Influence of Indoor Residual Spray on An. Gambiae S.L. and An. Funestus Abundance, Resting Behavior and Community Perception on Irs Intervention In Kagera-Tanzania

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Research

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INFLUENCE OF INDOOR RESIDUAL SPAY ON *AN. GAMBIAE* S.L. AND *AN. FUNESTUS* ABUNDANCE, RESTING BEHAVIOR AND COMMUNITY PERCEPTION ON IRS INTERVENTION IN KAGERA-TANZANIA

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Abstract:

Background: There has been a persistence of malaria transmission in Kagera Region despite consecutive phases of indoor residual spraying (IRS) targeting malaria vectors. In addition to

that, there is limited information on the resting behaviors of *An. gambiae* s.l. and *An. funestus* and community perception on IRS in Kagera.

Methods: The study was conducted in two districts; Missenyi and Karagwe. Missenyi was IRS sprayed with Pirimiphos methyl insecticide and Karagwe was unsprayed. Mosquito collections were conducted using bucket traps and CDC (Centre for Disease Control) light traps. Mosquitoes were identified morphologically using taxonomical key. Sibling species were identified by polymerase chain reaction (PCR). Interviews and focused group discussions (FGDs) were conducted in order to obtain information on community perception with regard to IRS interventions.

Results: A total of 5,777 mosquitoes were collected indoors by CDC light traps. *An. gambiae* s.l. and *An. funestus* accounted for 13% (n=749) of all mosquitoes collected. Unexpectedly, large proportions of *An. funestus* were collected indoors in Missenyi district, despite the fact that, the area was sprayed. In Karagwe district, *An. gambiae* s.l. was collected in large proportion. Results have showed that, *An. arabiensis* was the dominant sibling species among the *An. gambiae* s.l. in Karagwe and Missenyi districts with 82.8% (n=24) and 98.8% (n=166), respectively. Study participants agreed that, IRS campaign is useful for control of malaria vectors. They also reported that, malaria transmission have declined by comparing before and after IRS. Challenges reported which the IRS campaign poses to them, including the emergence of other insects in their houses and increase of mosquitoes later after IRS. They also reported some mosquito resting places which are not a target during spray operations.

Conclusion: The present study has revealed the abundance of malaria vectors and community perception on IRS intervention efficacy and sustainability. Surprisingly, large proportions of *An. funestus* were collected indoors, despite the fact that, Missenyi district was sprayed. This situation calls for further studies on *An. funestus* behaviors and possible reasons for tolerance in

sprayed area. Community sensitization before, during and after IRS application needs to be strengthen for getting intended results.

Keywords: Indoor residual spray, *An. gambiae* s.l., *An. funestus*, resting behaviors, community perception, Pirimiphos-methyl, Kagera, Tanzania.

Introduction

Malaria is among the most burdening public health problem globally. It is the leading cause of morbidity and mortality in many third world countries. Children are more at risk and they accounted for more than two thirds of the global deaths [1]. Malaria remains a serious vector-borne disease across sub-Saharan Africa where transmission is maintained by *Anopheles* species such as; *An. gambiae sensu stricto*, *An. coluzzii*, *An. arabiensis* and *An. funestus* [2]. In Tanzania, malaria transmission is still high especially around Lake Victoria regions whereby the main vectors include the *An. gambiae s. s.*, *An. arabiensis* and *An. funestus s.s.* The Ministry of Health of Tanzania through the National Malaria Control Programme (NMCP) has been implementing a number of interventions to control malaria. These interventions includes preventive vector control interventions which involve the use of long lasting Insecticide Nets (LLINs), indoor residual spraying (IRS), and larval source management (LSM) Also treatment of malaria case with artemisinin- combination therapies (ACTs), diagnostic testing with malaria rapid diagnostic tests (mRDTs), Intermittent preventive treatment during pregnancy (IPTp), behavioral change communication (BCC) campaigns and surveillance, monitoring and evaluation activities [3]. Among the interventions implemented by NMCP, the IRS has been implemented in selected districts in the Lake zone regions due to high malaria prevalence. In 2011, malaria prevalence in

children under five years of age was 32% in Geita region, which was the highest prevalence compared to other regions in Tanzania [4].

A number of factors interact to influence the transmission of malaria. These factors includes the quality of insecticides, human behaviors and practices such as fishing, agriculture and staying outside while getting dinner/supper, effectiveness of IRS and LLINs, mosquito feeding behaviors and weather conditions of a specific area interact altogether to influence the resting behaviors of malaria vectors [5]. *Anopheles* species have different resting and feeding behaviors [6]. Chemicals used as insecticides such as Dichloro Diphenyl Trichloroethane (DDT) are irritant to malaria vectors; therefore force them to exit houses quickly after entering them. When mosquitoes cannot obtain blood meals indoors, they will obtain it outdoors in which they may either feed on people outside houses or on animals these mosquitoes will continue surviving and will account for residual malaria transmission [7]. Insecticides used for IRS and LLINs have been reported to influence the malaria vectors' abundance, resting behaviors and species composition [8,9].

