Impact of insecticide resistance in *Anopheles arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation

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Insecticide-based interventions have contributed to ∼78% of the reduction in the malaria burden in sub-Saharan Africa since 2000. Insecticide resistance in malaria vectors could presage a catastrophic rebound in disease incidence and mortality. A major impediment to the implementation of insecticide resistance management strategies is that evidence of the impact of resistance on malaria disease burden is limited. A cluster randomized trial was conducted in Sudan with pyrethroid-resistant and carbamate-susceptible malaria vectors. Clusters were randomly allocated to receive either long-lasting insecticidal nets (LLINs) alone or LLINs in combination with indoor residual spraying (IRS) with a pyrethroid (deltamethrin) insecticide in the first year and a carbamate (bendiocarb) insecticide in the two subsequent years. Malaria incidence was monitored for 3 y through active case detection in cohorts of children aged 1 to <10 y. When deltamethrin was used for IRS, incidence rates in the LLIN + IRS arm and the LLIN-only arm were similar, with the IRS providing no additional protection [incidence rate ratio (IRR) = 1.0 (95% confidence interval [CI]: 0.36–3.0; $P = 0.96$)]. When bendiocarb was used for IRS, there was some evidence of additional protection [interaction IRR = 0.55 (95% CI: 0.40–0.76; $P < 0.001$)]. In conclusion, pyrethroid resistance may have had an impact on pyrethroid-based IRS. The study was not designed to assess whether resistance had an impact on LLINs. These data alone should not be used as the basis for any policy change in vector control interventions.

malaria | pyrethroid | resistance | insecticide | Anopheles

To date, the 21st century has seen an unprecedented reduction in the global burden of malaria. While still unacceptably high, disease incidence in sub-Saharan Africa has decreased by 42% from an average of 427 cases per 1,000 persons at risk in 2,000 to 246 cases per 1,000 persons at risk in 2015; infection prevalence with *Plasmodium falciparum* in children aged 2–10 y has halved from 33 to 16% over the same period (1). This has resulted in an estimated 1.2 billion fewer malaria cases and 6.2 million fewer malaria deaths. Recent estimates show that the widespread deployment of insecticide-based interventions has been, overwhelmingly, the driver of the reduction in malaria in Africa (2). Of the 663 million clinical cases estimated to have been averted due to malaria control interventions since 2000, 78% were attributable to insecticide-treated nets or indoor residual spraying (IRS). These two interventions have been massively scaled up since 2000 (1).

Emerging insecticide resistance in the anopheline malaria vectors could presage a catastrophic rebound in disease incidence. At present, there are only four insecticide classes available to malaria control programs; pyrethroids, organochlorines, carbamates, and organophosphates, with pyrethroids being the only class currently recommended by the WHO for use on long-lasting insecticidal nets (LLINs). Pyrethroids and the organochlorine dichlorodiphenyltrichloroethane (DDT) share the same target site, the voltage-gated sodium channel, while carbamates and organophosphates are acetylcholinesterase inhibitors. Resistance to pyrethroids is extensive throughout sub-Saharan Africa, while resistance to the three nonpyrethroid chemical classes used for IRS is simultaneously emerging in many regions (3–5). Resistance arises mainly from a combination of mutations within mosquitoes at the target site of the insecticide and enhanced detoxification/excretion of the insecticide.

While conclusive evidence that resistance is directly impacting epidemiological indicators of malaria is scanty, by the time such data are available, it may well be too late. To address the

Significance

Emerging insecticide resistance in malaria vectors could presage a catastrophic rebound in malaria morbidity and mortality. In areas of moderate levels of resistance to pyrethroids, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) with a carbamate insecticide were significantly more effective than IRS with pyrethroid insecticide. The impact on the effectiveness of LLINs could not be quantified. The incremental cost of using a carbamate insecticide to which vectors are susceptible was US $0.65 per person protected per year, which is considered acceptable by international standards. While the WHO recommends that different interventions, where possible, should use different insecticide classes, these data alone should not be used as the basis for a policy change in vector control interventions.

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problem, the WHO developed the Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM), which was designed to forestall a disastrous increase in malaria burden (6). Unfortunately, most malarious countries have yet to amend their vector control strategies to align with the GPIRM despite widespread resistance to pyrethroids (7). While this is partially due to the absence of compelling data on the impact of insecticide resistance, it also reflects the lack of viable alternatives to LLINs and current insecticides.

A five-country study was established to provide quantitative estimates of the impact of resistance on malaria burden (8). One study was conducted in Galabat, Sudan. In Sudan, almost the entire population lives in areas with low to moderate transmission. Malaria transmission is seasonal and unstable. Countrywide, the majority of the population (80%), including urban populations, live in areas with <1% *P. falciparum* infection risk in children aged 2–10 y, while the remaining 20% of the population, residing mainly in the south of the country, experience meso- to hyperendemic risk of infection (9). Reported malaria cases were reduced from an estimated 7.5 million in 1990 (10) to 1.2 million in 2014. *P. falciparum* accounts for 95% of the malaria burden (9, 10), with *Anopheles arabiensis* being the main malaria vector (11).

In Sudan, artemisinin-based combination therapy has been used for malaria treatment since 2004, with artesunate plus sulfadoxine/pyrimethamine as first-line treatment, artemether/lumefantrine as second-line therapy, and quinine for the treatment of complicated malaria (10). Malaria diagnosis is based on microscopy and rapid diagnostic tests (RDTs) (10). Between 2013 and 2015, 13 million LLINs were distributed, resulting in overall coverage of 92% of households with one LLIN per two persons in 2015 according to Ministry of Health reports. Therefore, the emergence of insecticide resistance (12, 13) is a major concern as it could severely compromise the effectiveness of vector control in Sudan.

Galabat, Gedarif State, was considered suitable for this study since malaria is highly seasonal and more intense there than in many other parts of Sudan, and it had no previous history of IRS. Human settlements also facilitate the formation of well-separated clusters. Twenty-six villages were selected as study clusters and received universal coverage of LLINs. Thirteen of these clusters were randomly selected to receive IRS in addition to LLINs. In 2012, deltamethrin was used for IRS, which was replaced by bendiocarb in 2013 and 2014. The protective effect of bendiocarb, to which there was no resistance, could therefore be compared with the effect of deltamethrin, to which there was insecticide resistance, with the LLIN-only clusters acting as controls. For ethical reasons, it was not feasible to assess the impact of insecticide resistance on the overall effectiveness of LLINs since this would require a neutral arm with no vector control.

