Title: Biting and resting preferences of malaria vectors in The Gambia

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Abstract

Background

The scale-up of indoor residual spraying and long-lasting insecticidal nets, together with other interventions have considerably reduced the malaria burden in The Gambia. This study examined the biting and resting preferences of the local insecticide-resistant vector populations few years following scale-up of anti-vector interventions.

Method

Indoor and outdoor-resting *Anopheles gambiae* mosquitoes were collected between July and October 2019 from ten villages in five regions in The Gambia using pyrethrum spray collection (indoor) and prokopack aspirator from pit traps (outdoor). Polymerase chain reaction assays were performed to identify molecular species, insecticide resistance mutations, *Plasmodium* infection rate and host blood meal.

Results

A total of 844 mosquitoes were collected both indoors (421, 49.9%) and outdoors (423, 50.1%). Four main vector species were identified, including *An. arabiensis* (indoor: 15%, outdoor: 26%); *An. coluzzii* (indoor: 19%, outdoor: 6%), *An. gambiae s.s.* (indoor: 11%, outdoor: 16%), *An. melas* (indoor: 2%, outdoor: 0.1%) and hybrids of *An. coluzzii-An. gambiae* (indoors: 3%, outdoors: 2%). A significant preference for outdoor resting was observed in *An. arabiensis* (Pearson X^2=22.7, df=4, P<0.001) and for indoor resting in *An. coluzzii* (Pearson X^2=55.0, df=4, P<0.001). Prevalence of the voltage-gated sodium channel (*Vgsc*)-*1014S* was higher in the indoor-resting (allele freq. = 0.96, 95%CI: 0.78–1) than outdoor-resting (allele freq. = 0.82, 95%CI: 0.76–0.87) *An. arabiensis*

68 population. For *An. coluzzii,* the prevalence of most mutation markers were higher in the outdoor
69 (allele freq. = 0.92, 95%CI: 0.81–0.98) than indoor-resting (allele freq. = 0.78, 95%CI: 0.56–0.86)
70 mosquitoes. Sporozoite positivity rate was 1.3% (95% CI: 0.5–2%). Indoor-resting *An. coluzzii*
71 had mainly fed on human blood while indoor-resting *An. arabiensis,* animal blood.
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73 # Conclusion

74 The indoor-resting behavior of *An. arabiensis* that preferred animal blood and had low sporozoite
75 rates, may be determined by the *Vgsc-1014S* mutation. Control interventions may include
76 complementary vector control approaches such as zooprophylaxis.
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Introduction

Successful implementation of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) has hugely contributed to the malaria decline observed in sub-Saharan Africa [1]. These interventions reduce transmission by primarily limiting human contact with human-feeding (anthropophagic), indoor-feeding (endophagic) and indoor-resting (endophilic) vectors [2]. Unfortunately, these measures also induce selection for physiological and behavioral resistance in vector populations, resulting in reduced mosquito susceptibility to most of the current insecticides used for LLINs and IRS [3], as well as increased exophilic behavioral phenotypes in primarily endophilic vectors [4]. Moreover, residual transmission where LLINs and IRS use is extensive, is maintained by vectors with physiological and behavioral resistance [5]. Therefore, studying the behavioral dynamics of vector populations during the scale up of vector control interventions will assist in determining the appropriate response to emerging behavioral changes.

Malaria burden in The Gambia has declined significantly over the last decades with vector control approaches being a major component of intervention, coordinated and implemented by The Gambia National Malaria Control Program (GNMCP). Following the World Health Organization (WHO) Global Plan for Insecticide Resistance Management (GPIRM), the GNMCP has consistently implemented rotational use of different classes of insecticides for IRS, to curtail dichlorodiphenyltrichloroethane (DDT) and deltamethrin resistance. For IRS, DDT was replaced initially by deltamethrin and bendiocarb, and since 2017 by pirimiphos-methyl (actellic 300CS) [6]. Similarly, LLINs intervention has been stable over the years and Gambia has recorded successful LLINs coverage as high as 90% [7,8].