It was estimated that, 124 million people were protected by IRS, representing 4% of the global population at risk of malaria in 2013[10]. Indoor residual spraying (IRS) in mainland Tanzania began in 2006 [11]. In 2018, in Tanzania mainland and Zanzibar a total of 15 district councils were already implementing IRS. The districts were Ngara, Misenyi, Bukoba rural, Chato, Musoma rural, Butiama, Sengerema, Kwimba and in Pemba and Unguja, Central, North A, North B, South, West, Chakechake and Micheweni [11]. During the National IRS campaign in 2016, a total of 543,865 house structures were targeted out of these 515,217 house structures were sprayed equal to 94.7%. IRS was implemented in Misenyi in December, 2017, by using

Primiphos-methyl (Actellic 300CS) sprayed on indoor wall surfaces. Primiphos-methyl (Actellic 300CS) efficacy is up to 8 months post spray [12]. In Missenyi district, 45,344 structures were targeted and 44,111 sprayed whereby the coverage was 97.3% [11].

Previous studies conducted in Bioko island have reported that, indoor vector control interventions have led to changes in behavior of malaria vectors [13,14]. Assessment of IRS impact on primary malaria vectors in West, East and South Africa revealed a great increase of *An. arabiensis* with a decrease in *An. funestus*, *An. gambiae* and *An. coluzzi* [9]. This is also the case in lake zone regions in Tanzania [15]. The change in behavior among malaria vectors have reported to contribute to persistent malaria transmission [16]. Although the use of insecticides reduce mosquito density, it leads to the selection of resistant species that account for residual malaria transmission in intervention area [17]. There is limited information on resting behaviors of malaria vectors in Misenyi and Karagwe districts. Therefore, the present study focused on assessing the influence of indoor residual spraying on abundance, resting behaviors of malaria vectors and community perception on mosquito bite protection in Missenyi (indoor residual sprayed district) and Karagwe (non- indoor residual sprayed district) in Kagera-Tanzania.

Materials and methods

Study area

A cross-sectional study with both quantitative and qualitative approach was conducted from June to July, 2018. It was conducted in two districts of Missenyi and Karagwe (Figure 1). Missenyi district covers a geographical area of 270,875 hectares. Missenyi district is located on an altitude

between 31.45⁰ east Greenwich line and 0.9⁰ southern of equator, it has 2 rain zones, heavy rainfall zone: The rain falls to between 400 – 2000mm per annum. The district is comprised of Missenyi division and Kiziba division [18]. It has a population of 227,270 with growth rate of 1.1 Population per division, In Kiziba division, there are 98,754 people while in Missenyi division there are 128,516 people [19].

Karagwe district has a population of 332,020 [19]. Population distribution has a rural urban difference in which the rural population is higher than urban population of 307,573 and 24,447, respectively. The District is characterized by mountain ranges, which are separated by swampy valley bottoms and wet lands. The altitude ranges between 1500-1800 meters above sea level while valley bottoms and wet lands are 1150 meters to 1450 meters above sea level. Most of the District has a tropical highland climate. The annual average temperature is 26⁰C, rainfall distribution is bi-modal with peak rains from September to December and from March to May (Karagwe district profile, 2012). The main economic activity for both districts is agriculture.

Selection of districts for IRS implementation

In 2016, the NMCP in collaboration with President Malaria Initiative (PMI) and other key malaria vector control stakeholders selected the IRS intervention areas which among them, Missenyi district council was included. The criteria used were high malaria burden districts as indicated by the epidemiological data on malaria incidence in the DHIS2.

Site selection

In the present study, three villages were selected in each district, for Missenyi; Kassambya, Gabulanga and Mabuye villages while in Karagwe; Kihanga, Katanda and Kishoju villages were selected. In each village, eight households were obtained based on the presence of eaves in Karagwe and presence of eaves plus being sprayed during the December, 2017 in Missenyi for mosquito collection giving a total of twenty-four (24) households [20]. The procedure was the same in both study areas where a total of 48 households were obtained. Selection of villages in Missenyi district was based on having high number of confirmed malaria cases (DHIS2 and top ten diseases from the health facilities), villages with many houses having open eaves and having many breeding sites of mosquitoes as per the report from District Malaria Vector Surveillance Officer (DMVSO). The same criteria were used to obtain the three villages in Karagwe district.

Mosquito collection

Mosquitoes were collected from six selected villages using bucket traps (BT) for collection of mosquitoes both indoors and outdoors and CDC-light trap for indoors collection [21]. Mosquitoes were trapped for three consecutive nights per house until all 24 houses in each district were covered [15]. For each house structure, six bucket traps were used per house; three indoors and the other three outdoors. A single CDC-light trap was located per house at night in a room with no bucket trap. Daily mosquito collections were carried out from 6.00 pm to 6.00am[22]. All collected mosquitoes were stored to species level using morphological identification key [23]. Culicines were recorded and discarded. *An. gambiae* s.l. and *An. funestus* group were identified to sibling species by method developed by Scott [24] and Koekemoer and others, respectively [25].