This study aimed to quantify:

i) The impact of switching to a nonpyrethroid IRS insecticide on the incidence and prevalence of malaria infection in an area of moderate pyrethroid resistance
ii) The impact of phenotypic and genotypic insecticide resistance on the incidence and prevalence of malaria infection
iii) The impact of the addition of nonpyrethroid IRS insecticide on the evolution of pyrethroid resistance in an area of universal LLIN coverage
iv) The cost of using nonpyrethoid IRS in addition to LLIN coverage to mitigate the negative consequences of pyrethroid resistance on the incidence of malaria infection

Results

Intervention Coverage. Following a census to determine the number of households and population size of the study area, 72,714 LLINs (PermaNet 2.0; Vestergaard) were distributed in the 26 clusters in April 2011 to protect 139,566 individuals based upon a universal coverage approach of one net for two people. Nets were replaced in June 2014 with 72,098 new LLINs for 122,647 people. An annual intervention assessment survey showed that household net ownership was 99.6% in 2012, 82.1% in 2013, and 98.6% in 2014. LLIN usage, defined as the proportion of affirmative responses to the question "Did this child sleep under an LLIN last night?", was generally high and very similar in study arms (Table 1), but varied by season throughout the year (data not tabulated). IRS was conducted in August and again in late December of each year. In 2012, both spray rounds utilized deltamethrin (25 mg of active ingredient per square meter; Chema Industries), while in 2013 and 2014, bendiocarb (Ficam 80% WP; Bayer; 200 mg of active ingredient per square meter) was sprayed. IRS spray coverage was 99%, 82%, and 83% in the years 2012, 2013, and 2014, respectively, as determined by annual cross-sectional surveys. The IRS coverage reported in the LLIN arm is from the householder questionnaires. It is possible that this was from private spraying, but more likely that it represents householder misreporting.

During the 3-y period from June 1, 2012–May 31, 2015, there were 643 episodes of malaria in 7,529 cohort children who were followed up cumulatively for 17,284 person-years. The mean age of cohort children was similar in the two study arms in each year, but rose gradually from 5.2 y in the first study year to 6.25 y in the third year (Table 1).

Incidence of Malaria and Prevalence of Malaria Infection by Study Arm and by IRS Insecticide. Mean overall incidence was 37.2 per 1,000 person-years [95% confidence interval (CI): 24.3–56.9], declining from 49.9 per 1,000 person-years in year 1, to 36.1 per 1,000 person-years in year 2, to 26.8 per 1,000 person-years in year 3.

Mean incidence in the LLIN + IRS study arm was 47.2 per 1,000 person-years when deltamethrin was used (2012), but was almost halved to an average of 24.6 per 1,000 person-years during the 2 y when bendiocarb was used (2013 and 2014) (Table 2). Over the same period in the LLIN-only arm, incidence remained virtually unchanged, from 44.4 per 1,000 person-years in year 1 to 42.1 per 1,000 person-years in years 2–3. Comparing incidence between study arms, the incidence rate ratio (IRR) for LLIN + deltamethrin compared with LLIN alone (2012) was 1.0 (95% CI: 0.36–2.97; $P = 0.96$); for LLIN + bendiocarb versus LLINs alone (2013), it was 0.60 (95% CI: 0.39–0.91; $P = 0.017$), while for 2014 (LLIN + bendiocarb versus LLIN), it was 0.69 (95% CI: 0.31–1.50; $P = 0.35$). For the two bendiocarb years combined, the IRR for IRS + LLIN versus. LLIN alone was 0.65 (95% CI: 0.44–0.96; $P = 0.032$). There was strong evidence that the change in insecticide modified the effect of IRS + LLIN versus LLIN alone: The IRR for comparing the effect of IRS + LLIN in 2013 and 2014 (bendiocarb) versus LLIN alone with the effect of IRS + LLIN in 2012 (deltamethrin) versus LLIN alone was 0.51 (95% CI: 0.35–0.73; $P < 0.001$) and 0.62 (95% CI: 0.42–0.93; $P = 0.020$), respectively (overall interaction $P = 0.001$; Table 2). The interaction IRR comparing the effect of IRS + LLIN in 2013–2014 combined (bendiocarb) versus LLIN alone with the effect of IRS + LLIN in 2012 (deltamethrin) versus LLIN alone was 0.55 (95% CI: 0.40–0.76; $P < 0.001$).

In a sensitivity analysis to test the robustness of the results to the possibility of undue influence exerted by participants who had multiple episodes of malaria, follow-up was restricted to the 550 first episodes observed (i.e., censoring follow-up after the first positive test result). This analysis produced very similar findings to those obtained from the full dataset (results not tabulated).

In the three cross-sectional prevalence surveys that were conducted from September to October of each of the three study years, 2,518, 3,445, and 3,841 children were tested by RDTs, of whom 214 (8.5%), 146 (4.2%), and 162 (4.2%) tested positive

Table 1. LLIN usage, IRS coverage, insecticide resistance, prevalence of infection, and malaria incidence in cohort children by study arm and study year

Variable	LLIN-only arm			LLIN + IRS arm		
	2012	2013	2014	2012	2013	2014
LLIN usage, % (child nights)	79 (73,375)	74 (75,040)	82 (78,918)	79 (73,738)	75 (74,612)	82 (78,888)
IRS coverage, % (*N*)	9 [1–45] (1,320)	1 [0–2] (1,954)	4 [1–27] (2,195)	99 [96–100] (1,314)	82 [75–87] (1,816)	83 [68–91] (2,032)
Mean age, y	5.1 [4.9–5.4]	5.4 [5.2–5.5]	6.2 [6.1–6.3]	5.2 [5.0–5.4]	5.5 [5.4–5.7]	6.3 [6.1–6.4]
Malaria cases	117	155	98	126	82	65
Malaria incidence*	45 [24–87]	52 [26–101]	33 [14–78]	47 [20–110]	27 [15–50]	21 [10–43]
Prevalence of infection, % (*N*)	7 [3–14] (1,272)	5 [2–10] (1,791)	5 [3–9] (1,961)	10 [6–16] (1,246)	4 [2–7] (1,654)	3 [2–5] (1,880)
Deltamethrin mortality (clusters), % (references)	65 [49–81] (6)	90 [85–95] (6)	56 [48–64] (11)	60 [44–76] (5)	84 [71–96] (6)	68 [61–75] (13)

95% CIs are shown in brackets.
*Cases per 1,000 child-years.

in 2012, 2013, and 2014, respectively. Analogous results were recorded to those comparing malaria incidence (Table 3). In the IRS + LLIN study arm, mean prevalence of infection fell from 10.4% when deltamethrin was used for IRS (2012) to 3.4% when bendiocarb was used (years 2013/2014) ($P = 0.002$). Over the same period, mean prevalence in the LLIN-only arm declined more moderately and nonsignificantly from 6.7 to 5.0% ($P = 0.41$). Comparing prevalence between study arms, the odds ratio (OR) for LLIN + deltamethrin versus LLIN alone in 2012 was 2.11 (95% CI: 0.36–2.97; $P = 0.96$); for LLIN + bendiocarb versus LLINs alone (2013), it was 1.39 (95% CI: 0.32–6.14; $P = 0.66$), while for 2014, it was 0.37 (95% CI: 0.18–0.77; $P = 0.007$). For the two bendiocarb years combined, the OR for LLIN + IRS versus LLIN alone was 0.61 (95% CI: 0.29–1.27; $P = 0.19$). There was strong evidence that the change in insecticide modified the effect of IRS with an overall interaction of $P = 0.001$ for interaction tests applied to individual years (left-hand side of Table 3) and $P < 0.001$ for interaction tests applied to 2012 versus 2013/2014 combined (right-hand side of Table 3). The interaction OR for comparing the effect of IRS + LLIN in 2013 and 2014 (bendiocarb years) versus LLIN alone with the effect of IRS + LLIN in 2012 (deltamethrin) with LLIN alone was 0.55 (95% CI: 0.35–0.87; $P = 0.01$) and 0.30 (95% CI: 0.19–0.47; $P < 0.001$), respectively. The interaction OR comparing the effect of IRS + LLIN in 2013–2014 combined (versus LLIN alone) with the effect of IRS + LLIN in 2012 (deltamethrin) (versus LLIN alone) was 0.40 (95% CI: 0.27–0.59; $P < 0.001$).

