

Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use

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Summary

OBJECTIVE To measure pyrethroid susceptibility in populations of malaria vectors and nuisance-biting mosquitoes in Tanzania and to test the biological efficacy of current insecticide formulations used for net treatment.

METHODS *Anopheles gambiae* Giles s.l., *An. funestus* Giles s.l. and *Culex quinquefasciatus* Say were collected during three national surveys and two insecticide-treated net (ITN) studies in Tanzania. Knockdown effect and mortality were measured in standard WHO susceptibility tests and ball-frame bio-efficacy tests. Test results from 1999 to 2004 were compared to determine trends in resistance development.

RESULTS *Anopheles gambiae* s.l. and *An. funestus* s.l. were highly susceptible to permethrin (range 87–100%) and deltamethrin (consistently 100%) in WHO tests in 1999 and 2004, while *Culex quinquefasciatus* susceptibility to these pyrethroids was much lower (range 7–100% and 0–84% respectively). Efficacy of pyrethroid-treated nets was similarly high against *An. gambiae* s.l. and *An. funestus* s.l. (range 82–100%) while efficacy against *Cx. quinquefasciatus* was considerably lower (range 2–100%). There was no indication of development of resistance in populations of *An. gambiae* s.l. or *An. funestus* s.l. where ITNs have been extensively used; however, susceptibility of nuisance-biting *Cx. quinquefasciatus* mosquitoes declined in some areas between 1999 and 2004.

CONCLUSION The sustained pyrethroid susceptibility of malaria vectors in Tanzania is encouraging for successful malaria control with ITNs. Continued monitoring is essential to ensure early resistance detection, particularly in areas with heavy agricultural or public health use of insecticides where resistance is likely to develop. Widespread low susceptibility of nuisance-biting *Culex* mosquitoes to ITNs raises concern for user acceptance of nets.

keywords *Anopheles gambiae*, *Culex quinquefasciatus*, insecticide-treated nets, pyrethroids, resistance, Tanzania

Introduction

A key component of the global strategy to reduce malaria is the promotion of insecticide-treated nets (ITNs). In several regions of Tanzania, social marketing has proven effective as a means of increasing ITN coverage (Premji *et al.* 1995; Schellenberg *et al.* 2001; Hanson & Worrall 2002), with

resulting impacts on malaria morbidity and mortality similar to those observed in randomized trials in other areas of Africa (Lengeler 2004).

However, the widespread development of resistance to pyrethroid insecticides in malaria vectors, now recorded from West Africa (Elissa *et al.* 1993; Chandre *et al.* 1999; Awolola *et al.* 2002; Etang *et al.* 2003), East Africa

(Vulule *et al.* 1994; Ranson *et al.* 2000; Stump *et al.* 2004) and South Africa (Hargreaves *et al.* 2000), raises concern over the sustainability of ITNs for malaria control.

Both public health and agricultural use of pyrethroids may contribute to the development of resistance in mosquito populations. In Kenya, use of permethrin-treated nets was associated with reduced permethrin susceptibility in *An. gambiae* s.s. (Vulule *et al.* 1994), while in South Africa indoor spraying with deltamethrin resulted in pyrethroid resistance in *An. funestus* (Hargreaves *et al.* 2000). Agricultural use of pyrethroids, primarily in cotton-growing areas, has contributed to selection for resistance in *An. gambiae* s.s. in Cote d'Ivoire, Benin and Burkina Faso (Akogbeto & Yakoubou 1999; Chandre *et al.* 1999; Diabate *et al.* 2002). It is unclear how resistance will affect the level of malaria control achieved by ITNs, as this may vary with the molecular mechanism(s) of resistance present in the vector population (Chandre *et al.* 2000; Corbel *et al.* 2004) and different results have been found in ITN trials in areas with resistance in West Africa (Henry *et al.* 2005; N'Guessan *et al.* 2007); however, the potential threat posed by development of resistance in vector populations has long been recognized (Curtis *et al.* 1998).

While randomized controlled trials across Africa demonstrated high ITN efficacy (Lengeler 2004), it was uncertain whether the high small-scale coverage achieved by free delivery of nets in a trial setting could be replicated on a larger scale and in a more easily sustainable way. The social marketing approach was developed as a step towards a feasible and sustainable large-scale delivery strategy. The success of this approach was then used to plan for national-scale initiatives. Here we define the term 'social marketing' to mean 'using approaches from commercial marketing for a social, rather than financial, gain' (Andreassen 1995), recognizing the differences in social marketing strategies between different organisations and over time. An early ITN effectiveness study in Tanzania, the Bagamoyo Bednet Project (BBNP), was conducted in 13 villages in the Bagamoyo district from 1991 to 1994 (Makemba *et al.* 1995; Premji *et al.* 1995). While this study used a relatively simple community-based distribution system for nets (Makemba *et al.* 1995), the larger Kilombero and Ulanga Treated Net Project (KINET), 1996–1999, developed a network of wholesale and retail sales agents to supply nets to all 109 villages in the Kilombero and Ulanga districts of Tanzania (Schellenberg *et al.* 1999). Social marketing was applied on an even larger scale in the Social Marketing of Insecticide-Treated Nets (SMITN) project, which was initiated in 1998 in Dodoma, Morogoro, Mtwara and Dar es Salaam regions, and expanded in 2000 to cover all regions of Tanzania (Hanson & Worrall 2002), becoming SMARNET from 2002 onwards. Like KINET, the SMITN

project used a combination of public and commercial sector distribution systems. Other ITN projects that have been operating in the Tanga region of Tanzania since 1987 have used a system of free net delivery and regular re-treatment. A timeline of ITN projects in Tanzania is presented in Figure 1. The number of ITNs sold in Tanzania through the SMITN and SMARTNET projects gives an indication of the evolution of the standing crop of ITNs in the country. Assuming an average 5-year lifespan of a net and annual net re-treatment, Figure 2 shows that the standing crop of ITNs in Tanzania (estimated from cumulative net sales) has increased steadily from 1 million in 2002 to more than 9 million in 2006.

