Composition of Mosquito Fauna and Insecticide Resistance Status of *Anopheles gambiae* complex in Itang special woreda, Gambella, Southwestern Ethiopia

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Research

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Abstract

Introduction

Malaria is a leading cause of morbidity and mortality mainly in sub-Saharan African countries. *Plasmodium falciparum* and *P. vivax* are the dominant malaria parasites responsible for the majority of malaria cases in Africa. The aim of this study was to investigate composition of mosquito fauna and insecticide resistance status of *Anopheles* mosquito in Itang special woreda (district), Gambella, southwestern Ethiopia.

Materials and methods

Adult mosquitoes were sampled from September 2020 to Feburary 2021 using Centers for Disease Control and Prevention (CDC) light trap and Pyrethrum Spray Catch (PSC). Moreover, mosquito larvae were collected from different breeding sites and reared to adults. Susceptibility tests were conducted on adult two to three days old non blood fed female *Anopheles gambiae* s.l following world health organization (WHO) standard susceptibility test procedure. Insecticide impregnated papers with deltamethrin (0.05%), alpha-cypermethrin (0.05%), propoxur (0.1%), pirimiphos-methyl (0.25%) and bendiocarb (0.1%) were used to assess susceptibility status of *Anopheles gambiae* s.l populations in the study area. Moreover, molecular diagnostics were done for the identification of member species of *Anopheles gambiae* s.l and detection of knockdown resistance (*kdr*) using species specific polymerase chain reaction (PCR) and allele specific PCR.

Results

In total, 468 adult mosquitoes were collected from different houses. *Culex* mosquitoes were the most dominant *(*80.4%*)* followed by *Anopheles* mosquitoes. Three species of *Anopheles* mosquitoes (*An. coustani, An. pharoensis*, and *An. gambiae* (s.l.)) were identified, of which *An. coustani* was the dominant *(*8.1%) species. Out of 468 adult mosquitoes, 294 were blood fed while 46 were half-gravid and gravid. The WHO bioassay tests revealed that the populations of *An. gambiae* s.l in the study area are resistant against alpha-cypermethrin and deltamethrin whereas, susceptible to bendiocarb, pirimiphos-methyl and propoxur. Out of the total 86 *An. gambiae* s.l specimens assayed, 79 (92%) successfully amplified and all were identified as *An. arabiensis*. West African *Kdr* (L1014F) mutation was detected with high *Kdr* allele frequency ranging from 67–88%.

Conclusion

The detection of target site mutation, *kdr* L1014F allele, coupled with the phenotypic resistance against alpha-cypermethrin and deltamethrin call for continuous resistance monitoring.

Background

Malaria is vector-borne disease caused by *five Plasmodium* species namely; *Plasmodium falciparum, P. ovale, P. malariae, P. vivax and P. knowlesi.* The parasite transmitted to human through the bite of infective female *Anopheles* mosquito [1]. There are over 445 recognized species of *Anopheles* mosquito of which around 70 species are potential malaria vectors [2]. In Africa, the major malaria vectors are *Anopheles gambiae* and *An. funestus* species complexes, but there are also a number of primary and secondary vectors that contribute to the malaria transmission [2].

In Ethiopia, there are more than 45 documented *Anopheles* mosquito species [3], of which only four species are malaria vectors. *Anopheles arabiensis*, member species of the *Anopheles gambiae* complex, is the primary vector of malaria widely distributed in the country[4, 5]. *Anopheles funestus An. pharoensis and An. nili* are secondary vectors occurring with varying population densities, limited distribution and vector competence [6]. Very recently, a new invasive *Anopheles* species, *An. stephensi*, has been documented in the country [7] which might complicate the malaria elimination effort of the country.

Chemical based vector control intervention is the pillar strategy to combat malaria. Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) are instrumental for the significant reduction of malaria morbidity and mortality [8]. However, the emergence and widespread of insecticide resistance in the major malaria vectors particularly in Africa might compromise effectiveness of chemical based (IRS and LLINs) interventions against malaria control and elimination efforts [8–14].

Insecticide resistance has been reported in more than 500 insect species worldwide among which over 70 *Anopheles* species (Diptera: Culicidae) are responsible for the transmission of malaria parasites to humans (Hemingway and Hilary, 2000). Insecticide resistance has become a serious challenge for the control and elimination of malaria due to the fact that malaria vectors are resistant to the four commonly used insecticide classes (pyrethroids, organochlorines, carbamates and organophosphates) [8, 14]

In Ethiopia, DDT resistance by *An. arabiensis* was reported in the1990s, since then widespread DDT resistance documented throughout the country[9, 10, 16–18]. Moreover, resistance against other classes of insecticides such as carbamates (bendiocarb), organophosphates (malathion) and pyrethroids (permethrin, deltamethrin) has been reported from various regions of the country [18, 19]

In the last decade the number of malaria cases has declined due to a high coverage of IRS and scaling up of LLINs [8, 14]. This result initiated the national malaria control and elimination program of Ethiopia to develop national malaria elimination road map to eliminate malaria from the country by 2030 [20]. However, this plan might be compromised as the magnitude of resistance against several insecticides is increasing in the *An. arabiensis* populations [8, 18, 19]

Insecticide resistance largely caused by two major mechanisms. The first one is due to target-site insensitivity as a result of mutations in the target site of the insecticide that changes binding. The second mechanism is metabolic based resistance, where the insecticide is either degraded, sequestered or transported/excreted out of the cell before binding to the target site [21, 22]