Association Between Resistance and Incidence and Prevalence of Malaria Infection. During the course of the study, 4,680 female *An. arabiensis* mosquitoes were phenotyped for deltamethrin susceptibility using WHO discriminating dose tests (14), with evidence of resistance to deltamethrin in both study arms (Fig. 1). The mean percentage mortality in the LLIN arm (65.0%, 95% CI: 44.6–85.3) was not significantly different ($t = 0.425$; df, 9; $P = 0.68$) from that of the LLIN + IRS arm (60%, 95% CI: 38.2–82.2) during year 1. The assay for the *Vgsc-1014F* mutation was successfully conducted in 1,847 of 1,872 specimens (Fig. 1). There was a subsequent decrease in allelic frequency (two-way ANOVA, $P < 0.001$), but no evidence of an association between allelic frequency and the study arm (two-way ANOVA, $P > 0.05$; Fig. 1). There was no evidence of nonnormality of the mortality or the allele frequency data.

The association between cluster- and year-specific bioassay survivorship (phenotypic resistance) and cluster- and year-specific malaria incidence in cohort children was assessed using multiple

Table 2. Effect of year, study arm, and IRS insecticide on malaria incidence, Galabat, Sudan, 2012–2014

Effects by individual study years							Effects comparing 2013/2014 (bendiocarb) with 2012 (deltamethrin)				
Year	Study arm	Cases	Mean incidence (range)	Unadjusted rate ratio (95% CI)	Adjusted rate ratio*, (95% CI)†	Bendiocarb effect‡	Period	Study arm	Mean incidence	Adjusted rate ratio*, (95% CI)‡	Bendiocarb effect‡
2012	LLIN	117	45 (24–87)	1	1	1	2012	LLIN	45 (4–87)	1	
	LLIN + Delta	126	47 (20–110)	1.0 (0.36–2.95); $P = 0.96$	1.0 (0.36–2.97); $P = 0.96$			LLIN + Delta	47 (20–110)	1.0 (0.36–2.97); $P = 0.96$	1
2013	LLIN	155	52 (26–101)	1	1	0.51 (0.35–0.73); $P < 0.001$	2013/2014	LLIN	42 (21–85)	1	
	LLIN + Bend	82	27 (15–50)	0.53 (0.21–1.32); $P = 0.17$	0.60 (0.39–0.91); $P = 0.017$						
2014	LLIN	98	33 (14–78)	1	1	0.62 (0.42–0.93); $P = 0.020$					
	LLIN + Bend	65	21 (10–43)	0.62 (0.20–1.95); $P = 0.42$	0.69 (0.31–1.50); $P = 0.347$			LLIN + Bend	25 (13–47)	0.65 (0.44–0.96); $P = 0.032$	0.55 (0.40–0.76); $P < 0.001$

*Adjusted for age at time of visit and, for 2013 and 2014, the rate in 2012.
†Test to determine if the effect of IRS was different between years ($P = 0.001$).
‡Test comparing the effect of bendiocarb IRS and deltamethrin IRS ($P < 0.001$).

Table 3. Effect of year, study arm, and IRS insecticide on malaria prevalence, Galabat, Sudan, 2012–2014

Effects by individual study years						Effects comparing 2013/2014 (bendiocarb) with 2012 (deltamethrin)				
Year	Study arm	Prevalence, % (*N*)	Unadjusted OR	Adjusted OR*	Bendiocarb effect†	Period	Study arm	Mean prevalence, %	Adjusted OR*	Bendiocarb effect‡
2012	LLIN	7 [3–14] (1,272)	1	1		2012 Delta	LLIN	7 [3–14] (1,272)	1	
	LLIN + IRS	10 [6–16] (1,246)	1.61 [0.60–4.35]; $P = 0.33$	2.11 [0.85–5.22]; $P = 0.11$			LLIN + IRS	10 [6–16] (1,246)	2.11 [0.85–5.22]; $P = 0.11$	1
2013	LLIN	5 [2–10] (1,791]	1	1		2013–2014 Bendiocarb	LLIN	5 [2.8–8.6] (3,752)	1	
	LLIN + IRS	4 [2–7] (1,654)	0.86 [0.31–2.42]; $P = 0.77$	1.39 [0.32–6.14]; $P = 0.66$	0.55 [0.35–0.87]; $P = 0.01$					
2014	LLIN	5 [3–9] (1,961)	1	1		LLIN +I RS	3.4 [2.1–5.4] (3,534)	0.61 [0.29–1.27]; $P = 0.19$	0.40 [0.27–0.59]; $P < 0.001$	
	LLIN + IRS	3 [2–5] (1,880)	0.54 [0.26–1.13]; $P = 0.098$	0.37 [0.18–0.77]; $P = 0.007$	0.30 [0.19–0.47]; $P < 0.001$					

95% CIs are shown in brackets. Delta, deltamethrin.
*Adjusted for age, study arm, and study period.
†Overall likelihood ratio test, $P < 0.0001$ (test to determine if effect of IRS was different between years).
‡Overall likelihood ratio test, $P < 0.0001$ (test comparing the effect of bendiocarb IRS and deltamethrin IRS).

variable Poisson regression models, adjusting for study arm and study year. Unadjusted and adjusted IRRs show no association between malaria incidence in cohort children and deltamethrin bioassay survivorship in mosquitoes from corresponding clusters (Fig. 2 and Table 4). To estimate any effect that resistance in a particular year and a particular cluster may have had on the prevalence of infection as determined by annual cross-sectional surveys, logistic regression was carried out, again adjusting for study arm and study year. Unadjusted and adjusted ORs showed no evidence of any association between resistance as measured through bioassay survivorship and infection prevalence (Table 3).

Similar analysis was carried out to investigate whether malaria incidence and infection prevalence were associated with *Vgsc-1014F* frequency, measured in mosquito specimens collected in corresponding clusters and years. There was no evidence of any association between malaria incidence or infection prevalence on the one hand and *Vgsc-1014F* frequency on the other (Fig. 2 and Table 4).

Subgroup analysis was carried out restricted to the combined data from the LLIN-only arm for all 3 y and to the LLIN + IRS arm for the year in which deltamethrin was sprayed. There was again no association between malaria incidence and *Vgsc-1014F* frequency ($P = 0.59$), between infection prevalence and *Vgsc-1014F* frequency ($P = 0.39$), between malaria incidence and bioassay survivorship ($P = 0.85$), and between infection prevalence and bioassay survivorship ($P = 0.98$).