Given the potential selection pressure for insecticide resistance from exposure of mosquitoes to ITNs or agricultural insecticides, this study aimed (1) to monitor the insecticide susceptibility status of local mosquito populations in areas of Tanzania where ITN projects have operated or where there is a relatively high use of pyrethroids for agricultural purposes and (2) to test the efficacy of the pyrethroid insecticides currently marketed for net re-treatment. The major vectors of malaria, *Anopheles gambiae* s.l. and *An. funestus* s.l., and the widespread nuisance-biting mosquito and vector of Bancroftian filariasis, *Culex quinquefasciatus*, were included in the study.

Entomological data were compiled from three national surveys to assess the level and distribution of mosquito pyrethroid susceptibility in Tanzania. Two surveys were commissioned by Population Services International (PSI), Tanzania, in 1999 and 2004, which provided data on mosquito insecticide susceptibility and net bio-efficacy from areas of Tanzania with increasing ITN coverage. In 2004–2005 a National Malaria Control Programme (NMCP)-commissioned survey provided additional insecticide susceptibility data from eight regions of Tanzania. To further assess the impact of long-term ITN use, entomological data from two major ITN study areas, in the Kilombero valley of southern Tanzania and the Tanga region of northern Tanzania, were interpreted in the context of trends in ITN coverage and resistance development.

Methods

Figures 3 and 4 show the regions of Tanzania where mosquitoes were collected in 1999. For the PSI-commissioned survey four sites were visited between August and September 1999 in the four regions of Tanzania where the Social Marketing of Insecticide Treated Nets (SMITN) project introduced ITNs in 1998. Both urban and rural areas were included: Mvumi and Igunguli villages,

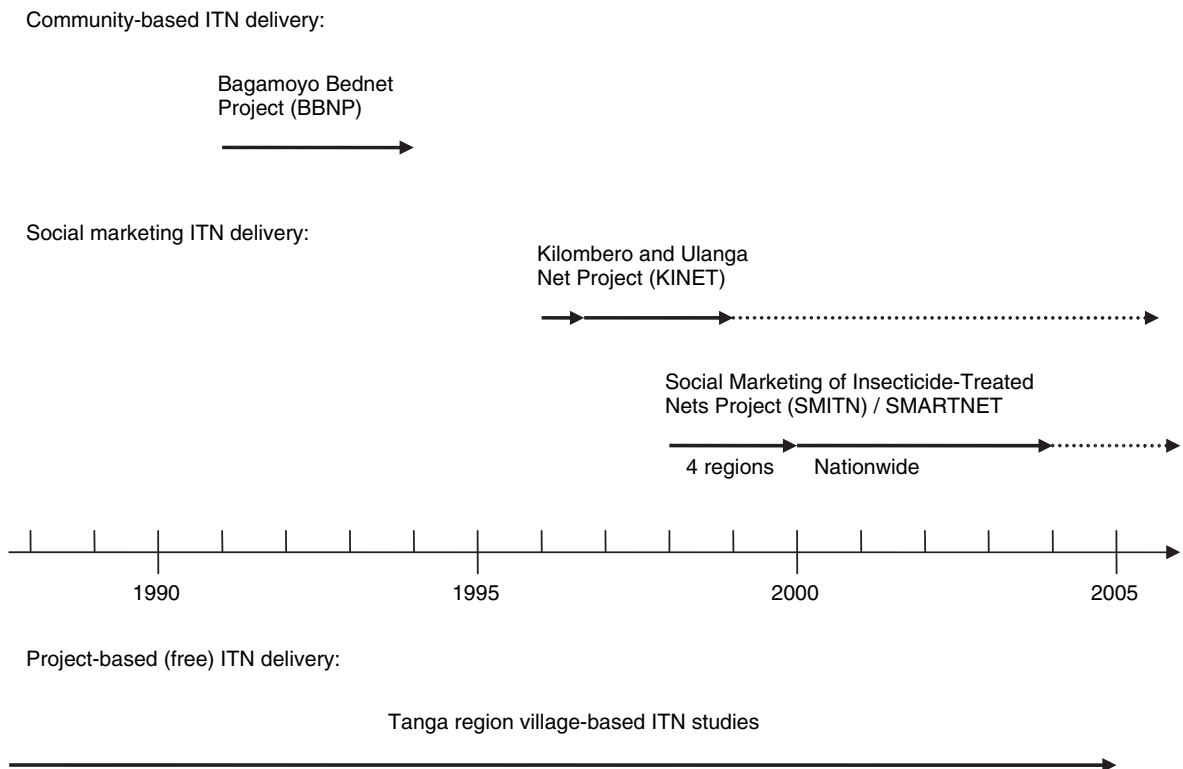
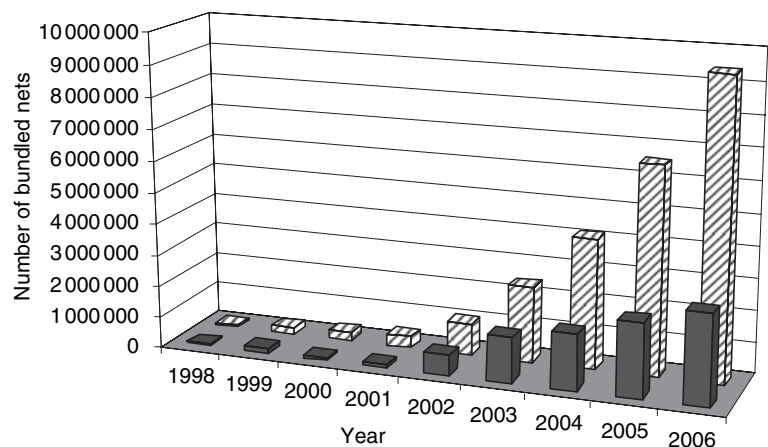


Figure 1 Timeline of insecticide treated net (ITN) distribution in Tanzania through community-based, social marketing and project-based (free) delivery. Solid arrows represent project implementation and duration; dotted arrows represent continued ITN distribution through established social marketing systems.

Figure 2 Sales of nets (bundled with insecticide treatment kits) in Tanzania since 1998 by Population Services International (PSI) in the SMITN and SMARTNET projects. Annual net sales are shown in solid columns; cumulative net sales (adjusting for average 5-year lifespan of a net) are shown in striped columns. The cumulative number of nets is an indication of the standing crop of insecticide-treated nets in Tanzania in a given year.



Dodoma region; Changarawe village and Morogoro town, Morogoro region; Mtoni kwa Azizi Ali area, Dar es Salaam region; Mikindani village and Mtwara region.

