academicJournals

Vol. 6(7), pp. 98-103, August, 2014
DOI: 10.5897/JEN2014.0100
Article Number: 5C0292647196
ISSN 2006-9855

http://www.academicjournals.org/JEN

Journal of Entomology and Nematology

Full Length Research Paper

Susceptibility test of female anopheles mosquitoes to ten insecticides for indoor residual spraying (IRS) baseline data collection in Northeastern Nigeria

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Received 23 June, 2014; Accepted 28 August, 2014

Malaria is a major public health problem in Nigeria, accounting for about 60% of all outpatient attendances and 30% of all hospital admissions. Indoor residual spraying (IRS) was scaled up in Nigeria to supplement long lasting insecticide treated nets (LLINs) for malaria vector control. The success of IRS partly defends on the susceptibility of local anopheles mosquitoes to insecticides. The WHO standard insecticides-impregnated papers and tubes were used to conduct bioassay tests against local populations of *Anopheles* species in Misau Bauchi State Nigeria with a view of selecting the suitable insecticides for IRS. The tests papers include: Cyfluthrin (0.15%), DDT (4%), Deltamethrin (0.05%), Lambdacyhalothrin (0.05%), Malathion (5%), Permethrin (0.75%), Propoxur (0.01%), Alpha-cypermethrin (0.75%), Bendiocarb (0.1%), Bifenthrin (0.15%) and untreated (control). Twenty (20) two to three day-old, female Anopheles species, glucose fed, none blood fed, were exclusively used in the bioassay per treatments which was replicated three times. The post exposure 1 h knockdown and 24 h mortality was assessed. The results of the knockdown assessment indicate that Alphacypermethrin had the lowest KD_{50} (time taken to knockdown fifty percent of the exposed mosquitoes) value of 4.8 min. Relatively moderate KD_{50} values (minutes) were obtained with Propoxur (11.34), Deltamethrin (13.20), Malathion (15.82), Bendiocarb (17.29), Permethrin (18.43), Cyfluthrin (20.28) and Lambdacyhalothrin (23.11). Relatively higher KD_{50} values were obtained with Bifenthrin (27.29) and DDT (32.12) impregnated papers. The results of mortality assessment indicate that *Anopheles* mosquitoes were susceptible to Alphacypermethrin, Malathion and Propoxur with 100% mortality. The *Anopheles* species were less susceptible to Bifenthrin, Lambdacyhalothrin, Permethrin, Deltamethrin, Bendiocarb, Cyfluthrin and DDT. The Anopheles species used in the tests were morphological identified as *Anopheles gambiae, Anopheles funestus* and *Anopheles nili*. The public health significance of these findings is discussed.

Key words: Nigeria, *Anopheles* mosquitoes, resistance, Misau, Bauchi State, indoor residual spraying (IRS).

INTRODUCTION

WHO current estimates show that malaria mortality rates were reduced by about 42% globally and by 49% in the WHO African Region between 2000 and 2012 and during the same period, malaria incidence rates declined by 25% around the world, and by 31% in the African Region (WHO, 2013a).

In Nigeria, malaria accounts for 60% of outpatient visits to health facilities, 30% of childhood deaths, 25% of death in children under one year and 11% maternal death in addition to about 132 billion naira financial loss in the form of treatment costs, prevention, loss of man-hours, etc in Nigeria (FMoH/ NMCP, 2009). In Nigeria, the economic impact of malaria can be attributed to low gross national income per capital (GNI) of US$260 (FMoH, 2005).

In recent times, IRS is being adopted and scaled up to protect the entire household and community members who possibly have no access to treated bed nets in Africa (Beier et al., 2008).

The Federal Government Policy on Malaria Control in Nigeria focuses on LLINs, IRS, intermittent preventive treatment (IPT) and environmental management (NMCP, 2014). In line with these strategies, the National Malaria Elimination Programme (NMEP) in Nigeria has scaled up indoor residual spraying (IRS) to achieve 85% coverage in 20% of eligible structures in Nigeria in 2014. To achieve these target, IRS activities was progressively expanded in the seven World Bank Supported Malaria Booster States of Bauchi, Gombe, Kano, Jigawa, Rivers, Anambra, Akwa Ibom states, Nigeria from 2009 to 2014 to supplement LLIN and environmental management.

Currently, WHOPES recommends 12 insecticide compounds and formulations, belonging to four chemical classes, for deployment in IRS program (WHO, 2009). The major challenge in use of these insecticides in malarial vector control has been the development of resistance to insecticides among the vector populations. *Anopheles* mosquitoes resistance to insecticides is spreading rapidly across African countries (Awolola et al., 2002, 2005, 2007; Ndams et al., 2006; Oduola et al., 2010; Ranson et al., 2011; Kabula et al., 2012; Natacha et al, 2013; Ibrahim et al., 2014) and could reduce the impact of malaria prevention interventions using IRS and LLINs, particularly in sub-Sahara Africa (NGuessan et al., 2007; Awolola et al., 2008).

