs under field conditions (Supplementary Table 1), corroborating the research findings reported by Gimnig et al.[5] The fact that these nets retained bioefficacy despite repeated washings makes this intervention appropriate and sustainable particularly for population groups most at risk due to an inadequate health infrastructure. Using a variety of LLINs, similar data on wash resistance and extended bioefficacy against prevailing vector species have been reported in malaria-endemic countries including India,[6] Sudan,[7] Cote d'Ivoire[8] and Tanzania.[9] Since the majority of ethnic communities of northeastern states reportedly washed their nets quarterly (>50% of inhabitants surveyed),[10] data on residual efficacy for 20 serial washes should serve as an evidence base for longer efficacy until the estimated life span of the net of 5 years. However, this remains to be established by an extended follow-up study for at least 3 years of use in field conditions as defined by the WHO Pesticide Evaluation Scheme (WHOPES).[11] Among other concerns is the depletion of residual insecticide subject to serial washings as well as nets in extended use in field conditions quantified by a significant decrease in knockdown mosquitoes for 3 min exposure from baseline observations, which calls for monitoring over extended use to confirm its long-term efficacy (Table 2; Supplementary Table 1). Furthermore, the data presented here are focused on *A. minimus*, the major vector in the study area, but for *A. baimaii* the study needs to be validated probably in another geographical area with a predominance of the latter. Admittedly, the bulk of the data in the present study relate to the low transmission period, which warrants extended follow-up investigations given the transmission heterogeneities.

Based on the available data, an Interceptor net-based intervention appears to hold good against *A. minimus*-transmitted malaria given the terrain and transmission intensities. What is vital to the success of a control programme is community perceptions and compliance. In the Interceptor net-based intervention villages, this was forthcoming, with diminishing malaria cases and decreased nuisance due to biting mosquitoes and other household pest insects being reported. LLINs are becoming increasingly popular and widely accepted by the ethnic communities of the northeastern states and are being accorded priority for larger population coverage under the National Rural Health Mission to address the healthcare needs of the poor (State Health Directorate of Assam). What is needed now is the scaling-up of availability of these LLINs, prioritising high-risk population groups with provision of free supply for families below the poverty line to ensure equity and social justice in health.[12] To achieve these targets, it is vital to prepare roadmaps, which should include advocacy, planning and information exchange, mobilisation of additional resources, human resource development, and much needed monitoring and evaluation.[13,14] It is high time to forge partnerships between governments, non-governmental organisations, donors and the commercial sector in meeting increasing demand at affordable prices. Rolling back malaria is becoming a reality with the appropriate mix of available technologies such as LLINs, rapid diagnostic tests and artemisinin-based combination therapies as well as strengthening of healthcare services.[15–17] Still there is a need for continued research to identify and evaluate newer tools for vector control that can be integrated with existing biomedical strategies within national malaria control programmes.[18–21]

Authors' contributions: KR conceived and designed the study, performed the interim analyses and revised the

manuscript; SPS and SP collected the field-based data, compiled the data files and helped draft sections of the manuscript; VD analysed the data and drafted the manuscript; APD co-ordinated the study and contributed to the data analysis and presentation; KK co-ordinated the study at the state level and critically revised and improved the manuscript. All authors read and approved the final manuscript. VD is guarantor of the paper.

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Conflicts of interest: None declared.

Ethical approval: National Institute of Malaria Research, Delhi, India.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.trstmh.2009.08.010.

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