High level of resistance in the mosquito Anopheles arabiensis to pyrethroid insecticides from low malaria transmission zone of Moroto district, Karamoja region, Uganda: Implication for malaria vector control

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Richard Echodu
Gulu University Bioscience Research Laboratories

Juliet Anena
Gulu University

Tereza Iwiru
Gulu University

Paul Mireji
Kenya Agricultural and Livestock Research Organization

Geoffrey Maxwell Malinga
Gulu University Faculty of Science

Elizabeth A. Opiyo
Gulu University Faculty of Science

Julius Iga
Gulu University Faculty of Science

Onanyang David
Gulu University Faculty of Science

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Abstract
Background Karamoja region of Uganda previously classified as low malaria transmission zone is currently experiencing significant upsurge of malaria incidences. Long lasting insecticidal nets (LLINs) impregnated with pyrethroids constitute a major tool for malaria control in this region. Efficacy of this tool can be hampered by resistance to the pyrethroids in the Anopheles mosquito vectors. Resistance status of these mosquitoes in this region is poorly understood, effectively hampering better understanding of the impact of LLINs in the malaria control initiative. Here, we assessed susceptibility of the Anopheles arabiensis from the region to deltamethrin, permethrin (pyrethroids) and pirimiphos-methyl (organophosphate) insecticides. Method We collected anopheline mosquito larvae from their natural habitats and reared them to adult emergence in situ field insectary in Karamoja region. We then identified them morphological to species level and exposed 513 emerge adult female An gambiae s.l., mosquitoes to diagnostic dosages of deltamethrin (0.05%), permethrin (0.75%) and pirimiphos-methyl (0.25%) pyrethroids exposure using the standard WHO insecticide susceptibility test assay. Synergic assays using piperonyl butoxide (PBO) were done to check for the involvement of detoxification enzymes in pyrethroid resistant populations. We then screened for knockdown resistance (KDR) and mosquito species diversity using Polymerase Chain Reaction (PCR). Results Majority (96%) of the mosquitoes we sampled were identified as An. arabiensis and 4% as An. gambiae sensu stricto. We observed cross-resistance to both deltamethrin (11.9%) and permethrin (47%) but susceptibility (100% mortality) to pirimiphos-methyl in An. arabiensis. The pre-exposure to PBO ameliorated the resistance to both pyrethroids. We detected homozygous KDR -eastern variant in 1.8 and 50% of the An. arabiensis and An. gambiae s.s. respectively. Conclusion Anopheles arabiensis and An. gambiae s.s. are the malaria vector in Karamoja region with An. arabiensis predominating. Both species are susceptible to pirimiphos-methyl but resistant to both deltamethrin and permethrin, through a metabolic process (phenotype). Mosquitoes with genetic (kdr) mutations for resistance were minimal and hence have minimal contribution to the pyrethroid resistance profile. An. arabiensis can thus be controled in Karamoja region using deltamethrin and/or permethrin impregnated mosquito nets integrated with PBO and/or through indoor residual spraying of sprayable human
dwellings with pirimiphos-methyl.

Background
Malaria is a leading cause of morbidity and mortality in the tropics despite reduction in global malaria burden [1, 2] The decline has been attributed to use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), introduction of artemisin-based combination therapy (ACT) for malaria treatment and intermittent preventive treatment (IPTp) during pregnancy [2]. Despite these significant gains, long-term effectiveness of malaria vector controls using LLINs and IRS is currently being challenged by widespread insecticide resistance in mosquito populations [3, 4]. World Health Organization (WHO) recommends expansion of insecticide resistance monitoring and surveillance within national and regional control programs [5]. This initiative can 1) provide insights into mechanisms that can drive resistance, 2) characterize effectiveness of control efforts and 3) identify causes of any failures within the controlled areas for effective implementation and sustainable vector control [6, 7] WHO recommend organochlorines, organophosphates, pyrethroids and carbamates for control of malaria vectors, among which pyrethroids are the only WHO prequalified insecticides for LLINs, and are also extensively used for IRS due to their low cost, human safety and long duration of residual action [5]. The LLINs and IRS are widely promoted and implemented as public health intervention tool for malaria control in most malaria-endemic countries including Uganda [8]. The LLINs is selectively implemented in the different regions, with an average operational coverage of over 95% [9]. The control programmes are concentrated in high/medium transmission zones while low malaria transmission zones, including Karamoja region have largely been neglected [10, 11]. Despite extensive LLINs implementation, over the recent years Karamoja experienced significant (> 60%) malaria incidences between 2015 to 2017 [9, 12] with Moroto district registering 334.5 cases per 1000 children under 5 years [13]. This translates into about 33 % disease prevalence and may be linked to factors such as pyrethroid resistance that might be impeding efficacy of the LLINs

