Effectiveness of Transfluthrin-impregnated Insecticide (Paper Rambo) and Mechanical Screening Against Culicine and Anopheline Mosquito Vectors in Kumbotso, Kano, Nigeria

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Abstract The level of personal protection acquired from the use of mechanical screening of windows and doors alone or the screening in compliment with burning transfluthrin- impregnated paper was assessed. The specific objectives were to compare the indoor resting densities, and man biting rates of the Culicine and Anopheline mosquitoes, with and without the interventions. Pyrethrum spray collection (PSC) technique was used to collect mosquitoes from 30 randomly selected houses, 10 each from the two interventions and control areas, monthly, between May, 2010 to April 2011. The Culicine and Anopheline species were sorted morphologically and An. gambiae s.l were identified to sibling species by PCR. A total of 1592 Cx. quinquefasciatus were collected from the houses, out of which 57.2%, 27.6%, and 15.2% were from the control, screened only and the screened houses in which transfluthrin-impregnated papers were used, respectively. Statistically higher number of Anopheline mosquitoes, 363 were collected from the control houses compared to 49 from the screened and 40 from the screened-transfluthrin treated houses, χ²(2,N=452)=68.66 p<0.05. The indoor resting density was 33.6 in the control compared to 2.7 in the screened and 3.0 in the screened-transfluthrin treated houses while the number of female Anopheline per person was 6.7, 1.3 and 1.0 respectively. An.gambiae ss, and An. arabiensis were identified by PCR. Mechanical screening compares favorably with conventional use of treated impregnated fabrics for personal protection against mosquitoes.

Keywords Personal protection; Transfluthrin; Impregnation; Mechanical-screening; Anopheline; Culicine

Introduction

Despite the decrease in global mortality (42%) due to malaria (World Health Organisation, 2013), the disease kills 627,000 individuals in 2012 alone, mostly children. Control of malarial vectors relies greatly on the use of insecticide formulations, for wide-coverage as in Insecticide Treated Nets (ITNs) (World Health Organisation, 2013), Long-Lasting Insecticide Treated Nets (LLINs) and indoor residual spraying (IRS) (Hemingway, 2014). However, pyrethroids are the safest insecticides and the only class fully approved by WHO for impregnation of LLINs and ITNs (WHO, 2013). ITN repels or kills mosquitoes coming into contact with insecticide on its netting material (World Health Organization, 2007), and is increasingly deployed into WHO malaria region for universal coverage, with community-wide benefits established (Killeen et al., 2007). LLINs have been developed because they last longer, for its netting material has insecticide incorporated within or bound around its fibers been associated with reduced prevalence (51%) of P. falciparum infection (Clarke et al., 2001). Screening of windows can also be very effective in keeping out mosquitoes, provided the screening is without tears (Curtis, 1989). Insects repellents are also used to deter biting mosquitoes, especially N, N-diethyl-meta-toluamide (DEET) which
Transfluthrin is a pyrethroid similar to the compounds used on nets but evaporates at much lower temperature. The active ingredient at doses of between 0.02 – 0.04% is used preferentially for development of innovative anti-mosquito products such as indoor strips, coils and vaporizers which work at room temperature (WHO, 2002) www.who/WHOOPES/transfluthrin. As a pyrethroid specified for use in public health (http://www.who.int/whopes/quality/newspecif/en/) the effectiveness of transfluthrin to protect against biting mosquito has been shown in India and Tanzania (Sharma et. al. 1993; Pates, 2003). Recently, in a lab setting it was shown that transfluthrin as a vapour-phase special repellent, deter An. arabiensis mosquitoes (Ogoma et al., 2012). Rambo insecticide paper contains 0.45% transfluthrin and 2.5% essential oil and the product is used widely for personal protection against mosquitoes in both urban and rural communities within Kano State, and other places in Nigeria. Also common in the area, is the use of metal wire mesh to screen windows and doors a “mechanical screening” to prevent mosquitoes from entering bed rooms. However, the effectiveness of these personal protection measures on malaria transmission is yet to be established in Kano. Here, we conducted a household, randomized, controlled trial to compare the effectiveness of Paper Rambo insecticide with mechanical screening of houses, on malaria incidence and mosquito density. Specifically, the efficacy of the mechanical screening alone and in combination with insecticide Paper Rambo on indoor resting densities and man biting rates of populations of Cx. quinquefasciatus, An. funestus and An. gambiae s.l. in Panshekara town, of Kano state, was investigated.

1 Methods and Materials
1.1 Study area
The study was conducted at Panshekara town, Kumbotso LGA of Kano State, Nigeria. Panshekara is located within latitude 11° 51’ N to 11° 53’ N and longitude 8° 27’E to 8° 29’E, and is a peri-urban settlement in Kumbotso LGA. The houses are made of mud and concrete bricks with corrugated zinc roofs. The people of Panshekara are low income earners, mainly traders, industry workers, farmers and public servants. Farming is dominated by rain-fed cropping but active irrigation during dry season is practiced in some neighboring towns. Malaria is endemic in the densely populated area, with characteristic perennial transmission pattern, with peaks during the rainy months of May to September.

