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Preliminary Evaluation of Village-Scale Insecticide-Treated Durable Wall Lining Against *Anopheles gambiae* s.l in Akorede, Kwara State, Nigeria

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ABSTRACT

Insecticide-treated durable wall lining (DL) was designed for user-independent and longer-lasting malaria vector control lacking in insecticide-treated bed nets and indoor residual spraying, respectively. Field efficacy studies involving pyrethroid-treated DL in Africa have been limited to experimental huts and mobile population settings. This study reports a preliminary assessment of village-scale DL installation against *An. gambiae* s.l in Akorede, Kwara State, Nigeria. Two similar villages were selected as DL intervention and control sites. Subsamples of installed DL were assessed for bio-efficacy every six months while window exit trap and monthly pyrethrum spray mosquito collections were conducted in both sites. *Anopheles gambiae* s.l collected were identified with PCR and tested for human blood and *Plasmodium falciparum* sporozoite using ELISA. Compared to baseline, the number of mosquitoes reduced significantly ($p = 0.008$) with 0% sporozoite rate in the intervention village but remained the same or increased significantly ($p = 0.003$) with 2.59% post-baseline sporozoite rate in the control. Bio-efficacy of DL remained 100% (mortality) with significantly ($p = 0.04$) increased mortality (53%) of free-flying indoor and exiting mosquitoes in the intervention village compared to control (1.8%). The results provide preliminary evidence to justify the need for randomized controlled trials in rural communities with pyrethroid-susceptible mosquitoes. Recent reports of widespread pyrethroid resistance in the *Anopheles gambiae* s.l also call for development and assessments of non-pyrethroid insecticide incorporated control tools.

Keywords: *An. gambiae* s.l, insecticide-treated durable wall lining, malaria

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INTRODUCTION

Malaria vector control currently thrives on the use of long-lasting insecticidal nets (LLIN) and indoor residual spraying (IRS) strategies. The widely deployed LLIN is limited by poor nightly user compliance (Von Seidlein et al., 2012; Koenker et al., 2013; Obembe et al., 2014; Watiro & Awoke, 2016; Nkamedjie et al., 2017) in malaria endemic African communities where high indoor temperature prevails (Von Seidlein et al., 2012). Moreover, IRS implementation is not sustainable in the African region due to unavailability of infrastructural and manpower requirements (Kolaczinski et al., 2007) added to possible user fatigue following recurrent conventional insecticide spraying rounds (Rowland, 1999). Insecticide-treated wall lining technology (durable lining) offers controlled and longer-lasting insecticide delivery system independent of nightly behavioral compliance (Messenger et al., 2012). However, efficient resistance management requires that pyrethroid-based IRS or DL should not accompany pyrethroid-LLIN use in the same community. Therefore, current concerns are that widespread (49%) access to LLIN among global malaria-prone population (WHO, 2014) could preclude the use of pyrethroid-based DL in the same communities. Additionally, evidence (Chandre et al., 2010) has shown that pyrethroid-DL installation may not provide additional protection over current best practice of universal LLIN coverage. However, researchers comparing combination of DL installation and LLIN use with that of LLIN alone usually ensure constant use of the distributed bed nets so as to objectively determine and compare the benefits derived from stand-alone and combined treatment arms. Such close monitoring and maintenance of LLIN fabric integrity and utilization hardly follow most net distribution campaigns conducted to achieve universal LLIN coverage (Asidi et al., 2012; Obembe et al., 2014). In these current situations of poor net utilization and care rates caused by heat and perceived confinement (Asidi et al., 2012; Obembe et al., 2014; Watiro & Awoke, 2016; Nkamedjie et al., 2017), durable lining may serve almost as a stand alone tool, lasting beyond the damage and human resistance prone LLIN. The idea will then be to have at least one functional and user independent vector control tool in the community. Besides, other reports have attested to the efficacy of pyrethroid-based DL alone under experimental huts and refugee camps in Africa (Diabate et al., 2006; Burns et al., 2012; Ngufor et al., 2014).

To date, community-based report from Africa on the impact of pyrethroid-based DL on malaria entomological risks is not available to our knowledge. The closest to this was the study in a refugee camp setting in Sierra Leone showing significantly reduced *Plasmodium falciparum* incidence rate in children up to three years of age (Burns et al., 2012). A study from Nigeria observed a high level of deltamethrin-treated durable lining (ZeroVector® DL, Vestergaard Frandsen, Switzerland) acceptability among rural inhabitants as against the urban residents (Messenger et al., 2012). Here, we report a preliminary assessment of village-scale ZeroVector® DL installation against *An. gambiae* s.l in Akorede, Kwara State, Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted in the Akorede (N 08°40.048 E 004°31.370) and Lumoh (N 08°38.001 E 004°33.740) villages in the Moro Local Government Area of Kwara State, Nigeria. Houses in the villages were of the mud type and all lacking LLINs or window nets. The inhabitants were mostly crop farmers, and the predominant *Anopheles* mosquito vector in the area was *An. gambiae* s.l.