Pyrethroids exert their insecticidal effect on the voltage-gated sodium channel (VGSC) located on the membrane of neurons [14]. When pyrethroids bind an open channel, they block its closure, thus
extending the action potential and resulting in the insect’s rapid paralysis, a phenomenon known as “knockdown” (kdr) [15]. However, mosquito resistance to pyrethroids is an emerging challenge to efficacy of otherwise successful insecticide-treated nets (ITN) based malaria control intervention against adult vector [16]. This is particularly of great concern to sub-Saharan Africa, with high malaria transmission levels and major vectors (An. funestus, An. gambiae s.s. and An. arabiensis) resistant to pyrethroids (Mnzava et al., 2015) [17]. The resistance is mediated through knockdown resistance (kdr) mutations, enhanced detoxification of pyrethroids by the mosquito enzymes (esterases, monooxygenases and glutathione S-transferases [3, 18] and probably other unknown mechanisms such as behavioral and penetration [19, 20] known to occur in other vectors [21, 22]. This present study was initiated to establish distribution of major malaria vectors and their resistance status to deltamethrin, permethrin (pyrethroids) and pirimiphos-methyl (organophosphate) insecticides in Karamoja region of Uganda. Our findings, reported herein, will contribute to designing suitable control interventions and improve implementation of resistance management strategies across Karamoja region of Uganda.

Methods

Study area

Karamoja lies between 1°30′ to 4° 06′ N and 33° 30′ to 35° covering an area of 29,430 km² forming the north-eastern part of Uganda with a human population of about 1.1million [23]. This region encompasses Abim, Amudat, Kaabong, Karenga, Kotido, Moroto, Nabilatuk, Napak and Nakapiripit districts. The region has 53.8 to 63.5% Human Poverty Indices (HPI) compared to 37.5 % Uganda national average [24]. Most of the population are nomadic pastoralist. The region is characterized by savannah woodlands and semi-desert vegetation with 1200 and 1500m plateaus and Kadam, Akisim and Napak Mountains and Moroto mountain (about 3083m above sea level) [25]. The region is drained by Turkwel River, part of internal drainage basin of Lake Turkana in Kenya. Karamoja experiences only one rain season which occurs from May to July. The mean annual rainfall level is between 350 and 750 mm [26]. Ambient temperatures range from 16ºC to 30ºC. The species of Anopheles mosquitoes in the and Anopheles gambiae sensu stricto Giles 1902 [27]. Malaria
transmission in the region is absolutely (100%) attributed to *Plasmodium falciparum* parasite [28]. Malaria prevalence in the region has increased by 30% with average incidence ranging from 166 in 2015 to 295 in 2018 per 1000 people [9]. The LLINs which is the only malaria vector control strategy employed in the region with 57% households owning more than one ITN with ratio of use and access of 0.95 [11, 29] This indicate low ratio of people sleeping inside nets.

**Mosquito collections**

We collected mosquito larvae stages 3, 4 and pupae from their aquatic breeding habitats in Moroto (N02°32.0′ E34°40.0′) in the rainy month of July 2019 using dipping technique. The sites included brick pits, puddles, road side drains and excavations, sand pits and open gardens. Immediately after sampling, the larvae were transferred to a field insectary where they were maintained at a density of 500 larvae per three liters in their natural water that contained dissolved natural foods. We kept the emergent adult mosquitoes in standard 30×30×30 cm cages in under the ambient insectary environmental conditions. We identified *An. gambiae s. l* among emerged mosquito adults using morphological keys [30] offered them 10% glucose solution *ad libitum* and tested the females for insecticide resistance 2-5 days post emergence. We obtained ethical clearance for mosquito collection from Uganda National Council for Science and Technology (authorization No SS 4610).

