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Indoor Use of Plastic Sheeting Impregnated with Carbamate Combined with Long-Lasting Insecticidal Mosquito Nets for the Control of Pyrethroid-Resistant Malaria Vectors

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Abstract. The combined efficacy of a long-lasting insecticidal net (LLIN) and a carbamate-treated plastic sheeting (CTPS) or indoor residual spraying (IRS) for control of insecticide-resistant mosquitoes was evaluated in experimental huts in Burkina Faso. *Anopheles gambiae* from the area is resistant to pyrethroids and to a lesser extent, carbamates. Relatively low mortality rates were observed with the LLIN (44%), IRS (42%), and CTPS (52%), whereas both combinations killed significantly more mosquitoes (~70% for LLIN + CTPS and LLIN + IRS). Blood feeding by *An. gambiae* was uninhibited by IRS and CTPS compared with LLIN (43%), LLIN + CTPS (58%), and LLIN + IRS (56%). No evidence for selection of the *kdr* and *ace-1^R* alleles was observed with the combinations, whereas a survival advantage of mosquitoes bearing the *ace-1^R* mutation was observed with IRS and CTPS. The results suggest that the combination of the two interventions constitutes a potential tool for vector-resistance management.

INTRODUCTION

Malaria represents one of the most critical public-health challenges for Africa.[1] With the absence of effective vaccine, prompt access to diagnosis and treatment with artemisinin-based combination therapy (ACT) remains the mainstay of malaria control. In addition to that, the use of long-lasting insecticide-treated nets (LLIN) and indoor residual spraying (IRS) represents the main approach to malaria prevention.[2] A recent systematic review of different randomized controlled trials conducted in a range of transmission settings worldwide in children and adults showed that insecticide-treated nets (ITNs) reduced uncomplicated malaria episodes by 50% in stable areas and 62% in unstable areas.[3] Until now, IRS has always been recommended for areas of low-to-moderate transmission, although the World Health Organization (WHO) has changed the policy and is now promoting a wider application of IRS, even in highly endemic areas.[1] However, many authors have questioned the global efficacy of IRS in high malaria-transmission settings, because a great reduction in transmission level is needed to achieve a significant reduction in malaria prevalence.[4] To be sustainable in malaria-endemic countries, IRS with conventional insecticides must be re-applied frequently and continued indefinitely, requiring long-term predictable financing mechanisms that many African countries lack.[4] For this reason and because LLINs seem to be more cost effective than IRS,[5] scaling up of LLINs coverage in Africa has become the cornerstone of malaria prevention.

To date, pyrethroids are the only insecticides used for net impregnation because of their strong efficacy, their fast-acting effect at low dose, and their low toxicity for mammals.[6] Unfortunately, pyrethroid resistance in malaria vectors has spread across Africa and is now present in most of the countries where the National Malaria Control Programs (NMCP) implemented a large-scale distribution of LLINs to the population at risk (i.e., children under 5 years and pregnant women).[7] Until now, there was no evidence to support the fact that pyrethroid resistance reduced the effectiveness of LLIN for controlling malaria at an operational level.[8] However, comparative experimental hut trials of lambdacyalothrin as ITN and IRS treatments in the coastal area of Benin, Ladji and Malanville in the north, indicated a major loss of efficacy associated with pyrethroid resistance in *Anopheles gambiae* Giles at Ladji, Benin.[9]

Carbamates (CARs) and organophosphates (OPs) are considered as promising alternatives to pyrethroids for vector control.[10] Their twin characteristics of non-excitorepellency and toxicity may combine to make them stronger than pyrethroids in reducing adult densities, survival rates, and hence, vectorial capacity.[11] Unfortunately, the use of such non-irritant insecticides would not inhibit blood feeding of mosquitoes and therefore, would provide much lower personal protection than pyrethroids.[12] In addition, they have a less favorable toxicological profile than pyrethroids that renders their use on mosquito bed nets unlikely.