Figure 1: Map of Kagera region showing Missenyi and Karagwe districts.

Data collection using questionnaires and interview guide

A total of 396 heads of households were randomly selected and interviewed using structured questionnaires. Four focused group discussions (FGDs) were conducted two in each district. Each group was composed of eight members aged 18 years and above.

Data analysis

The number of mosquitoes and variables on the questionnaire were entered in SPSS software version 20 and analyzed to obtain the proportions and frequencies. Statistical tests such as t-test, Anova and Chi-Squared tests were performed. Identification of Mosquitoes to sibling species was achieved by molecular technique (PCR) which was conducted at NIMR, Amani centre in Muheza, Tanzania.

Results

Mosquito abundance in Missenyi and Karagwe districts

A total of 5,777 mosquitoes were collected. Majority of mosquitoes (95.7%, n=5530) were collected in Missenyi (indoor residual sprayed district). A total of 247 mosquitoes were collected in Karagwe (non- indoor residual sprayed district). Among them, 5679 were collected indoors and 98 were collected outdoors by using bucket traps. Majority of mosquito collected were *Culex* species. Malaria vectors; *An. gambiae* s.l. and *An. funestus* accounted for 13% (n=749) of all mosquitoes collected. In Missenyi district, *An. funestus* were collected in large proportions while in Karagwe district, *An. gambiae* s.l. were collected in large proportion. A total of 98 mosquitoes were collected by bucket traps outdoors in Missenyi and Karagwe districts, majority of them (88.8%, n=87) were collected in Missenyi district. All *An. funestus* were collected by CDC light traps indoors in Missenyi district (Table 1).

Table 1: Proportion of mosquitoes collected using CDC-light traps and the bucket traps indoors and outdoors in Missenyi and Karagwe districts

Species	Missenyi district, n (%)			Karagwe district, n (%)		
	CDC-light trap indoors	Bucket trap outdoors	Bucket trap indoors	CDC-light trap indoors	Bucket trap outdoors	Bucket trap indoors
<i>An. gambiae</i> s.l.	3 (0.1)	6 (60.0)	4 (40.0)	29 (13.1)	2 (18.2)	9 (81.8)
<i>An. funestus</i>	683 (12.6)	0 (0.0)	0 (0.0)	11 (5.0)	2 (100.0)	0 (0.0)
<i>An. coustani</i>	0 (0.0)	0 (0.0)	0 (0.0)	63 (28.4)	0 (0.0)	0 (0.0)
<i>An. rufipes</i>	4 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Culex</i> species	4,277 (78.8)	81 (88.0)	11 (12.0)	108 (48.6)	7 (58.3)	5 (41.7)
<i>Mansonia</i> species	461 (8.5)	0 (0.0)	0 (0.0)	11 (5.0)	0 (0.0)	0 (0.0)
Total	5,428	87	15	222	11	14

***An. gambiae* s.l. and *An. funestus* sibling species**

A total of 211 female *Anopheles* mosquitoes; 40 from Karagwe district and 171 from Missenyi district were analysed by polymerase chain reaction (PCR) to reveal the composition of the sibling species. A total of 29 *An. gambiae* s.l. and 11 *An. funestus* were obtained from Karagwe district. In Missenyi district, *An. gambiae* s.l. and *An. funestus* were 168 and 3, respectively. The PCR results have showed that, *An. arabiensis* was the dominant sibling species among the *An. gambiae* s.l. in Karagwe and Missenyi districts with 82.8% (n=24) and 98.8% (n=166), respectively. Higher proportions of *An. gambiae* s.s. were from Karagwe district than in Missenyi district. The PCR results have revealed that, all *An. funestus* were *An. funestus* s.s. in both districts (Figure 2).

Figure 2: Sibling species of *An. gambiae* s. l. and *An. funestus* in Karagwe and Misenyi districts

Social demographic characteristics of study participants

A total of 396 study participants were interviewed from Misenyi and Karagwe districts. Misenyi contributed 50% (n=198) of the study participants. Majority of the study participants were males 59.6% (n=236). The age of respondents was categorized whereby age group 21-30 years made up the highest percentage (27.3%, n=108). Majority of the participants (73.7%, n=292) were married. The economic activity of most of the participants in both districts was farming (83.8%, n=332). When education level was assessed, many of the participants had a primary level of education (57.3%, n= 227) (Table 2).