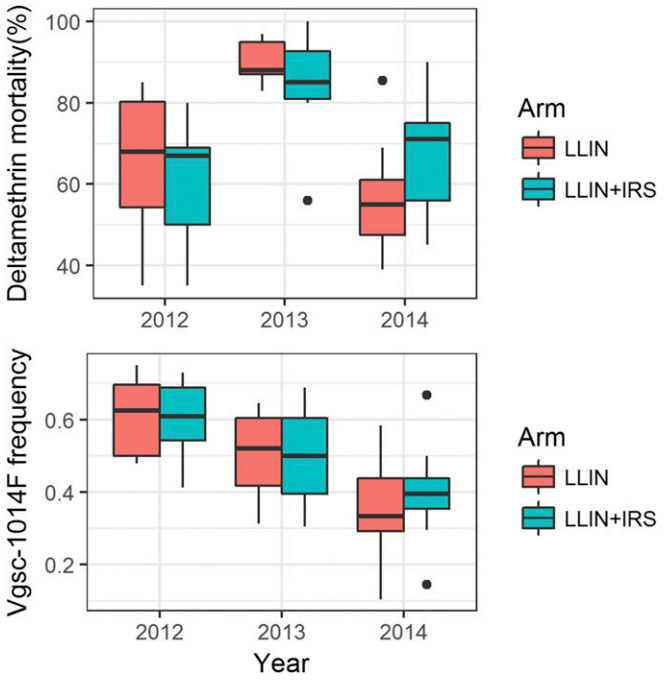

Fig. 1. Change in deltamethrin mortality (*Upper*) and *Vgsc-1014F* (*Lower*) across study years and between single (LLIN) and dual (LLIN + IRS) intervention arms. Box whisker plots show the median (bold line) and interquartile range (boxes). Phenotypic data were available from six LLIN and five LLIN + IRS clusters in 2012, six LLIN and six LLIN + IRS clusters in 2013, and 11 LLIN and 13 LLIN + IRS clusters in 2014. Genotypic data were available for all 26 clusters for all years. In 2014, there was significantly ($P = 0.038$) higher mortality (less resistance) in the LLIN + IRS arm ($n = 13$; mortality = 68%; 95% CI: 60.0–76.0) compared with the LLIN-only arm ($n = 11$; mortality = 56.1%; 95% CI: 47.1–64.9).

Association Between the Addition of Nonpyrethroid IRS Insecticide and the Evolution of Insecticide Resistance to Pyrethroids in an Area of Universal LLIN Coverage. It was only possible to conduct resistance phenotyping in 11 and 12 clusters in 2012 and 2013, respectively; this figure rose to 24 in 2014. In 2012 as well as in 2013 (the first year of bendiocarb spraying), there was no significant difference in mosquito deltamethrin susceptibility between intervention arms. However, in 2014, there was significantly higher mortality (less resistance) in the LLIN + IRS arm ($n = 13$; mortality = 68%; 95% CI: 60–76) compared with the LLIN arm ($n = 11$; mortality = 56%; 95% CI: 47–65) ($P = 0.038$) (Fig. 1). In all clusters across all years, *An. arabiensis* populations were susceptible to bendiocarb.

Cost and Cost-Effectiveness. The cost of protection with LLIN only in Galabat was estimated to be US $2.16 per person-year,

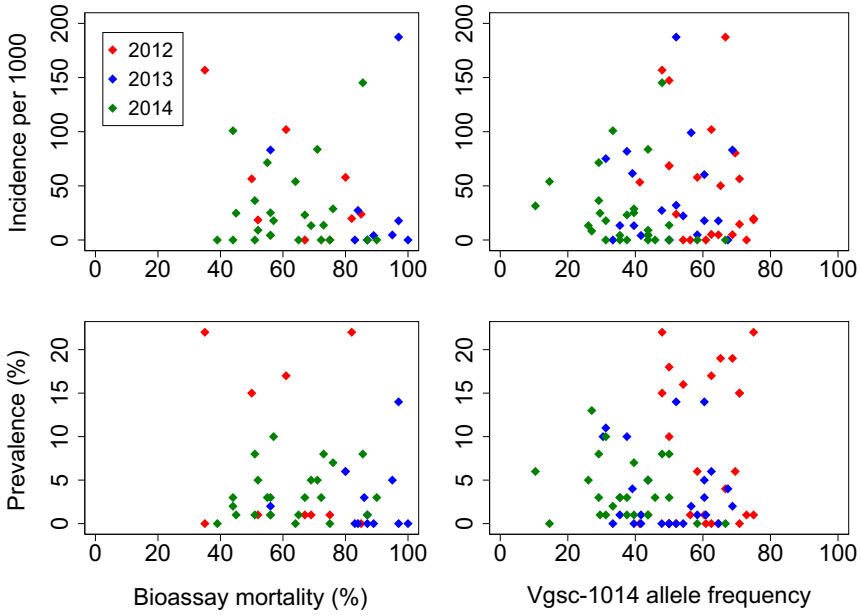

Fig. 2. Cluster-specific malaria case incidence and cluster-specific malaria infection prevalence plotted against cluster-specific phenotypic resistance (bioassay mortality after standard exposure to deltamethrin) and against cluster-specific *Vgsc-1014F* allele frequency for 2012, 2013, and 2014 in Galabat, Sudan.

while the cost of protection per person-year with LLIN + IRS (deltamethrin) was estimated to be US $4.36. The cost per person-year of protection with LLIN + IRS (bendiocarb) was estimated to be US $5.01. These cost estimates translate into an incremental cost of switching from IRS with deltamethrin to IRS with bendiocarb in this study of US $0.65 per person-year. With the year 1 incidence of 49.9 episodes per 1,000 person-years, this translates to an incremental cost per case averted of US $29. Assuming a case fatality rate for malaria of 0.005, the incremental cost per death averted was approximately US $6,400 and an incremental cost per disability adjusted life year (DALY) averted of US $195. These results are well within accepted standards for highly cost-effective interventions for Sudan established by the WHO (15). Sensitivity analysis indicated that changes to discount rate, prices of LLINs, or allocation of shared costs did not affect the incremental costs of the insecticide switch and that reduction of the cost of bendiocarb to the cost of deltamethrin would have resulted in nearly identical costs per person protected, indicating that there were only minor differences in delivery costs for the intervention with bendiocarb. Sudan is an area with low malaria incidence, and it is likely that in other areas with a higher incidence and more severe pyrethroid resistance, such a switch is likely to be associated with even greater cost-effectiveness.