Despite such successes, residual transmission has become increasingly spatially heterogeneous, with its intensity increasing from western to eastern Gambia, and could have been driven by

111 specific vector population dynamics [9]. The major vector species, namely *Anopheles arabiensis*,
112 *An. coluzzii* and *An. gambiae sensu stricto* (*s.s.*) are variably distributed throughout the country.
113 *An. arabiensis* is most prevalent in the eastern Gambia while *An. coluzzii* and *An. gambiae s.s.*
114 inhabit the western region [10,11]. However, *An. arabiensis* has been recently found throughout
115 the country [12], indicating possible replacement due to successful control of other sibling species
116 [13,14]. Moreover, the population prevalence of each vector species varies by season, whereby
117 *An. arabiensis* and *An. coluzzii* are dominant throughout the rainy season, while *An. gambiae s.s.*
118 become rarest early in the onset of dry season [10,11]. DDT and pyrethroid resistance has been
119 reported at various degrees in all vectors, that continue to be highly susceptible to carbamates and
120 organophosphates [12,15,16].

121 Host seeking and resting behavior of vectors are important metrics to evaluate the impact of control
122 and resistance management strategies [17]. Vector behavioral adaptation, resistance selection and
123 persistent transmission could increase during extensive scale-up of interventions, and this
124 information can only be captured by real-time surveillance [18,19]. Hence, national malaria control
125 programs should actively monitor behavioral dynamics in the local vector population, to inform
126 decisions.

127 In The Gambia, DDT and pyrethroid resistance is widespread and associated with residual
128 transmission [12,15]. However, the effect of control activities on vectors feeding and resting
129 behavior remains unclear. The biting and resting preferences of *An. gambiae sensu lato* (*s.l.)*
130 populations was investigated in The Gambia following few years of intensive vector control
131 interventions.

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Materials and methods

Anopheles gambiae s.l. collection

Indoor and outdoor-resting adult mosquitoes were sampled from July to October 2019, during the malaria transmission season across five administrative regions in The Gambia, namely Central River Region (CRR), Lower River Region (LRR), North Bank Region (NBR), Upper River Region (URR) and West Coast Region (WCR). WCR is a coastal area characterized by mangrove swamps. The remaining regions are mainly inland and have forest vegetation. Rice is mainly cultivated in CRR while cereals farming is common in all regions. Two villages were selected from each region and most of the villages are GNMCP surveillance sites with high LLIN and IRS coverage. Malaria transmission is highest in URR compared to other regions in The Gambia [7].

Indoor-resting mosquitoes were collected from sleeping rooms using pyrethrum spray collection (PSC). Twenty houses per village, at least 50m apart from each other, were randomly selected. In each village, collections were done for two consecutive days, with ten houses sampled per day. Outdoor-resting mosquitoes were sampled from pit shelter traps using prokopak aspirator. Three pit shelter traps that were 10m away from the selected compounds, were placed at different parts in each village. Both indoor and outdoor collections were conducted from 06.00 am to 09.00am in every collection day.

Mosquito identification

Morphological identification of female *An. gambiae s.l.* was done using identification keys as described by Gillies & Coetzee [20]. Afterwards, mosquitoes were stored individually in 96%

ethanol in 1.5ml Eppendorf tube until DNA extraction. DNA was extracted separately from abdomen and head/thoraces of individual mosquitoes using Qiagen QIAxtractor robot. Species-specific genotyping PCR to identify *An. arabiensis*, *An. melas* and *An. gambiae* was performed as previously described [21]. This was followed by restriction enzyme digestion to specifically identify *An. coluzzii*, *An. gambiae s.s*. and their hybrids (*An. coluzzii-An. gambiae s.s*.) [22].