219 **Table 2: Social demographic characteristics of study participants (n=396)**

Characteristics	Missenyi district, n (%)	Karagwe district, n (%)	Total
Age			
<20	10 (5.1)	12 (6.1)	22
21-30	48 (24.2)	60 (30.3)	108
31-40	39 (19.7)	54 (27.3)	93
41-50	41 (20.7)	32 (16.2)	73
51-60	23 (11.6)	18 (9.1)	41
>60	37 (18.7)	22 (11.1)	59
Sex			
Male	118 (59.6)	118 (59.6)	236
Female	80 (40.4)	80 (40.4)	160
Education Level			
Never Attended	0 (0.0)	0 (0.0)	0
Primary	137 (69.2)	90 (45.5)	227
Secondary	28 (14.1)	55 (27.8)	83
University	1 (0.5)	24 (12.1)	25
Marital Status			
Single	16 (8.1)	19 (9.6)	35
Married	131 (66.2)	161 (81.3)	292
Divorced	41 (20.7)	18 (9.1)	59
Widow/Widower	10 (5.1)	0 (0.0)	10
Occupation			
Fishing	11 (5.6)	0 (0.0)	11
Farming	169 (45.4)	163 (82.3)	332
Animal Keeping	4 (2.0)	12 (6.1)	16
Market(<i>gilio</i>)	5 (2.5)	7 (3.5)	12
Business	9 (4.5)	16 (8.1)	25

220

221

222 **Human behaviors in relation to Indoor residual spray protection**

223 A total of 396 participants were interviewed. In Missenyi 2.1% (n=24) reported to carry out
 224 fishing activities from 08:00 am to 12:00 noon. Participants were also interviewed on malaria
 225 diagnosis in Missenyi and Karagwe those reported to be diagnosed positive for malaria were
 226 36.9% (n=73) and 34.8% (n=69), respectively. There was no association between where they sit
 227 while having dinner and malaria diagnosis. Participants were also interviewed about the time

they go to sleep. Majority (59.6%, n=118) in Missenyi reported going to bed from 8:00 to 10:00 pm which is earlier than in Karagwe where majority (45.5%, n=90) go to sleep from 10 p.m. to 12 midnight. In both Missenyi and Karagwe, majority 84.3% reported staying inside their houses after the evening meal and only 4% and 2% reported to have dinners outdoors in Missenyi and Karagwe, respectively.

Most of the interviewed participants 95.9% (n=187) in Missenyi reported to leave the walls of their rooms as they are after they were sprayed while 1.5% (n=3) reported to plaster them after they were sprayed. Majority of participants, 51.5% (n=102) from Karagwe reported to go out at night for a refreshments. Majority 84.8% (n=168) and 89.4% (n=177) of the respondents in Missenyi and Karagwe, respectively do not use any measure to protect themselves against mosquito bites while to go out at night for a refreshments. In Missenyi, only 13.6% (n=27) wear long sleeved shirts and trousers for protection and 1.5% (n=3) respondents reported using mosquito repellants (Table 3).

Table 3: Showing frequency of participant responses to various variables regarding community behaviors in relation to IRS protection in Missenyi and Karagwe districts

Variables	Frequency (n)		Percentage (%)	
	Missenyi	Karagwe	Missenyi	Karagwe
Fishing time	n = 198	n = 198		
4:00 a.m to 6:00 a.m	2	0	1.0	0
4:00 p.m to 6:00 p.m	5	4	2.5	2
06:00 p.m to 08:00 p.m	2	0	0.1	0
08:00 am to 12:00 noon	24	0	12.1	0
Not applicable	165	194	83.3	98

Hours spent when farming?	n =198	n = 198		
Less than 2 hours	21	13	10.6	6.6
Two to four hours	92	49	46.5	24.7
Four to 6 hours	28	52	14.1	26.3
Six to eight hours	3	17	1.5	8.6
More than eight hours	0	14	0.0	7.1
Not applicable	54	53	27.3	26.8
Sitting place when having dinner or supper	n = 198	n = 198		
Outside the house	8	4	4	2
In the kitchen	6	39	3	19.7
Inside the house	184	155	92.9	78.3
Sleeping time at night	n= 198	n = 198		
8 p.m to 10 p.m	118	55	59.6	27.8
10 p.m to 12 midnight	69	90	34.8	45.5
12 midnights to 2 am	2	16	1.0	8.1
2 am to 4 a.m	9	37	4.5	18.7
Where do you stay after dinner before sleeping?	n= 198	n = 198		
Outside the house	16	26	8.1	13.1
Go out for a drink	15	5	7.6	2.5
Watch TV/radio inside	167	167	84.3	84.3
Do you go out during the night?	n = 198	n = 198		
YES	56	102	28.3	51.5
NO	142	92	71.7	48.5
Hours spent out at night	n = 198	n = 198		
Less than 2 hours	29	61	14.6	30.8
3 hours to 5 hours	31	42	15.7	21.2
Not applicable	138	95	69.7	48
Protective measures	n = 198	n = 198		
Wear long sleeved shirts and trousers	27	21	13.6	10.6
Mosquito repellents				
I don't use any measure	3	0	1.5	0.0
	168	177	84.8	89.4

245

246 **Qualitative analysis from Focused Group Discussion (FGD)**

247 A total of 4 focused group discussions were conducted; two in both districts. Themes identified
248 were; community members' acceptance of IRS campaign as the useful control measure against
249 malaria vectors though reported a number of challenges, targets for mosquito control

interventions, risky community behaviors for mosquito bites, complementary mosquito control measures, and risk of outdoor mosquito bites and influence on the efficiency and effectiveness of IRS. Author's note: F indicates female and M indicates male and numbers are identity given to members of the group.