Discussion

Association Between Malaria Incidence/Prevalence and Switching Active Ingredients. Malaria incidence was similar and malaria prevalence was higher in the study arm in which deltamethrin IRS was sprayed in addition to LLIN use, compared with LLINs alone. The higher prevalence in the LLIN + IRS arm was likely to be the result of an imperfect balance in the two study arms. In the following 2 y when IRS with bendiocarb replaced deltamethrin, there was a significant reduction in the LLIN + IRS arm compared with the year in which deltamethrin was used, while incidence and prevalence remained stable in the clusters that only had LLINs. Compared with the LLIN-only arm, incidence was significantly lower in the IRS + LLIN arm in the 2 y in which bendiocarb was used [IRR = 0.65 (95% CI: 0.44–0.96; $P = 0.032$)], and this effect differed significantly from the effect of IRS + LLIN with deltamethrin [interaction IRR = 0.55 (95% CI: 0.40–0.76; $P < 0.001$)]. Prevalence of infection was only lower in the IRS + LLIN arm compared with the LLIN-only arm of the study in 2014; however, again, there was very strong evidence that bendiocarb significantly modified the effect of IRS when comparing the OR between study arms during the years in which bendiocarb was used with the OR between study arms when deltamethrin was used [interaction OR = 0.40 (95% CI: 0.27–0.59; $P < 0.001$)]. Deltamethrin has been shown to be effective when used for IRS in a variety of settings (16–19). While we cannot completely discount differential longevity of the deltamethrin and bendiocarb IRS formulations used in Sudan, the expectation from previous studies is that the deltamethrin formulation would have a marginally longer residual life than the bendiocarb formulation. We conclude that the presence of pyrethroid resistance is a plausible explanation for the loss of effectiveness of deltamethrin IRS, resulting in substantially suboptimal malaria control. To date, there are few studies assessing the impact of insecticide resistance as the design of such studies is problematic because (*i*) the exposure of interest, resistance to an insecticide in the local vector, cannot be randomly allocated and (*ii*) it is unethical to knowingly protect a human population with IRS using an insecticide that is unlikely to be effective if alternatives are available. Evidence of the impact of insecticide resistance on IRS has thus far always been generated by retrospective studies (20, 21). Our data, when considered with two retrospective studies from South Africa and Uganda, where the primary vectors were *Anopheles funestus* and *Anopheles gambiae*, respectively, provide further evidence that pyrethroid resistance is highly likely to be compromising the effectiveness of IRS.

The impact of resistance in KwaZulu-Natal, South Africa, was more marked than that observed in the current study, with a near 10-fold increase in annual malaria cases witnessed from 1995 to 2000 following a switch from DDT to deltamethrin (21). Insecticide susceptibility tests later showed that there was resistance to pyrethroids in *An. funestus*, a vector that had previously been driven to near extinction in KwaZulu-Natal but that resurged following the change to pyrethroid IRS (22). Subsequent reversion back to IRS with DDT in 2000 was followed by a rapid decline in cases that was maintained in subsequent years (23). In

Table 4. Association between malaria prevalence and malaria incidence with genotypic and phenotypic resistance, respectively, from 2012–2014

Resistance status	Unadjusted OR/ rate ratio (95% CI)	Adjusted OR/rate ratio* (95% CI)
Malaria prevalence		
L1014F linear, per 1% mutation	1.009 (0.99–1.03); $P = 0.35$	0.992 (0.97–1.02); $P = 0.51$
Deltamethrin linear, per 1% survivorship	0.999 (0.970–1.029); $P = 0.91$	1.007 (0.98–1.04); $P = 0.67$
Malaria incidence		
L1014F linear, per 1% mutation	1.462 (0.411–5.196); $P = 0.56$	0.415 (0.065–2.665); P = 0.35
Deltamethrin linear, per 1% survivorship	0.995 (0.959–1.032); $P = 0.78$	0.991 (0.947–1.037); $P = 0.70$

*Adjusted for variations in year and study arm. Note that for phenotype data, there is interyear variation in the number of clusters.

a study by Kigozi et al. (20) in Uganda, routinely collected malaria health facility data were compared temporally in relation to the insecticide used in IRS: five rounds of IRS spraying DDT ($n = 1$), pyrethroid ($n = 1$), or bendiocarb ($n = 3$). There was known resistance to both pyrethroids and DDT, but not to bendiocarb, in the area. Small decreases in the odds of malaria infection were observed following both DDT and pyrethroid spraying, with the decrease in malaria infection being much greater following the bendiocarb spray round.

The strength of the study described here was the ability to compare the change in incidence in the IRS clusters with changes in incidence in 13 contemporaneous control group clusters, which had been randomly selected and which were similar in most respects to the intervention group. The reduction in both malaria incidence and prevalence after the introduction of the carbamate insecticide may therefore be attributable to this change in insecticide. Since IRS with deltamethrin and IRS with bendiocarb are effective insecticides in settings with susceptible vectors (17, 18, 24, 25), the lack of impact of the deltamethrin IRS may be due to the presence of deltamethrin resistance in the study area. As would be expected from earlier studies (26, 27), despite resistance, some protection against malaria was provided in both study arms by high coverage with LLINs.

The IRS with bendiocarb in this study provided very similar protection in addition to LLINs to that estimated in a recent cluster randomized trial in Tanzania (28). This study serves as reminder of the potential consequences of using failing insecticides, particularly if no other preventive measures are in place, and the need for insecticide policy to be informed by appropriate susceptibility testing (6).

Association Between Resistance and Malaria Incidence/Infection Prevalence. There was no evidence of an association between malaria rates and insecticide resistance measured phenotypically or genotypically for target-site resistance (as *Vgsc-1014F* frequency). An association between malaria burden and pyrethroid insecticide resistance was expected in clusters with pyrethroid-treated LLINs only or in clusters receiving LLINs and IRS with pyrethroid. The study lacked power to detect such an association due to a number of factors: (*i*) the range in pyrethroid resistance in the study area may have been too small; (*ii*) the effect of insecticide resistance on the effectiveness of LLINs may be relatively modest in settings of moderate pyrethroid resistance; (*iii*) bioassay data were not available in all clusters in each study year; and (*iv*) bioassay survivorship is an imprecise measure of the strength of resistance (29), despite its wide use for detecting the presence of resistance in a mosquito population. Our analysis of malaria incidence and prevalence in relation to insecticide resistance relied on detecting a dose–response association between the two variables; if the latter is inadequately quantified through bioassay survivorship or *Vgsc-1014F* frequency, then such a trend is unlikely to be significant.

Previous studies have shown that insecticide-treated nets still provide protection against malaria infection even in settings of vector resistance to pyrethroids. A meta-analysis using entomological data (26) concluded that insecticide treated nets are a more effective form of vector control than untreated nets despite insecticide resistance. A study in Malawi found that children sleeping under LLINs suffered significantly fewer malaria episodes than their counterparts who did not sleep under nets in an area where the predominant malaria vector species, *An. funestus* and *An. arabiensis*, showed moderate to high levels of pyrethroid resistance (27).

Cost and Cost-Effectiveness. The current study also demonstrated the relative cost-effectiveness of switching active ingredients to mitigate the potential effects of insecticide resistance on malaria incidence. It is, to date, the only study of which we are aware that does so using direct field evidence. Even in a relatively low transmission area, such as Galabat, a switch to the marginally more expensive bendiocarb insecticide is justified.

Insecticide Combination and the Evolution of Resistance. A positive finding was modest evidence of retardation in the speed of evolution of insecticide resistance when two active ingredients with differing modes of actions were used in the LLIN + IRS arm. This is important for malaria control program managers as they struggle to develop plans for the monitoring and management of insecticide resistance in line with WHO GPIRM recommendations (6). Curiously, across our study site, there was a significant decrease in the *Vgsc-1014F* resistance marker frequency. While there are a number of instances of *kdr* markers sweeping rapidly to fixation (30–32), the obverse trend shown here has not been reported elsewhere. There are numerous studies showing that in *An. arabiensis*, *Vgsc-1014F* is a strong predictor of pyrethroid resistance (33), so this may suggest a decline in its importance in conferring a resistant phenotype due to the emergence of additional resistance mechanism(s).