**Insecticide susceptibility bioassay**

We conducted insecticide susceptibility tests on 2-5 days old non-fed adult female *An. gambiae s.l.* mosquitoes using the standard WHO tube bioassay protocol for assessing potential insecticide resistance [31] at Gulu University mosquitoes insectary. To simulate natural conditions, we maintained an average temperature in the insectary at 29 ± 2°C (day) and 24 ± 2°C (night), with relative humidity (RH) ranging from 57 to 70 and a photoperiod of 12:12 h (L–D). Our overall maintenance of the colony followed standard operating procedure for rearing *Anopheles* mosquitoes [32]. We exposed 492 female *An. arabiensis* to 0.05% deltamethrin, 0.75% permethrin or 0.25% pirimiphos-methyl concentrations on insecticide-impregnated papers or control (oil-treated) (Vector Control Research Unit, Universiti Sains Malaysia) for 60 minutes. During this exposure duration, we recorded the number of mosquitoes knocked-down at 10, 15, 20, 30, 40, 50 and 60 minutes intervals.
post exposure. After the exposure period, mosquitoes were transferred to holding tubes and maintained on 10% glucose solution. The final mortalities were determined after 24 h post exposure. After the bioassays, we collected dead (susceptible) and alive (resistant) mosquitoes, and stored them individually in separate Eppendorf tubes with silica gel for subsequent molecular laboratory analysis.

**Evaluation of potential impact metabolism on resistance to the pyrethroids in the mosquitoes**

To evaluate if metabolism of the pyrethroids by the mosquitoes was responsible for the insecticide resistance we observed, we assessed relative mortality of the mosquitoes exposed to the pyrethroids with or without piperonyl butoxide (PBO) that prevents pyrethroid catabolism by Cytochrome P450 oxidase that can detoxify the active ingredient before an insecticidal effect can occur [33]. This inhibition produces a synergistic effect [34]. Briefly, we assessed this effect using WHO protocol [31] where we pre-exposed adult female *An. gambiae* s.l to WHO papers impregnated with 4% PBO an oxidase inhibitor for 1 hour. This was followed immediately with exposure to discriminating doses of 0.05% deltamethrin and 0.75% permethrin on impregnated papers for 60 minutes. Five replicated were performed for each exposure set. We recorded the knock-down rates at 10, 15, 20, 30, 40, 50- and 60-minutes intervals during the one-hour exposure to synergist and to insecticides (deltamethrin or permethrin). We immediately assessed the resultant mortality of the flies from the three treatments (PBO + deltamethrin or permethrin exposed or unexposed mosquitoes). The live mosquitoes were fed on 10% glucose solution. Mortality rates from assays conducted with and without exposure to PBO (synergist) were scored after 24 hours of exposure. Mortality rates were compared between PBO deltamethrin or permethrin exposed and unexposed mosquitoes. Resistance was also compared with pirimiphos-methyl to ascertain which test mosquitoes were fully susceptible. All mosquitoes tested were identified to species level by PCR as described in mosquitoes species identification subsection below.

**PCR identification of members of *An. gambiae* s.l and *An. funestus* s.l.**

We identified our mosquitoes (post exposure) to their *An. gambiae* s.l sibling species status using PCR
methods of [35], and for members of the *An. funestus s.l.* group using a method of Koekemoer et al., [36]. We extracted total genomic DNA (gDNA) from whole flies using the DNeasy blood and tissue kits (Qiagen, Valencia, CA) following the manufacturer’s protocols and assessed the quality of the DNA on 1.5% agarose gel as visualized on Gel Doc Imaging System (UVITEC, Cambridge). Our primers included those specific to *An. gambiae s.s, An. arabiensis* as well as the universal *An. gambiae s.l.* complex primer [35]. Similarly, specific primers for *An. funestus s.s.* and universal primer for the *An. funestus* group were also used for the *An. funestus s.l.* [36]. In the PCR reaction, we amplified 2.5µl gDNA with 1 unit of GoTaq Green Master Mix (Promega, Madison, MO) in the buffer in a total volume 12.5 µl. For *An. funestus complex*, conditions remained the same except that we added 0.5 mM of MgCl₂. We run the reactions in touch screen thermal cycler (SimpliAmp, Applied Biosystems, Life Technologies, Singapore). The first cycle included five minutes at 95°C, 30 seconds at 50°C, and 30 seconds at 72°C. Subsequent cycles involved 1 minute at 94°C, 30 seconds at 50°C, and 1 minute at 72 °C for 30 cycles. We also run positive and no-sample negative controls. We loaded The PCR products onto 1X SYBR safe (Invitrogen, 5791 Allen Way Carlsbad CA 92008, USA) 2 % agarose gels (AppliChem GmbH Ottoweg Damstadt Germany) in a TBE (40mM Tris-HCl pH 8.3, 45 mM boric acid, and 1mM EDTA) buffer and run a 100 bp DNA ladder molecular weight marker (Life Technologies, Rockville, MD) to confirm expected molecular weights of the amplification products. We documented our PCR products using GelDoc Imaging System (UVITEC, Cambridge).