To overcome these limitations, we have investigated and tested a new concept for malaria vector control consisting of the use of an LLIN plus a carbamate-treated plastic sheeting (CTPS) or an IRS using non-pyrethroids in the same dwelling to improve the personal protection and killing effect against pyrethroid-resistant mosquitoes. The rationale behind this concept is that neither ITNs nor IRS-like treatments will be sufficiently effective alone to achieve and maintain interruption of transmission in stable malaria areas of Africa. It is indeed essential to assess if combining the two interventions would maximize the impact of malaria vector control and offer opportunities for the management of insecticide resistance (i.e., insecticide resistance management [IRM] strategy).

A previous study conducted under laboratory- and field-simulated conditions showed a good efficacy and wash resistance of bendiocarb-treated polypropylene plastic sheeting mixed with a binding agent against susceptible and pyrethroid-resistant *An. gambiae* and *Culex quinquefasciatus* Say.[13] Here, the combined efficacy of an LLIN (PermaNet 2.0) plus a CTPS or an IRS using bendiocarb at 400 mg/m^2 was investigated in the Vallée du Kou (Burkina Faso) where *An. gambiae* shows resistance to pyrethroids and to a lesser extent,

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carbamates[14] with mortalities of 24% and 86% for deltamethrin (0.05%) and bendiocarb (0.1%) (Dabiré R, unpublished data). Molecular genotyping of mosquitoes collected in each hut was also performed to assess the pressure of selection induced by each intervention on the *kdr* and *ace-1^R* alleles.

MATERIALS AND METHODS

Study area. The experimental hut trial was carried out at the Vallée du Kou (VK7), a huge rice field area located near Bobo-Dioulasso, Burkina Faso,[15] between October and December 2008. Both molecular M and S forms of *An. gambiae* coexist in sympatry, but the M form is mostly present during the dry season.[14] The *kdr* mutation occurred in M and S molecular forms but at different allelic frequencies.[14] The *ace-1^R* mutation was found essentially in the S molecular form.[14]

Experimental huts, volunteer participants, and mosquito collections. The treatments and control were evaluated in six experimental huts according to the WHO procedures.[16] Local adult male volunteers were recruited to sleep in the huts. They provided informed consent before enrolment. They received malaria chemoprophylaxis and medical surveillance during and 3 weeks after the trial. The ethical committees of the Institut de Recherche pour le Développement (IRD) and the Ministry of Health of Burkina Faso formally approved the protocol. At 6:00 PM, before the start of the tests, the volunteers removed spiders and other mosquito predators. Then, they slept from 8:00 PM to 5:00 AM, at which time they closed the entry baffles, lowered the curtain separating the sleeping room from the veranda-trap, and collected all mosquitoes, dead and alive, from the room, bed net, and veranda. Female mosquitoes were scored by location as dead or alive and fed or unfed; species was identified according to morphologic characteristics. To minimize bias related to mosquito attractiveness of each volunteer and spatial variation in mosquito densities, the volunteers and bed nets were rotated between huts each day. Mosquito collection began 3 days after huts treatments. All six treatments were investigated on concurrent nights, and 57 replicates were performed for each experimental hut.

Mosquito nets and treatments. The following treatments were compared in six huts: (1) control, untreated polyester mosquito net; (2) LLIN, PermaNet 2.0 alone; (3) IRS, carbamate alone; (4) LLIN + IRS, Permanet 2.0 plus carbamate; (5) CTPS, carbamate-treated plastic sheeting alone; and (6) LLIN + CTPS, Permanet 2.0 plus carbamate-treated plastic sheeting.