Limitations. For ethical reasons, it was not possible to conduct a trial with a neutral control arm; therefore, the effectiveness of the IRS alone could not be directly assessed. A trial randomizing clusters to the two insecticides would have provided a more direct contemporaneous comparison of deltamethrin to bendiocarb. Instead, the opportunity of comparing each insecticide against the same LLIN-alone control arm arose when insecticide needed to be switched due to the presence of pyrethroid resistance in the area. This design lacks the strength of evidence provided by a direct comparison; however, this shortcoming was compensated for, at least in part, by the contemporaneous comparison with the randomly selected LLIN-only clusters to control for temporal change in malaria transmission. The evidence of a change in the effect of IRS coinciding with the switch to bendiocarb was statistically very strong.

Conclusion

In summary this trial has shown the following: (*i*) loss of effectiveness of pyrethroid IRS is likely to be associated with pyrethroid

resistance in malaria vectors; (*ii*) IRS effectiveness can be restored by switching to an insecticide to which vectors are fully susceptible for an incremental cost that is considered attractive by international standards; and (*iii*) modest retardation to the speed of pyrethroid resistance development may be achieved with appropriate combinations of LLINs and nonpyrethroid IRS, compared with using LLINs alone.

Materials and Methods

Study Site. Galabat is located ∼80 km from Gedarif town and borders Ethiopia. A baseline household census estimated that the area comprised ∼119,000 households in 197 villages with a total of 600,000 inhabitants who are predominantly dependent on rain-fed agriculture. Climatically, the area is within the dry savannah region, with annual rainfall ranging between 700 mm and 1,200 mm and concentrated in a short rainy season from June to September. Average daily temperatures range between 31 °C and 44 °C (34). Malaria transmission is seasonal from September to November, with *P. falciparum* prevalence between 1% and 10% in 2- to 10-y-old children (9). *An. arabiensis* is the main vector of malaria in the area, with *An. funestus* implicated as having a minor role in malaria transmission (34). Before this study, LLINs were the only form of vector control in Galabat.

Treatment for malaria in the area is provided by 101 public sector health facilities, including 7 referral hospitals, 24 health centers, 70 health units, and 20 villages with home-based management of malaria. Diagnosis of suspected cases is based on RDTs at the majority of health and home-based management facilities, while microscopy is used at 20 facilities in the area.

Study Design. The Galabat study is loosely linked to a larger multicountry study on the implications of insecticide resistance that has been described previously (8). The opportunity to compare the two insecticides arose when deltamethrin IRS had to be replaced with bendiocarb IRS. The original objective of the Galabat trial was to investigate whether IRS in combination with LLINs provided additional protection against malaria compared with LLINs alone. With 13 clusters per arm and 200 children per cluster followed up for 3 y, the trial had 80% power to observe a 38% reduction in malaria incidence or more in the LLIN + IRS arm compared with the LLIN-only arm, assuming a mean incidence of 30 per 1,000 person-years and a coefficient of variation of 0.3 between study clusters. Twenty-six villages were selected from a total of 197 to form clusters, each consisting of at least 500 households, and with the distance between the edges of adjoining clusters being at least 3 km (Fig. 3). In 2010, a baseline malaria indicator survey testing for *P. falciparum* infection using malaria RDTs (SD BIOLINE-Malaria Ag P.f/P.v.; Standard Diagnostics, Inc.) was carried out on a sample of 100 children <10 y of age in each of the 26 clusters. At the same time, pyrethrum spray catches were made in houses, from which a sample of 24 *An. arabiensis* mosquitoes from each cluster was screened for the pyrethroid resistance-associated mutations in the voltage-gated sodium channels *Vgsc-1014F* and *Vgsc-1014S* using established molecular diagnostic techniques (33).

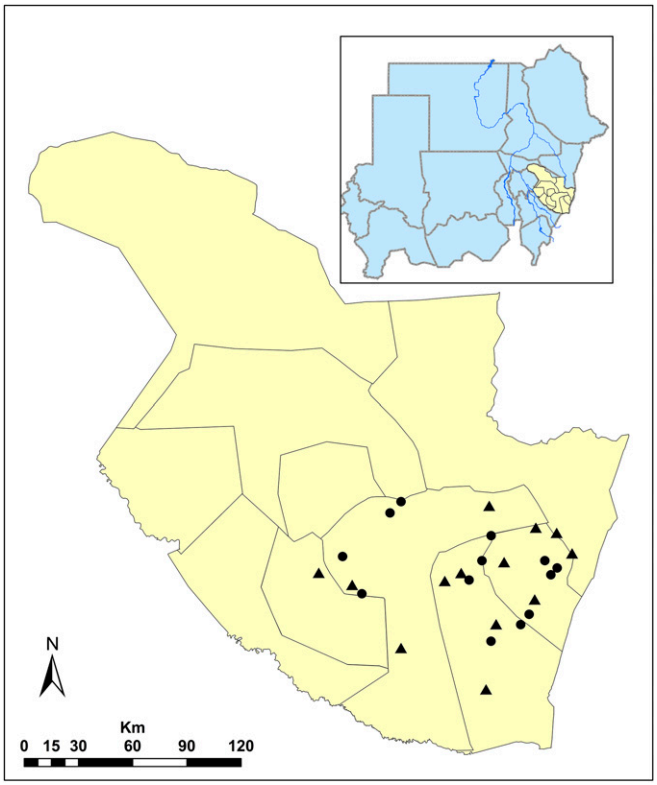

Fig. 3. Map of the study area in Galabat, southeastern Sudan. Triangles denote clusters with LLIN only, and circles denote clusters with LLIN + IRS.

LLINs with a deltamethrin concentration of 55 mg of active ingredient per square meter (PermaNet 2.0) were distributed in April 2011 in all 26 study clusters to reach levels required for universal coverage, defined as one net for every two people. The LLINs were replaced with new nets of the same type in all clusters in June 2014. In 2012, clusters were randomly allocated to two study arms using restricted randomization (35, 36) to ensure that the two study arms were balanced on a number of criteria, including baseline frequency of the *Vgsc-1014F* mutation, baseline prevalence of infection, baseline use of LLINs, and access to health facilities. One study arm ($n = 13$ clusters) retained universal coverage of LLINs only, while the second arm ($n = 13$ clusters) received two rounds of IRS in addition to LLINs. The first round of IRS was in August of each year to cover the main transmission season (September to November), with the second round in late December. In 2012, deltamethrin insecticide (Wettable Powder 25%) was used in both IRS rounds; in 2013 and 2014, bendiocarb (Ficam WP 80%) was sprayed. Bendiocarb, a carbamate, is an acetylcholinesterase antagonist, as opposed to deltamethrin, which targets the mosquito *Vgsc*. While LLINs target mosquitoes that are seeking a blood meal, IRS targets mosquitoes that rest indoors either before or after blood-feeding. Quality assurance of both IRS and LLIN interventions was conducted following WHO guidelines (14) using a susceptible laboratory strain of *An. arabiensis* raised in the insectaries of the Sennar Malaria Research and Training Centre.