Major themes discussed

Theme 1 Community perception on IRS campaign

Community members accept the IRS campaign as the useful control measure against malaria vectors though reported a number of challenges the campaign pose to them, including emergence of insects not formerly present in their houses and increase of mosquitoes later after IRS.

Negative perceptions: *"You know after these chemicals are sprayed many insects not formerly present in ours houses come and are very troublesome to kill"* (Member 03 F Karagwe).

"To be sincere, in the first three months after spraying, the mosquitoes are few but thereafter, the numbers of mosquitoes increase even more" (Member 01 F Missenyi).

"Last year after spraying, mosquitoes did not die" (Member 05 F Karagwe).

Both females and male reported that malaria transmission have declined by comparing before and after IRS.

"You know in the past every minute you go to hospital they tell you that you have malaria but nowadays it is not easy, or even if they say it is malaria, it is not severe as that of the past before these chemicals" (Member 07 F Missenyi).

Theme 2 Targets for mosquito control interventions

274 Members in all the groups were asked on where they find many mosquitoes inside and outside
275 their houses, most of them reported to find mosquitoes outside the house in places with water,
276 grasses, coffee trees, near animal sheds and in bathrooms. Inside the house, they reported to find
277 mosquitoes behind doors, in bed rooms and in dark places like under the bed.

278 *“The challenge with mosquitoes is that during the rainy season especially when there are*
279 *planted beans and maize and presence of stagnant water usually are many”* (Member 02
280 M Missenyi).

281 *“Please this is how I see; many mosquitoes are hiding in the coffee trees, banana trees,*
282 *and in grasses around our houses”* (Member 02 M Karagwe).

283 *“Inside the houses they like to hide behind doors, in bed rooms, and in dark places like*
284 *under beds but they fear the kitchen because of smoke”* (Member 08 M Missenyi).

285

286 **Theme 3 Risky community behaviors for mosquito bites**

287 Members in all the groups mentioned the following behaviors that expose them to mosquito
288 bites; bathing children outside in the evening, not closing doors and windows early in the
289 evening, not cleaning the environment like not pruning banana leaves, while going to the farm,
290 while farming, while cooking outside the house if no kitchen.

291 *“You know us people in Kagera we grow bananas, sometimes we leave our farms with*
292 *grasses, weeds and banana leaves where mosquitoes hide”* (Member 07 M Missenyi).

293 *“While going to the farm early in the morning there are many mosquitoes and they are*
294 *biting like bees”* (Member 04 F Karagwe).

295 *“While bathing children outside at night mosquitoes are many you know we fear going to*
296 *the bath rooms”* (Member 07 M Missenyi).

297

298 **Theme 4 complementary mosquito control measures**

299 Community members reported few of other chemicals apart from IRS and protective measures
300 against mosquito bites, most of the members declared not using other chemicals while few
301 reported using example Rungu® and Doom® (pyrethroid insecticide sprays) while few of the
302 members reported to burn eucalyptus tree leaves to serve as mosquito repellents.

303 *“I used Rungu® the time when mosquitoes were many but I stopped using them after*
304 *spraying these chemicals”* (Member 07 M Karagwe).

305 *“When mosquitoes are too many we burn leaves from eucalyptus trees on the door so*
306 *when mosquitoes smell the smoke they run away, challenge is mosquitoes go inside when*
307 *smoke is over”* (Member 03 M Missenyi).

308

309 **Theme 5 Risk of outdoor mosquito bites**

310 Most members of the groups reported being outdoors at night before going to sleep without
311 protection which makes them prone to mosquito bites outdoors. However most of them reported
312 not being aware of protection measures against mosquito bites.

313 *“Now life is difficult, we stay out doing business”* (Member 02 M Karagwe).

314 *“It is time to prepare food and bath children and all these are done outside”* (Member 07
315 F Missenyi).

316 *“You know in our traditions men do not stay at home watching their wives cook, we*
317 *normally go out at night making stories with our fellow men”* (Member 08 M Karagwe).

318 *“Me I usually catch them and kill them because I don’t see any means to avoid them”*
319 (Member 07 F Karagwe).

Theme 6: Influence on efficiency and effectiveness of IRS

Most of the community members reported on a number of factors that might influence the effectiveness and efficiency of IRS, which either emanates from the community itself.

“Some people do not want their houses to be sprayed” (Member number 04 M Missenyi).

“Sometimes it happen that, village leaders and the rich are not involved except us the poor who have few items in the house to take out during the spray exercise”. (Member number 5 F Karagwe).