Study Design

During the study site selection in September 2012, many of the rural communities visited were without LLIN despite the LLIN distribution campaign that was conducted in the state in March 2011. Six relatively isolated communities in the Moro Local Government area were visited, out of which two villages—Akorede and Lumoh—were selected for the study based on similarities in housing patterns, human population, and baseline *Anopheles* mosquito indices (Table 1). The village (Akorede) with the slightly higher number of houses and human population (Table 1) was chosen for DL installation (intervention site) after confirmation of deltamethrin susceptibility in the *Anopheles gambiae* s.l population collected from the site. Indoor resting adult mosquitoes were collected once in September 2012 to serve as baseline in each village. The single baseline mosquito collection was adopted because of the observed similarities in the two study communities (Table 1). Thereafter, monthly mosquito collections were conducted in each village between October 2012 and September 2013. To determine the mortality rates of mosquitoes in the intervention and control villages, live indoor and exiting mosquitoes from the rooms in both sites were collected using aspirators and window exit traps and monitored for 24 hours. The control community did not represent a full control treatment because of the unavailability and non-installation of untreated DL in the houses. Therefore, entomological indices such as number of mosquitoes, man-biting, and sporozoite rates were not compared between the intervention and control communities. Rather, initial and post-baseline values of these indices were compared within each village. The idea behind this is that if the post-intervention changes in the intervention village are due to weather variations, such post-baseline changes may also show in the neighboring control village. The only values compared between the intervention and control villages were the mortality rates. This is because the presence of untreated DL in the control community could not have killed the vectors resting thereon.

Durable Lining Installation and Tracking

Ninety-four percent of households in the intervention village were covered (Fig. 1) with the deltamethrin-treated (4.4 g/kg ± 15% *a.i*) Polyethylene Zero Vector® DL to achieve the required 80% minimum coverage for IRS programs (WHO, 2006a). The three households not covered had moved out of the village

Table 1. Housing, Human Population, and DL Coverage in the Study Sites

Community Name	Number of Houses	Number of Human Occupants	Number of *An. gambiae* s.l Collected from 10 Rooms at Baseline	Number of Households with ZVDL *n* (%)
Akorede (intervention)	14	167	153	47 (94)
Lumoh (control)	12	155	151	0

before the study commenced. Except for dust gathering on the linings, all wall-installed DLs in the rooms were intact with no evidence of tear throughout the period of the study.

Baseline Insecticide Susceptibility and DL Bio-Efficacy Tests

Prior to DL installation in September 2012, adult female *Anopheles gambiae* s.l samples reared from larval collections in the intervention site were exposed to WHO test papers with diagnostic concentrations of pyrethroid (0.05% deltamethrin and 0.75% permethrin) according to standard procedures (WHO, 1998). Durable lining bio-efficacy was assessed by collecting subsamples of installed DL materials (50 × 50 cm) immediately after installation and at 6-month intervals from 10 randomly selected sleeping rooms in the intervention village. The subsamples were transported to the laboratory in well-labeled aluminum foil, and the wall spaces were replaced with new DL materials. Cone bioassays were conducted on each DL subsample with 3 minutes exposure of 45 (5 mosquitoes per cone, 9 replicates per DL) non-blood-fed, 2- to 3-day-old female *Anopheles gambiae* s.s Kisumu susceptible strain (*Kss*) following standard procedures (WHO, 2006b). Knockdown was noted after the exposure period while the mosquitoes were removed and kept in netted paper cups supplied with 10% sugar solution and observed for 24-hour post-holding mortality rates.

Endophilic Mosquito Collection

Endophilic *Anopheles* mosquito samples were collected indoors at baseline (before DL installation—September 2012) and subsequently once a month in intervention and control villages between October 2012 and September 2013 using the pyrethrum spray

Figure 1. Rooms with window exit trap (C–D) and DL (A–B) in the intervention village.

catch (PSC) method (WHO, 2003). Ten houses from the intervention and control communities were selected for monthly mosquito sampling throughout the period of the study while the number of individuals who slept in such rooms overnight was noted for indirect man-biting rates estimations as described by Githeko et al. (1993). The baseline mosquito collection was conducted during study site selection in September 2012 to identify villages with similar entomological parameters and also for comparison with subsequent data obtained within each village.

Mosquito Mortality Rate Estimations

Window exit traps (Fig. 1) modelled after Service (1977) were mounted in five sleeping rooms in each village for seven consecutive days in November 2012 and July 2013. This was short-lived to reduce the number of days the room occupants were denied access to ventilation through the blocked windows. Exiting mosquitoes trapped overnight were collected from the traps the following mornings while the remaining mosquitoes resting indoors were collected with manual aspirators for 10 minutes. Collected samples were identified visually as dead/alive for appropriate recording according to house and community. Live mosquitoes from the rooms and traps were transferred to netted paper cups, provided with 10% sucrose solution, and observed for mortality after a 24-hour holding period.