Insecticide Resistance. Over the course of the study, two estimates of insecticide resistance were made yearly in each cluster: One, termed phenotypic resistance, was reliant upon collecting live mosquitoes and ascertaining their susceptibility to standard dosages of deltamethrin and bendiocarb; the other, termed genotypic resistance, involved screening for *Vgsc-1014F* (reviewed in ref. 33). *Anopheles* larvae and pupae were collected annually (2012–2014) during the rainy season. All larvae and pupae were reared to adults in a field insectary until used for insecticide susceptibility tests. Pyrethrum spray catches were also performed to collect adult *Anopheles* mosquitoes. Phenotypic assays for deltamethrin and bendiocarb were performed following the standard WHO discriminating dose tests (14, 37). All susceptibility tests were conducted under laboratory conditions at temperatures ranging from 24.8 to 27.1 °C and relative humidity ranging from 75.4 to 79.8%.

An. gambiae complex species, the main vector, were identified to species status, with *An. arabiensis* the only species observed, using a standard PCR assay (38). Twenty-four *An. arabiensis* females per cluster were selected at random for *Vgsc-1014F* genotyping to estimate a cluster-specific resistance marker frequency (39).

Active Case Detection. In each cluster, a community health worker (CHW) was appointed, and ∼200 children aged between 6 mo and <10 y were recruited into cohorts after explaining the study procedures to caregivers and after obtaining written informed consent. Older children were asked to assent to recruitment. CHWs visited cohort members weekly during the peak of the malaria season (September to November) and fortnightly during the remainder of the year, for a total of 30 annual visits. Cohort children who were reported to be febrile at the time of a visit had their temperature taken. Children who had a confirmed fever at the time of the visit, or a reported fever during the period since the last visit, were referred to the local health facility to be tested for malaria parasites, or were tested by the CHW using an RDT when no local facility was available. Local health facilities used either RDTs or microscopy to test for malaria parasites. If caregivers, upon questioning, reported that a child had visited the health facility for a febrile illness during the period since the last visit, the CHW visited the health facility to determine if the child had a blood test that confirmed a diagnosis of malaria. Each visit by a CHW and each clinic attendance resulting in a definitive diagnosis of malaria were recorded in the cohort register, which was collected at regular intervals for entry into an MS Access database at a central location. A study coordinator carried out regular supervisory visits to CHWs to verify the quality of data collection. Upon reaching the age of 10 y,

cohort children were replaced by younger children from the same household or from another household if no younger sibling was available.

Prevalence of Infection. Once each year, during September to October, cohort children were tested for *P. falciparum* infection using RDTs (SD BIOLINE-Malaria Ag P.f/P.v.), irrespective of symptoms. A random sample of 50% of cohort members was selected in 2012, while all cohort children who were present at the time of the survey were screened in 2013 and 2014. Any child who tested positive was referred to the local health facility for treatment.

Statistical Analysis. Malaria case incidence was estimated as the number of cases per child-year of follow-up. IRRs were calculated comparing incidence between study arms for each study year, and for the 2 y combined during which bendiocarb IRS was used. Effect modification (interaction) between study arm and study year was investigated to determine the differential effect of IRS + LLINs with bendiocarb compared with IRS + LLINs with deltamethrin versus LLINs alone. Multiple variable Poisson regression was used to adjust the effect of study arm for age of child at time of visit and, for 2013 and 2014, for incidence rate in 2012. To assess whether malaria incidence was associated with insecticide resistance, IRRs were calculated per 1% change in cluster-specific vector susceptibility (mosquito mortality) and per 1% change in cluster specific *Vgsc-1014F* allele frequency. For this analysis, year and cluster-specific insecticide resistance measurements were linked to year and cluster-specific incidence.

For cross-sectional prevalence of infection data, analogous analyses were carried out using logistic regression to estimate ORs.

Multiple episodes of malaria in the same child were rare; any consecutive positive test results were counted as one episode since the second positive test result could be either a false-positive result from RDTs that measure parasite protein retained from an already cleared infection or the result of a treatment failure. To test the robustness of these results against the possibility of undue influence exerted by participants who had multiple episodes of malaria, the analysis was repeated but restricted to the 550 first-time episodes that were observed in the same cohort over the same period (i.e., excluding children from further follow-up after their first positive test result). To calculate appropriate SEs of estimated means, a robust variance estimator using the first-order Taylor-series linearization method was used to account for variation between clusters (40, 41). Poisson regression and logistic regression were performed using random effects models.

To compare the differences in mean mortalities and *Vgsc-1014F* allele frequencies between the two intervention arms, *t* tests were used, while ANOVA was used to compare the differences between years. The Shapiro–Wilks test was used to assess whether mortality and allele frequency data deviated from the normal distribution.

Cost Data Collection Tools and Indicators. A microcosting (ingredients approach) activities-based framework was applied to the development of cost-collection tools. Key-informant interviews and record reviews were conducted to identify all of the activities and resources needed that were expected to be utilized during the course of the trial. Care was taken to exclude activities that were specifically related to research and not necessary for the provision or performance of the intervention; these included enhanced case finding and enhanced vector surveillance beyond what was necessary for routine use of IRS or LLINs. A standardized instrument for the collection of resource quantities and prices was developed for use at the national (central), state, and locality levels. The instrument was employed by staff of the Federal Ministry of Health Integrated Vector Management Unit to collect information on resource usage at each level of the health system.

Analysis of Cost Data. Resource use was quantified and valued in Sudanese pounds (SDG) in the year during which the resource use occurred. Costs were converted to US dollars using the prevailing average exchange rate for the period. All costs were valued in 2011 US dollars, after adjusting for inflation using the consumer price index for Sudan. Prices derived from the WHO-CHOICE (choosing interventions that are cost effective) database (15) were converted from international dollars using a purchasing power parity (PPP)-to-local currency ratio (1 international dollar to 1.28 SDG) for 2009.

In all cases, economic costs are presented, which are also known as opportunity costs. Economic costs represent the value of a given resource in its next most appropriate use. As such, capital costs, including vehicles, buildings, LLINs, and spray equipment, were annualized and discounted using assumed lifetimes and a social discount rate of 3%.

Cost Outcomes Sensitivity Analysis. Two types of outcome were measured: a process measure, numbers of persons living in clusters with vector control per year (or person-years of protection), and the effectiveness of the interventions in terms of incident cases of malaria prevented.

Because most cost models and assessments are dependent on assumptions about quantities of resources used, prices of resources, and allocation of shared costs, it is necessary to conduct a sensitivity analysis to attempt to determine the robustness of the cost assessment to various assumptions made during development of the model. A one-way sensitivity analysis was conducted to determine the robustness of the cost model to various assumptions made during the assessment. Parameters, which were varied, included discount rate, prices of LLINs and insecticides used, allocation of shared costs, numbers of persons protected by the interventions, and baseline malaria incidence.

Ethics. The study was approved by the Ethics Committees of the London School of Hygiene and Tropical Medicine (approval no. 5825) and Federal Ministry of Health, Sudan (approval no.116-12-09). The study was registered on ClinicalTrials.com (registration no. NCT01713517).