**Detection of East African kdr resistance mutations in An. gambiae s.l.**

We also used the methods described by Ranson et al., [37] to detect single base pair specific single nucleotide polymorphisms (SNPs) leucine to serine substition TTA/TCA mutation in the voltage-gated sodium channel known as knockdown resistance to DDT and pyrethroids in East Africa. Briefly, we amplified 5 µl gDNA, 0.2 µM of the specific primers (AgD1, AgD2, AgD4 and AgD5) with 1 unit of GoTaq Green Master Mix (Promega, Madison, MO) in the buffer in a total volume 25µl. We run the reactions in touch screen thermal cycler (SimpliAmp, Applied Biosystems, Life Technologies, Singapore). We used touch down PCR conditions with the initial denaturation at 95°C for 5 minutes followed by 10 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 30 seconds and
extension of 72°C for 30 seconds. This was followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 47°C for 30 seconds and extension at 72°C for 30 seconds with the final extension of 72°C for 10 minutes and holding at 4°C until when collected. We also run positive and no-sample negative controls. PCR products analysis was carried out as described in mosquito species identification subsection above.

Data analysis

We corrected the knockdown rates for testing the toxicity of each insecticide by Abbott’s formula [38] and then transformed them to Probits [38] for linear regression analysis and the determination of 50% knockdown (KD$_{50}$). For Probit analysis, we used GraphPad Prism version 7.00 for Mac (GraphPad Software, La Jolla California USA). We used weighted mean summarize knockdown due to different insecticides, and adopted WHO criteria of interpretation of our results. Consequently, we considered mosquito population to be 1) resistant (confirmed) to a particular insecticide if mortality rate was 0-79%, 2) resistant suspected if mortality was 80-97% and more investigations are required, and 3) susceptible when the mortality was 98-100%.

Data for the metabolic tests were summarized as mean percent mortality of the five replicates, and the 95% confidence intervals were calculated. The final mortality observed 24-hour post-exposure was compared between samples with and without pre-exposure to PBO, using paired sample t-test. Pearson’s chi-square was used to evaluate the association of kdr frequency with WHO assay results. All statistical analyses were performed in SPSS v25 with level of significance set at $\alpha = 0.05$.

Results

Susceptibility of An. arabiensis to pyrethroids

Results of bioassay of adult female An. gambiae s.l. (N= 513) for susceptibility to the three insecticides are summarized in Figure 1 and Table 1. Based on WHO criteria, [31], resistance (< 95% mortality) of An. arabiensis to deltamethrin and permethrin was observed in all the samples, with mortality varying from 12% to 91% in Moroto (Table 2). Susceptibility of An. arabiensis to pirimiphos-methyl was observed in Moroto (Table 2).

Impact of PBO on toxicity permethrin and deltamethrin to An. arabiensis
An hour pre-exposure of *An. arabiensis* to PBO enhanced toxicity of deltamethrin (from 11.8 to 90%) or permethrin (from 47 to 89.2%) in Moroto, compared to cohorts directly exposed to each of the two candidate insecticides without PBO pre-exposure (Figure 1), suggesting potential cytochrome P450 oxidase mediated resistance [39] to these insecticides in the mosquito. However, our observations that the pre-exposure to PBO partially abolished resistance to deltamethrin and permethrin in Moroto still suggests partial role of the cytochrome P450 oxidase in the resistance phenotype while other mechanisms might also play a role (Figure 1 and Table 2).

In the absence of PBO, the LT$_{50}$ for deltamethrin and permethrin on *An. arabiensis* were similar (Table 3 and Figure 3). However, the PBO significantly reduced the LT$_{50}$ for both deltamethrin and permethrin on *An. arabiensis* relative to their respective native formulations (without PBO). Additionally, incorporation of PBO reduced the LT$_{50}$ in permethrin more than it did with deltamethrin (Table 3).