PermaNet 2.0 (Vestergaard Frandsen SA, Lausanne, Switzerland) is an LLIN made of multifilament polyester (100 denier) fabric, factory treated with deltamethrin at 55 mg/m^2 (1.4 g/kg for a 100-deniers net). The untreated (control) net was a polyester fabric (SiamDutch Mosquito Netting Co., Bangkok, Thailand). Both untreated and PermaNet 2.0 were deliberately holed (six holes, 4 × 4 cm each) to simulate torn nets.[15]

The carbamate used for the treatment of the plastic sheeting (CTPS) and walls (IRS) was bendiocarb wetable powder (WP) concentrate 80W (Ficam) at a single dose of 400 mg active ingredients (ai)/m^2. Ficam and the resin were provided by Bayer (Leverkusen, Germany). This dose has been recommended by the WHO for classical IRS.[17] For the IRS, bendiocarb was applied one time using a hand-operated compression sprayer (Berthoud N°2000 PRO model; Vermorel, Villefranche sur Saône, France) equipped with a flat fan nozzle with 80° swath and 0.76 L/minute flow rate.

The polypropylene plastic sheeting was obtained from Filtisac SA (Abijan, Côte d'Ivoire). Treatment procedure for the huts containing CTPS was done as per Djenontin and others.[13] This involved evenly applying bendiocarb to the polypropylene plastic sheeting, letting the treatment dry horizontally in the shade for 1 day, and covering just the upper one-third of the walls to minimize contact with sleepers. A binding resin (20% concentrated) prepared at 12 mL/m^2 was added to the bendiocarb-polypropylene plastic sheeting preparation to improve residual activity and wash resistance.[13]

Residual activity of insecticide treatments. To evaluate residual activity, WHO cone bioassays were undertaken 7, 10, 21, and 120 days post-treatment using a laboratory-susceptible *An. gambiae* s.s., Kisumu strain. Adult females, 3–5 days old, were introduced into cones fixed to LLIN for 3 minutes and to sprayed walls (IRS) or treated plastic sheeting (CTPS) for 30 minutes according to WHO guidelines.[16] Approximately 50 mosquitoes in 5 replicates of 10 mosquitoes were tested on each substrate.

Molecular analysis. After scoring field-collected Anopheles mosquitoes in each hut and identifying each specimen to species by polymerase chain reaction (PCR),[18] the presence and relative frequency of the molecular M and S forms of *An. gambiae* s.s. was determined according to the method of Favia and others.[19] The methods of Martinez Torrez and others[20] and Weill and others[21] were used for the molecular detection of the *kdr* and *ace-1^R* alleles, respectively, in individual mosquitoes collected, alive or dead, from the control and treated huts.

Statistical analysis. Data from *in situ* bioassays were compared between each hut using a χ^2 test at a 95% confidence interval with the Minitab software version 12.2. The Kruskal–Wallis non-parametric test was used to assess deterrence by comparison of the number of mosquitoes entering each hut. Proportional data from the hut trial (exophily, blood feeding, and mortality) were analyzed using logistic regression (XLSTAT software, Paris, France).[22] For each treatment, differences in genotypes between live and dead mosquitoes were analyzed using the exact test of Fisher with the software GENEPOP (Montpellier, France).[23]

RESULTS

Residual activity of treatments. Residual activity of LLIN and CTPS, as measured by cone bioassays on the Kisumu strain, showed no decline over 60 days. By contrast, mortality on walls (IRS) decreased significantly from 100% after 10 days to 29% after 60 days ($P < 0.05$).

Efficacy of treatments in huts. The summary results for exophily are shown in Figure 1, whereas those for blood feeding and mortality are presented in Figures 2 and 3, respectively.

A total of 1,374 *An. gambiae* were collected within the six experimental huts. Fewer *An. gambiae* entered the huts with LLIN, alone or in combination with IRS and CTPS, than the untreated hut ($P < 0.05$). Natural exophily in the control hut was 21% (Figure 1). All treatments induced significantly higher exophily than the control ($P < 0.05$); however, the best effect was observed with the LLIN alone (79.7%; $P < 0.01$) or combined with IRS (60.5%; $P < 0.01$) and CTPS (50.2%; $P < 0.01$). The IRS and CTPS treatments induced exophily < 40%, and the difference between them was not significant ($P = 0.10$). Blood-feeding rates of *An. gambiae* in the control, CTPS, and IRS huts were not significantly different (69–88%; $P > 0.05$) (Figure 2), suggesting that IRS and CTPS do not