The successful implementation of IRS program partly depends on availability of insecticide(s) susceptible Anopheles mosquitoes in the local environment. Therefore, it is imperative to periodically conduct bioassays tests to assess the susceptibility status of local mosquito species to IRS interventional insecticides. The susceptibility of Anopheles mosquitoes against insecticides was fairly evaluated in southern parts of Nigeria (Olayemi et al., 2011; Oduola et al., 2012) but there was dearth of information in the northern Nigeria (Molta and Ali, 1998; Ndams et al., 2006). No documented evidence on the susceptibility status of *Anopheles* mosquitoes to guide procurement of IRS insecticide in Northeast Nigeria is available. Therefore, the presents study was conducted to provide baseline data on insecticides susceptibility status of local *Anopheles* mosquito in Misau, Bauchi State, Nigeria.

MATERIALS AND METHODS

Study area and period

The study was conducted in August 2010 in Misau town, Misau L.G.A located at latitude 11.31897 and longitude 10.47587, human population of 263,487 as at the 2006 census with an area of 1,226km^2. IRS was scaled up in 2009 in the three wards of Misau (Gundari, Kukadi A and Kukadi B) where Lambdacyhalothrin, Deltamethrin and Bifentrin respectively were used. The total coverage for insecticides was 52,000 households.The community has been using LLINs since 2002 till date. The farmers in the suburb cultivate vegetables, rice and wheat on the wetlands where agrochemicals (cypermethrin, lambdacyhalothrin, deltamethrin, dichlovos and primiphos-methyl) are used in pest control. The wetlands also has number of tube bore holes to supplement provision of portable water to Misau community. Pools of standing water from the wetlands and tube bore holes provide active breeding sites for the *Anopheles* mosquitoes.

Mosquito collection and rearing

The *Anopheles* species larvae were collected in naturally infested waterbodies in Misau using entomological ladles. When culicine larvae were collected, they were separated from the Anopheline larvae and discarded on the land. The emerging pupae were sucked out of the larval containers using pipette and kept in plastic cups inside a mosquito cage made from five(5) litres white plastic bucket, fastened with cone shape white mosquito netting with its rear end tied in to a knot to prevent escape of emerging adult mosquitoes. The adult that emerged in 1-3 days were reared according to methods of Umar et al. (2008).

Test kits and insecticide impregnated papers

The WHO susceptibility test kits (WHO tubes and accessories) and insecticide impregnated papers (0.75% Alpha-cypermethrin, 0.1% Bendiocarb, 0.15% Bifenthrin 0.15% Cyfluthrin, 4% DDT, 0.05% Deltamethrin, 0.05% Lambdacyhalothrin, 5% Malathion, 0.75%, Permethrin, 0.01% Propoxur and untreated control) were provided by the National Malaria Elimination Program (NMEP), Federal Ministry of Health, Abuja.

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Bioassay techniques

Insecticide susceptibility tests were carried out using the WHO standard procedures and test kits for adult mosquitoes (WHO, 1998). The bioassay was conducted using 2-3 days old, glucose-fed but non-blood fed female Anopheles mosquitoes.

For each insecticidal paper and the control, a three replicates of 20 adult female *Anopheles* mosquitoes were exposed to tubes and allowed to stand for 1 h and numbers of knocked-down mosquitoes were recorded at intervals of 10 min. After the exposure, mosquitoes were then transferred to a recovery tubes and fed with a pad of cotton wool soaked in 10% glucose solution. The holding tubes were kept for 24 h in a secluded, shaded and sterile place. Adult mortality was assessed after 24 h post-exposure by inability to stand upright and walk when probed with glass rod. The dead and survived mosquitoes at the end of experiment were separately kept in labeled 1.5 mL Eppendorf tubes containing silica gel, for species identification. The susceptibility tests were conducted in laboratory under fluctuating temperature (25-33°C) and relative humidity (90-95%).

Identification of *Anopheles* mosquitoes

Morphological keys of Gillies and DeMeillon (1968) and Gillies and Coetzee (1987) were used in morphological identifications of adult *Anopheles* mosquitoes.

Data analysis

The knockdown data was subjected to probit analysis using a statistical software (Statsdirect, 2013) to compute the KDT_{50} and KDT_{90} (time taken to knockdown 50 and 90% of the exposed mosquitoes) and their 95% confidence intervals. The 24 h mortality was manually assessed. The susceptibility of *Anopheles* mosquitoes to insecticides was assessed using the current WHO (2013b) criteria: A mortality in the range 98-100% indicates susceptibility and a mortality of less than 98% is suggestive of the existence of resistance. The adult mortality in control experiments were less than 5% and hence were not corrected for (Abbott, 1925).