Njagi village in the Kilombero district of Tanzania was included in the KINET study area, where between

1997 and 1999 ITN coverage of infants increased from less than 10% to more than 50% through implementation of social marketing strategies (Schellenberg *et al.* 2001). The study area is described in detail by Schellenberg *et al.* (1999).

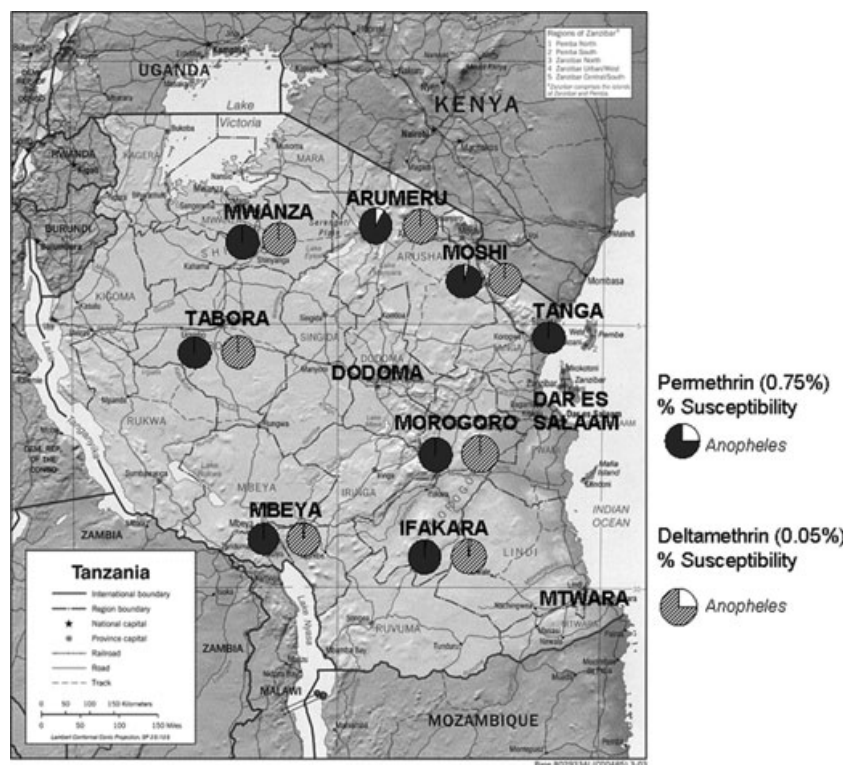


Figure 3 Map of Tanzania showing susceptibility of *Anopheles* spp. to permethrin (0.75%) and deltamethrin (0.05%) in WHO susceptibility tests, 2004–2005. Percentage mortality is represented by the shaded portion of the pie chart.

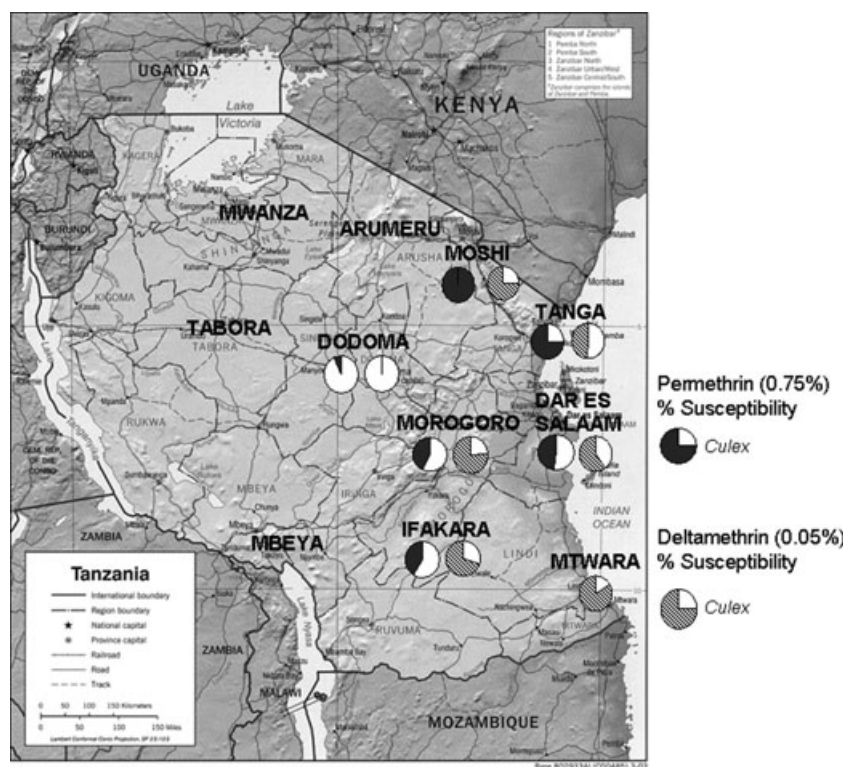


Figure 4 Map of Tanzania showing susceptibility of *Culex quinquefasciatus* to permethrin (0.75%) and deltamethrin (0.05%) in WHO susceptibility tests, 2004–2005. Percentage mortality is represented by the shaded portion of the pie chart.

Mn'gaza village is situated in the Muheza district of Tanzania. For the Tanga Study ITNs were first introduced in 1987 (Curtis *et al.* 1998), since which time regular insecticide re-treatment and replacement of torn nets has maintained 70–90% ITN coverage in this village (Maxwell *et al.* 2006).

2004–2005 Study sites

For the PSI-commissioned survey the four SMITN project study sites as well as two other sites where long-term ITN projects have operated (KINET, Ifakara and BBNP, Bagamoyo) were revisited between November 2004 and January 2005. Urban and rural areas were included in the survey: Mvumi village, Dodoma region; Mlali, Mindu and Lupiro villages, and Morogoro and Ifakara towns, Morogoro region; Keko and Gerezani areas, Dar es Salaam region; Mikindani village, Mtwara region; Mangesani and Kigongoni vitongoji (subvillages) of Bagamoyo, Pwani region.