1.2 Study Design
The study was designed to measure and compare entomological parameters with and without interventions. The design permitted comparison between mechanical screenings alone and the combination of screening and use of Rambo paper, with internal control (Figure 1). After a detailed mapping and house numbering of the community, 150 houses were randomly selected. The doors and windows of all the selected houses were netted with mesh-wire. Rambo paper or a placebo were given randomly to 20 houses (intervention group) to use monthly and 10 houses unscreened (internal control) were allowed to use other personal protection measures of their choice.

1.3 Entomological Methods
1.3.1 Pyrethrum Spray Collection (PSC)
Adult mosquitoes were collected between May, 2010 to April, 2011 from houses, using pyrethrum Spray Collection (PSC) technique. Mosquitoes were collected by trained persons (housewives) between 4.30 -6.30 GMT. The PSC was conducted as outlined by WHO (1998) The technique involves collection of indoor resting mosquitoes on white sheets after knocking them down by space spraying with a pyrethrum aerosol. White sheet of cloth about 2 x 4 m were used to cover the entire floor surface. Doors and windows were closed and room sprayed with a pyrethrum insecticide for 2 minutes. After 10 minutes, all the mosquitoes which were knocked down were collected, transferred into labeled petri-dishes and transported to laboratory for further analysis.

1.3.2 Species identification
Mosquitoes were counted and sorted into the genera: Culex and Anopheles based on taxonomic characters
The genus *Anopheles* was further identified into *An. gambiae* s.l., and *An. funestus* group using the morphological keys (Gillies, 1987).

1.3.3 Molecular identification
Polymerase chain reaction (PCR) assay was used to identify members of the sibling species of *An. gambiae* complex. Genomic DNA (gDNA) was extracted from single specimen using the LIVAK extraction method described by Collins et al. (1987). 1µl of the gDNA was used in a PCR reaction with the following species – specific primers: UN (GTC TGC CCC TTC CTC GAT GT), AR (AAT TGT CCT TCT CCA TCC TA), GA (CTG GTT TGG TCG GCA CGT TT) and ME (TGA CCA ACC CAC TCC CTT GA), respectively for identification of GA, following the established protocol (Scott et al., 1993).

**Figure 1** A diagram illustrating the design for the study

**2 Results**

2.1 Indoor resting density (IRD)
The indoor resting densities and man biting rates of the Anopheline mosquitoes estimated from the PSC during 12 month period is shown (Table 1). The highest number of Anopheline malaria vectors 339, feeding indoors during the peak transmission period (Aug – Oct.), was recorded in the control compared to 27 in the houses with screening only and 30 in the screen plus Rambo paper. The difference between the numbers caught in the houses with screening and those screened and treated with the insecticide is not significant $X^2$ (1, N=57) = 3.34. The number of blood fed Anopheline female per person in the period, was 15 in the control, and 1.3 and 1 for the netting and netting plus Rambo respectively. The seasonal distribution and IRDs for *An. funestus* group and *An. gambiae* s.l, the major malaria vectors is presented (Table 2). *An. gambiae* s.l. and *An. funestus* s.l. are the primary vectors abundant throughout the year. In the dry season (May – July) *An. funestus* s.l. is more predominant than the *An. gambiae* s.l., while in the rainy season (August – October) *An. gambiae* s.l. population is higher. the
variation of the two species differs significantly across the two seasons $X^2 (1,N=497)=74.45$, p<0.05. The populations of both two major malaria vectors decline in the periods Nov. – Jan. and Feb.-April.

Table 1 Percentage and number per person blood fed Anopheline vectors in Panshekara estimated from PSC: MAY, 2010 – APRIL, 2011

| Period   | Type of Intervention | No of Anoph Caught | No of Anoph. BF | % Anoph. BF | No. of BF Anoph./person |
|----------|----------------------|--------------------|-----------------|-------------|------------------------|
| May-Jul  | NONE                 | 16                 | 15              | 93.8        | 0.8                    |
|          | NETTING              | 17                 | 09              | 53.0        | 0.5                    |
|          | RAMBO + NET          | 08                 | 07              | 87.5        | 0.4                    |
|          | NONE                 | 339                | 298             | 88.1        | 15                     |
| Aug-Oct  | NETTING              | 27                 | 26              | 96.3        | 1.3                    |
|          | RAMBO + NET          | 30                 | 20              | 66.7        | 1.0                    |
|          | NONE                 | 06                 | 06              | 0.3         |                        |
| Nov-Jan  | NET                  | 03                 | 03              | 0.2         |                        |
|          | NET + RAMBO          | 02                 | 02              | -           | 0.1                    |
|          | NONE                 | 02                 | 02              | 0.1         |                        |
| Feb-Apr  | NET                  | 02                 | 02              | 0.1         |                        |
|          | NET + RAMBO          | 00                 | 00              | 00          |                        |

