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Research

Efficacy of Long-lasting Insecticidal Nets With Declining Physical and Chemical Integrity on *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Fitting long-lasting insecticidal nets (LLIN) as screens on doors/windows has a significant impact on indoor-adult *Aedes aegypti* (L.), with entomological reductions measured in a previous study being significant for up to 2 yr post-installation, even in the presence of pyrethroid-resistant *Aedes* populations. To better understand the mode of LLIN protection, bioassays were performed to evaluate the effects of field deployment (0, 6, and 12 mo) and damage type (none, central, lateral, and multiple) on LLIN efficacy. Contact bioassays confirmed that LLIN residual activity (median knockdown time, in minutes, or MKDT) decreased significantly over time: 6.95 (95% confidence interval [CI]: 5.32–8.58) to 9.24 (95% CI: 8.69–9.79) MKDT at 0- and 12-mo age, respectively, using a pyrethroid-susceptible *Aedes* strain. Tunnel tests (exposing human forearm for 40 min as attractant) showed that deployment time affected negatively *Aedes* passage inhibition from 54.9% (95% CI: 43.5–66.2) at 0 mo to 35.7% (95% CI: 16.3–55.1) at 12 mo and blood-feeding inhibition from 65.2% (95% CI: 54.2–76.2) to 48.9% (95% CI: 26.4–71.3), respectively; both the passage/blood-feeding inhibition increased by a factor of 1.8–2.9 on LLINs with multiple and central damages compared with nets with lateral damage. Mosquito mortality was 74.6% (95% CI: 65.3–83.9) at 0 mo, 72.3% (95% CI: 64.1–80.5) at 6 mo, and 59% (95% CI: 46.7–71.3) at 12 mo. Despite the LLIN physical integrity could be compromised over time, we demonstrate that the remaining chemical effect after field conditions would still contribute to killing/repelling mosquitoes.

Key words: *Aedes aegypti*, long-lasting insecticidal net, insecticide-treated screening, house screening

Insecticide-treated materials are a simple, safe, and effective tool with the potential to protect from a variety of vector-borne diseases (Wilson et al. 2014). Long-lasting insecticidal nets (LLIN) have the insecticide (mostly pyrethroids) already incorporated to the fabric to retain insecticidal activity for 1–3 yr (World Health Organization [WHO] 2005). LLIN act as a physical barrier to mosquitoes, preventing access to human hosts and reducing human–vector contacts, and also have a mosquitocidal/deterrence effect causing mosquito mortality or reducing of their longevity (Takken 2002, Vanlerberghe et al. 2011).

Research on the efficacy of LLIN to control diurnally active *Aedes aegypti* (L.) has been encouraged by the WHO (McCall et al. 2009). LLINs, used singly or in combination with other interventions, have been field evaluated in different settings worldwide as an integrated environmental management/housing improvement approach to complement and enhance existing dengue vector control actions (Vazquez-Prokopec et al. 2016). Some degree of success of LLINs against dengue vectors has been reported: 1) when used as a physical barrier on breeding sites to block oviposition (Kroeger et al. 2006, Seng et al. 2008, Tsunoda et al. 2013); 2) to reduce human contact and provide personal protection in the home as bednets (Lenhart et al. 2008); 3) as curtains hanged on windows and doors (; Igarashi 1997; Kroeger et al. 2006; Vanlerberghe et al. 2011, 2013; Nguyen et al. 1996Rizzo et al. 2012; Lenhart et al. 2013); and, more

recently, 4) as insecticide-treated screening (ITS; Che-Mendoza et al. 2015, 2018; Manrique-Saide et al. 2015).

ITS can provide simple, safe, and low-tech *Aedes* control. LLINs affixed as ITS in doors and windows can act as a physical/chemical barrier and confer sustained protection for indoor-female *Ae. aegypti* (Che-Mendoza et al. 2015, 2018; Manrique-Saide et al. 2015). Phase II randomized controlled trials in two endemic localities for *Ae. aegypti* and *Aedes* transmitted diseases of south Mexico showed that ITS conferred both rapid and sustained (~2 yr) impact on indoor-female *Ae. aegypti* infestations, even in the presence of locally high pyrethroid resistance and a decrease of insecticide activity and retention (Che-Mendoza et al. 2018). ITS was very well accepted by the community, with a perceived efficacy on reductions on mosquito abundance and biting, and, furthermore, reduction in other domestic insect pests (Jones et al. 2014). However, one of the problems identified with the screens once installed was fragility (Jones et al. 2014).