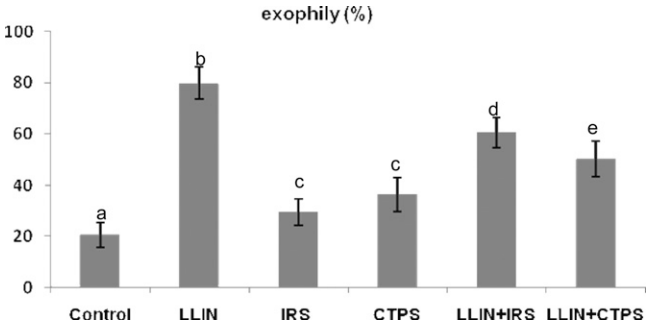

FIGURE 1. Exophily (95% confidence interval) of *An. gambiae* exposed to LLIN alone and in combination with IRS and CTPS in experimental huts (Vallée du Kou, Burkina Faso). Treatments carrying the same letter are not significantly different ($P > 0.05$).

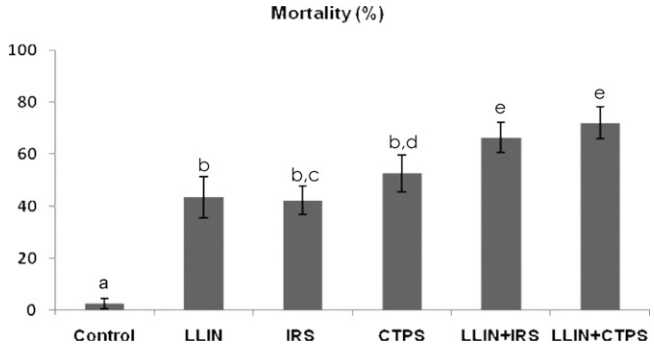

FIGURE 3. Mortality rate (95% confidence interval) of *An. gambiae* exposed to LLIN alone and in combination with IRS and CTPS in experimental huts (Vallée du Kou, Burkina Faso). Treatments carrying the same letter are not significantly different ($P > 0.05$).

constitute an effective barrier against mosquito bites. By contrast, significant blood-feeding inhibition was observed in huts with LLIN (43%), LLIN + IRS (56%), and LLIN + CTPS (58%) compared with the control hut ($P < 0.01$). Differences between the monotreatment and the combinations were not significant ($P > 0.05$).

Mortality rate in the control hut was less than 3%, indicating that no contamination occurred during the evaluation. Relatively low mortality rates were observed with the LLIN (44%), IRS (42%), and ITPS (53%), whereas both combinations killed significantly more pyrethroid-resistant mosquitoes ($P < 0.01$); the LLIN + IRS killed 67%, and the LLIN + CTPS had 73% mortality (Figure 3).

Effect of treatments on insecticide-resistance genotypes. A total of 325 *An. gambiae* females were genotyped for their sibling species and molecular forms as well as for the *kdr* and *ace-1^R* alleles. About 98% of the specimens analyzed (321 of 325) belonged to the M molecular form. The frequency of the resistance alleles in the control hut with untreated net was 90% for the *kdr* and 11% for the *ace-1^R* mutations.

The results of *kdr* and *ace-1^R* genotyping performed on mosquitoes caught in the treated huts are shown in Table 1. There was no significant differences in the *kdr* allele frequency between mosquitoes that died or survived each treatments ($\chi^2 < 0.00$; degree of freedom [df] = 2; $P > 0.53$). By contrast, the frequency of the *ace-1^R* allele was significantly higher among the live than the dead mosquitoes from both IRS and CTPS huts (IRS: $\chi^2 = 7.05$; df = 2; $P = 0.03$; CTPS: $\chi^2 = 7.60$; df = 2; $P = 0.02$). This finding suggests a survival advantage for *An. gambiae* bearing the *ace-1^R* mutation in the presence of carbamate-treated substrate. Conversely, there was no evidence for a significantly higher *ace-1^R* allelic frequency among the live mosquitoes from LLIN + IRS ($\chi^2 = 2.62$; df = 2; $P = 0.27$) and LLIN + CTPS huts ($\chi^2 = 3.24$; df = 2; $P = 0.20$), which suggests the benefits of using combined pyrethroid and carbamate treatments to prevent the selection of *ace-1^R* allele. No significantly higher *ace-1^R* allelic frequency was noted among live mosquitoes from LLIN treatment alone ($\chi^2 = 3.63$; df = 2; $P = 0.16$).