Insecticide resistance markers identification

Screening for molecular markers of target-site resistance to carbamates, DDT, pyrethroids and organophosphates was done on all samples using a probe-based assay (TaqMan SNP genotyping) [23]. The following markers were investigated: voltage-gated sodium (*Vgsc*)-*1014F, Vgsc-1014S* and *Vgsc-1575Y* associated with target-site mutation to DDT and pyrethroids [24–26]. Acetylcholine esterase (*Ace*)-*119S*, marker for carbamate and organophosphate resistance [27] and glutathione-S-transferase epsilon 2 (*Gste2*)-*114T*, involved in metabolic resistance to DDT [28] were also assayed. The TaqMan allelic discrimination assay is a multiplex real time PCR with primers and probes specific for each insecticide target gene and discriminate susceptible (wild type) and resistant (mutant) alleles based on probe fluorescence signals [29].

Plasmodium sporozoite detection

DNA extracted from mosquito head and thoraces was used to detect sporozoites of *Plasmodium falciparum*, *P. ovale*, *P. malariae* and *P. vivax* species, employing TaqMan SNP genotyping protocol [30] which enables discriminatory identification of circum-sporozoites (CSPs) of *P.*

falciparum from *P. ovale*, *P. malariae* and *P. vivax* CSPs. Genomic DNA specific to each of these *Plasmodium* species were analyzed in each assay as positive controls.

Blood meal identification

Extracted DNA from engorged mosquito abdomens were amplified using modified multiplex PCRs with primers targeting cytochrome B genes of human and animal hosts including chicken, cow, dog, donkey, goat, horse and pig [31,32].

Statistical analyses

The proportion of each mosquito species in relation to the total number of mosquitoes captured from each region was calculated in percentage, as well as allele frequencies of indoor and outdoor-resting mosquitoes. Sporozoite positivity rate was the proportion of PCR positive mosquitoes among all mosquitoes tested. Human (HBI) and animal blood meal indices were estimated as the proportion of mosquitoes positive for human or animal hosts among those positive for all hosts. Mean differences between HBI and animal blood meal indices by vector species and resting locations were analyzed by ANOVA. Statistical analyses were done using Stata/IC 15.0 (2017 StataCorp LP).

Results

Anopheles species distribution and their resting behavior

A total of 844 *An. gambiae s.l.* mosquitoes were collected from the five regions. Four main vector species were identified, namely *An. arabiensis* (N=350, 41%); *An. coluzzii* (N=214, 25%), *An. gambiae s.s.* (N=224, 27%) and *An. melas* (N=17, 2%). Hybrids of *An. coluzzii-An. gambiae s.s.* were also detected (N=39, 5%). Most mosquitoes were collected from URR (642, 76%), followed by LRR (97, 11%) and then the other regions (Fig 1).

Fig 1: Distribution of *Anopheles gambiae s.l* by region as collected indoors and outdoors.

*An. col*uzzii-An. *gambiae s.s.* are the hybrids of *An. coluzzii* and *An. gambiae s.s*. Mosquitoes were collected from 5 regions: CRR- central river region, LRR-lower river region. NBR- north bank region. URR-upper river region and WCR- West coast region.

Overall, the number of mosquitoes resting indoors (421, 49.9%) and outdoors (423, 50.1%) were similar. Nevertheless, the resting preference varied by species. A significantly higher proportion of *An. arabiensis* were found outdoor (26.1%) than indoor (15.4%) (Pearson X^2=22.7, df=4, P<0.001) while both *An. coluzzii* (19.1% indoor and 6.3% outdoor, Pearson X^2=55.0, df=4, P<0.001) and *An. melas* (1.9% indoor and 0.1% outdoor, Pearson X^2=13.3, df=4, P<0.01) preferred resting indoor. For *An. gambiae s.s.* (10.9% indoor and 15.6% outdoor, Pearson X^2=7.0, df=4, P<0.1) and *An. coluzzii-An. gambiae s.s.* hybrids (2.6% indoor and 2% outdoor, Pearson X^2=0.7, df=4, P<0.95), there was no significance difference between resting indoor and outdoor. In URR, the region with the highest malaria transmission in The Gambia, *An. arabiensis* was most abundant vector (45.8%, 294) (indoor: 14.5%, outdoor: 31.3%), followed by *An. gambiae s.s.* (28.4%, 182)