Discussion

In many part of Africa, the main malaria vectors are the *An. gambiae s. s.*, *An. arabiensis* and *An. funestus* [26], this is also the case in Tanzania [27–29]. This study has revealed the abundance of *An. gambiae s.l.* and *An. funestus* in Missenyi (indoor residual sprayed district) protection and Karagwe (non-indoor residual sprayed district) in Kagera, Tanzania. Missenyi district was sprayed with pirimiphos-methyl in 2016 and 2017 with 93.7% coverage [30]. Pirimiphos-methyl (Actellic® 300CS) has been reported to have a residual efficacy beyond 9 months on oil paint wall surfaces post-IRS [31]. Unexpectedly, large proportions of *An. funestus* were collected indoors, despite the fact that, Missenyi district was IRS sprayed. Our findings contrast with the previous finding conducted in the lake zone Tanzania by Kakilla and others [30] which reported very low *An. funestus* indoor density in most sites including Missenyi district in the as evidenced by monthly mosquito collection for a period of one year in 2017. The findings of the present study were also dissimilar to a study conducted in western Kenya whereby IRS using pirimiphos-methyl was associated with 88% reduction in *An. funestus* numbers in intervention areas

compared to non-intervention areas [32]. Indoor residual spray with pirimiphos-methyl has reported to maintain sporozoite rates at low levels hence an important tool for malaria vector control in the area [33]. However, there is limited information on insecticides susceptibility status among *An. funestus* in Tanzania [34]. Missenyi district usually receive high amount of rainfall from March to May hence creates and maintain permanent and semi-permanent breeding sites which are favorable for *An. funestus*. The proportions of *An. funestus* collected indoors were lower in Karagwe district than in Missenyi district, although the area was not sprayed.

The density of adult mosquitoes is determined by the productivity of larval habitats and their proximity to human hosts where they can obtain blood meal [35]. In the present study, the proportion of *An. gambiae* s.l. collected indoors in Missenyi was very low compared to Karagwe district. These findings contrast with the previous study conducted in the lake zone, Tanzania which reported the high density of *An. gambiae* s.l. was collected in Missenyi district post-spray with pirimiphos-methyl [30]. This disparity could be due to rapid species shift as a result of LLINs and IRS interventions implementation [36,37].

A better understanding of mosquito behavioral patterns such as breeding, resting and feeding are essential in selection of the mosquito control interventions [38]. In the present study, large proportion of *An. gambiae* s.l. was collected resting outdoors in Missenyi district while in Karagwe district, majority of *An. gambiae* s.l. was collected resting indoors. Indoors vector control intervention such as LLINs and IRS may result to malaria vectors' behavior change [14,39]. The malaria vectors that were known to feed and rest indoors may prefer to feed and rest

outdoors in order to avoid contacts with insecticides inside houses and repellent effect of IRS chemicals on the resting behaviors of malaria vectors [13,40]. No *An. funestus* were collected outdoors in Missenyi district. Our findings is comparable with a study conducted in Burkina Faso which reported the *An. funestus* prefer to feed indoors than outdoors [41].

In the present study, polymerase chain reaction (PCR) results have showed that, *An. arabiensis* was the dominant sibling species among the *An. gambiae* s.l. in Karagwe and Missenyi districts with 82.8% and 98.8%, respectively. This zoophilic species primarily feed and rest outdoors also play important role in malaria transmission [42]. This is in line with a previous study that reported a lesser effect of indoor-based insecticide control on *An. arabiensis* [9]. Higher proportions of *An. gambiae* s.s. were from Karagwe district which was unsprayed areas. This species primarily prefer to feed and rest indoors [42]. All *An. funestus* were *An. funestus* s. s. in both districts. These findings are in line with a previous study conducted in the northeastern Tanzania [28,43]. However, the reason for recent changes in malaria vectors sibling species composition is less documented.

Human behaviors in relation to IRS protection in Missenyi has revealed that, most of the residents in Missenyi and Karagwe are crop farmers and only a few are animal keepers though most households were observed to keep few animals like pigs, goats, chickens and cows at home. Presence of animals in the compound favors persistence of malaria vectors in the areas under IRS [7]. Most interviewed residents of both Missenyi and Karagwe reported, staying outdoors from 8:00 pm to 10:00 pm. For this case, the time between 8:00 to 10:00 pm, is the particular time when they are protected by IRS intervention. Additionally, majority of people go to sleep

from 10 p.m. to 12 midnight in Karagwe. According to a previous study conducted in Dar es Salaam this is a peak outdoor biting time for *An. arabiensis* [5]. However, in the present study, *An. arabiensis* is the dominant sibling species in *An. gambiae* complex in Missenyi. Despite the fact that, Missenyi district was sprayed, these findings suggest a lowered community protection against malaria vectors offered by IRS.