For the NMCP-commissioned survey, nine sites across Tanzania were included in a resistance monitoring survey commissioned by the National Malaria Control Programme in 2004–2005: Mvomero and Ifakara, Morogoro region; Kyela, Mbeya region; Magu, Mwanza region; Arusha and Babati, Arumeru region; Manolo village, Tabora region; Lower Moshi, Kilimanjaro region and Ilala, Dar es Salaam.

Mosquito collection

Early morning indoor-resting catches were carried out in all locations with the exception of Mn'gaza village. Freshly blood-fed, female *Anopheles* and *Culex* mosquitoes were aspirated from their resting sites on the walls and other surfaces inside houses and transported to a field laboratory or other suitable test location for susceptibility tests and bio-efficacy tests. In Mn'gaza village in 1999 larvae were sampled and reared to the adult stage for testing.

Susceptibility tests

Standardized test papers impregnated with diagnostic concentrations of insecticides were used for susceptibility tests in all surveys and studies. WHO-recommended diagnostic concentrations of pyrethroids were increased in 2000 following a multi-centre trial (WHO 1998), therefore susceptibility tests carried out in 1999 or earlier used the former diagnostic concentrations of permethrin (0.25%) and deltamethrin (0.025%), while the increased concentrations of permethrin (0.75%) and deltamethrin (0.05%) were used in later surveys. DDT (4.0%) was tested in

addition to pyrethroids in the NMCP-commissioned survey.

Susceptibility of mosquitoes to diagnostic concentrations of permethrin, deltamethrin and DDT was assessed using standard WHO (1992) methods. Control and test replicates with batches of 20–25 mosquitoes were carried out simultaneously. The number of mosquitoes knocked down was recorded at 5-min intervals during a 1-h exposure period. Mortality was scored after a 24-h holding period, during which time mosquitoes were provided with a 10% sugar solution. Resistance was defined according to WHO guidelines which state that 98–100% mortality indicates susceptibility, 80–97% indicates the possibility of resistance that needs to be confirmed, and <80% indicates resistance (WHO 1998).

Bio-efficacy tests

Bio-efficacy tests conducted during the KINET study and the 1999 and 2004 PSI commissioned surveys used standard polyester bednets (KINET study: Siamdutch Ltd., Bangkok, Thailand; PSI surveys: A to Z Textiles, Arusha, Tanzania) that were treated with pyrethroid formulations according to the instructions enclosed within the insecticide treatment kit (NgaoTM, PSI, Dar es Salaam, Tanzania). Deltamethrin (KO-tabTM, Bayer Environmental Science, Johannesburg, South Africa; 25 mg ai/m²) was tested in both 1999 and 2004. In addition, the newer alphacypermethrin formulation (FendonaTM BASF, Gravelines, France 6% SC; 40 mg ai/m²) was tested in 2004.

Mosquitoes were exposed to a piece of ITN stretched over a wire ball-frame of approximately 15 cm diameter (WHO 1998). Ten mosquitoes were introduced into the test chamber and their exposure timed for 3 min; the number knocked-down was recorded at 3, 20, 30 and 60 min. Mosquitoes were provided with a 10% sugar solution and mortality was scored after 24 h. The ball-frame was repositioned after each replicate to allow for variation in insecticide coverage; control replicates using an untreated net were run simultaneously.

Statistical analysis

Percent mortality was corrected by Abbott's formula when mortality in control replicates was >5% (Abbott 1925). Tests where control mortality exceeded 20% were excluded from analysis. Time taken for 50% knockdown of mosquitoes (KT₅₀) and 95% confidence intervals were determined by probit analysis using the computer program PoloPlus (Version 1.0, LeOra Software) (Finney 1971). Lethal dose ratios (LD₅₀) were calculated using estimates of the slopes and intercepts of two probit lines to determine

the relative susceptibility of mosquito populations to different insecticide treatments or in different years. Probit lines were tested for equality by chi-squared (χ^2) tests of slopes and intercepts.

Results

Mosquito collection

Seasonal fluctuations in mosquito densities made it difficult to consistently collect large numbers of mosquitoes from all sites. During the 2004–2005 study period, heavy rains in the coastal areas of Bagamoyo and Mtwara and drought conditions in Dodoma hampered efforts to collect large numbers of *Anopheles* spp. Dar es Salaam was visited twice during the 2004–2005 PSI-commissioned survey, but very few (<10) adult *Anopheles* were collected from households despite the visible presence of larvae. Efforts to rear field-collected larvae to the adult stage for testing in Dar es Salaam were not successful.

Survivors and dead mosquitoes from susceptibility tests and bio-efficacy tests in the 2004–2005 PSI-commissioned survey were stored desiccated in microcentrifuge tubes with silica gel and refrigerated when possible. Unfortunately, due to technical difficulties during fieldwork leading to poor preservation of specimens it was not possible to perform molecular analysis to identify *An. gambiae* s.l. sibling species or to test for knockdown resistance (*kdr*) alleles.

Susceptibility tests

Table 1 lists the results of susceptibility tests done in 1999 and 2004 from PSI- and NMCP-commissioned surveys. In the majority of sites *An. gambiae* s.l. was the only malaria vector species tested, while *An. funestus* s.l. was tested in Morogoro and Mtwara. Susceptibility >85% of *An. gambiae* s.l. and *An. funestus* s.l. to pyrethroids was observed in all areas. Complete susceptibility of these *Anopheles* species to deltamethrin was observed; however, slightly reduced levels of susceptibility to permethrin were noted in Dodoma (90%) and Morogoro (87%) in 1999 and in Ifakara (97%), Arumeru (91%) and Moshi (96%) in 2004. Lower susceptibility in 1999 may reflect the lower diagnostic concentration used at this time, since complete susceptibility was observed in tests using the increased dosage in 2004. *Anopheles* spp. susceptibility to DDT (4.0%) was generally high (98–100%), with slightly reduced mortality in Ifakara (93%) and Tabora (95%). *Cx. quinquefasciatus* susceptibility was generally much lower in all sites, ranging from 7% to 100% for permethrin (0.75%) and 49–84% for deltamethrin (0.05%). A map of

2004–2005 sampling sites is shown in Figures 3 and 4, indicating the level of pyrethroid susceptibility of *Anopheles* spp. and *Cx. quinquefasciatus*, respectively, in each area.