Mosquito Species Identification

All samples collected were preserved individually in 1.5-mL Eppendorf tubes containing desiccated silica gel for identification using morphological keys (Gillies & Coetzee, 1987). Samples belonging to *An. gambiae* complex were characterized using species-specific PCR (Scott et al., 1993) and PCR-RFLP (Favia et al., 1997) assays. All laboratory processing was conducted at the Molecular Entomology and Vector Control Research Laboratory of the Nigerian Institute of Medical Research, Lagos.

Detection of Human Blood and *P. falciparum* Sporozoites in Mosquitoes

The head and thorax of each female *An. gambiae* s.l mosquito sample identified were separated from the abdomen and assayed for *P. falciparum* infection using sporozoite ELISA test (Wirtz et al., 1987) conducted with monoclonal antibodies and positive controls obtained from the Center for Disease Control, Atlanta, USA. Blood meal ELISA (Beier et al., 1988) was also conducted to determine the presence of human blood in the abdomen of each blood-fed mosquito sample using capture and conjugated monoclonal antibodies from Kikergaard and Perry Laboratories, as well as human serum from Rockland Immunochemicals.

Data Analysis

Man-biting rates (MBR) were determined as the number of blood-fed female *Anopheles* samples collected divided by the number of persons sleeping in the rooms the night preceding the collection multiplied by proportion of mosquitoes with human blood (Githeko et al., 1993). Sporozoite rate (SPR) was taken as percentage of *Anopheles* mosquitoes found with *Plasmodium falciparum* (Pf) sporozoites. Mortality rate was also determined as the percentage of female *Anopheles* found dead immediately and after 24 hours in the rooms and traps in each village. Numbers of mosquitoes collected were transformed [$\sqrt{n+0.5}$] (Ogbeibu, 2005) to normal distribution, and the means were compared within each site (baseline and afterwards) using Student's t-test ($p < 0.05$). Proportional data such as mortality rates were compared using logistic regression.

Ethical Considerations

Ethical approval for the study was obtained from the Ethical Review Board of the Nigerian Institute of Medical Research (NIMR) under the NIMR/National Malaria Elimination Program (NMEP) project. Informed consent of village and household heads were obtained during social advocacy visits to the communities in the company of officials of the Kwara State Roll Back Malaria Office.

RESULTS AND DISCUSSION

The results of the WHO susceptibility tests conducted before DL installation in the intervention site indicated that the *An. gambiae* s.l population in the village was susceptible to both deltamethrin and permethrin (100% mortality) insecticides. Likewise, observations from the residual efficacy cone bioassays conducted on DL after the 6th and 12th months showed that all installed DL subsamples collected from the house walls after installation induced 100% knockdown and 100% mortality against the reference *An. gambiae* Kisumu susceptible strain. Molecular characterization of *An. gambiae* s.l from the sites showed the preponderance of *An. gambiae* (> 80%) over *An. coluzzii* in both villages. The mortality rates of female mosquitoes in the intervention site (52.51% [95% CI = 42.70–62.32]) were significantly (F = 5.74, p = 0.04) higher compared to the control (1.8% [95% CI = 1.78–2.63]) village for the whole period of collection (Fig. 2). An important observation that could lend credence to the mosquito mortality results in this study was that some of the mosquitoes in the intervention village were found with erratic flight or were already dead before collection from the traps. The 53% mortality rates of *Anopheles* mosquitoes observed is comparable with the results of 40% and 45% mortality rates in DL experimental hut (Ngufor et al., 2014) and permethrin insecticide treated plastic sheeting (ITPS) trials (Diabate et al., 2006), respectively.