In this study, very few residents in both Missenyi and Karagwe districts reported using alternative protective measures such as use of mosquito repellents, wearing long sleeved shirts and insecticidal sprays for protecting themselves from mosquito bite while they are outside their houses at night. It is important to device vector control interventions which target outdoor and early biting behavior such as application of larvicides in mosquito breeding sites [44]. This is consistent with a study by [5], and is in contrast to findings in cities in Kenya that reported high usage of personal of protective measures which was reported to be due to their high economic status [45]. Few of the members reported to burn eucalyptus tree leaves to serve as mosquito repellents. A member from Missenyi district stated “*When mosquitoes are too many we burn leaves from eucalyptus trees on the door so when mosquitoes smell the smoke they run away, challenge is mosquitoes go inside when smoke is over*”. Our findings are supported by the previous studies with regard on the use eucalyptus extracts to repel mosquito vectors [46,47].

Residents in both Missenyi and Karagwe were also asked on where they sit while having evening meal; most of them reported having these meals indoors. This was reported to be influenced by their traditions that they are not allowed to get food outdoors as reported by the residents. The findings are opposite to those by [5] in Dar es Salaam. No any resident reported sleeping

outdoors in both Missenyi and Karagwe which might be due to the fact that these areas have lower temperatures compared to Dar es Salaam so residents are forced to sleep indoors in search for warmth. Those who reported resting indoors after dinner were 84.3%. In Missenyi contrast to findings from studies in Dar es Salaam which showed only 47.8% rested indoors.

All the studied house structures were traditional being muddy with open eaves, no window screens, unsealed window/door frames and no ceiling. A high number of mosquitoes were collected from these houses. This is in line with a study by [5] in Dar es Salaam that reported a reduced mosquito biting rates of malaria vectors under improved housing standard especially mosquito-proof screening, closed eaves, ceiling and sealed window/door frames.

The present study has revealed the behaviors that predispose community member to mosquito bites which included; bathing children outdoors in the evening, not closing doors and windows early in the evening, not cleaning their environment like pruning banana leaves, coffee trees, slashing of grasses and shrubs in the compound and removal of stagnant water. Poor environment creates mosquito resting places and breeding sites [48]. They also acknowledged being bitten by mosquitoes while going to the farm early in the morning also while in farms as a man from Karagwe declared “*Now life is difficult, we stay out doing business*”. Also a woman from Karagwe insisted that “*While going to the farm early in the morning there are many mosquitoes and they are biting like bees*”. Our findings concur with a previous study which reported the occurrence of early morning mosquito biting among malaria vectors [49]. Furthermore, women without kitchens reported being bitten by mosquitoes while cooking outdoors as supported by a woman from Missenyi “*It is time to prepare food and bath children and all these are done outside*”. In addition to that, most of the men reported staying outdoors

before going to sleep at night without any protection against mosquito bites. A member from Karagwe added that *“You know in our traditions men do not stay at home watching their wives cook, we normally go out at night making stories with our fellow men”*. This situation also reveals how traditional attributes lead people at risk of being bitten by mosquitoes [50].

In high malaria transmission like the study area, health education is deemed important for residents to adopt informed healthy choices. Complementary outdoor protective measures such as; wearing long sleeves, socks, use of mosquito repellents and environmental management should be considered during health promotion strategies. These will help in reducing outdoor malaria transmission.

In the present study the community members in Missenyi and Karagwe districts agreed that, IRS implementation is a useful control measure against malaria vectors. All participants reported that malaria transmission have declined by comparing before and after IRS. As a participant from Missenyi added that *“You know in the past every minute you go to hospital they tell you that you have malaria but nowadays it is not easy, or even if they say it is malaria, it is not severe as that of the past before these chemicals”*. Our findings are similar to previous studies conducted in Tanzania and elsewhere [50–53]. Although IRS and LLINs has contributed to the decline in malaria in the past decade [54], the continue community sensitization in paramount for sustainability of such interventions [50,55].

On the other hand, participants reported a number of challenges with regard to the IRS campaign posed to them, including emergence of insects such as fleas, bedbugs and lice which were not formerly present in their houses. A participant from Karagwe insisted that, *“You know after these chemicals are sprayed many insects not formerly present in ours houses come and are very troublesome to kill”*. Our findings are comparable to a previous study conducted in Tanzania by Kaufman and others [50]. The insecticides used for IRS may have irritated the hidden insects on the walls which lead them to increase mobility and become visible to house occupants [50,56]. Furthermore, our study contrast with a previous study which reported IRS reduce other insects and rodents [57].

The present study has revealed the perceived low efficacy of the insecticides used during IRS. The participants reported an increase of mosquitoes later after IRS campaign as pointed out by a woman from Missenyi *“To be sincere, in the first three months after spraying, the mosquitoes are few but thereafter, the numbers of mosquitoes increase even more”* and also a woman from Karagwe who added that *“Last year after spraying, mosquitoes did not die”*. These findings are unlike to the previous studies which reported that, IRS reduce mosquitoes and lower the cost of malaria prevention at household level [51,57]. The perceived benefit of IRS such as reduction in mosquito bites, incidental benefits were also reported [52].