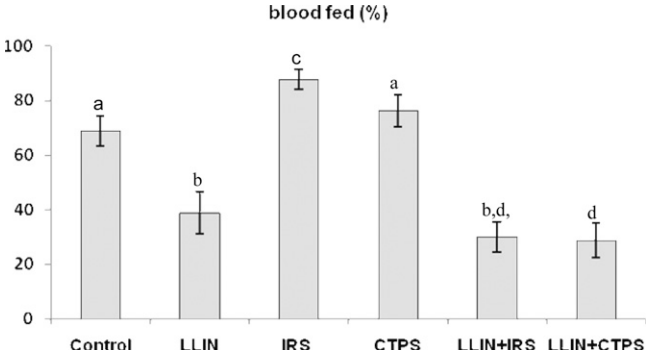

FIGURE 2. Blood-feeding rate (95% confidence interval) of *An. gambiae* exposed to LLIN alone and in combination with IRS and CTPS in experimental huts (Vallée du Kou, Burkina Faso). Treatments carrying the same letter are not significantly different ($P > 0.05$).

DISCUSSION

In this study, we investigated the combined efficacy of LLIN (PermaNet 2.0) plus an IRS or a CTPS (400 mg/m^2 bendiocarb) against pyrethroid-resistant *An. gambiae*. The results showed a clear benefit for combining the two interventions in terms of blood-feeding inhibition and mortality, which confirms previous laboratory findings.[13] The use of carbamates or organophosphates on treated materials or walls against pyrethroid-resistant *An. gambiae* has been the subject of several studies.[24,25] These insecticides have proved to be good alternatives to pyrethroids against pyrethroid-resistant malaria vectors, but they do not confer the same level of personal protection because of lower irritancy.[11] However, the present study suggests that their use as a complementary method to LLIN within a community may achieve a mass-killing effect of malaria vectors; for example, the estimated overall killing effect (see WHO guidelines 2006 for details) was 23% for LLIN alone compared with 62% for LLIN + IRS and 53% for LLIN + CTPS.

This study also showed that coverage of the upper one-third of the walls of huts with a CTPS provided equal or better efficacy than a full coverage with IRS. This is particularly interesting for human safety and user perception, because the risk of chronic occupational exposure to the CTPS would be reduced. Conventionally, IRS with carbamates lasts 2–6 months,[2] although some studies showed lower persistence under field conditions.[26,27] As suggested by Djènontin and others,[13] binding bendiocarb onto the plastic sheeting strongly increased the residual life of the insecticide and prevented the insecticide from wearing off quickly after washing. The development of long-lasting technology for CTPS is, however, required to ensure cost-effectiveness of this IRM strategy for malaria control.

TABLE 1
Comparative frequencies of two resistance genes (*kdr* and *ace1^R*) in *An. gambiae* after exposure to different treatments*

Treatment	Live *kdr* frequency (*n*)	Dead *kdr* frequency (*n*)	*P*	Live *ace1^R* frequency (*n*)	Dead *ace1^R* frequency (*n*)	*P*
LLIN	0.92 (30/87)	0.92 (30/67)	1.00	0.12 (30/87)	0.03 (30/67)	0.16
IRS	0.91 (40/174)	0.88 (20/128)	0.53	0.13 (40/174)	0.00 (20/128)	0.03
CTPS	0.92 (33/94)	0.92 (26/104)	1.00	0.15 (33/94)	0.02 (26/104)	0.02
IRS + LLIN	0.90 (30/86)	0.93 (29/170)	0.74	0.09 (29/86)	0.03 (30/170)	0.27
CTPS + LLIN	0.89 (26/56)	0.87 (34/145)	1.00	0.14 (25/56)	0.06 (34/145)	0.20

