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A preliminary test of the protective efficacy of permethrin-treated bed nets in an area of *Anopheles gambiae* metabolic resistance to pyrethroids in north Cameroon

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Summary A trial of permethrin-treated nets (PTNs) versus untreated nets (UTNs) was conducted in Pitoa (north Cameroon), where the main malaria vectors, *Anopheles gambiae* s.l. and *Anopheles arabiensis*, show metabolic-based permethrin resistance. The deterrent effect of permethrin greatly reduced *A. gambiae* biting rate inside rooms where PTNs were installed. After 3 months of net use, malaria reinfection rate was significantly lower in children sleeping under a PTN, but no such effect was observed after 6 months. Parasitaemia was not significantly different between the two arms. These findings suggest good, although transitory, personal protection against malaria conferred by PTNs in an area of metabolic-based permethrin resistance.

1. Introduction

Insecticide-treated mosquito nets (ITNs) are widely advocated for malaria prevention in Africa. Their efficacy in reducing malaria transmission, morbidity and all-cause

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Table 1 Malaria incidence in Pitoa children before and after installation of permethrin-treated bed nets (PTNs) and untreated bed nets (UTNs)

	Children sleeping under PTNs			Total ($n = 65$)	Children sleeping under UTNs			Total ($n = 57$)
	Age group (years)				Age group (years)			
	0–4 ($n = 22$)	5–9 ($n = 20$)	10–15 ($n = 23$)		0–4 ($n = 19$)	5–9 ($n = 20$)	10–15 ($n = 18$)	
Baseline (M0)								
Ni	8	7	3	18	9	8	6	23
Prev. (%)	36.4^{a0}	35.0^{a0}	13.0^{a0}	27.7^{a0}	47.4^{a0}	40.0^{a0}	33.3^{a0}	40.4^{a0}
(95% CI)	(17.2–59.4)	(15.4–59.2)	(2.8–33.6)	(17.3–40.2)	(24.4–71.1)	(19.1–64.0)	(13.3–59.0)	(27.6–54.1)
GMPD	390.8^{b1}	480.2^{b1}	497.4^{b1}	512.2^{b1}	1104.3^{b1}	632.1^{b1}	438.8^{b1}	683.5^{b1}
(95% CI)	(127.7–1195.2)	(267.7–863.6)	(399.0–620.1)	(304.9–862.6)	(385.4–3164.1)	(319.2–1251.6)	(138.1–1139.0)	(391.5–1187.9)
Third month (M3)								
Ni	1	2	4	7	3	5	8	16
Prev. (%)	4.5^{c0}	10.0^{c0}	17.4^{c0}	10.8^{d2}	15.8^{c0}	25.0^{c0}	44.4^{c0}	28.1^{c0}
(95% CI)	(0.0–22.8)	(1.2–31.7)	(5.0–38.8)	(4.4–20.9)	(3.4–39.6)	(8.7–49.1)	(21.5–69.2)	(17.0–41.5)
GMPD	560.0^{e1}	454.5^{e1}	138.5^{e1}	301.1^{e1}	69.6^{e1}	124.3^{e1}	1121.0^{e1}	385.2^{e1}
(95% CI)	(–)	(281.4–734.0)	(40.7–471.2)	(154.5–584.0)	(32.3–149.6)	(81.0–190.8)	(347.5–3816.5)	(165.6–888.9)
Sixth month (M6)								
Ni	7	4	5	16	3	4	7	14
Prev. (%)	31.8^{f0}	20.0^{f0}	21.7^{f0}	24.6^{f0}	15.8^{f0}	20.0^{f0}	38.9^{f0}	24.6^{f0}
(95% CI)	(13.9–54.9)	(5.7–43.7)	(7.5–43.7)	(14.8–36.9)	(3.4–39.6)	(5.7–43.7)	(17.3–64.3)	(14.1–37.8)
GMPD	1241.2^{g1}	699.4^{g1}	380.4^{g1}	811.6^{g1}	1474.7^{g1}	231.2^{g1}	203.8^{g1}	568.8^{g1}
(95% CI)	(481.7–3197.5)	(333.8–1465.2)	(110.9–13004.0)	(441.4–1495.2)	(1318.0–1650.0)	(92.7–576.4)	(72.4–573.9)	(244.7–1326.1)

All infections were due to *Plasmodium falciparum*, and two cases of co-infection with *P. malariae* were recorded. *n*: number of children; ni: number of infected children; Prev.: *P. falciparum* prevalence, compared using χ^2 test and Monte Carlo simulations for small sample sizes ($n = 5000$ permutations); GMPD: geometric mean of parasite density, compared using Kruskal-Wallis test; (95% CI): 95% confidence interval built by bootstrapping (10 000 replications). Values in the same row sharing a letter superscript do not differ significantly at the 5% level. Values in the same column sharing a number superscript do not differ significantly at the 5% level.

infant mortality has been extensively demonstrated (Henry et al., 2005; Lengeler, 2004). However, the rapid spread of pyrethroid resistance in major mosquito vector species might jeopardize the public health benefit expected from ITNs (N'Guessan et al., 2007). Furthermore, the diversity of insecticide resistance mechanisms that have evolved prompt consideration when it comes to insecticide resistance management for a sustained efficacy of ITNs.

