SURE Rapid Response

Pyrethroid Resistant Anopheles gambiae: Pyrethroid Impregnated or Synergistic LLINs?

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This rapid response was prepared by the Uganda country node of the Regional East African Community Health Policy Initiative.

Key messages

- Several studies from the country and the region have confirmed presence of pyrethroid resistance to malaria vectors including *Anopheles gambiae.*

- The resistance is through several mechanisms and its effect on the nets depends on whether the mosquitoes with the reduced susceptibility phenotypic trait reduce the impact of permethrin-treated nets on the vectorial capacity of *Anopheles gambiae*

  It is not clear whether this is the case in Uganda.

- There is still mixed evidence about additional benefit or effectiveness of synergistic LLINs compared to mono-treated LLINs with several studies reporting a lack of a significant difference between the two.

  Additional research may be needed/is warranted in this area if a policy shift is to be considered.

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SURE Supporting the Use of Research Evidence

REACH Regional East African Community Health Policy Initiative

EVIPNet EVIDENCE-INFORMED POLICY NETWORK

Who requested this rapid response?

This document was prepared in response to a specific question from a policy maker in Uganda.

! This rapid response includes:

- Key findings from research
- Considerations about the relevance of this research for health system decisions in Uganda

X Not included:

- Recommendations
- Detailed descriptions

What is SURE Rapid Response?

SURE Rapid Responses address the needs of policymakers and managers for research evidence that has been appraised and contextualised in a matter of hours or days, if it is going to be of value to them. The Responses address questions about arrangements for organising, financing and governing health systems, and strategies for implementing changes.

What is SURE?

SURE – Supporting the Use of Research Evidence for policy in African health systems - is a collaborative project that builds on and supports the Evidence-Informed Policy Network (EVIPNet) in Africa and the Regional East African Community Health (REACH) Policy Initiative (see back page). SURE is funded by the European Commission's 7th Framework Programme.
www.evipnet.org/sure

Glossary

of terms used in this report:
www.evipnet.org/sure/rr/glossary

How this Response was prepared

After clarifying the question being asked, we searched for systematic reviews, local or national evidence from Uganda, and other relevant research. The methods used by the SURE Rapid Response Service to find, select and assess research evidence are described here:

www.evipnet.org/sure/rr/methods

Background

There is growing and warranted concern about resistance of malaria vectors to the existing control agents especially pyrethroid. This is coupled with a slow pace for new agents to replace the current ones fast enough to keep up with the rate of increasing resistance. Malaria was and is still a leading killer in Uganda and the continent generally and so there is cause to worry about the implications of this resistance (1). Currently the World Health Organization Global Malaria Programme (WHO/GMP) recommends the following three primary interventions for effective malaria control, which must be scaled up if countries are to move towards achieving the United Nations Millennium Development Goals by 2015 (2):

- diagnosis of malaria cases and treatment with effective medicines;
- distribution of insecticide-treated nets (ITNs), more specifically long-lasting insecticidal nets (LLINs), to achieve full coverage of populations at risk of malaria; and
- indoor residual spraying to reduce and eliminate malaria transmission

The National Malaria Control Program (NMCP) in Uganda currently uses the strategies of controlling vectors with Indoor Residual Spraying and Long Lasting Insecticidal Nets, and effective case management. A recent (unpublished) study by Michael Okia et al sanctioned by the NMCP was done to assess the bio-efficacy of five World Health Organization-recommended Long Lasting Insecticidal Nets against pyrethroid resistant *Anopheles gambiae* field populations from Uganda with the aim of guiding decisions on the most appropriate choice of Long Lasting Insecticidal Nets for specific regions (3). This paper will look at the findings of this study with the aim of placing these in the context of research done within the region.

Michael Okia et al. (2011) Pyrethroid resistance status and susceptibility to long-lasting insecticidal nets of *Anopheles gambiae* populations from different malaria transmission zones in Uganda.