RESULTS

The results of knockdown assessment of female *Anopheles* mosquitoes exposed to ten different insecticide impregnated papers is presented in Table 1. The results indicates that Alphacypermethrin has the lowest KDT_{50} and KDT_{90} values of 4.84 and 24.58 min while Bifenthrin had the highest KDT_{50} and KDT_{90} value of 27.29 and 85.95 min among all the pyrethroids tested. Among the cabamates, propoxur was most effective with KDT_{50} and KDT_{90} values of 11.35 and 17.30 min than bendiocarb with KDT_{50} and KDT_{90} values of 17.87 and 30.68 min, respectively. Malathion and DDT had lower KDT_{50} and KDT_{90} values of 15.82 and 29.22 min and higher 32.12 and 65.31 min, respectively.

The results of the 24 h post-exposure mortality presented in Table 2 indicate that the local *Anopheles* mosquito species were susceptible to Alphacypermethrin, Propoxur and Malathion with 100%. The tested *Anopheles* mosquito were resistant to Cyfluthrin (55.00%), DDT (78.33%), deltamethrin (83.33%), Lambdacyhalothrin (93.33%), Bifenthrin, Permethrin and Bendiocarb (96.67% each). The morphological identifications of stored *Anopheles* mosquito revealed *A. gambiae, A. funestus* and A*. nili*. The members of the *Anopheles gambiae* and *Anopheles funestus* were not identified using polymerase chain reactions (PCR) techniques.

DISCUSSION

The present study presents for the first time baseline data on the susceptibility status of *Anopheles* mosquitoes to WHOPES approved IRS insecticides in Misau, Bauchi State, Northeastern Nigeria to guide procurement of IRS insecticides in the state.

The results of knockdown assessment showed that the tested insecticidal papers induced knockdown of adult *Anopheles* mosquitoes suggesting that knockdown mechanism could be operating in the local *Anopheles* mosquito populations. This confirm earlier studies which separately indicates the knockdown effects of impregnated papers against *Anopheles* mosquitoes in Nigeria (Awolola et al., 2005; 2007; Oduola et al., 2010; Olayemi et al., 2011; Oyewole et al., 2011; Ibrahim et al., 2014). The knockdown of *Anopheles* mosquitoes exposed to insecticidal papers indicates the presence of KDR resistance mechanism (Kristan et al., 2003; Awolola et al., 2007; Ibrahim et al., 2014) operating in the populations of *Anopheles* mosquitoes in Misau.

Using the WHO (2013b) criteria for insecticides susceptibility or resistance assessment of mosquitoes, the 24 h post-exposure results indicates that the local *Anopheles* mosquito species were susceptible to alphacypermethrin, propoxur and malathion each with 100% mortality. Other Principal Investigators for IRS working in Northern Nigeria shown that local *Anopheles* mosquito species were particularly susceptibility to alphacypermethrin (Mwansat, 2012; Manu, 2013, Yoriyo, 2013).

The local Anopheles mosquito species were resistant to Cyfluthrin, Deltamethrin, Permethrin, Lambdacyhalothrin, Bifenthrin, Bendiocarb and DDT. Previous reports have documented evidence on resistant of *Anopheles* mosquitoes to Cyfluthrin (Coetzee et al., 2006); Deltamethrin (Betson et al., 2009; Oduola et al., 2012; Awolola et al., 2014); Permethrin (Abdalla et al., 2007; Awolola et al., 2007, 2012, 2014; Ramphul et al., 2009; Kemabonta et al., 2013); Lambdacyhalothrin (Awolola et al., 2014); Bendiocarb (Ibrahimet al., 2013) and DDT (Betson et al., 2009; Oduola et al., 2010, 2012). Sustainable insecticide resistance management strategy is imperative to avoid control failures when the resistant insecticides are used for IRS program in Bauchi State. There is need for periodic monitoring of insecticide resistance in malaria control programmes in Bauchi State, as it affects ITNs and IRS interventions across Africa (Awolola et al., 2008).

Table 1. Knockdown periods of anopheles mosquitoes exposed to ten insecticide impregnated papers in Misau, Bauchi State, Nigeria.