Note: Anoph = Anopheles, None = Without intervention

The number of *An. gambiae s.l* and *An. funestus s.l* caught are pooled to estimate the indoor resting densities of the malaria vectors in the intervention houses and the controls. Catches are grouped to reflect the seasonal variation in malaria transmission in the area. The highest indoor resting density (33.19) was recorded in the control houses during the rainy season compared to 3.0 in house where net intervention were used in addition to transfluthrin-impregnated Paper Rambo

2.2 Effect of the interventions on Culicine

A total of 1592 Culicine mosquitoes, identified as *Cx. quinquefasciatus* were collected out of which 57.2% from the control, 27.6% and 15.1% from the screened only and screened plus transfluthrin (Rambo) paper respectively. The effect of the two interventions compared to the control on the indoor resting densities of Culicine mosquitoes is shown (Figure 2).

![Figure 2](image-url)

2.3 Molecular identification of *An. gambiae* complex mosquitoes

Out of the 40 specimens tested by PCR, 22 were successfully amplified. 16 of the specimens were identified as *An. gambiae s.s.* (390 bp) while six were identified as *An. arabiensis* (315 bp) (Figure 3).

![Figure 3](image-url)
3 Discussion

The effect of two interventions, mechanical screening of windows and doors with metal wire mesh and the screening in compliment with burning of (Rambo) paper impregnated with transfluthrin on entomological parameters of malaria transmission was measured in Panshekara town.

3.1 Indoor resting density (IRD)

In this study, mosquitoes were collected by trained house wives between (4 – 6 GMT) before the time endophilic mosquitoes were expected normally to exit the rooms. Appropriate measures were taken to prevent mosquitoes from outside to enter the rooms at the time of the collections to reduce confounding effects; hence the number caught was a fair representation of endophilic fraction of the population.

There was a significant drop in the number of the Anopheline malaria vectors caught resting in the houses which have been mechanically screened only and in those screened and complimented with transfluthrin impregnated paper compared to the control houses. There was also a corresponding decrease in the number of the malaria vector species feeding per person in the rooms in the interventions than in the control houses (Table 1). The average number of the Anopheline malaria vector mosquitoes feeding per person (18) in control houses was similar to that reported in unprotected sleepers in the transfluthrin trial studies in Tanzania (Pate et al., 2002). The indoor resting densities of the Anopheline malaria vectors have not differed significantly $X^2= 3.34$ between the houses which have been screened only (27) compared to those complimented with burning of transfluthrin impregnated paper (30). Lines et al. (1987) reported considerable reductions in the number of mosquitoes which have fed on persons in experimental huts whose eaves were covered with permethrin – treated curtains but such effects have not been observed when treated netting was placed around the eaves of dwelling houses. The absence of additional effects of transfluthrin in Panshekara could be due to the formulation of the product. Transfluthrin vapour is usually released from the smoke of a smouldering paper (Rambo) which burns slowly for 2 minutes. The concentration of the insecticide in the room and its effect on mosquitoes would decrease proportionately from the time the paper burns out in contrast to other slow release systems such as mosquito coil which release continually throughout the night (WHO, 2002). Transfluthrin released from the flame of typical African kerosene lamp burning for 4 hours was reported to be effective against biting mosquitoes (Pates et al., 2002).

3.2 Distribution of malaria vector species

An. gambiae s.l and An. funestus were collected in the ratio of 1: 2.3 and 1: 2.6 respectively (Table 2). Molineaux and Grammiccia (1980) have reported the preponderance of An. gambiae s.l over An. funestus in the rainy season and have demonstrated the expected contribution of each to malaria transmission in the Garki district in northern Nigeria. Our results suggested the potential of increasing dominance of An. funestus over An. gambiae probably due to the major ecological and climatic changes experienced in the region over the years (Louis and Mckenzie 2009). Similarly, the molecular analysis revealed the dominance of An. gambiae s.s over An. arabiensis in peri-urban Panshekara town and could be attributed to suitability of microclimate in Sudan savanna which seems to favour the occurrence of the former over the latter species (Colluzi et al., 1979). Of course, we recently have reported the dominance of An. coluzzii over An. arabiensis in the Sudan savannah of Jigawa states, of Nigeria (Ibrahim et al., 2014).