The excito-repellent properties of some pyrethroids (especially irritancy/deterrence) elicit a behavioral avoidance of treated nets, in addition to the toxicological (knockdown and mortality) effects (Bayili et al. 2017, Sahu et al. 2017, Massue et al. 2019). For *Aedes*, it has been hypothesized that these properties contribute to the effectiveness of LLIN (deployed as window curtains) in operational field conditions, preventing the entrance of mosquitoes into the house (Loroño-Pino et al. 2013). In any case, because the durability of LLIN could be compromised and affect their effectiveness in field conditions, WHO recommends monitoring of physical integrity and evaluation of the bioefficacy in laboratory tests (WHO 2013). This is important because many nets already used in the field with different levels of damage could still remain effective against adult mosquitoes (Massue et al. 2019).

In a study our team carried out in Merida, Mexico (Che-Mendoza et al. 2018), we found that a house protected with ITS on doors and windows had at least a 50% lower probability of having *Ae. aegypti* females in comparison with a nonscreened house throughout a 2-yr study period. However, when we determined the residual effect of nets, after their deployment and operational conditions with WHO cone bioassays, aged LLIN did not show very satisfactory results: bioefficacy indeed was high with new nets using a susceptible strain, but the knockdown effect (54–65%) and mortality (71–80%) decreased with the time of post-installation of the LLIN in the field (Che-Mendoza et al. 2018). Even more, there was evidence of pyrethroid resistance in the local *Aedes* population (Che-Mendoza et al. 2018). Considering that the field efficacy of an LLIN may be underestimated if based only on standard WHO cone bioassays (Itoh 2005), it remains to be identified whether the protective effect we estimated was due to an insecticidal effect, a mechanical effect (nets acting as a physical barrier) or a combination of both. Following on our previous study (Che-Mendoza et al. 2018), we performed laboratory assays using field-deployed LLINs to quantify how their bioefficacy varies as a function of the time after installation in houses of the Mexican city of Merida.

Materials and Methods

Study Design

Samples of LLIN exposed to real field conditions were obtained from houses screened (LLIN mounted on aluminum frames and fixed to windows and external doors) as part of a 2-yr study performed in Merida, Mexico in 2012 (Che-Mendoza et al. 2018). The LLIN tested was DuraNet (0.55% w.w. alpha-cypermethrin-treated nonflammable polyethylene netting [145 denier; mesh = 132 holes/sq. inch]; Clarke Mosquito Control, IL; WHOPES approved), used commonly for bednets. Twenty houses with LLIN screening from the neighborhood Juan Pablo II in Merida (Yucatan State, Mexico) were randomly selected at 6 and 12 mo after the installation. Entire nets from these houses were removed from the frames and separately packaged in aluminum foil and transported to the laboratory, where they were cut immediately (undamaged sections of 25 cm × 25 cm), washed once gently with tap water for removing the dirt, and dried at room temperature (WHO 2013). Then, these pieces were wrapped in aluminum foil and stored at 3°C in the dark before used in all tests (no more than 7 d after washing).

To record the most frequent physical damages in field conditions, between May 2016 and November 2016 (6 mo post-installation of LLIN), we visited 120 premises intervened, randomly selected from five field trial clusters (24 houses per cluster) considered as part of the study from Che-Mendoza et al. (2018). Most LLINs (64%) were damaged: central damage 25.83% (unique hole, located in the central area of the net), lateral damage 23.95% (unique hole, located in one of the corners of net), small multiple damages 6.25% (two or more holes less than 2 cm, distributed throughout the net), big multiple damages 5.62% (two or more holes less than 2 cm, distributed throughout the net), and complete damage 2.29% (aluminum frame without net).

For bioassays, we simulated the most common physical damages recorded in the field (Fig. 1). Briefly, for each combination of screen age and damage type, groups of LLIN samples (n = 5 per group, randomly assigned from the 20 screens of each age collected from the field) were deliberately damaged with 1) a central damage, consisting of one hole with oval shape in the center of the net (6 cm^2); 2) lateral damage, consisting in a right triangle shape in one corner of the net (18 cm^2); and 3) multiple damages, with nine holes (1 cm in diameter), one hole located in the center of the square, and eight equidistant holes located at 5 cm from the border.