(indoor: 12.8%, outdoor: 15.6%) and *An. coluzzii* (21.5%, 138) (indoor: 13.6%, outdoor: 7.9%). No *An. gambiae s.s.* was collected in CRR while *An. melas* was mainly found in LRR (N=15). All mosquitoes collected from LRR and NBR were resting indoors. The hybrids of *An. coluzzii* and *An. gambiae s.s.* were mainly found in URR (indoor: 2.3%, outdoor: 1.9%) and WCR (indoor: 10%, outdoor: 8.3%).

Distribution of voltage-gated sodium channel (Vgsc) mutation markers in the vectors

Vgsc point mutations associated with DDT and pyrethroid resistance were highly prevalent and detected at varying frequencies in all vector species across all regions. Overall, *An. arabiensis* was found resting indoors when resistance allele frequency was higher in the indoor population, whereas *An. coluzzii* were resting outdoors with higher outdoor resistance. No consistent resting preference was observed in *An. gambiae* in the presence of mutations.

Vgsc-1014S mutation was found predominantly in indoor-resting vector populations (Table 1). In *An. arabiensis*, the mutation was more frequent in the indoor-resting than outdoor-resting mosquitoes regardless of the region. V*gsc-1014S* was also the only mutation identified in *An. gambiae s.s.* and *An. melas* when found resting indoors.

239 **Table 1: Frequencies of insecticide resistance alleles on VGSC, GST and AChE loci in *Anopheles gambiae s.l.* populations from**
240 **all study regions**
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Region	Anopheles species	*Vgsc-1014F*		*Vgsc-1014S*		*Vgsc-1575Y*		*GSTe2-114T*		*Ace1-119S*	
		Indoor	**Outdoor**	**Indoor**	**Outdoor**	**Indoor**	**Outdoor**	**Indoor**	**Outdoor**	**Indoor**	**Outdoor**
URR	***An. arabiensis* (N=294)**	0.05	0.02	0.91	0.82	0	0.004	0	0.01	0	0
	***An. coluzzii* (N=138)**	0.74	0.92	0.25	0.04	0.68	0.9	0.78	0.9	0	0
	***An. gambiae ss* (N=182)**	1	0.99	0	0.01	0.96	0.98	0.98	0.99	0.05	0.04
	***An. col./ gam* (N=27)**	0.93	1	0	0	0.87	1	0.87	0	0.07	0
LRR	***An. arabiensis* (N=10)**	0	-	0.9	-	0	-	0.1	-	0	-
	***An. coluzzii* (N=71)**	0.66	-	0.3	-	0	-	0	-	0	-
	***An. gambiae ss* (N=1)**	0	-	1	-	0	-	0	-	0	-
	***An. melas* (N=15)**	0	-	1	-	0	-	0	-	0	-
WCR	***An. arabiensis* (N=8)**	-	0.13	-	0.88	-	0	-	0	-	0
	***An. coluzzii* (N=1)**	-	1	-	0	-	0	-	0	-	0
	***An. gambiae ss* (N=40)**	0.25	0.13	0.75	0.84	0.13	0.06	0	0	0	0
	***An. col./ gam* (N=11)**	0	0	1	1	0	0	0.17	0	0	-
CRR	***An. arabiensis* (N=34)**	0	1	0.96	0	0	0	0.1	0.27	0	0
	***An. coluzzii* (N=3)**	-	1	-	0	0	0	0	0	0	0
	***An. melas* (N=1)**	0	-	1	-	0	-	0	-	-	0

Vgsc- voltage-gated sodium channel. *GSTe2*-glutathione-s-transferase epsilon 2. Ace1-Acetylcholine esterase1.