3.3 Personal protection

The Culicine and the Anopheline mosquito populations in the area responded to the control methods differently. The mechanical screening of doors and windows alone provided 51% protection against nuisance Culicine mosquitoes, while netting complimented with transfluthrin – impregnated paper gave 73%. The early biting habit of the Culicine mosquitoes in contrast to the midnight peaks of the local Anopheline populations might have made the Culicines more vulnerable to the excito – repellent effects of the transfluthrin –impregnated paper (Ogoma et al., 2014).
3.4 Side effects

People tend to recognize and recon with the beneficial and untoward effects of vector control operations. Irritation of skin, eyes and mucous membranes, manifesting as asthma – like symptoms have been caused by pyrethroid treated nets and some brands of mosquito coil (Paulin and Ozaki, 2015). We have not observed such effects with the transfluthrin impregnated paper, possibly because of the fast smoldering of the burning paper. The Rambo paper was unlikely to have toxic side – effects on domestic animals that have sometimes been noted to interfere with house-spraying (Curtis and Mnzava, 2000). The beneficial side – effects, in the form of additional protection against the nuisance mosquitoes could have more immediate public appeal than effect on vector – borne disease (Curtis et al., 1990).

Table 2 Seasonal distribution and IRDs of An. gambiae and An. funestus estimated from PSC in Panshekara Kumbotso LGA

| Months  | An. gambia s.l | An. gambia s.l /room | A. gambia s.l /person | An. funestus | An. fun/ room | An. fun./ person |
|---------|----------------|----------------------|-----------------------|--------------|--------------|-----------------|
| May-Jul |                |                      |                       |              |              |                 |
| Control | -              | -                    | -                     |              |              |                 |
| NET     | -              | -                    | -                     |              |              |                 |
| NET + RAMBO | 01   | 01                   | 01                    | 01           | 01           | 01              |
| Aug-Oct |                |                      |                       |              |              |                 |
| C       | 151            | 15.1                 | 7.6                   | 133          | 13.3         | 6.6             |
| N       | 10             | 1                    | 0.5                   | 17           | 1.7          | 0.9             |
| NR      | 15             | 1.5                  | 0.8                   | 15           | 1.5          | 0.8             |
| Nov-Jan |                |                      |                       |              |              |                 |
| C       | -              | -                    | -                     | 06           | 0.6          | 0.3             |
| N       | -              | -                    | -                     | 03           | 0.3          | 0.2             |
| NR      | -              | -                    | -                     | 02           | 0.2          | 0.1             |
| Feb-Apr |                |                      |                       |              |              |                 |
| C       | -              | -                    | -                     | 02           | 0.2          | 0.1             |
| N       | -              | -                    | -                     | 02           | 0.2          | 0.1             |
| NR      | -              | -                    | -                     | 00           | 0.2          | 00              |

Note: C= Control, N= netting, NR= netting + Rambo. Relative abundance of An. gambiae s.l. and An. funestus group collected in Panshekara between May 2010 – April 2011. An. funestus is preponderant and responsible for the biting in the dry season (May – July)

3.5 Mechanical screening and use of Paper Rambo in public health

Malaria vector control by conventional spraying of houses with residual insecticides and mass distribution of LLINs require large funding, intensive organization and often depended on foreign aids which are not readily available in many malaria endemic regions (Lines et al., 1987). Over the last five years, Nigeria has distributed over 60 million nets free of charge. (MIS, 2010). However, low utilization of bed nets has been recorded all over the country and in many places the nets were sold to hawkers at low prices due to the abject poverty at house – hold level (MIS, 2010). Resistance to the insecticides approved by WHO has been recorded in most of the ecological zones of Nigeria and we have established resistance to Permethrin, Lamdacyhalothrin and Deltamethrin in malaria vectors at Bunkure LGA which is adjacent to the study area (Ibrahim et al., 2014). It is possible that there may be cross – resistance with transfluthrin. The use of metal wire – mesh on windows and doors as mechanical screening against mosquitoes is increasingly becoming popular especially among the urban and semi – urban dwellers. The wide spread use of mechanical screening can reduce the selection for multiple resistance a problem which the insecticide based malaria control methods could pose in future. The government can make a policy supporting mechanical screening and encourage people by providing materials for the screening free of charge.

3.6 Limitations

Initially the entomological component was designed to compare Panshekara town (a treated community) and
Danbare village as a control but the two are epidemiologically dissimilar and an internal control has to be included in Panshekara.

The resistance status of the local mosquito populations to transfluthrin has not been detected. This is important in the interpretation of the result. It is important to know which of the two malaria vectors, *An. gambiae*s.l and *An. funestus* is more susceptible to transfluthrin. There was inadequate supervision for logistical reasons to ensure compliance of the participants with proper use of Rambo and maintenance of intact screening during the period of study.

4 Conclusion

Mechanical screening compares favorably with conventional methods in protection against mosquitoes and additional benefits of transfluthrin was demonstrated significantly on the culicine mosquitoes. The effects of the screening and transfluthrin impregnated paper on prevalence of malaria need to be investigated.

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