**An. gambiae s.l. species identification**

Our subsequent PCR of sibling species on most of the mosquitoes (N = 342) post exposure revealed that most of the mosquitoes were *An. arabiensis* (96%) with the rest being *An. gambiae* s.s. (4%). No *An. funestus* species was detected.

Table 1: Knockdown summary during 60 minutes exposure

| Insecticide Tested | No. of Knocked down Mosquitoes | Total Exposed | KT50 (min) | KD60 (%) |
|--------------------|--------------------------------|---------------|------------|----------|
|                    | 10 | 15 | 20 | 30 | 40 | 50 | 60 |                |                     |
| Pirimiphos-methyl  | 0  | 1  | 3  | 9  | 15 | 30 | 56 | 110           | 60 | 51 |
| Deltamethrin       | 1  | 3  | 11 | 25 | 51 | 79 | 84 | 101           | 40 | 83 |
| Deltamethrin PBO   | 23 | 63 | 76 | 90 | 96 | 10 | 10 | 100           | 15 | 100 |
| Permethrin         | 2  | 12 | 18 | 45 | 65 | 89 | 95 | 100           | 40 | 95 |
| Permethrin PBO     | 34 | 86 | 94 | 95 | 98 | 10 | 1 |

*KT50*: Time after which 50% of the Anopheles tested are knocked down.

*KD60*: Proportion of Anopheles knocked down after 60 minutes.
Table 2: Mean percentage mortalities following exposure of 2-5 day old *An. arabiensis* to pirimiphos-methyl, deltamethrin and permethrin. Mortalities were recorded 24 hours post exposure (for 60-minutes)

| Insecticide tested | EXPOSED Mortality Total Exposed | % Mortality in exposed | CONTROL Mortality Total Exposed | % Mortality in control |
|--------------------|---------------------------------|------------------------|---------------------------------|------------------------|
| Pirimiphos-methyl  | 110                             | 110                    | 100                             | 00                     |
| Deltamethrin       | 12                              | 101                    | 12                              | 00                     |
| Deltamethrin PBO   | 90                              | 100                    | 90                              | 00                     |
| Permethrin         | 47                              | 100                    | 47                              | 00                     |
| Permethrin PBO     | 93                              | 102                    | 91                              | 00                     |

Table 3: Median Lethal Time for knockdown in Adult female *An. arabiensis* mosquitoes (from Moroto district, Uganda) by various formulations of pyrethroids.

| Pyrethroid     | PBO | LT$_{50}$, Min | 95% CI            | Slope ($\beta \pm SE$) |
|----------------|-----|----------------|-------------------|------------------------|
| Deltamethrin   | -   | 39.13          | 34.03 - 70.73     | 4.18 ± 0.90            |
|                | +   | 13.44          | 11.95 - 15.24     | 3.43 ± 0.46            |
| Permethrin     | -   | 37.02          | 30.69 - 63.59     | 2.73 ± 0.43            |
|                | +   | 11.03          | 10.47 - 11.67     | 6.09 ± 0.62            |

PBO - Piperonyl Butoxide, (+) - with PBO, (-) - Without PBO, CI- Confidence Interval, LT - Median lethal Time.

We have summarized our distribution of L1014S mutations from genotyping of *An. arabiensis* (n=328) and *An. gambiae* s.s. (15) in Table 4. Of the 328 *An. arabiensis* tested for kdr east allele, 66.2% (n=217) were homozygous for wild type alleles (SS) and 1.2% (n=4) were homozygous for mutation alleles (RR). Of the 15 *An. gambiae* s.s., 33.3% (n=5) were homozygous for the susceptible wild type (SS), 13.3% (n=2) were homozygote mutation alleles (L1014S) and only 6.7% (n=1) was heterozygous (HR).