The deployment times evaluated in bioassays were as follows: new, nonexposed LLIN samples and samples of LLIN deployed 6 and 12 mo under field conditions. The effect of damage type was tested at the samples of LLIN collected in field at different deployment times. In all cases, untreated (without insecticide) nets were used as negative controls. For each treated net combination tested, we ran a control.

Bioassays

Two bioassay methods were performed to assess the LLIN bioefficacy under different physical conditions of field-exposure time and damage.

First, contact bioassays were carried out with the aim to evaluate the impact of field-exposure time on LLIN residual insecticidal activity (measured as median knockdown time or MKDT). One negative control net (without insecticide) was conducted for every set of 10 contact bioassays. Contact bioassays for continuous exposure were performed using the device described by Skovmand et al. (2008) with modifications by Santamaria et al. (2016). The device consisted of a transparent acrylic base (two sheets between which the netting material is placed) and a circular exposure chamber (diameter: 90 cm, height: 2 mm). A movable lid, made up of a circular sheet which can be moved horizontally with a central hole, connected to a short tube for transferring mosquitoes. The mosquitoes do not have space to fly on another surface, so they stayed in permanent contact with LLIN surface. The MKDT was determined exposing batches of 11 female *Ae. aegypti* mosquitoes to the LLIN and then recording the observed knockdown time of the median (corresponding to the sixth) mosquito (WHO 1998, Graham et al.