Insecticide group	Insecticidal paper	Concentration (%)	Number exposed	KD_{50} (min)	95% Confidence interval	KD_{90} (min)	95% Confidence interval
Pyrethroids	Bifenthrin	0.15	60	27.29	22.83-32.52	85.95	63.09-126.73
	Lambdacyhalothrin	0.05	60	23.11	19.14-27.34	49.11	40.10-62.43
	Alphacypermethrin	0.75	60	4.84	3.148 - 6.47	24.58	19.78- 32.53
	Permethrin	0.75	60	18.43	16.51-20.34	38.09	34.00-43.79
	Cyfluthrin	0.15	60	20.28	17.63-22.66	40.48	36.17-46.74
	Deltamethrin	0.05	60	13.20	10.12-16.89	36.79	27.45-51.13
Cabamates	Bendiocarb	0.1	60	17.87	14.25-21.78	30.68	25.28-38.24
	Propoxur	0.01	60	11.35	10.34-12.43	17.30	15.46-20.30
Organochlorine	DDT	4.0	60	32.12	29.21-35.01	65.31	57.29-78.54
Organophosphate	Malathion	5.0	60	15.82	14.02-17.53	29.22	26.20-33.45

Table 2. Mortality and susceptibility status of anopheles mosquitoes exposed to ten insecticide impregnated papers in Misau, Bauchi state, Nigeria.

Insecticide group	Insecticidal paper	Concentration (%)	Number Exposed	No Dead	Mortality (%)	Susceptibility status*
Pyrethroids	Bifenthrin	0.15	60	58	96.67	Resistance
	Lambdacyhalothrin	0.05	60	56	93.33	Resistance
	Alphacypermethrin	0.75	60	60	100	Susceptible
	Permethrin	0.75	60	58	96.67	Resistance
	Cyfluthrin	0.15	60	33	55.00	Resistance
	Deltamethrin	0.05	60	50	83.33	Resistance
Cabamates	Bendiocarb	0.1	60	58	96.67	Resistance
	Propoxur	0.01	60	60	100	Susceptible
Organochlorine	DDT	4.0	60	47	78.33	Resistance
Organophosphate	Malathion	5.0	60	60	100	Susceptible

*WHO scoring for resistance (WHO, 2013b).

The multiple insecticide resistances of *Anopheles* mosquitoes to the tested pyrethroids, carbamates and organochlorine insecticides may have grave implications for the malaria control programme. It may compromise the efficacy of interventions and potentially lead to the failure of IRS and ITNs based vector control (Awolola et al., 2008).

The resistance of *Anopheles* mosquitoes to bifenthrin, lamdacyhalothrin and deltamenthrin may be linked to use of these insecticides in 2009 IRS intervention in the communities. It is established that prior exposure of mosquitoes to insecticides may induced selection pressure (Kerah-Hinzoumbé et al., 2008). Pyrethroids based aerosols and coils are used for control of mosquitoes and domestic pests and it might contribute to the development of resistance as reported elsewhere (Kristan et al., 2003). The farmers in the community also use cypermethrin, lambdacyhalothrin, deltamethrin, dichlovos and primiphos-methyl for agricultural crop protection. Previous researchers have reported that exposure of malarial vectors to crop protection insecticides could result in development of insecticide resistance (Etang et al., 2003; Awolola et al., 2007; Müller et al., 2008; Chouaibou et al., 2008; Bigoga et al., 2012; Philbert et al., 2014). LLIN was used in Misau for protection against mosquitoes since 2002 to date and it may induce selections to pyrethroids insecticides. Previous studies revealed that use of LLIN could result in development of insecticide resistance in Anopheles mosquitoes (Kabula et al., 2011).

The morphological analysis of preserved mosquito samples showed populations of *A. gambiae, A. funestus a*nd *A. nilin* were used in the bioassays. *A. gambiae* is the principal vector of malaria in sub-Saharan Africa (Gillies and Coetzee, 1987; Samdi et al., 2006; Sinka et al., 2010). The fauna of *A. gambiae, A funestus and A nili* was earlier documented in northern Nigeria (Molineaux and Gramiccia, 1980; Gadzama, 1983; Molta et al., 1999; Samdi et al., 2006; Ahmed, 2013). The *A gambiae* and *A. funestus* are major malarial vector in Nigeria (Molineaux and Gramiccia, 1980) and have great implication in malaria transmission in Bauchi State. Therefore, periodic monitoring of insecticides resistance in this mosquito species is imperative to avoid vector control failures.

Conclusion

It is concluded that procurement of IRS insecticide(s) in the state should be guided by the results of the present study until new susceptibility status is established and resistance management strategies should be considered when using the less susceptible insecticides. It is recommended that future studies should focus on investigation on the *A. gambiae* and *A. funestus* complexes and elucidations of resistance mechanisms in these mosquito species.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENTS

The authors sincerely thank the World Bank Supported Malaria Booster Project for supporting Bauchi State Malaria Control Booster Project and the National Malaria Elimination Programme for coordinating roles.

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