Bio-efficacy tests

Mortality after 3-min exposure to alphacypermethrin- and deltamethrin-treated nets in 1999 and 2004 is presented in Table 2. In general pyrethroid-treated nets were highly efficacious against *An. gambiae* s.l. and *An. funestus* s.l., although between-replicate variability in tests in Ifakara in 2004 decreased the calculated mean mortality. In 2004, no significant difference in efficacy was observed between the pyrethroids in Dodoma ($P > 0.05$) while in Mtwara deltamethrin produced more rapid knockdown and higher mortality than alphacypermethrin ($P < 0.05$). Susceptibility of *Cx. quinquefasciatus* was lower than that of *Anopheles* spp. in all sites in 2004; however, higher levels of mortality were achieved with deltamethrin in Bagamoyo and Mtwara compared to alphacypermethrin ($P < 0.05$).

Changes over time

Data from the 1999 and 2004 PSI-commissioned surveys on susceptibility of *Cx. quinquefasciatus* to deltamethrin-treated nets in Dar es Salaam and Mtwara are compared in Figure 5. A slower knockdown rate was observed in Mtwara in 2004 relative to 1999 ($P < 0.05$) although 24-h mortality remained high. A dramatic reduction in insecticidal power occurred in Dar es Salaam over the 5-year time span: KT_{50} increased from 18.2 min (95% CI: 15.4–21.2) in 1999 to an inestimable length of time in 2004 ($P < 0.05$).

An increase of the WHO diagnostic concentrations of pyrethroids in 2000 confounded the trends in mosquito susceptibility between 1999 and 2004. Nonetheless, between 1999 and 2004 there was an apparent increase in susceptibility in several sites as would be expected given the higher test dosage (Table 1). However, in Dodoma, the susceptibility of *Cx. quinquefasciatus* to permethrin and deltamethrin declined despite exposure to higher insecticide concentrations. The same occurred for *Cx. quinquefasciatus* in Dar es Salaam with respect to deltamethrin. These results highlight areas where resistance may be developing.

In the Kilombero district of Tanzania, where the KINET project operated from 1996 to 2000, we observed 82% (95% CI: 66–98) susceptibility to alphacypermethrin-treated nets in bio-efficacy tests and 97% (95% CI: 93–100) susceptibility of *An. gambiae* s.l. to permethrin in susceptibility tests in 2004. Data from bio-efficacy tests in

Table 1 Susceptibility of Tanzanian populations of malaria vectors, *Anopheles gambiae* s.l. and *An. funestus* s.l., and *Culex quinquefasciatus* mosquitoes to diagnostic concentrations of pyrethroids and DDT in 1999 and 2004. Mean percentage mortality in WHO susceptibility tests and number of mosquitoes exposed (*n*)

| Species | Insecticide | Site | 1999† | | 2004‡ | |
|-------------------------------|--------------|---------------|--------------------------|----------|--------------------------|----------|
| | | | % Mortality (95% CI)* | <i>n</i> | % Mortality (95% CI)* | <i>n</i> |
| <i>Anopheles</i> spp. | Deltamethrin | Dodoma | 100 | 40 | – | – |
| | | Mtwara | 100 | 60 | – | – |
| | | Morogoro | 100 | 75 | 100 | 66 |
| | | Ifakara | 100 | 50 | 100 | 100 |
| | | Mwanza | – | – | 100 | 79 |
| | | Tabora | – | – | 100 | 80 |
| | | Mbeya | – | – | 100 | 128 |
| | | Arumeru | – | – | 100 | 150 |
| | | Moshi | – | – | 100 | 102 |
| | Permethrin | Dodoma | 90 | 20 | – | – |
| | | Mtwara | 100 | 60 | – | – |
| | | Morogoro | 87 (88–93) | 75 | 100 | 76 |
| | | Ifakara | 100 | 50 | 97 (93–100) | 50 |
| | | Tanga | 98 | 100 | 100 | 80 |
| | | Mwanza | – | – | 100 | 79 |
| | | Tabora | – | – | 100 | 80 |
| | | Mbeya | – | – | 100 | 149 |
| | | Arumeru | – | – | 91 (90–93) | 150 |
| | | Moshi | – | – | 96 (93–99) | 104 |
| | | Morogoro | – | – | 100 | 105 |
| | | Ifakara | – | – | 99 (97–100) | 120 |
| | | Mwanza | – | – | 98 (97–100) | 79 |
| | | Tabora | – | – | 95 | 20 |
| | | Mbeya | – | – | 99 (96–100) | 122 |
| <i>Culex quinquefasciatus</i> | Deltamethrin | Arumeru | – | – | 100 | 150 |
| | | Moshi | – | – | 100 | 100 |
| | | Dodoma | 33 (28–37) | 50 | 0 | 20 |
| | | Mtwara | 75 (51–100) | 60 | 84 (73–96) | 40 |
| | | Morogoro | 38 (35–50) | 75 | 71 (54–89) | 75 |
| | | Dar es Salaam | 75 (65–85) | 60 | 46 (26–66) | 55 |
| | | Tanga | – | – | 49 (28–69) | 101 |
| | | Bagamoyo | – | – | 59 (55–63) | 37 |
| | | Ifakara | – | – | 70 (63–77) | 160 |
| | | Moshi | – | – | 76 (64–88) | 111 |
| | Permethrin | Dodoma | 20 (13–22) | 50 | 7 | 15 |
| | | Mtwara | 65 (50–85) | 60 | – | – |
| | | Morogoro | 8 | 50 | 43 (33–53) | 144 |
| | | Dar es Salaam | 40 (20–60) | 60 | 48 (44–51) | 40 |
| | | Tanga | – | – | 74 (58–90) | 65 |
| | | Ifakara | – | – | 67 (38–96) | 160 |
| | | Moshi | – | – | 100 | 100 |
| | DDT | Dar es Salaam | – | – | 4 | 75 |
| | | Moshi | – | – | 8 (5–11) | 121 |

*Confidence intervals not applicable where <2 replicates or no between-replicate variation.

†1999 WHO diagnostic concentrations: deltamethrin 0.025%, permethrin 0.25%.

‡2004 WHO diagnostic concentrations: deltamethrin 0.05%, permethrin 0.75%, DDT (4.0%).