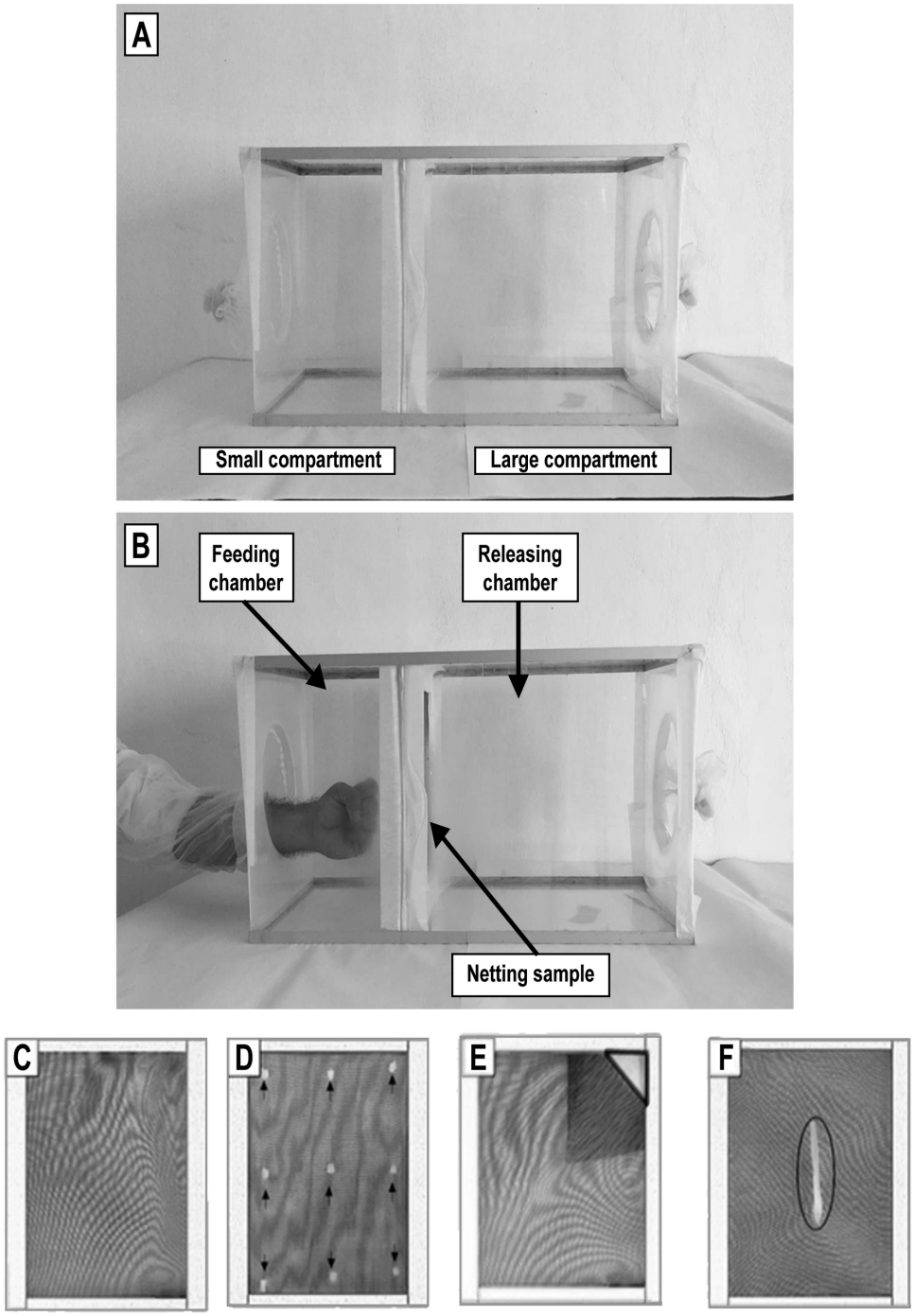

Fig. 1. Design and operation of the tunnel device (A and B). Types of damages on LLIN: (C) nondamage, (D) multiple damages, (E) lateral damage, and (F) central damage.

2005, Kayedi et al. 2007a). Ten replicates (each one using one batch of 11 mosquitoes) per deployed time were performed to calculate the mean value of MKDT. Knockdown in the control was zero in all tests, confirming no knockdown effect due to manipulation.

We also assessed the impact of both physical net damage (simulating the types of damage recorded in the field) and field-deployment time on mosquito passage/blood-feeding inhibition, knockdown, and mortality using tunnel bioassay method (WHO 2013). Two tunnel devices were used simultaneously for each replicate, one tunnel with LLIN and one with negative control net (untreated). The tunnel consists of a cubic acrylic tunnel device (square section 25 cm × 25 cm, 60 cm length) with two access sleeves fitted at the ends covered with polyester netting (WHO 2013). The tunnel has two compartments: a large section 40 cm long (releasing chamber) and a small section 20 cm long (feeding chamber), divided by a disposable cardboard frame where the netting sample (400 cm^2) is accessible to the mosquitoes (Fig. 1).

As *Ae. aegypti* is an anthropophilic species, we used a human forearm from two volunteers directly involved in the study (JHB&ETP), ensuring that the volunteers (separated by up to 5 m from each other) were only present in the test room. Briefly, once the volunteer introduced the forearm into the feeding chamber, 40

female mosquitoes were released into the releasing chamber and let fly freely for 40 min. The criterion of 40 min of exposure was based on a set of tunnel tests (five replicates per each of the three types of damages evaluated on nets) carried out with the susceptible strain, and it represents the time it took a released mosquito to pass through the net and attempt a blood meal (n = 40 mosquitoes per tests). After the 40-min observation period, all mosquitoes were removed from the chambers, and female mosquitoes grouped into status: fed/not fed, knocked down/dead/alive, and transferred to recovery cups with sugar solution for mortality observations at 24 h. The procedure was repeated five times per combination of deployment time and damage type (n = 60 bioassays). The following parameters were calculated: passage inhibition (proportion of mosquitoes passing to feeding compared with the control tests), blood-feeding inhibition (the reduction of blood-fed females—alive or dead—relative to the control tests), knockdown effect at 40 min, and mortality at 24 h (measured by pooling the knockdown effect and mortalities of mosquitoes from the two sections of the tunnel). Tests were discarded if control mortality exceeded 10% or control blood-feeding success was less than 50% (WHO 2011).

All bioassays were performed in a temperature-controlled room with artificial lights (28–30°C, RH 70–80%) belonging to the Laboratory of the Collaborative Unit for Entomological Bioassays of the Universidad Autonoma de Yucatan (UCBE-UADY) in Merida, Mexico.

Mosquito Strains

Two strains of *Ae. aegypti* mosquitoes were used: the susceptible strain New Orleans (NO) and the field-derived resistant strain Juan Pablo II (JP). The NO strain was obtained from a colony established in UCBE since 2012, originally provided by the Centers for Disease Control and Prevention (CDC), Atlanta, GA. The JP local strain was obtained from ovitraps placed at the study site in the Juan Pablo II neighborhood (Yucatan State, Mexico) in 2012 and kept under insectary conditions since then. This strain has been previously characterized as knockdown resistance (allele frequency of I1016 of 82% and C1534 of 93%) to pyrethroids (Che-Mendoza et al. 2018). Groups of 2- to 4-d-old, nonblood-fed *Ae. aegypti* females (both NO and JP local strains) were used for all tests.