The above researchers carried out a study to assess the bio-efficacy of five World Health Organization-recommended LLINs against pyrethroid-resistant *Anopheles gambiae* field populations from Uganda. They carried out their research in four districts (Lira, Tororo, Kanungu, Wakiso), one each from the different regions of the country. They found:

i. *Anopheles gambiae* species predominant in all of these places to a tune of 98%.

ii. From WHO susceptibility tests:
    a. confirmed resistance to both permethrin and deltamethrin for the populations from the northern and eastern districts of Lira and Tororo
    b. confirmed resistance to permethrin and possible resistance to deltamethrin for the population from Kanungu
    c. possible resistance to both pyrethroids for the population from Wakiso
    d. At all four sites, higher resistance to permethrin was identified than for deltamethrin at the standard tested dosages

iii. All LLIN sub-samples had optimal bio-efficacy (100% KD and 100% MT) against the susceptible *Anopheles gambiae* s.s. strain with the exception of Interceptor and Olyset.

iv. Chemical analyses confirmed that all LLINs exceeded the specified lower cut-off level for insecticide concentration (at two instances LLIN sub-samples slightly exceed the upper limits i.e., roof of PermaNet 2.0 and sides of Interceptor)

v. There was no significant difference observed in the "deltamethrin plus PBO" roof and the "deltamethrin-only" sides of the combination LLINs, presumably because bioefficacy of the three sections was high against all four populations

vi. Reduced susceptibility to LLINs was observed for all four field populations of An. Gambiae

vii. With Cone bioassays:
    a. The combination LLINs exhibited the highest bio-efficacy against all the four populations (98.5 – 100%)
    b. When data were analyzed via multiple comparison methods
        i. The combination LLINs performed significantly better than the mono-treated LLINs in Lira and Wakiso
        ii. Equal performance for combination LLINs with given mono-treated LLINs (NetProtect in Kanungu and Olyset in Tororo).
    c. Each of the mono-treated LLINs varied in bioefficacy for the four different populations. However there was no identifiable difference in bioefficacy against the four population when using the combination LLINs.

viii. With Wireball assays:
    a. Differences in LLIN bioefficacy between net types for each of the four field populations, with the combination LLINs resulting in the highest bio-efficacy (76.5 – 91.7%)
    b. Bioefficacy also varied against the susceptible Kisumu strain and was highest for the combination LLIN followed by PermaNet 2.0 and then the other LLINs
    c. Bioefficacy of specific net types also varied against the different populations for the mono-treated LLINs,

d. Bioefficacy (in contrast to the cone bioassay data) also varied across populations for the combination LLINs (with the lowest bioefficacy observed in Kanungu (76%)
e. Bioefficacy of the net sections differed only for the combination LLINs in Kanungu and for one of the mono-treated LLINs (Olyset) in Lira.

ix. Overall:
a. PermaNet 3.0 exhibited high bioefficacy in both assay types and performed best or equally best against all four populations
b. NetProtect performed well in Kanungu (cone bioassays) population and in Lira (wireball assays)
c. Olyset performed well in Tororo (cone bioassays) and three mono-treated LLINs (wireball assays). Tororo population was overall the most susceptible to LLINs.
d. LLINs had the lowest efficacy against the Lira and Wakiso populations (cone bioassays); however wireball assays indicated lowest efficacy against the Kanungu population (55.2% KD30).
Bioefficacy was highest against the Tororo population for both bioassays.

Summary of the literature

Okia et al in context

Reduced susceptibility to pyrethroids in Uganda and the region

Pyrethroid resistance is a real threat in the country and the region. The Atlas of insecticide resistance in malaria vectors of the WHO African region notes that eastern Africa is one of the areas in which this resistance by *Anopheles gambiae* species to Pyrethroid has already been documented (4). It also notes that this resistance is evolving and will continue to do so because of the massive use of pyrethroids for malaria control but also for agricultural purposes.