1999 and susceptibility tests carried out in 1997–1999 (while ITN coverage was increasing) were compared to the 2004 data to detect changes over time. Bio-efficacy tests

from Njagi village showed 100% susceptibility of *An. gambiae* s.l. to deltamethrin in 1999 while susceptibility to alphacypermethrin was 82% in 2004; however,

Table 2 Bio-efficacy of nets treated with deltamethrin (KO-tab™, 25 mg/m²) and alphacypermethrin (Fendona™, 40 mg/m²) against Tanzanian populations of malaria vectors, *Anopheles gambiae* s.l. and *An. funestus* s.l., and *Culex quinquefasciatus*. Mean percentage mortality and number of mosquitoes exposed (*n*) are presented for sites visited during surveys commissioned by PSI in 1999 and 2004

| Species | Insecticide | Site | 1999 | | 2004 | |
|-------------------------------|-------------------|---------------|--------------------------|----------|--------------------------|----------|
| | | | % Mortality (95% CI)* | <i>n</i> | % Mortality (95% CI)* | <i>n</i> |
| <i>Anopheles</i> spp. | Deltamethrin | Dodoma | – | – | 100 | 20 |
| | | Mtwara | – | – | 100 | 24 |
| | | Ifakara | 100 | 99 | – | – |
| | Alphacypermethrin | Dodoma | – | – | 100 | 23 |
| | | Mtwara | – | – | 94 (85–100) | 24 |
| | | Morogoro | – | – | 100 | 49 |
| <i>Culex quinquefasciatus</i> | Deltamethrin | Ifakara | – | – | 82 (66–98) | 50 |
| | | Dodoma | – | – | 7 | 30 |
| | | Mtwara | 100 | 50 | 91 (82–100) | 68 |
| | | Dar es Salaam | 92 (85–100) | 50 | 8 (0–16) | 30 |
| | | Bagamoyo | – | – | 68 (50–85) | 40 |
| | | Dodoma | – | – | 2 (0–6) | 30 |
| | Alphacypermethrin | Mtwara | – | – | 39 (24–53) | 68 |
| | | Morogoro | – | – | 27 (8–47) | 48 |
| | | Ifakara | – | – | 73 (65–81) | 50 |
| | | Dar es Salaam | – | – | 17 (8–26) | 30 |
| | | Bagamoyo | – | – | 20 (8–32) | 40 |

*Confidence intervals not applicable where <2 replicates or no between-replicate variation.

wide between-replicate variation in mortality was observed in 2004 (range 60–100%) that may reflect uneven distribution of insecticide on the net. Susceptibility tests carried out in Njagi village showed a progressive shift in knockdown time between 1997 and 1999 for permethrin and deltamethrin (Figure 6; Table 3); however, 24-h mortality was consistently 100% for both pyrethroids. In 2004 this knockdown rate returned to that seen in 1997 (data for deltamethrin only), probably due to the increased diagnostic concentrations. In permethrin tests, a slight decrease in 24-h mortality from 100% in 2000 to 97% in 2004 was observed despite the increased diagnostic concentration.

In the Tanga region, in a village where ITN coverage was maintained at 70–90% between 1987 and 2004, 98–100% susceptibility of *Anopheles* spp. to permethrin was observed in 1999 and in 2004.

Discussion

This study demonstrates the continued efficacy of two pyrethroids against malaria vectors in areas of Tanzania where ITNs have been used for up to 17 years. At recommended treatment dosages, both deltamethrin- and alphacypermethrin-treated nets had strong knockdown and lethal effects on the major malaria vectors *An. gambiae* s.l. and *An. funestus* s.l. These results are promising given

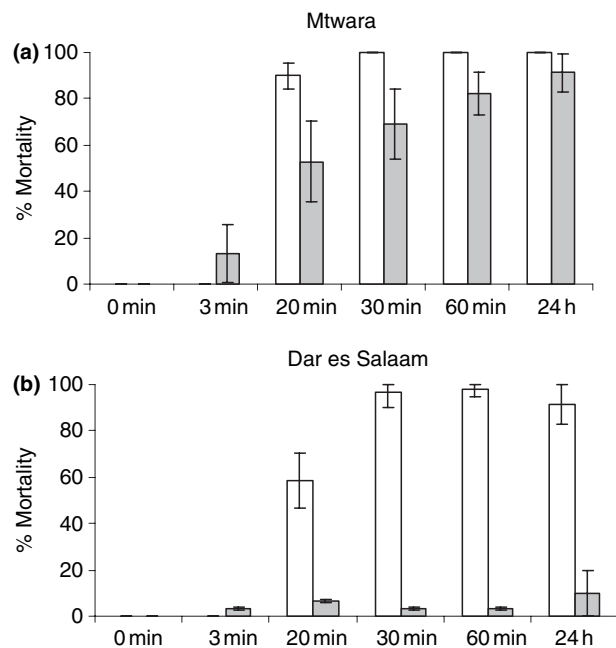


Figure 5 Mortality of *Culex quinquefasciatus* in (a) Mtwara and (b) Dar es Salaam after 3-min exposure to deltamethrin-treated net (25 mg/m²) in ball tests carried out in 1999 (open columns) and 2004 (shaded columns). Error bars represent 95% confidence intervals.

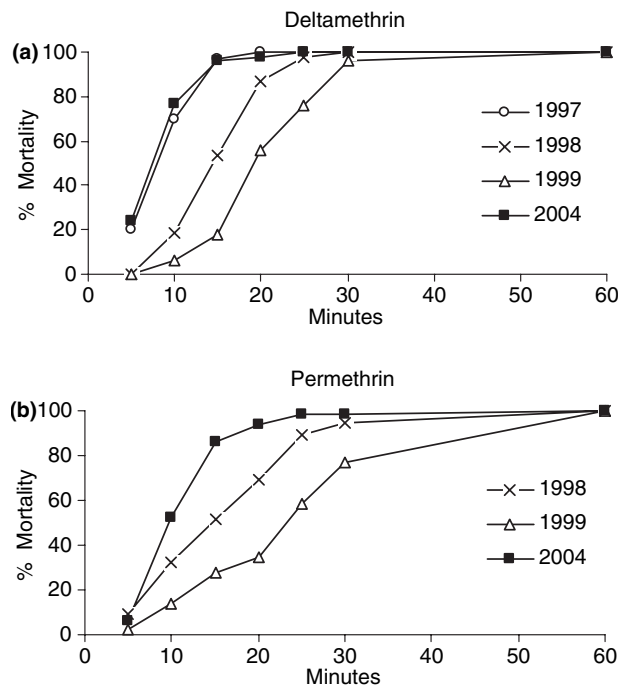


Figure 6 Mortality of *Anopheles* spp. during 1-h exposure to (a) Deltamethrin and (b) Permethrin in WHO susceptibility tests, 1997–1999 and 2004, Njagi village, Kilombero district, Tanzania, showing shift in knockdown time. Diagnostic concentrations: 1997–1999, deltamethrin (0.025%) and permethrin (0.25%); 2004, deltamethrin (0.05%) and permethrin (0.75%).