Data Management and Analysis

To estimate the effect of LLIN deployment time on net residual activity, linear regression models were constructed with MKDT as the dependent variable and field-deployment time of LLIN as the independent variable. To estimate the effect of net exposure factors (deployment time and damage type) on mosquito passage/blood-feeding inhibition, knockdown, and mortality, Poisson regression models were constructed with these parameters as dependent (passage/blood-feeding inhibition, knockdown, and mortality) and one of the exposure factors as independent variables (deployment time and damage type). Rate ratios estimated with Poisson regression model were also calculated. Analyses were performed using STATA 12.0 (Stata Corp, College Station, TX). In the case of mortality rates, the average observed mortalities were corrected according to Abbott (1925) when mortality was observed in the control group.

Results

Residual Activity

Contact bioassays showed that MTKD for both strains increased dramatically with the deployment time of LLIN from 6.95 min (95% confidence interval [CI]: 5.32–8.58) in new, nonexposed LLIN to 9.24 min (95% CI: 8.69–9.79) at 12-mo age in the susceptible strain and from 8.56 min (95% CI: 7.71–9.40) in new, nonexposed LLIN to 15.43 min (95% CI: 14.25–16.60) at 12-mo age in the resistant strain, suggesting that residual activity of the insecticide was significantly reduced over time and exposure under field conditions (Table 1). In all deployment times of LLIN, the field resistant strain showed 1.2–1.7 times higher MTKD compared with the susceptible strain (Table 1).

Passage and Blood-Feeding Inhibition

Both LLIN and untreated nets without any damage were highly effective (99–100%) in preventing mosquito passing through and then impeded blood feeding in both *Aedes* strains, irrespective of the time after installation. Blood-feeding success in control tests (untreated nets with different damages/times post-installation) recorded an average of 68.5% (13.8 ± SD, minimum value 50.7%, maximum value 88.3%) in all treatments and strains, suggesting that the tests were valid (WHO 2011).

The type of damage was the main exposure factor affecting both the passage (pseudo-R^2 = 0.23, Coef. = 0.51, $P < 0.001$ in the susceptible strain; pseudo-R^2 = 0.11, Coef. = 0.33, $P < 0.001$ in the field-derived strain) and blood feeding (pseudo-R^2 = 0.06, Coef. = –0.23, $P < 0.001$; pseudo-R^2 = 0.12, Coef. = 0.33, $P < 0.001$, respectively). Nets with 'lateral damage' showed the lowest passage inhibition (32.4%, 95% CI: 17.1–47.7 in the resistant strain and 20.9%, 95% CI: 9.9–31.8 in the susceptible strain); in other words, blocked fewer mosquitos (Table 2). When compared with 'lateral damage', passage inhibition of field-derived *Aedes* significantly increased 1.5 times in 'multiple damage' (48.7%, 95% CI: 34.5–62.9) and 1.9 times in 'central damage' (63.9%, 95% CI: 45.2–82.5). In the susceptible strain, the passage inhibition increased 2.1 and 2.9 times in 'multiple' (43.5%, 95% CI: 27.7–59.2) and 'central damaged' nets (62.4%, 95% CI: 49.6–75.2), respectively, compared with nets with a 'lateral damage'.

A similar trend was also observed for blood-feeding inhibition (Table 2). Blood-feeding inhibition values were closely related to the passage inhibition values in the resistant strain, but in the susceptible strain, blood-feeding inhibition was higher in comparison to passage inhibition, suggesting that feeding behavior is more affected in the susceptible strain.

LLIN deployment time showed a lower association to both the passage (pseudo-R^2 = 0.06, Coef. = –0.23, $P < 0.001$ in the susceptible strain; pseudo-R^2 = 0.02, Coef. = 0.05, $P = 0.049$ in the derived-field strain) and blood feeding (pseudo-R^2 = 0.06, Coef. = –0.23, $P < 0.001$; pseudo-R^2 = 0.01, Coef. = 0.06, $P = 0.013$, respectively). These results show that, when compared with untreated nets, the

Table 1. Mean values for the median knockdown times (MKDT) in minutes observed when two *A*edes *aegypti* strains were continuously exposed to different LLIN deployment times, Merida, Mexico (n = 10 replicates for each deployment time)