In *An. arabensis* resting indoors in URR, *Vgsc-1014S* frequency was higher in the indoor- (allele freq. = 0.91, 95%CI: 0.84–0.96) than outdoor-resting (allele freq. = 0.82, 95%CI: 0.76–0.87) mosquitoes. Moreover, *Vgsc-1014S* was the only mutation identified in this species when found resting indoors (allele freq. = 0.96, 95%CI: 0.78–1) in CRR. Similarly in URR, the *Vgsc-1014S* mutation in *An. coluzzii* was higher in the indoor (allele freq. = 0.25, 95%CI: 0.17–0.36) than outdoor-resting mosquitoes (allele freq. = 0.04, 95%CI: 0.005–1.3). In LRR, the mutation was found only in indoor-resting mosquitoes (allele freq. = 0.3, 95%CI: 0.19–0.42). Conversely in WCR, the mutation was common in *An. gambiae s.s.* and higher among outdoor- (allele freq. = 0.84, 95%CI: 0.67–0.95) than indoor-resting (allele freq. = 0.75, 95%CI: 0.35–0.97) mosquitoes.

Vgsc-1014F was almost fixed in most mosquitoes, except *An. arabiensis*. It was also more common in the outdoor- than indoor-resting mosquitoes. More specifically in URR, the mutation was found higher in outdoor-resting (allele freq. = 0.92, 95%CI: 0.81–0.98) than the indoor-resting *An. coluzzi* population (allele freq. = 0.74, 95%CI: 0.63–0.82). Likewise, in the hybrid population of *An. coluzzi* and *An. gambiae s.s.*, the mutation was fixed and higher in the outdoor-resting (allele freq. = 1, 95%CI: 0.74–1) than indoor-resting (allele freq. = 0.93, 95%CI: 0.80–1) mosquitoes. The mutation was similarly fixed in both the indoor (allele freq. = 1, 95%CI: 0.96–1) and outdoor (allele freq. = 0.99, 95%CI: 0.95–1) *An. gambiae s.s.* populations. Conversely in WCR, *Vgsc-1014F* was more frequent in *An. gambiae s.s.* resting indoors (allele freq. = 0.25, 95%CI: 0.03–0.65) than the outdoor population (allele freq. = 0.13, 95%CI: 0.04–0.29). Whereas in LRR, where only mosquitoes resting indoors were caught, this mutation was most common in *An. coluzzii* (allele freq. = 0.66, 95%CI: 0.81–0.98).

Vgsc-1575Y and *GSTe2-114T* were found mostly in URR and were more frequent in outdoor-resting mosquitoes. The mutations were almost fixed in *An. gambiae s.s.* regardless of resting place (allele freq. = 0.96-1, 95% CI: 0.92–1.2). When *An. coluzzii* was found resting outdoors also in this region, these mutations were higher (allele freq, = 0.9, 95% CI: 0.79–0.97) than in their indoor-resting counterpart (allele freq, = 0.68-0.78, 95% CI: 0.56–0.86). The hybrids of *An. coluzzii* and *An. gambiae s.s.* with higher and fixed *Vgsc-1575Y* mutation were equally resting outdoors (allele freq. = 1, 95% CI: 0.74–1) while those found resting indoors were carrying only the *GSTe2-114T* mutation (allele freq. = 0.87, 95% CI: 0.60–0.98).

The carbamate and organophosphate resistance marker, acetylcholine esterase (*Ace*)-*119S* was detected only in 8 (4 indoor and 4 outdoor) *An. gambiae s.s.* and in one hybrid specimen in URR.

Sporozoite infection rate

Plasmodium falciparum sporozoites were detected in 11 out of 844 mosquitoes (Table 2), representing a 1.3% (95% CI: 0.5–2%) infection rate. All the infected mosquitoes were caught in URR, of which six were resting indoors and five resting outdoors. Outdoor-resting *An. arabiensis* were mostly infected (36%, 4/11), followed by indoor-resting *An. gambiae s.s.* (27%, 3/11) and *An. arabiensis* (18%, 2/11). One each of outdoor-resting *An. coluzzii* and *An. coluzzii*-A*n. gambiae s.s.* hybrid were also infected.