Rubaihayo et al in a study to determine the prevalence of pyrethoid insecticide resistance in malaria vectors in western Uganda found that the trend showed an increasing mosquito resistance status with cross-resistance against all the three pyrethroid insecticides (deltamethrin, cyfluthrin and cypermethrin) which are what are common in the commercially available nets (5). Another study was done aimed at surveying *Anopheles gambiae* species from eastern Uganda to determine their resistance levels against chemicals that represent all four WHO-recommended classes of pesticides against adult vectors (dichlorodiphenyltrichloroethane (DDT), permethrin, deltamethrin, bendiocarb and malathion), in two ecologically distinct field sites representing temporary (Tororo) and permanent (Butaleja) larval breeding sites (6). It found that the mosquitoes tested were susceptible to bendiocarb and malathion but there was reduced susceptibility to DDT and the pyrethroids. Samples collected from Tororo also showed resistance to permethrin. Survival rates were higher in Tororo than in Butaleja although the survival rates of *Anopheles gambiae* s.l. to deltamethrin exposure at the two sites were not different.

During a study by Verhaegen and colleagues to develop and test a new assay on field collected *Anopheles gambia*e s.s. and *Anopheles arabiensis* specimens from Uganda did in fact report the presence of knockdown resistance (kdr) alleles in Ugandan field populations of *Anopheles gambiae* s.s., the main malaria vector in Uganda (7). This presence of resistance was further confirmed in a later study to assess the pyrethroid and DDT resistance status of the major malaria vectors and the role of one of the known kdr mutations identified from the earlier study (7) in providing pyrethroid and DDT resistance (8).

The findings in the different areas of Uganda are not in isolation; indeed they match with what has been observed in the region. Kawada and colleagues in a study whose one of two objectives was to monitor the pyrethroid susceptibility in 3 major malaria vectors including *Anopheles gambiae s.s*. in a highly endemic area in western Kenya, found a high level of resistance shown to be caused by a high frequency of point mutations in the mosquitoes (9).

Vulule and colleagues noted in their study in western Kenya about two decades ago that susceptibility of the malaria vector *Anopheles gambiae* to permethrin decreased following the installation of mosquito nets impregnated with permethrin in four villages (10). This is similar to the results reported by Curtis and others in Tanga, Tanzania at about the same time (11, 12) and even a decade later (13).

Such findings are also in keeping with several in Central and West Africa whose conditions that have contributed to resistance are not very different from those in the Eastern part of the continent. Insecticide susceptibility was investigated in *Anopheles gambiae s.l* from an area of large scale Insecticide Treated Nets distribution in south-western Chad (14). Increased tolerance to pyrethroids was detected in almost all samples (8/9) with mortality rates ranging from 70.2 to 96.6% for deltamethrin and from 26.7 to 96.3% for permethrin. By 1999, Chandre and colleagues had confirmed the presence of pyrethroid resistance among *Anopheles gambiae s.l* mosquitos in Côte d'Ivoire and had reported the observation of such resistance in Benin and Burkina Faso (15). These similar trends have also been observed in Cameroon (16).

Efficacy of nets in the face of increasing pyrethroid resistance

The Africa Network for Vector Resistance Atlas referred to earlier recommends that because of the evolving resistance patterns, it is essential to regularly assess the resistance status of local vector populations in order to select the appropriate insecticide for use. And when possible the potential resistance on the efficacy of interventions should be assessed.

As early as two decades ago, studies were pointing towards reduced efficacy of pyrethroid impregnated nets in Eastern, Central and Western Africa. Vulule et al, as seen earlier, reported this in western Kenya. Reduced efficacy associated with pyrethroid resistance in *Anopheles gambiae* has also been noted in Benin. A study to compare the impact of IRS and ITNs against both pyrethroid resistant and susceptible populations was done in Benin (17). N'guessan and colleagues reported that from findings in their study whose objective was to compare the impact of IRS and ITN against pyrethroid resistant population in the northern and southern parts of that country, pyrethroid resistance in *Anopheles gambiae* appeared to threaten the future of ITNs and IRS in Benin. Marie Claire et al noted the same in Cote d'Ivoire in the year 1999 and again in 2005 (18, 19).