Table 4: KDR allele frequencies
| Mosquitoes species | Insecticide | Survival status after exposure | # mosquitoes tested | Homozygote mutation (RR) | Heterozygote mutation (RS) | Homozygote wild type (SS) |
|-------------------|-------------|--------------------------------|---------------------|--------------------------|---------------------------|--------------------------|
| An. arabiensis    | Deltamethrin + PBO Exposed | Dead                   | 55                  | 1                        | 0                         | 39                       |
| An. arabiensis    | Deltamethrin + PBO Exposed | Dead                   | 4                   | 0                        | 0                         | 3                       |
| An. gambiae s.s.  | Deltamethrin + PBO Exposed | Live                   | 81                  | 1                        | 0                         | 76                       |
| An. arabiensis    | Deltamethrin + PBO Exposed | Live                   | 2                   | 1                        | 0                         | 0                       |
| An. arabiensis    | Permethrine + PBO Exposed | Dead                   | 110                 | 0                        | 0                         | 80                       |
| An. gambiae s.s.  | Permethrine + PBO Exposed | Dead                   | 3                   | 0                        | 1                         | 2                       |
| An. arabiensis    | Permethrin               | Live                   | 40                  |                          |                           |                          |
| An. gambiae s.s.  | Permethrin               | Live                   | 1                   |                          |                           |                          |
| An. arabiensis    | Primiphos methyl         | Dead                   | 42                  | 2                        | 0                         | 22                       |
| An. gambiae s.s.  | Primiphos methyl         | Dead                   | 4                   | 1                        | 0                         | 0                       |
| An. arabiensis    | Primiphos methyl         | Live                   | 0                   | 0                        | 0                         | 0                       |
| An. gambiae s.s.  | Primiphos methyl         | Live                   | 0                   | 0                        | 0                         | 0                       |

KDR - knockdown resistance, PBO – Piperonyl Butoxide, # - number

**Discussions**

We report the first results of resistance in *An. arabiensis* against pyrethroid in Karamoja region. We established *An. arabiensis* as the major major malaria vector in Karamoja areas followed by *An. gambiae s.s.* These two vectors have different genetics and behavior that can have important implications for the epidemiology of malaria and their control. The current use of LLIN in Karamoja region for malaria vector control might be less effective against *An. arabiensis*. This is mainly due to resistance of the mosquito to the insecticides (deltamethrin and permethrin), more exophagic, exophilic and zoophilic nature of *An. arabiensis*.

*An. arabiensis* is being less killed by they two insecticides deltamethrin and permethrin commonly used for the treatment of LLIN. Similar studies have been reported in Kenya and Tanzania [40, 41] This happens as a result of the more exophagic, exophilic and zoophilic tendencies of *An. arabiensis* compared to *An. gambiae s.s.* behaviors. The exophagic and exophilic nature of *An. arabiensis* similarly can affect the performance of IRS since these mosquitoes tend to rest and feed outdoors.

In terms of malaria epidemiology, because of the exophagic and exophilic nature of *An. arabiensis*,
this increases the outdoor biting and resting of these mosquitoes leading to high malaria transmission in the area which concedes with what is currently seen in Karamoja region. Besides, An. arabiensis, bite early evening or morning when the population is not protected by LLIN potentially sustaining residual malaria in area with high coverage of net [42, 43] Additionally communities in Karamoja region thrive on pastoralism looking for pasture and water for their livestock which pre-expose them to outdoors activities when keeping the livestock and increasing the chances of more outdoor mosquitoes’ bites and thus more malaria in the area.

At present, we confirm existence of both mechanisms of insecticide resistances: knock down resistance (kdr) and metabolic resistance due to elevated levels of detoxifying enzymes in the An. arabiensis in Karamoja region. Future planned roll out of LLINs enhanced with PBO might not be a magic bullet since more than one resistance mechanisms were observed in An. gambiae s.l. in Karamonja region.

While the two pyrethroids seems to be having similar performance, PBO significantly enhances the performance of both deltamethrin and permethrin and further enhances that of permethrin over that of deltamethrin (Table 3). This suggests that permethrin incorporated with PBO would have a much better performance in addressing the issues of pyrethroids resistance in Moroto than deltamethrin. These insecticide resistances could as well have important implications for the epidemiology of malaria as well as malaria vector control in the low malaria transmission zones of Karamoja areas.

Pyrethroids are the only class of insecticides used in LLINs currently recommended by the World Health Organization. Pyrethroid resistance of malaria vectors is widespread in Africa as well as other classes of insecticides [3, 31, 44] Increased resistance has been attributed to selection pressure from the scale-up of LLINs and IRS [3] and use of similar classes of insecticides in agriculture [45], although the relative contribution of these mechanisms varies by area [46].