Deployment time	MKDT (95% CI)	P value
NO susceptible strain		
New, nonexposed	6.95 (5.32–8.58)	Reference
6 mo	7.51 (7.16–7.85)	0.422
12 mo	9.24 (8.69–9.79)	0.001*
JP resistant strain		
New, nonexposed	8.56 (7.71–9.40)	0.022*
6 mo	9.36 (8.74–9.98)	0.001*
12 mo	15.43 (14.25–16.60)	0.001*

Table 2. LLIN exposure factor analysis using Poisson regression models constructed with passage/blood-feeding inhibition as dependent variables, and deployment time/damage type as independent variables, Merida, Mexico (n = 5 replicates for each combination deployment time/damage type)

Strain/net exposure factor	Passage Inhibition		Blood-feeding inhibition	
	Mean (95% CI)	RR (95% CI)	Mean (95% CI)	RR (95% CI)
Resistant strain				
Deployment time				
New, nonexposed	49.1% (29.8–68.4)	1	49.6% (30.0–69.2)	1
6-mo use	41.7% (24.9–58.5)	0.85 (0.8–0.9)*	44.7% (28.1–61.4)	0.9 (0.8–1.0)[&]
12-mo use	54.1% (37.8–70.5)	1.1 (0.9–1.2)	56% (39.4–72.6)	1.1 (1.0–1.2)*
Type of damage				
Lateral	32.4% (17.1–47.7)	1	32.9% (17.3–48.4)	1
Multiple	48.7% (34.5–62.9)	1.5 (1.3–1.7)*	51.8% (38.1–65.5)	1.6 (1.4–1.8)*
Central	63.9% (45.2–82.5)	1.9 (1.8–2.2)*	65.7% (47.0–84.3)	1.9 (1.8–2.2)*
Susceptible strain				
Deployment time				
New, nonexposed	54.9% (43.5–66.2)	1	65.2% (54.2–76.2)	1
6-mo use	36.1% (20.9–51.4)	0.66 (0.6–0.7)*	44.7% (27.0–62.4)	0.69 (0.6–0.8)*
12-mo use	35.7% (16.3–55.1)	0.65 (0.6–0.7)*	48.9% (26.4–71.3)	0.75 (0.7–0.8)*
Type of damage				
Lateral	20.9% (9.9–31.8)	1	29.5% (14.8–44.2)	1
Multiple	43.5% (27.7–59.2)	2.1 (1.8–2.4)*	53.2% (34.1–72.2)	1. 8 (1.6–2.0)*
Central	62.4% (49.6–75.2)	2.9 (2.6–3.4)*	76.1% (67.2–84.9)	2.6 (2.3–2.9)*

'No damage' net category was not considered in the analysis. RR = rate ratio, estimated with Poisson regression models. CI = confidence interval.
*$P < 0.001$.
[&]$P = 0.052$.

LLIN always recorded the highest passage and blood-feeding inhibition in both mosquito strains, suggesting a deterrent/repellent effect of LLIN.

Knockdown and Mortality

Knockdown in all the control tests was zero. Nine out of 120 control tests (6 for susceptible strain and 3 for resistant strain) recorded mortalities less than 10% (between 7 and 8%). Both deployment time (pseudo-R^2 = 0.38, Coef. = –0.63, $P < 0.001$ for resistant strain; pseudo-R^2 = 0.01, Coef. = –0.04, $P = 0.013$ in the susceptible strain) and, in lesser degree, the damage type (pseudo-R^2 = 0.1, Coef. = –0.15, $P < 0.001$ for resistant strain; pseudo-R^2 = 0.04, Coef. = –0.03, $P = 0.03$) of the net affected the knockdown mainly in the *Ae. aegypti* resistant strain (Table 3). The knockdown recorded for the resistant strain was 51.9% (95% CI: 40.5–63.3) when exposed to new nonexposed LLIN, and decreased significantly from 24.5 (95% CI: 18.5–30.7) to 15.3% (95% CI: 11.1–19.5) when mosquitoes were exposed to LLIN with 6–12 mo, respectively (Table 3). With the susceptible strain, the initial knockdown of 81.3% (95% CI: 73.9–88.7) decreased to 78.5% (95% CI: 71.6–85.5) with LLINs of 6 mo and until to 74.3% (95% CI: 62.4–86.4) with LLINs of 12 mo post-installation. Undamaged nets recorded the highest knockdown in both the resistant 43.6% (95% CI: 28.3–58.9) and susceptible strains 84.2% (95% CI: 76.9–91.4) and decreased significantly, mostly in LLIN with central and lateral damages from 26% (95% CI: 16.0–35.9) to 23.5% (95% CI: 13.9–33.0) in the resistant strain and from 72.1% (95% CI: 55.2–89.1) to 70.8% (95% CI: 64.9–76.8) in the susceptible strain; Table 3).