However one thing to note is that there are several mechanisms of vectors' pyrethroid resistance. When it is induced by the knock down rate (kdr) mutation, it does not dramatically reduce efficacy of insecticide treated nets, such that even in areas with a very high prevalence of this resistance, the nets would still efficiently prevent malaria (1). However it is also important to keep in mind that the potential impact of resistance mechanisms other than kdr has not yet been fully assessed. Furthermore, LLINs impregnated with permethrin can continue to be efficacious even in a region with pyrethroid resistance. The effect on the nets depends on whether the mosquitoes with the reduced susceptibility phenotypic trait reduce the impact of permethrin-treated nets on the vectorial capacity of *Anopheles gambiae s.s* (10). Insecticides can and would produce large reductions in vectorial capacity when all the target vectors in the area have the same probability of acquiring a lethal dose. However, if vectors with reduced susceptibility have a higher than average probability of avoiding a toxic dose of permethrin during the process of biting humans, then the impact of the nets on vectorial capacity will be less. If this is the case in Uganda then reduced susceptibility to permethrin in anopheles gambiae has serious implications in the country and the region where there is widespread use of permethrin-treated bednets for malaria control.

In line with Okia et al's findings, several countries have also reported different resistance and susceptibility levels of Pyrethroid across different regions within the same country. For example, in a study to determine insecticide resistance status in Anopheles species in Madhya Pradesh, central India, susceptibility to DDT, malathion and deltamethrin was tested in nine different districts using standard WHO adult susceptibility kit and methods (20). The districts selected for assessment of susceptibility to insecticides had almost similar ecotype, vector prevalence and employ same vector control strategies. In the results, for example, to deltamethrin, the same species registered resistance in 2 Districts (Mandla and Dindori), were tolerant in 6 Districts (Sidhi, Shadol,Balaghat, Betul, Chhindwara and Jhabua) but were still susceptible in 1 district, Guna district.

Another example is a study done in the Greater Accra region of Ghana, involving eight localities. The study reported on the susceptibility of Anopheles gambiae s.s. exposed for 1 hour to the pyrethroid insecticide permethrin and the carbamate insecticide propoxur, in these different areas. The observed mortality rates ranged between 21–92% to permethrin suggesting that permethrin may not be effective in all areas (21). There are similar findings in Kenya too (22).

Much of the literature is silent on handling different resistance or susceptible levels differently. It does not seem to matter what the different levels of susceptibility are but the mechanisms of resistance for one to be able to make a choice of control. However the study from the Greater Accra region of Ghana suggests that where pyrethroid resistance is a problem in different levels, alternative agents like propoxur could be used for indoor residual spraying and for insecticide-treated materials such as curtains and eave screen, while pyrethroids are used where they are effective (21).

Combination LLINs vs Mono-treated LLINs for Pyrethroid resistant areas

One of the latest campaigns in the effort to control malaria vectors in the face of pyrethroid resistance is to use Long Lasting Insecticidal Nets that are laced with a combination of pyrethroids and another agent, for example, carbamates. A recently introduced combination or synergistic LLIN is Permanet 3.0 which has been tested fairly widely in a very short time. PermaNet 3.0 is a long-lasting combination net laced with deltamethrin on the sides and a mixture of deltamethrin and piperonyl butoxide (an oxidase synergist) on the top panel or roof, currently manufactured by Vestergaard Frandsen in Switzerland (23). The addition of piperonyl butoxide to the deltamethrin is expected to increase bioefficacy of these nets. An unpublished report (not peer reviewed) from a survey done by the Ministry of Health in Zambia in 2011 showed high bioefficacy against kdr resistant strains of Anopheles gambiae on the roof compared to the sides of the synergistic nets, following twelve months of field usage (24). The efficacy of the synergistic nets is not in doubt as seen from all the studies done to assess this including that by Vincent Corbel and colleagues and Adeogun et al. (16, 25). However from peer-reviewed work, there are mixed results about the added efficacy and effectiveness of these synergistic nets as compared to the mono-treated nets, on the control of malaria vectors. No systematic reviews were found that have synthesized these findings. However several trials have been done and some of the results of these are presented here.
In an experimental study comparing unwashed and 20 times-washed PermaNet 3.0 and PermaNet 2.0 (a long-lasting insecticidal net incorporating deltamethrin as a single active ingredient), Olyset