In north Cameroon, the major malaria vectors, *Anopheles gambiae* and *A. arabiensis*, have developed metabolic resistance to pyrethroids in the absence of known target-site mutations such as *Kdr* (Etang et al., 2007). We assessed whether permethrin-treated nets (PTNs) maintain their efficacy in such an entomological setting, by conducting a trial throughout the 2002 rainy season in Pitoa (9°24′ N, 13°31′ E), a village where malaria transmission occurs from April to November. Two areas of similar geographical size and human population characteristics (census, ethnicity) were identified, and 50 households were randomly selected in each area. All sleeping mats where children under 15 years old were sleeping were identified and covered with either a PTN or an untreated net (UTN). Signed informed consent was obtained from both parents of each participating child. All children in a household were included in the study after initial parasitaemia was cleared (Maxwell et al., 1999).

The trial started with a baseline survey conducted in April (M0), at the onset of the malaria transmission season. Nets were treated (500 mg/m^2) and distributed, and subsequent follow-up was conducted in July (M3) and October (M6). At each survey, thick blood films were made in the field for parasitological check-up. Mosquitoes were collected by human landing catches during two consecutive nights, in two randomly selected houses per area (PTN and UTN), in the room where the net was installed and outdoor in the same compound. The same compounds were used at every survey.

2. Impact on mosquito behaviour

At M0, only two mosquitoes were captured; both were *A. gambiae* s.l. During the two subsequent surveys, a total of 308 *A. gambiae* s.l. specimens was collected, representing 83.2% of all anophelines caught (including *A. pharoensis*, *A. funestus*, *A. nili* and *A. ziemanni*). The total number of specimens collected in the UTN compounds ($n = 257$) was considerably higher than in the PTN compounds ($n = 51$), but lack of preliminary data on vector density in both areas precluded further interpretation of this result. However, the rate of exophagy (the proportion of mosquitoes biting outdoors) was significantly higher in the PTN arm (38/51, 74.5%) than in the UTN arm (134/257, 52%; $P = 0.003$). This demonstrates a strong deterrent effect of permethrin on metabolic resistant *A. gambiae* and suggests that PTNs maintain their protective effect against the bite of metabolic resistant *A. gambiae*. Furthermore, our study design suggests that, unlike UTNs, this protective effect might benefit unprotected peoples sleeping in the same room.

3. Impact on reinfection and parasitaemia

Overall, 122 children were successfully monitored throughout the survey (Table 1). At M0, the overall prevalence of *Plasmodium* infection was 33.6% (41/122) and was not significantly different between the two groups of children, overall as well as within each age group ($P > 0.05$). At M3, the overall reinfection rate was significantly lower in the PTN group (10.8%) than in the UTN group (28.1%; $P = 0.01$). However, at M6, a significant increase in *Plasmodium* prevalence was observed in the PTN group, and no significant difference was revealed between the PTN and UTN groups ($P = 0.99$), nor between the three age groups ($P > 0.3$) at the end of the trial. This transient reduction in the reinfection rate in the PTN group at M3 was not accompanied by any significant reduction in parasite density, whatever the age group considered.

4. Conclusion

Our results provide evidence for sustained, although transitory, efficacy of PTNs in an area where major malaria vectors have developed metabolic resistance to pyrethroids. The benefit of permethrin treatment of the nets compared with untreated ones essentially relied on the strong deterrent effect of the insecticide, to which metabolic resistant vectors are still susceptible. The lack of efficacy in preventing reinfection of children at M6 may have a number of causes that were not tested within the frame of this preliminary trial, including relaxed adherence to the proper use of nets, increased transmission intensity at the end of the rainy season, seasonal variation in vector resistance level and permethrin residue on the net fabrics. Subsequent field trials should be planned with higher levels of net coverage and the use of long-lasting impregnated nets (LLINs), in which permethrin is industrially incorporated into the fabrics. Such nets were demonstrated to be effective in reducing malaria incidence in net users in areas of Cote d'Ivoire with high *Kdr* mutation frequency (Henry et al., 2005). Furthermore, scaling up net coverage would allow the assessment of any mass protection effect, an issue of paramount importance for a global impact of the use of ITNs as an efficient malaria prevention strategy.

Authors' contributions: JE, OF and LM designed the study protocol; JE, MC, JCT, and PAA carried out field and laboratory work; JE, MC and FS carried out data analyses and interpretation, and drafted the manuscript; ASE and FS coordinated the work and revised the manuscript. All authors read and approved the final manuscript. JE and MC are guarantors of the paper.

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Conflicts of interest: None declared.

Ethical approval: The study protocol was reviewed and approved by the WHO ethical committee during a workshop organized in Dakar (Senegal) in 2001 and received the agreement of all administrative, religious and traditional authorities of Pitoa.

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