288 **Table 2: Sporozoite positivity rate in the eleven vector species that were infected based on**
289 **their resting locations**

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	An. arabiensis **proportion (n)**	***An. coluzzii*** **proportion (n)**	***An. gambiae s.s.*** **proportion (n)**	***An. coluzzii-An. gambiae s.s.*** **proportion (n)**
Indoor	0.18 (2)	0.09 (1)	0.27 (3)	0
Outdoor	0.36 (4)	0	0	0.09 (1)

n= number of mosquitoes positive for sporozoite detection. Proportion = the number positive per species divided by overall positive (11).

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294 # Host blood meal preference

295 Host blood meal origin was determined in 251 randomly selected engorged mosquito abdomens.
296 Overall, animal and human blood meal indices were higher for indoor- than outdoor-resting
297 mosquitoes (Table 3). In all vector species, most blood meal (91%) had animal origin. Indoor-
298 resting *An. coluzzii* had the highest preference for human blood while indoor-resting *An. arabiensis*
299 had most preference for animal blood.

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301 **Table 3: Human and animal blood meal preferences of the indoor and outdoor-resting vector species in combined study sites.**

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	An. arabiensis		*An. coluzzii*		*An. gambiae ss*		*An. col./An. gambiae* **ss**	
	Indoor (n)	Outdoor(n)	Indoor(n)	Outdoor(n)	Indoor(n)	Outdoor(n)	Indoor(n)	Outdoor(n)
Human	0.01 (3)	0.004 (1)	0.03 (7)	0	0.01 (2)	0.01 (2)	0	0.004 (1)
Animal	0.23 (58)	0.16 (40)	0.12 (30)	0.09 (23)	0.12 (29)	0.13 (33)	0.04 (9)	0.02 (6)
Human + Animal	0.004 (1)	0.004 (1)	0.01 (3)	0	0.01 (2)	0	0	0
HBI	2	0.8	4	0	1	0.8	0	0.4
Animal blood indices	23	16	12	9	12	13	4	2

303 Proportion (number). HBI= Human blood index.

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Discussion

Insecticide resistance is currently widespread among malaria vectors in The Gambia [12,15], resulting in insecticide rotation for IRS and more recently the use of actellic, an organophosphate insecticide, as recommended by WHO [33]. It is unclear how such vector control interventions have influenced the vectors' feeding and resting behavior, and malaria transmission dynamics. In this study, *An. arabiensis* had a marked preference for outdoor resting and *An. coluzzii* and *An. melas* for indoor resting. Resting location was similar for *An. gambiae s.s.* and *An. coluzzii-An. gambiae s.s.* hybrid populations. Moreover, local vectors had a marked preference for animal blood. The sporozoite infection rate was low and infectious mosquitoes were mainly outdoor-resting *An. arabiensis*.

An. arabiensis tended to rest indoors if they had resistance mutations. This was particularly evident for the *Vgsc-1014S* which seems to influence the resting behavior of this vector species. Vectors with this mutation, which is not fixed yet, may prefer to rest indoors as it protects against the effect of IRS and LLINs [37]. It would be worthwhile to further explore how this mutation modulates resting behavior in vector species. Conversely, *Vgsc-1014F* seems to be fixed in most vector populations in West Africa [34,35], and this may explain why vectors with this mutation do not have a specific resting behavior [36].

An. coluzzii displayed an outdoor-resting behavior when genotypically more resistant than the indoor population. This was consistent across all mutation markers except *Vgsc-1014S;* and was observed mainly in URR, where most mosquitoes were caught. The association between genotypic resistance and outdoor-resting behavior in *An. coluzzii* was recently reported in Northern Ghana [36]. However, for *An. gambiae s.s.*, the mutation frequency did not vary by resting location. *Vgsc-*

1014F was the main mutation in *An. gambiae s.s* at fixed frequencies and did not have any significant effect on resting behavior.