Given that resistance has been reported in An. gambiae s.s and An. funestus [47– 50] that are the major malaria vector in Uganda and now An. arabiensis in this current study in Karamoja region, this is a major threat to LLINs and IRS use in malaria vector control programmes in the country and the whole of the neighbouring countries. This appears to reflect the need to monitor malaria vector
resistance.
Currently, WHO guidelines recommend combining ITNs and IRS in various transmission settings, especially in areas with holoendemic and epidemic malaria [6]. LLINs and IRS could be employed together in the same households in Karamoja region. Besides, insecticide resistance management should rely on tactical deployment of the active ingredients used for IRS and on LLINs in rotation, combinations (particularly LLINs), mosaics and mixtures as recommended in other studies [6]. What we observed would be important is, if LLINs are to be combined with IRS for malaria prevention and control, the selection of appropriate LLIN types and design should put in consideration the housing structures and cultural issues concerning in-house sleeping patterns and IRS chemicals should be done with caution to avoid further exacerbating existing resistance.

**Implication for future malaria vector control**

Nevertheless, the increasing intensity of resistance currently seen in *An. arabiensis* mosquito populations in Karamoja region will reduce the efficacy of pyrethroid-based interventions. The right course of action for malaria vector control program is therefore to adopt a proactive approach and modify current practices so as to delay the spread of resistance and preserve the effectiveness of deltamethrin and permethrin insecticides.

The high frequencies or intensities of resistance can lead to failure of IRS and can thereby have an epidemiologically significant effect on malaria incidence [51]. There is also cross-resistance between different classes of insecticides that share the same mode of action. The existence of cross-resistance and multiple resistance restricts the choice of alternative insecticides in situations where resistance has been detected. Besides, the impact of the observed spread of resistance will have effectiveness of current vector-control programmes. Therefore, a key element of effective resistance management is the use of alternations, rotations, or sequences of different insecticide mode of action classes. Besides other studies have shown that even in the presence of pyrethroid resistance, insecticide- treated mosquito nets (ITNs) perform better than untreated nets in terms of protection against blood-feeding, and ITNs can induce significant mosquito mortality [4].

We also recommend the use of Larval Source Management (LSM) in order to prevent the completion
of development of the immature stages. Mosquitoes breeding sites in Karamoja region can easily be mapped out during the dry season. LSM can be done through 1) Larviciding; the regular application of biological or chemical insecticides to water bodies and 2) biological control: the introduction of natural predators into water bodies.

**Limitations**

We acknowledge the limitations of the current study including:

The time constraints of conducting this research during. The sites sampled were predominantly suited for *gambiae* s.l breeding, thus findings don’t rule out possibility existence of *An. funestus* s.l in the area.

**List Of Abbreviations**

IRS: indoor residual spraying

LLINs: long lasting insecticide-treated nets

WHO: World Health Organization

KDR: knockdown resistance

PCR: Polymerase chain reaction assay

PBO: Piperonyl butoxide

MFOs: mixed-function oxidases

**Declarations**

**Authors’ contributions**

RE, JI and DO conceived, contributed design of the study, field collections, performed laboratory work, analyzed the data, and drafted an initial version of the manuscript. JA, PM, GMM and TI performed laboratory work and analyzed the data. EAO conceived, designed the study, coordinated fieldwork and provided guidance. All authors read and approved the final manuscript.

**Author details**

1Faculty of Science, Gulu University, P. O. Box 166,Gulu, Uganda,

2Gulu University Biosciences Research Laboratories, P. O. Box 166,Gulu, Uganda.

3Department of Biochemistry, Biotechnology Research Institute - Kenya Agricultural and Livestock Research Organization, Kikuyu, Kenya
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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The authors declare that all the main data supporting the findings of this study are available within the article. Any additional data sets are available from the corresponding author upon reasonable request.

Consent for publication
Not applicable.

Ethics approval and consent to participate
This study was approved by Gulu University Ethical Review Committee. Formal approval to conduct the study was granted by the Uganda National Council for Science and Technology and the Office of the Ugandan president (SS4610). Community leaders in Moroto district provided written informed consent.

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Additional File
Additional file 1: KDR Allele frequencies

Figures
Figure 1

Percentage of An. arabiensis knocked down during 60-minutes exposure to deltamethrin or permethrin and PBO using WHO tube assay in Moroto District in Uganda, July 2019 (Larval collections)
Figure 2

Percentage of An. arabiensis knocked down during 60-minutes exposure to pirimiphos-methyl, deltamethrin or permethrin using WHO tube assay in Moroto District in Uganda, July 2019 (Larval collections)
Figure 3

Temporal (log10 minutes) percentage knockdown of Adult female An. arabiensis mosquitoes
(from Moroto district, Uganda) by various formulations of pyrethroids.

Supplementary Files
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