As observed with knockdown, mortality in both strains was affected by the time of deployment (pseudo-R^2 = 0.45, Coef. = –0.68, $P < 0.001$ for resistant strain; pseudo-R^2 = 0.05, Coef. = –0.11, $P < 0.001$ in the susceptible strain) and in a lesser degree by the type of damage (pseudo-R^2 = 0.1, Coef. = –0.06, $P = 0.003$ for resistant strain; pseudo-R^2 = 0.09, Coef. = –0.03, $P = 0.012$) mainly in the resistant strain. The mortality effect was reduced over time and also affected by the degree of damage of nets (i.e., central and lateral damages; see Table 3).

The efficacy of LLIN is challenged by the pyrethroid resistance status of *Ae. aegypti*, i.e., knockdown and mortality observed with the resistant strain were lower than those observed with the susceptible strain (Table 3). Mortality was similar or higher than knockdown within the resistance strain, but always lower than knockdown in the susceptible strain (Table 3).

Discussion

Contact bioassays were carried out to evaluate the chemical integrity of nets over time (WHO 1998, 2006). The MKDT on new nonexposed LLIN was around 417 s in the *Ae. aegypti* susceptible strain. This time is comparable to those obtained in contact bioassays with a susceptible strain of *Anopheles* (Diptera: Culicidae) species using different types of LLIN, i.e. range, between 230 and 549 s (Graham et al. 2005; Kayedi et al. 2007a, 2008, 2009; Skovmand et al. 2008). Particularly for unwashed LLIN, an MKDT of 300 s has been reported on susceptible strains of *An. culicifacies* (G.) (Sood et al. 2014). No other published study about MKDT on LLIN using *Ae. aegypti* was found.

We observed a reduced insecticide activity over time, with MKDT increasing up to 9.24 min at 12 mo post-installation. The reduced insecticidal activity has also been reported on *Anopheles* mosquitoes using the same bioassay technique (Kayedi et al. 2007b). Our results confirmed that the time of exposure under field conditions is an important factor affecting the bioavailability of the insecticide active ingredient on the LLIN surface, in contrast with what was observed in other types of LLIN, which had consistent insecticide activity (in terms of knockdown and mortality rates) on susceptible *Ae. aegypti* strains over time but using WHO cone bioassays (Vanlerberghe et al. 2010, Rizzo et al. 2012).

Table 3. LLIN exposure factor analysis using Poisson regression models constructed with % knockdown at 40 min and % mortality at 24 h as dependent variables, and deployment time/damage type as independent variables, Merida, Mexico (n = 5 replicates for each combination deployment time/damage type)

Strain/net exposure factor	Knockdown		Mortality	
	Mean (95% CI)	RR (95% CI)	Mean (95% CI)	RR (95% CI)
Resistant strain				
Deployment time				
New, nonexposed	51.9% (40.4–63.3)	1	57.7% (47.2–68.2)	1
6-mo use	24.6% (18.5–30.7)	0.47 (0.4–0.5)*	29.0% (23.8–34.3)	0.50 (0.5–0.6)[&]
12-mo use	15.3% (11.1–19.5)	0.29 (0.3–0.3)*	14.8% (10.5–19.1)	0.26 (0.2–0.3)*
Type of damage				
No damage	43.6% (28.3–58.9)	1	43.3% (27.3–59.2)	1
Lateral	23.5% (13.9–33.0)	0.54 (0.5–0.6)*	23.1% (14.6–31.6)	0.53 (0.5–0.6)*
Multiple	29.4% (16.6–42.2)	0.67 (0.6–0.8)*	37% (21.3–52.7)	0.85 (0.8–0.9)*
Central	26% (16.0–35.9)	0.59 (0.5–0.7)*	32.1% (23.6–40.5)	0.74 (0.7–0.8)*
Susceptible strain				
Deployment time				
New, nonexposed	81.3% (73.9–88.7)	1	74.6% (65.3–83.9)	1
6-mo use	78.5% (71.6–85.5)	0.97 (0.9–1.0)	72.3% (64.1–80.5)	0.97 (0.9–1.0)
12-mo use	74.3% (62.4–86.4)	0.91 (0.8–0.9)*	59% (46.7–71.3)	0.79 (0.7–0.8)*
Type of damage				
No damage	84.2% (76.9–91.4)	1	79.9% (71.6–88.2)	1
Lateral	70.8% (64.9–76.8)	0.84 (0.8–0.9)*	55% (46.3–63.7)	0.69 (0.6–0.7)*
Susceptible strain				
Multiple	85.1% (78.4–91.7)	1.0 (0.9–1.1)	74.3% (64.0–84.5)	0.93 (0.9–1.0)
Central	72.1% (55.2–89.1)	0.86 (0.8–0.9)*	65.4% (48.7–82.0)	0.82 (0.7–0.9)*