The vector populations analyzed had a higher preference for animal than human blood meal and the overall sporozoite rate was also low. Given the current low malaria prevalence in The Gambia [9], a low sporozote rate is expected. These may reflect the impact of the scaled-up in IRS and LLINs program in the study sites which seems to successfully limit mosquito access to human blood meal indoors and consequently reducing transmission, as previously reported [39,40]. The observed choice of animal blood by majority of the vectors could lead to increase in vector population that may eventually resort to biting humans in the long run and become difficult to control.

Furthermore, the proportion of vectors resting indoors and that have taken a blood meal either from human and animal source, could be a concern for the effectiveness of vector control measures. This shows that the vectors took blood meal from animals outdoors and later went indoors to rest regardless of the presence of IRS and LLINs, indicating that these vectors are resistant to the insecticides being used. This behavior was as previously demonstrated where blood-fed mosquitoes were more resistant than their unfed counterpart [41]. Notably, alternative vector control methods such as treatment of animals with endectocides [42] and zooprophylaxis [43], could be promising tools that could be adopted by the Gambia National Malaria Control Program.

The significant preference for outdoor resting by *An. arabiensis* and indoor resting in *An. coluzzii* prevalent in URR may explain the high intensity of malaria transmission in this region [7,9], which may be driven by these vectors. *An. coluzzii* is highly anthropophagic, endophilic and an efficient

vector of malaria (44), traits that facilitate its contact with human indoors as observed here. A previous study in this setting corroborated the finding here where HBI as high as 80% was documented in *An. coluzzii* and *An. gambiae s.s.* [10]. Further, a relatively high vector parity rate was recently reported in the same vector species [9]. Moreover, *An. arabiensis* is known for its exophilic behavior that increases outdoor transmission in unprotected humans outside LLINs [45].

The composition of the vector species was consistent with previous studies in the Gambia where the most abundant vector was *An. arabiensis,* followed by *An. gambiae s.s.* and*, An. coluzzii* along with their hybrids [12,15,16]. Low density of *An. melas* found was as a result of our choice of villages in the West, which were not located in the coastal regions where this species breeds in salty water [46–48]. Remarkably, predominance of *An. arabiensis* could be as a result of its outdoor-resting preference to avoid insecticide used in IRS and LLINs [49]. This leaves the highly anthropophilic and endophilic species more exposed to vector interventions, possibly leading to relative advantage that maintains the exophilic population and malaria transmission [45].

Conclusion

The study observed an indoor-resting behavior in *An. arabiensis* that were carrying *Vgsc-1014S* mutation and outdoor-resting behavior in *An. coluzzii* populations having other mutations. However, preference for outdoor resting was predominant in *An. arabiensis* and indoor resting in *An. coluzzii* populations. No specific preference for indoor or outdoor-resting behavior was demonstrated in *An. gambiae s.s.* and remaining vector species. An overall high preference for

370 animal blood meal was found in the vector populations. Low rate of mosquito infectivity was
371 identified likely due to high coverage of LLINs and IRS in the study regions. As malaria
372 transmission remains low in The Gambia, which is in earnest preparation for pre-elimination
373 phase, the magnitude of genotypic resistance observed in this study suggests a serious threat to the
374 success of vector intervention in pre-elimination programs. Finally, the observed preference for
375 animal host by vector populations, recommends the consideration of veterinary endectocides and
376 zooprophylaxis as complementary vector control measures.