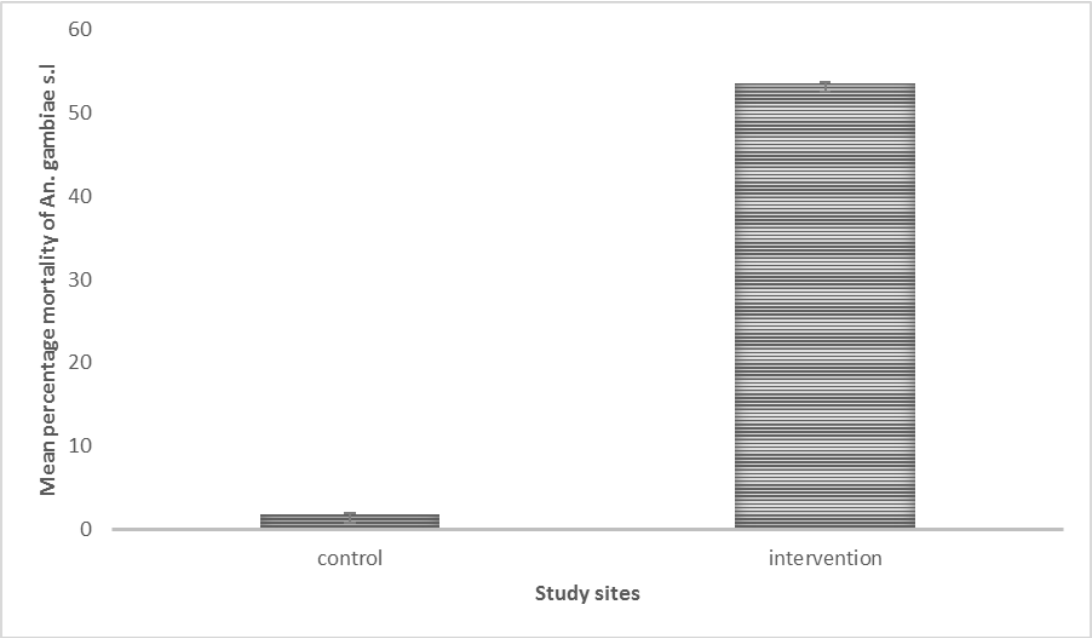

Figure 2. Mean (±S.D.) percentage mortality of female *An. gambiae* s.l samples in the study villages.

Mean numbers of female *An. gambiae* s.l in the intervention village reduced in September 2013 (1.85, $p = 0.008$) and between October 2012 and September 2013 (2.67, $p = 0.275$) compared to the September 2012 baseline (3.65) (Table 2). *Anopheles* sporozoite rate was 0% while man-biting rate was reduced by 4-fold (0.948) at the end of one year (October 2012–September 2013) compared to baseline (4.095) (Table 2). In the control village, the mean number of *Anopheles* mosquitoes remained similar (3.15, $p = 0.508$) in September 2013 but increased significantly after 1 year (6.12, $p = 0.003$) compared to the baseline (3.57). Man-biting and sporozoite rates increased from 3.99 and 4.64 at baseline to 4.76 and 4.67 in September 2013. Man-biting and sporozoite rates recorded after 1 year (1.69, 2.59) reduced by 1.8- to 2-fold in the control village compared to 4-fold and 100% reductions in the intervention village (Table 2).

Cumulative mortality effect of the DL probably accounted for the significant reductions in the number of mosquitoes and 0% sporozoite rates recorded during the post-intervention period in the intervention village. These results suggest efficacy of the installed DL in the intervention site. However, the results from this study, conducted in a community without LLIN, could be seen as less applicable now that 49% of malaria endemic areas reportedly have access to LLIN (WHO, 2014). Yet, other communities like the several ones in this study area still lack the tool outright or as a result of non-replacement of earlier distributed nets. Besides, emphasis should be placed on low utilization rates even in areas with a modest or high level of net ownership (Von Seidlein et al., 2012; Koenker et al., 2013; Obembe et al., 2014; Watiro & Awoke, 2016; Nkamedjie et al., 2017). All of these call for the need to explore other vector control tools that eliminate dependence on the sustained action or compliance of the community residents. This preliminary study is one of such efforts, and the results could serve as the basis for implementation of a randomized controlled trial to determine the effectiveness of the DL in malaria vector control.

Table 2. Comparison of Initial and Post-Baseline Entomological Indices Within Each of the Study Villages

	Intervention			Control		
Entomological Indices	**Sept. '12 (Baseline)**	**Sept. '13**	**Oct. '12–Sept. '13**	**Sept. '12 (Baseline)**	**Sept. '13**	**Oct. '12–Sept. '13**
Mean (±SD) number caught per room	3.65 ± 1.65[a]	1.85 ± 0.87[b]	2.67 ± 1.67[a]	3.57 ± 1.78[a]	3.15 ± 1.29[a]	6.12 ± 0.95[b]
Man-biting rates	4.09	1.19	0.29	3.99	4.76	1.69
Sporozoite rates (%)	5.23	0	0	4.64	4.67	2.59

Note. Values having the same letter superscript along the same row of each study site are not significantly different at the 5% level.

CONCLUSION

The results of significantly higher mosquito mortality, 0% sporozoite rates, and significantly reduced post-baseline number of mosquitoes in the intervention village could provide evidence to justify the need for more randomized controlled trials in rural communities with pyrethroid-susceptible mosquitoes. However, the reported increase in potency and spread of pyrethroid resistance in the *An. gambiae* mosquitoes in Sub-Saharan Africa (Strode et al., 2014) also call for development and assessments of non-pyrethroid insecticide incorporated vector control tools in Africa.

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