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1. World Health Organization (2015) *World Malaria Report 2015* (WHO, Geneva).
2. Bhatt S, et al. (2015) The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* 526:207–211.
3. Mitchell SN, et al. (2012) Identification and validation of a gene causing cross-resistance between insecticide classes in *Anopheles gambiae* from Ghana. *Proc Natl Acad Sci USA* 109:6147–6152.
4. Mitchell SN, et al. (2014) Metabolic and target-site mechanisms combine to confer strong DDT resistance in Anopheles gambiae. *PLoS One* 9:e92662.
5. Edi CVA, Koudou BG, Jones CM, Weetman D, Ranson H (2012) Multiple-insecticide resistance in Anopheles gambiae mosquitoes, Southern Côte d'Ivoire. *Emerg Infect Dis* 18:1508–1511.
6. World Health Organization-GMP (2012) *Global Plan for Insecticide Resistance Management in Malaria Vectors* (WHO, Geneva).
7. Ranson H, Lissenden N (2016) Insecticide resistance in African Anopheles mosquitoes: A worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol* 32:187–196.
8. Kleinschmidt I, et al. (2015) Design of a study to determine the impact of insecticide resistance on malaria vector control: A multi-country investigation. *Malar J* 14:282.
9. Noor AM, et al. (2012) Malaria risk mapping for control in the republic of Sudan. *Am J Trop Med Hyg* 87:1012–1021.
10. Malik EM, et al. (2006) From chloroquine to artemisinin-based combination therapy: The Sudanese experience. *Malar J* 5:65.
11. Petrarca V, et al. (2000) Cytogenetics of the *Anopheles gambiae* complex in Sudan, with special reference to *An. arabiensis*: Relationships with East and West African populations. *Med Vet Entomol* 14:149–164.
12. Abdalla H, et al. (2008) Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. *Trans R Soc Trop Med Hyg* 102:263–271.
13. Himeidan YE, Chen H, Chandre F, Donnelly MJ, Yan G (2007) Short report: Permethrin and DDT resistance in the malaria vector *Anopheles arabiensis* from eastern Sudan. *Am J Trop Med Hyg* 77:1066–1068.
14. World Health Organization (2012) *Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes* (WHO, Geneva).
15. World Health Organization (2003) *Making Choices in Health: WHO Guide to Cost-Effectiveness Analysis* (WHO, Geneva).
16. Bradley J, et al. (2016) A cluster randomized trial comparing deltamethrin and bendiocarb as insecticides for indoor residual spraying to control malaria on Bioko Island, Equatorial Guinea. *Malar J* 15:378.
17. Ratovonjato J, et al. (2014) Entomological and parasitological impacts of indoor residual spraying with DDT, alphacypermethrin and deltamethrin in the western foothill area of Madagascar. *Malar J* 13:21.
18. Hamainza B, et al. (2016) Incremental impact upon malaria transmission of supplementing pyrethroid-impregnated long-lasting insecticidal nets with indoor residual spraying using pyrethroids or the organophosphate, pirimiphos methyl. *Malar J* 15:100.
19. Kleinschmidt I, et al. (2006) Reduction in infection with Plasmodium falciparum one year after the introduction of malaria control interventions on Bioko Island, Equatorial Guinea. *Am J Trop Med Hyg* 74:972–978.
20. Kigozi R, et al. (2012) Indoor residual spraying of insecticide and malaria morbidity in a high transmission intensity area of Uganda. *PLoS One* 7:e42857.
21. Maharaj R, Mthembu DJ, Sharp BL (2005) Impact of DDT re-introduction on malaria transmission in KwaZulu-Natal. *S Afr Med J* 95:871–874.

22. Hargreaves K, et al. (2000) *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Med Vet Entomol* 14:181–189.
23. Maharaj R, et al. (2013) Epidemiology of malaria in South Africa: From control to elimination. *S Afr Med J* 103:779–783.
24. Kleinschmidt I, et al. (2007) Factors influencing the effectiveness of malaria control in Bioko Island, Equatorial Guinea. *Am J Trop Med Hyg* 76:1027–1032.
25. Sharp BL, et al. (2007) Seven years of regional malaria control collaboration–Mozambique, South Africa, and Swaziland. *Am J Trop Med Hyg* 76:42–47.
26. Strode C, Donegan S, Garner P, Enayati AA, Hemingway J (2014) The impact of pyrethroid resistance on the efficacy of insecticide-treated bed nets against African anopheline mosquitoes: Systematic review and meta-analysis. *PLoS Med* 11:e1001619.
27. Lindblade KA, et al. (2015) A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. *Malar J* 14:31.
28. West PA, et al. (2014) Indoor residual spraying in combination with insecticide-treated nets compared to insecticide-treated nets alone for protection against malaria: A cluster randomised trial in Tanzania. *PLoS Med* 11:e1001630.
29. Bagi J, et al. (2015) When a discriminating dose assay is not enough: Measuring the intensity of insecticide resistance in malaria vectors. *Malar J* 14:210.
30. Clarkson CS, et al. (2014) Adaptive introgression between anopheles sibling species eliminates a major genomic island but not reproductive isolation. *Nat Commun* 5: 4248.
31. Mathias DK, et al. (2011) Spatial and temporal variation in the *kdr* allele *L1014S* in *Anopheles gambiae s.s.* and phenotypic variability in susceptibility to insecticides in Western Kenya. *Malar J* 10:10.
32. Norris LC, et al. (2015) Adaptive introgression in an African malaria mosquito coincident with the increased usage of insecticide-treated bed nets. *Proc Natl Acad Sci USA* 112:815–820.
33. Donnelly MJ, Isaacs AT, Weetman D (2016) Identification, validation, and application of molecular diagnostics for insecticide resistance in malaria vectors. *Trends Parasitol* 32:197–206.
34. Hamad AA, et al. (2002) A marked seasonality of malaria transmission in two rural sites in eastern Sudan. *Acta Trop* 83:71–82.
35. Hayes RJ, Moulton LH (2009) Introduction. *Cluster Randomised Trials, Interdisciplinary Statistics* (Chapman and Hall/CRC, Boca Raton, FL), p 3.
36. Sismanidis C, et al. (2008) Restricted randomization of ZAMSTAR: A 2 x 2 factorial cluster randomized trial. *Clin Trials* 5:316–327.
37. World Health Organization (1998) Test procedures for insecticide resistance monitoring in malaria vectors, bio-efficacy and persistence of insecticides on treated surfaces (WHO, Geneva), Technical Report WHO/CDS/CPC/MAL/98.12.
38. Scott JA, Brogdon WG, Collins FH (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *Am J Trop Med Hyg* 49:520–529.
39. Bass C, et al. (2007) Detection of knockdown resistance (*kdr*) mutations in *Anopheles gambiae*: A comparison of two new high-throughput assays with existing methods. *Malar J* 6:111.
40. Rao JN, Scott AJ (1992) A simple method for the analysis of clustered binary data. *Biometrics* 48:577–585.
41. Stata Corporation (2013) *Stata Survey Data Reference Manual: Release 14* (Stata Press, College Station, TX).