Net and a conventional deltamethrin-treated net washed three times conducted in southern Benin where *Anopheles gambiae* are highly resistant to pyrethroids, the unwashed PermaNet 3.0 killed slightly more *Anopheles gambiae* PermaNet 2.0, indicating only partial synergism of resistance (26). However after washing there was significant loss of activity to a similar level, with PermaNet 3.0 and PermaNet 2.0 and the conventional net. Blood-feeding rates were partially inhibited for unwashed PermaNet 3.0 and Olyset Net. Personal protection against *Anopheles gambiae* derived from PermaNet 3.0 was similar to that from PermaNet 2.0 before washing, and after 20 washes it decreased by about 20%. This study found that Permanet 3.0 does not provide a solution to the problem of pyrethroid resistance in an area where it is prevalent.

This is corroborated by a study done by Tungu et al in Tanzania in which with closely similar methods they compared PermaNet 3.0, PermaNet 2.0 and a conventional deltamethrin-treated net using standard WHO Pesticide Evaluation Scheme (WHOPES) procedures (27). The PermaNet arms included unwashed nets and nets washed 20 times. The results showed that when tested against pyrethroid susceptible *Anopheles gambiae*, the unwashed PermaNet 3.0 showed no difference to unwashed PermaNet 2.0 in terms of mortality, but showed differences in blood-feeding rate (3% blood-fed with PermaNet 3.0 versus 10% with PermaNet 2.0). After 20 washes the two products showed no difference in feeding rate but showed small differences in mortality of about 8%. The authors concluded that both PermaNet products were highly effective against susceptible *Anopheles gambiae*, and that the negligible difference in mortality between PermaNet 3.0 and 2.0 against *Anopheles gambiae* either before or after washing would seem unlikely to provide additional control of *Anopheles gambiae* populations. The results seen here could be because the study was done on susceptible species however an experimental hut trial of insecticide treated nets between two adjacent sites with resistant and susceptible populations of Anopheles gambiae assessing the impact of resistance on entomological parameters, showed no apparent difference in the effectiveness of ITNs at the two sites (28).

Adeogun et al on the other hand, in a recent study concluded that there was indeed evidence on the increased efficacy of Permanet 3.0 against malaria vectors with kdr only and kdr plus metabolic-based pyrethroid resistance mechanisms under realistic LLIN use scenarios (29). The researchers in this study carried out a small-scale village trial carried at two localities where malaria vectors were resistant to pyrethroid insecticides and demonstrated that Permant 3.0 was well accepted by nets users and resulted in 8–11% and 34–37% reductions in blood feeding relative to the Olyset and the untreated control respectively. *Anopheles gambiae s.s*. mortality was also greater for PermaNetN 3.0 compared to the Olyset nets by 20%. It should be noted that the study was a small scale study and would need larger scale studies to reinforce or confirm these findings.

Bingham et al. carried out a study to assesss the bioefficacy of deltamethrin versus delatmethrin synergized by PBO against strains of Aedes aegypti (30). They showed that in the resistant strains deltamethrin alone showed limited bioefficacy and the addition of piperonyl butoxide significantly increased the bioefficacy. It is however not clear whether these results can be directly extrapolated to a region that is mainly infested with *gambiae* and *funestus* species, and also to real life settings as this was a laboratory study, warranting more research in the given fields.