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379 # Acknowledgements

380 We thank Messrs Musa Jawara, and Mamlie Touray for their assistance in the field work for this
381 study.

382

383

384 # Funding

385 This work was supported by funds from a Wellcome Trust DELTAS Africa grant (*DEL-15-007*:
386 *Awandare*). Majidah Hamid-Adiamoh was supported by a WACCBIP-Wellcome Trust DELTAS
387 PhD fellowship. The DELTAS Africa Initiative is an independent funding scheme of the African
388 Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA)
389 and supported by the New Partnership for Africa's Development Planning and Coordinating
390 Agency (NEPAD Agency) with funding from the Wellcome Trust (107755/Z/15/Z: Awandare)

and the UK government. Additional support was also provided by the H3Africa PAMGENe project, H3A/18/002, funded by the AAS. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, Wellcome Trust or the UK government.

Competing Interests:

The authors have declared that no competing interests exist.

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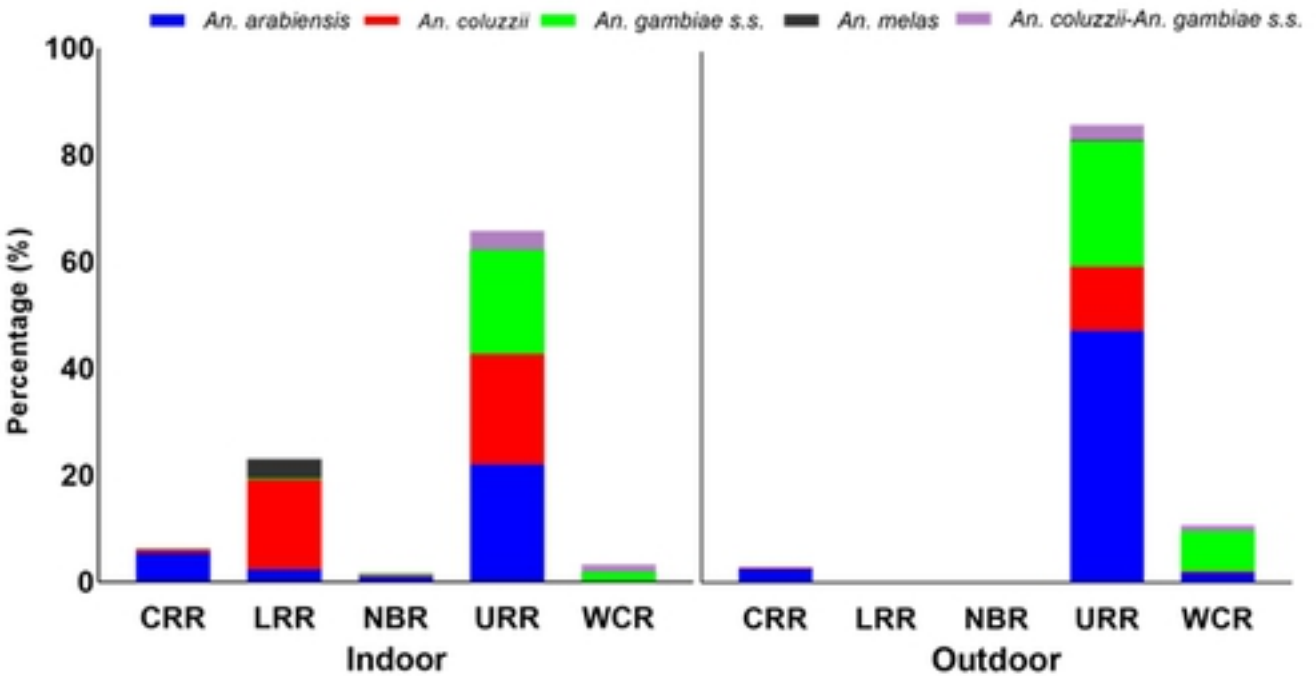
An. arabiensis
An. coluzzii
An. gambiae s.s.
An. melas
An. coluzzii-An. gambiae s.s.
Percentage (%)
100
80
60
40
20
0
CRR
LRR
NBR
URR
WCR
Indoor
CRR
LRR
NBR
URR
WCR
Outdoor

Figure 1