In the present study, few participants also mentioned some behavioral factors such as washing walls, repainting, plastering and covering walls using newspapers after IRS which may hinder the efficacy of the intervention. The behaviors are influence by the smell of the insecticide used during IRS. Our findings concur with the previous study whereby modification of wall surfaces post-spraying potentially reduce the tangible IRS coverage, effectiveness and impact hence offer

less protection [58]. Providing equate information before and during IRS implementation could raise the awareness about spraying, resulting in high acceptability and sustainability [50,51,58]. Participants also suggested that chemicals used for IRS should have less smell and less allergic to humans.

In this study, participants mentioned location/sites where they use to find mosquitoes. These location included outside houses in the areas with grasses, coffee trees, banana trees, water pools, near animal sheds and in bath rooms. These kinds of mosquito resting places are sometimes not a target for IRS. Indoors they reported finding mosquitoes behind doors, in bed rooms, and in darker areas like under beds. One member from Missenyi stated that, *“Inside the houses they like to hide behind doors, in bed rooms, and in dark places like under beds but they fear the kitchen because of smoke”*. These findings are in line with the previous finding conducted in south-eastern Tanzania [59]. Community awareness on these sites is a stepping stone towards influencing practices such as environmental cleaning like pruning of banana and coffee trees, slashing of grasses near houses, clearing of stagnant water in compounds especially during the rainy season when breeding sites are many. Sometime microclimate may favor malaria vector to rest in or outside the house [60]. In addition, in both Missenyi and Karagwe areas, residents reported using local herbs for instance burning eucalyptus tree leaves with intension of chasing mosquitoes from their houses using the smoke released. Others reported using insecticide sprays such as “Doom®” and “Rungu®”. The use of locally available herbs for repelling mosquitoes from entering their home have been reported in north-eastern Tanzania and elsewhere [61,62].

In the present study, most of the participants reported on a number of factors that might influence the effectiveness and efficiency of IRS, which are either due to the community itself or from spray operators. Participants reported cases of chemicals not being mixed in the required ratios, not spraying some houses, selling of chemicals to farmers to be used in gardens for killing pests. Our findings are similar to a previous study conducted in Tanzania and Mozambique with regard to quality and performance of the spray operators which affects their acceptability to the IRS intervention [50,55]. With regard to the community itself, few participants reported plastering sprayed walls to avoid bad smell. A man from Missenyi added that “*Some people do not want their houses to be sprayed*”. A member from Karagwe stated that, “*Sometimes it happen that, village leaders and the rich are not involved except us the poor who have few items in the house to take out during the spray exercise*”. Difficulties in moving items outside during spray and the lack of uniformity in the spray operation between the rich and the poor, unpleasant smell of the insecticide used were among the reason for them to perceive negatively on the intervention. These findings concur with the previous study conducted in Iran [63]. Community sensitization before and during IRS implementation has to strengthen [50,64]. In the present study, participants have agreed that, IRS intervention is important for malaria control. They also suggested that, close supervision should be in place in order to mitigate the mentioned behaviors that may compromise IRS efficiency and effectiveness.

Conclusion

The findings of the present study have revealed the abundance of malaria vectors in IRS and unsprayed districts. Unexpectedly, large proportions of *An. funestus* were collected indoors, despite the fact that, Missenyi district was sprayed. This situation calls for further studies on *An.*

funestus behaviors and possible reasons for tolerance in sprayed area. The findings have also revealed community perception on IRS intervention which may affects its efficacy and sustainability. Community sensitization before, during and after IRS application needs to be strengthen for getting intended results.

List of abbreviations

ACT: Artemisinin Combination Therapy, BCC: Behavioral Change Communication, CDC: Center for Disease Control, CI: confidence intervals; DDT: Dichloro Diphenyl Trichloroethane, DHIS: District Health Information System, DMVSO: District Malaria Vector Surveillance Officer, FGD: Focused Group Discussion, GEE: Generalized Estimating Equation, IRS: Indoor Residual Spraying, IPT: Intermittent Presumptive Treatment, LSM: Larval Source Management, LLIN: Long Lasting Insecticide Net, mRDT: Malaria Rapid Diagnostic Test, MoHCDGEC: Ministry of Health Community Development Gender Elderly and Children, MUHAS; Muhimbili University of Health and Allied Sciences, NIMR: National Institute for Medical Research, NMCP: National Malaria Control Program, NBS: National Bureau of Standards, PCR: Polymarase Chain Reaction, USAID: United States Agency for International Development, WHO: World Health Organization.

Ethics approval

The present study was approved by the Muhimbili University Health and Allied Science IRB, by the ethical clearance with reference number. DA.287/298/01.A/ dated 29th May, 2018.

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551

552 **Availability of data materials**

553 The data supporting the conclusion are included within the article.

554

555 **Authors' contributions**

556 **GK** conceived the study, design and performed the study, participated in the field, contributed to
557 interpretation of results and drafted the manuscript; **BE** contributed to overall study design and
558 critically reviewed the manuscript; **BN** contributed to overall study design and critical review of
559 the manuscript. All authors read and approved the final version of the manuscript.

560

561 **Competing interests**

562 The authors declare that they have no competing interests.

563 **Consent for publication**

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