Table 3 Time for 50% knockdown (KT_{50} in minutes, 95% CI) of *Anopheles gambiae* s.l. during 1-h exposure to WHO diagnostic concentrations of permethrin and deltamethrin in Njagi village, Kilombero district, Tanzania, in 1997–1999 and 2004

| Year | Permethrin (0.25%) | Deltamethrin (0.025%) |
|-------|--------------------|-----------------------|
| 1997 | n/a | 7.4 (6.8–8.0) |
| 1998 | 13.3 (11.7–15.0) | 13.9 (12.8–15.0) |
| 1999 | 25.3 (20.8–32.3) | 19.0 (17.8–20.2) |
| 2004* | 9.7 (8.8–10.6) | 7.0 (6.4–7.6) |

*Diagnostic concentrations in 2004: permethrin (0.75%) and deltamethrin (0.05%).

present malaria control strategies to increase ITN use in Tanzania (Magesa *et al.* 2005), and suggest that, with high ITN coverage, current insecticide treatments on ITNs can provide an effective means of malaria control.

While the high susceptibility of malaria vector mosquitoes to diagnostic concentrations of pyrethroids is encouraging, in several areas less than 100% mortality to permethrin was observed. Continued monitoring is therefore required, particularly in the areas where marginal (i.e.

80–97%) susceptibility is seen. It should be noted that two of the three areas where marginal susceptibility was observed are situated in regions with large-scale agricultural production, e.g. floriculture in Arumeru region and irrigated rice production in Moshi district, so the potential contribution of agricultural insecticide use to resistance development must be considered despite lack of evidence of resistance selection in areas where pyrethroids were used for cotton production in the 1990s (Curtis *et al.* 1999). In the third area with marginal susceptibility, in southern Tanzania, historical data suggest that while full susceptibility was observed during years of ITN scaling-up, there was a concurrent shift in knockdown time. While we do not have genetic measures of resistance frequency, such a shift has been found to correlate with early stages of resistance development in vector populations (Chandre *et al.* 2000), and thus this area should be closely monitored.

Interestingly, in the Tanga region of coastal Tanzania where ITNs have been distributed by research projects achieving high levels of coverage, similar high levels of susceptibility of *Anopheles* mosquitoes to pyrethroids have been observed.

Mortality of malaria vectors after exposure to DDT was greater than 97% indicating high susceptibility to this organochlorine insecticide. While marginally lower (95%) anopheline susceptibility to DDT was observed in Tabora, the number of vectors tested was insufficient to draw conclusions. High levels of resistance to DDT were observed in *Cx. quinquefasciatus* in the two areas where this species was tested.

Despite efforts to reduce variability in the results, several factors may have impacted on the precision of insecticide susceptibility and ITN bio-efficacy measurement. These factors include the variability between replicates associated with pyrethroid irritability and/or variable insecticide distribution on individual nets, the small numbers of mosquitoes tested in some locations, and the use of wild-caught mosquitoes (i.e. non-age-standardized). Pyrethroid resistance declines with increasing mosquito age (Hodjati & Curtis 1999), therefore the use of wild-caught mosquitoes could result in variability in the response to insecticides. However, these assays were representative of the biting mosquito population that nets are meant to protect against. The issue of numbers of mosquitoes tested may be overcome by more intensive and regular sampling of mosquito populations to account for seasonal changes in population density. In addition, testing of F1 progeny of field collected mosquitoes would likely produce more consistent results. However, this would require a field insectary or laboratory for rearing of larvae. In locations where such facilities exist continued testing should be

encouraged and such studies could be enhanced by molecular analysis.

Using standard bioassay methods we have found no evidence that high coverage with ITNs has selected for physiological resistance in malaria vector populations in Tanzania. Caution may be required, however, since the levels of coverage achieved in ITN projects may not have put sufficient pressure on the overall vector population to give evidence of resistance being selected. While the standing crop of nets in Tanzania is increasing the actual level of ITN coverage may be lower due to lack of regular insecticide re-treatment.

A recent innovation in ITNs is the development of long-lasting insecticide-treated nets (LLIN) (Guillet *et al.* 2001a). These wash-resistant nets eliminate the need for regular re-treatment, which has been a major barrier to maintaining sustained net efficacy in many areas (Schellenberg *et al.* 2002). Gradual replacement of untreated and conventionally-treated nets with long-lasting versions through increasing availability of long-lasting insecticide treatments and factory-treated LLIN will ensure wider coverage in the future.

The low levels of susceptibility of *Cx. quinquefasciatus*, and the low efficacy of treated-nets against this widespread, nuisance-biting species must be taken into account. While the reduced insecticide efficacy against culicine mosquitoes does not directly impact on the level of malaria control achieved by ITNs, net users often value the reduction in biting by nuisance insects over perceived health benefits (Zimicki 1996; Chandre *et al.* 1998; Schellenberg *et al.* 1999; Myamba *et al.* 2002); therefore, reduced public acceptance may have an indirect impact on ITN effectiveness. Our data suggest that certain pyrethroids may be more insecticidal against *Cx. quinquefasciatus* than others, as was observed in Mtwara and Bagamoyo; however the decline in efficacy of certain insecticides over time indicates the potential for rapid resistance development in this species. In addition to an innate tolerance to residual insecticide deposits (Brown & Pal 1973), resistance to pyrethroids has been widely recorded in *Cx. quinquefasciatus* (Khayrandish & Wood 1993; Chandre *et al.* 1998), often resulting in low efficacy of ITNs against this species in experimental huts (Curtis *et al.* 1996; Guillet *et al.* 2001b) and community-based trials (Maxwell *et al.* 1999).