RR = rate ratio, estimated with Poisson regression models. CI = confidence interval.

*$P < 0.001$.

[&]$P = 0.052$.

The second set of tests consisted of tunnel bioassays (WHO 2013), performed to provide additional information on the mortality, and blood-feeding inhibition of the LLIN. In general, results with susceptible *Ae. aegypti* showed blood-feeding inhibition and mortality < 90% in new nonexposed LLIN (WHO 2013). For this type of test, WHO establishes the use of live animals as baits (usually a guinea pig or rabbit for *Anopheles*) and exposure for at least 15 h (WHO 2011, 2013). Many of the published studies about LLIN efficacy evaluation using animal baits in tunnel tests are typically based on malaria vector populations (Bayili et al. 2017, Massue et al. 2019). As *Ae. aegypti* is a strongly anthropophilic mosquito species, we used the forearm of a volunteer as bait in our tunnel tests, to be as realistic as possible.

Similarly, Denham et al. (2015) proposed the use of human arms and breath as an attractant for *Ae. aegypti* and arms as a potential bloodmeal source in a two-directional tunnel test, providing both untreated versus treated netting as choices for the mosquitoes in the same tunnel test (the releasing chamber was located between the two feeding chambers). Using this technique, they found that *Ae. aegypti* blood-feeding success and mortality were significantly affected by LLIN (new unwashed) in comparison to untreated nets using a susceptible strain and that this is dramatically affected by pyrethroid resistance (Denham et al. 2015). Particularly for DuraNet LLIN, they observed a blood-feeding inhibition around 80 and 21% (relative to the untreated control) in the susceptible and resistant strains, respectively, after 10 min of bait exposure. They did not inform about knockdown and mortality in tunnel tests. In comparison with those of Denham et al. (2015), our results showed low levels of blood-feeding inhibition and mortality in the susceptible strain across different exposure factors (32–66% and 55–80%, respectively). The difference of our study compared with Denham et al.'s (2015) study is that we used only one forearm as bait during an increased exposure time (40 min); however, the addition of human breath surely provided a source of carbon dioxide (CO_2), a potent activator of mosquito host-seeking (Dekker et al. 2005), and probably stimulated to mosquitoes passage throughout holes in the nets.

Other exposure factor evaluated was the type of damage on LLIN. Lateral net damage showed the poorest performance for blood-feeding inhibition and mortality in tunnel tests. The lateral damages are probably large enough for mosquitoes to avoid contact with the LLIN, in contrast with the other types of induced damages tested. Interestingly, most of the damages (56%) found under field conditions at 6 mo were one or more hole smaller than 2 cm, so LLIN could have been exerting their lethal and repellent effects on mosquitoes that landed on the nets in their struggle to go through small holes.

Manrique-Saide et al. (2014) reported in Merida, Mexico, that the presence of untreated window screening significantly decreased both the odds of having *Aedes* adult mosquitoes inside the house and odds of the number of females found indoors. The benefits of house screening with LLIN, as a physical and chemical barrier, rely on its efficacy to exclude and kill mosquitoes and eventually protect against mosquito bites, which is epidemiologically relevant if most transmission occurs indoors. Although the physical integrity could be compromised over time, our results provided information on repellency, blood-feeding inhibition, and mortality to a greater or lesser degree depending on the level of damage of the LLIN. Though insecticide resistance can reduce LLIN efficacy, we showed that, after 1 yr in field conditions, the remaining chemical active ingredient in the nets would still contribute to killing/repelling mosquitoes.

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