Corbel and colleagues carried out a multi centre experimental hut trial in Benin, Burkina Faso and Cameroon to evaluate the performances of the new mosaic long-lasting insecticidal PermaNet 3.0, against wild pyrethroid-resistant *Anopheles gambiae* s.l. in West and Central Africa, which is prevalent with pyrethroid-resistance in malaria vectors (16). They found the personal protection and insecticidal activity of PermaNet 3.0 and PermaNet 2.0 were both excellent in the pyrethroid-tolerant area in Benin. In the pyrethroid-resistance areas of Cameroon and Burkina Faso, PermaNet 3.0 showed equal or better performances than PermaNet 2.0. although it should be noted that the deltamethrin content on PermaNet 3.0 was up to twice higher than that of PermaNet 2.0. Significant reduction of efficacy of both LLIN was noted after 20 washes. The authors concluded that although the use of combination nets for malaria control offers promising prospects, more investigations were needed to demonstrate the benefits of using PermaNet 3.0 for the control of pyrethroid resistant mosquito populations in Africa, over the current recommended Permanet 2.0.

Killeen et al. carried out a study using mathematical modeling to estimate the effects of PermaNet 3.0 versus the monotreated net, PermaNet 2.0 on malaria transmission levels (17). Experimental hut trials were done in Vietnam, Cameroon, Burkina Faso and Benin to investigate the effect of each net type on entomological inoculation rates. Results showed that direct estimates of mean personal protection against insecticide-resistant vectors in all four countries revealed no clear advantage for combination LLINs over deltamethrin-only LLINs unless both types of nets were extensively washed. However, simulations of impact at high coverage (80% use) predicted consistently better impact for the combination net across all four sites, regardless of whether the nets were washed or not. The degree of advantage obtained with the combination varied substantially between sites and their associated resistant vector populations. This is promising but at the current coverage rates in Uganda it leaves the responsibility of first increasing the coverage rates which are currently estimated to stand at about 47%, that is, of households owning an ITN (31) although according to the 2006 demographic and health survey this was at 22% (32). This is not to mention that actual use of these nets brings these percentages even lower (31).

Clearly there is need for more research in this area. This has been acknowledged by a review done by Hillary Ranson and colleagues who have tried to do a comprehensive literature review to answer the question on what implications pyrethroid resistance in African anopheline mosquitoes has for malaria control. They recommend further investigation to assess the impact of pyrethroid resistance on vector control effectiveness (33).

Additional options

It is of concern that continued use of insecticide treated nets would further exacerbate pyrethroid resistance and worsen the resistance situation (4). What is expected or anticipated is that personal protection might be maintained despite resistance but at the expense of community protection. However authorities' choices are limited and there is a need to work fast on other options. There are other recommendations for countries in addition to current efforts:

- To strengthen insecticide resistance monitoring as a component of the national malaria control plans.
- To fill gaps in the current knowledge of resistance in malaria vectors (distribution, mechanisms involved) and to start testing susceptibility to insecticides other than DDT and pyrethroids. Examples of these include carbamates and organophosphates.
- To select vector control interventions and insecticides taking into account, among other important factors, the resistance status of local vector populations.
- To ensure continuous resistance monitoring.
- To adopt insecticide resistance management as part of national policies for vector control.

In addition to these, the recently launched Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM) has outlined five activities or pillars that should be considered over the short, medium and long term, by countries and others at the global level (2). The GPIRM that was developed in response to requests from the World Health Assembly and the Roll Back Malaria program lays out a comprehensive strategy including short term action plans with clear responsibilities and even research priorities in a bid to fill gaps in knowledge on mechanisms of insecticide resistance and the impact of current insecticide resistance management strategies.

Conclusion

Michael Okia's report on pyrethroid resistance in Uganda is in keeping with research and findings from the region and even beyond, documenting reduced susceptibility to LLINs of *Anopheles gambiae*. The literature however suggests that identification of the mechanism of resistance would be helpful in determining the most effective method to be used in the face of resistance. The current literature is still mixed with much of it failing to show a significant difference in effectiveness between synergistic and mono-treated insecticide nets, further studies would be required to establish this extra benefit for policy change. In addition, it is recommended that regular monitoring of the resistance and susceptibility trends is done as these are likely to continue changing especially with widespread use of LLINs and other factors like use in Agriculture.

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None known.

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