Other classes of insecticides have been evaluated for their potential use on ITNs, and high levels of control have been achieved with certain carbamates and organophosphates (Miller *et al.* 1991; Kolaczinski *et al.* 2000). However, safety remains a concern with these insecticide classes, and formulations with low toxicity or methods of delivery that limit human contact may be potential options alone or in

combination with pyrethroid-treated nets. Combining two classes of insecticides on nets may present a method for managing resistance, by exposing mosquitoes to two insecticides with different modes of action (Curtis 1985; Guillet *et al.* 2001a,b; Hougard *et al.* 2003), a phenomenon similar to combination therapy (ACT) currently being advocated for antimalarials (Ashley & White 2005).

Close monitoring of insecticide resistance is essential, and while current insecticide treatments have demonstrated efficacy against malaria vectors in Tanzania there is no room for complacency: efficacy against non-vector 'nuisance' mosquito populations may also be needed to ensure public acceptability and sustained ITN use.

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Efficacité des filets traités aux pyréthroïdes sur les vecteurs de la malaria et les moustiques piqueurs nuisants en Tanzanie dans les endroits où les ITNs sont utilisés à long terme

OBJECTIF Mesurer la sensibilité aux pyréthroïdes de populations de vecteurs de la malaria et de moustiques piqueurs nuisants en Tanzanie et Examiner l'efficacité biologique des formulations actuelles d'insecticides utilisés pour le traitement des filets.

MÉTHODES *Anopheles gambiae* Giles s.l., *An. funestus* Giles s.l. et *Culex quinquefasciatus* Say ont été collectés au cours de trois surveillances nationales et de deux études sur les filets traités aux insecticides (ITNs) en Tanzanie. L'effet d'assomage et la mortalité ont été mesurés dans les essais standards de l'OMS pour la sensibilité et les essais de bio-efficacité de «ball-frame». Les résultats des essais de 1999 et de 2004 ont été comparés pour déterminer des tendances dans le développement de résistance.

RÉSULTATS *An. gambiae* s.l. et *An. funestus* s.l. étaient fortement sensibles au perméthrine (87 à 100%) et au deltaméthrine (tous à 100%) dans les essais de l'OMS en 1999 et 2004, alors que la sensibilité de *Culex quinquefasciatus* à ces pyréthroïdes était beaucoup faible (de 7 à 100% et 0 à 84% respectivement). L'efficacité des ITNs était pareillement forte sur *An. gambiae* s.l. et *An. funestus* s.l. (82 à 100%) tandis que l'efficacité sur *Cx. quinquefasciatus* était considérablement plus faible (2 à 100%). Il n'y avait aucune indication de développement de résistance dans les populations de *An. gambiae* s.l. ou *An. funestus* s.l. sur lesquelles les ITNs ont été intensivement utilisés. Cependant, la sensibilité des moustiques piqueurs nuisants, *Cx. quinquefasciatus*, déclinait dans certains endroits entre 1999 et 2004.

CONCLUSION La sensibilité constante des vecteurs de la malaria aux pyréthroïdes en Tanzanie est encourageant pour le contrôle efficace de la malaria avec l'utilisation des ITNs. La surveillance continue est essentielle pour assurer la détection tôt de la résistance, en particulier dans les endroits avec une utilisation massive des insecticides en agriculture et pour la santé publique où la résistance est susceptible de se développer. La faible sensibilité répandue des moustiques *Culex* piqueurs nuisants souligne le souci pour l'acceptation des ITNs par les utilisateurs.

mots clés *Anopheles gambiae*, *Culex quinquefasciatus*, filets traités aux insecticides, pyréthroïdes, résistance, Tanzanie

Eficacia de las redes mosquiteras impregnadas con piretroides frente a los vectores de malaria y otros mosquitos en Tanzania, en áreas con largo uso de redes mosquiteras

OBJETIVO Medir la susceptibilidad a piretroides en poblaciones de vectores de malaria y otros mosquitos responsables de picaduras molestas en Tanzania y evaluar la eficacia biológica de los métodos actuales de formulación de insecticida utilizados para el tratamiento de redes mosquiteras.

MÉTODOS Se recolectaron *Anopheles gambiae* Giles s.l., *An. funestus* Giles s.l. y *Culex quinquefasciatus* Say durante tres estudios nacionales y dos estudios de redes mosquiteras impregnadas (RMI) en Tanzania. El efecto 'Knockdown' y la mortalidad fueron medidas mediante pruebas de susceptibilidad estándar de la OMS y pruebas de bioeficacia tipo 'ball-frame'. Se compararon los resultados de las pruebas de 1999 con las de 2004, para determinar las tendencias en el desarrollo de resistencias.

RESULTADOS *Anopheles gambiae* s.l. y *An. funestus* s.l. eran altamente susceptibles frente a la permetrina (rango 87–100%) y la deltametrina (consistentemente 100%) en las pruebas de la OMS en 1999 y 2004, mientras que la susceptibilidad de *Culex quinquefasciatus* a estos piretroides era mucho menor (rango 7–100% y 0–84%, respectivamente). La eficacia de las RMI con piretroides era similar frente a *An. gambiae* s.l. y *An. funestus* s.l. (rango 82–100%) mientras que la eficacia frente a *Cx. quinquefasciatus* era considerablemente menor (rango 2–100%). No había indicación de desarrollo de resistencias en poblaciones de *An. gambiae* s.l. o *An. funestus* s.l. en lugares en los que las RMI habían sido usadas extensivamente; sin embargo, la susceptibilidad de mosquitos *Cx. quinquefasciatus* disminuyó en algunas áreas entre 1999 y 2004.

CONCLUSIÓN La susceptibilidad mantenida de los vectores de malaria frente a los piretroides en Tanzania es esperanzadora en lo que respecta a un posible control exitoso de la malaria con RMI. La monitorización continua es esencial para asegurar una detección temprana de resistencias, particularmente en áreas con un uso intensivo de insecticidas, bien sea agrícolas o por motivos de salud pública, en los cuales la resistencia podría desarrollarse. La baja susceptibilidad generalizada a RMI de los mosquitos *Culex* es preocupante, pudiendo influir en la aceptación que los usuarios tengan de las redes.

palabras clave *Anopheles gambiae*, *Culex quinquefasciatus*, redes mosquiteras impregnadas con insecticida, piretroides, resistencia, Tanzania