**kdr* = knockdown resistance allele; *ace1^R*= insensitive acetylcolinesterase; CTPS = carbamate-treated plastic sheeting; IRS = indoor residual spraying; LLIN = long-lasting insecticidal nets (Permanet 2.0).

This study also showed a strong decrease in pyrethroid-treated nets performance against pyrethroid-resistant malaria vectors, which confirms previous findings in Benin[9] and Cote d'Ivoire.[28] In our study site (Vallée du Kou, Burkina Faso), a rapid increase in *kdr* allelic frequency (from 0.4 to 0.9) was observed in the molecular M form of *An. gambiae* compared with previous resistance monitoring.[14] If the reason for this sudden increase is unknown, it seems to have a significant impact on LLIN, which is shown by another recent experimental hut trial.[29] In our study, no particular type of selection for the *kdr* resistance was observed. However, the susceptible genotypes were too rare to make conclusions about the effect of any treatment (alone or combined) on the *kdr* selection pressure (i.e., the allele reached a fixation level). Further experimental hut trials should be carried out in areas with lower *kdr* allelic frequency to confirm or deny this trend. Regarding carbamate resistance, we confirmed the presence of the *ace-1^R* allele (11%) in the molecular M form of *An. gambiae* in the Vallée du Kou.[14] Genotyping of live and dead mosquitoes collected from the huts showed evidence for a significantly higher frequency of *ace-1^R* allele in those that survived the IRS and CTPS treatments (i.e., *ace-1^R* heterozygotes showed a survival advantage). This is particularly worrisome considering that large-scale IRS campaigns using bendiocarb are being scaled up in several African countries through the President Malaria Initiative (PMI).[30] Previous hut trials carried out in Cote d'Ivoire showed similar types of selection of the *ace-1^R* allele by the use of carbamate-treated mosquito nets but not pyrethroid and carbamate "two-in-one" combination nets.[31] Here, the use of LLIN plus IRS or CTPS seemed to indifferently kill mosquitoes having the susceptible or resistant alleles, suggesting that combining the two interventions may be promising for managing carbamate resistance where the *ace-1^R* is present mainly in its heterozygous form.

Since the advent of pyrethroids in the 1970s, no new major class of active ingredients (AI) has appeared in the pipeline of public-health products. Suppliers estimate that developing a new AI today takes at least 10 years and costs more than $150 million; additionally, the cost of developing a new AI class might potentially reach $300 million given the probable higher rates of attrition.[32] This suggests that one cannot expect new public-health chemical classes soon; the key question is how do we maintain and sustain the efficacy of pyrethroids for malaria vector control?

In the short term, the use of non-pyrethroids for IRS over pyrethroids (e.g., bendiocarb, indoxacarb, or chlorfenapyr) should be a priority.[33,34] For example, on the island of Bioko on the West African coast, an IRS campaign with lambdacyhalothrin failed to curtail an increase in the population of *An. gambiae*, and it required switching to the carbamate bendiocarb before the mosquito population, and malaria, went into decline.[35] The use of synergists (e.g., piperonyl butoxide [PBO]) to improve the efficacy of LLIN[29,36] should be further explored. Integrated approaches for malaria vector control, either by mixing two unrelated insecticides (including synergists) on the same substrates[15,25] or combining spatially different interventions within dwellings (LLIN + IRS-like treatments), also represent key options for the management of insecticide resistance in malaria-endemic areas. This later strategy is currently being investigated in south Benin through a randomized controlled trial to assess the impact of LLIN alone and in combination with CTPS on malaria incidence and transmission as well as the management of insecticide resistance of malaria vectors.

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