Target-site and metabolic based resistance mechanisms operating in malaria vectors in several malaria endemic African countries have been documented. Knockdown resistance (*kdr*) is target site mutations in the voltage-gated sodium channel gene of mosquito nerve membranes is associated with DDT and pyrethroids resistance. In *Anopheles*, this involves the substitution of leucine (TTA) to phenylalanine (TTT) (*kdr*L1014F) or to serine (TCA) (*kdr*L1014S) [23, 24]. In addition, substitution of asparagine to tyrosine (N1575Y) is linked with resistance in *An. gambiae* s.s [25] but not in *An. arabiensis* [18]. There is also an acetylcholinesterase gene (ace-1R) mutation, substitution of glycine (GGC) to a serine (AGC) which confers resistance to organophosphates and carbamates [26]. In Ethiopia, target site resistance mechanism, *Kdr* L1014F (West Africa *Kdr*), in populations of *An. arabiensis* documented in several populations across the country [9, 10, 16, 18, 19]

Metabolic based resistance in *Anopheles* mosquitoes have been reported from several countries in Africa [27–30]. Moreover, modifications in the cuticle either through cuticle thickening and/or altering of the cuticle composition of arthropods which can slow down the penetration of chemical compounds [31–33] has also been reported in *Anopheles* populations [34].

Approximately 60% of the Ethiopian populations live in malaria risk areas [6]. The disease primarily occurs up to the 2000-meter *(*m*)* elevation but can also occasionally affect areas over 2000m elevation in response to the spatial and temporal changes [35, 36]. Malaria transmission is unstable and seasonal which produces little immunity in the community; hence malaria epidemics are common and lead to high mortality and morbidity [6]. Gambella is one of the malarious areas of Ethiopia with high malarial endemicity. Itang special woreda is known for its a stable form of malaria transmission [37]. Moreover, despite the current effort of the country malaria incidence rate in Itang did not decline unlike many other malarious areas of Ethiopia. [38]. Moreover, to the best of our knowledge composition of mosquito fauna and insecticide resistance status of *Anopheles gambiae* s.l in Itang, not yet studied, and the resistance mechanisms operating in the populations not known. Therefore, this study aimed to investigate mosquito fauna composition, insecticide resistance status of *Anopheles gambiae* s.l and detection of target site mutations associated with DDT and pyrethroid resistance.

Methods And Materials

Study area

Gambella is one administrative regional state among the ten regional states of Ethiopia. It's, located in south western Ethiopia, 777 km away from Addis Ababa (Fig. 1). Most of the region is flat, hot, and humid, with an altitude range of 300–2,300m above sea level and sloping westward. The annual average temperature of the region is 21.1°C–35.9°C, with an average annual rainfall of 600 mm .The region has a total area of 25,802 km^2 and administratively divided in to three Zones (Nuwer, Agnua, Mezeng) and one special woreda, Itang [39]. According to the 2017 Ethiopian population projection, Gambella is sparsely populated with the total population approximately 436,000 [40].

The study was conducted from September 2020-Feburary 2021 in Itang special woreda, which is situated 42 km to the west of the regional capital of Gambella. Itang has 23 kebeles, with estimated total population of 45,772 and 9,154 households [40]. The woreda's average annual temperature and rainfall are 29°C and 1,000 mm, respectively. The climatic conditions of the woreda are favorable for the existence of a stable form of malaria throughout the year [6]

Sampling collection

Anopheles **mosquito larvae collection**

Potential mosquito breeding habitats (Baro river fringes, paddle or farming field ditches, sewerage, and stagnant water pools) were first inspected for the presence of mosquito larvae visually and positive habitats were sampled with a 350 ml capacity mosquito scoop. Dipping was done following WHO guidelines and standard operating procedures for entomological surveillances [41].Third to fourth (late) instars anopheline larvae were sampled and put in enamel trays with water from the breeding sites from they were collected for rearing. They were reared and kept at constant 80%±10% RH and 27 ± 2^{o}c and fed with baking powder. The pupae were sorted and transferred with pipettes from the enamel trays to beakers with small amounts of water. Each beaker was placed inside a cage for rearing. Pupae were provided with 10% sugar in the cage. After three to four days, the pupae emerged to adults and the cages were put in safe place protected from contamination, ants, and other insects.

Adult Anopheles **mosquito collection**

Collection of adult mosquitoes was carried out after obtaining ethical clearance letter from research and ethical review board of College of Natural Sciences, Jimma University (Ref. No. RPG/ 056/2020). Moreover, informed consent was obtained from the head of the selected households. Adult *Anopheles* mosquitoes were collected using CDC light traps and PSC from selected houses in study area. CDC light traps were placed in three selected houses indoor and outdoor from 6:00PM to 06:00AM to collect female *Anopheles* mosquitoes for two subsequent days per month from September 2020 –February2021.

In addition to CDC light traps, indoor resting mosquitoes were collected using pyrethrum spray catches PSC in ten selected houses from 6:00AM to 9:00AM from October 2020-February 2021 once in a month. Before spray the whole house was covered by white sheet and closed every opening such as

windows, door, and others. After fifteen minute of spraying knockdown mosquitoes were collected and recorded and separately placed in Eppendorf tube.

Identification of Anopheles mosquito species

Anopheles mosquitoes were morphologically identified to the species using taxonomic keys [42, 43].

Insecticide susceptibility test

Two to three days old non blood fed adult females morphologically identified as *An. gambiae*s s.l were exposed to discriminating doses of deltamethrin (0.05%), alpha-cypermethrin (0.05%) propoxur (0.1%) pirimiphos-methyl (0.25%), and bendiocarb (0.1%). The insecticide impregnated and control papers were obtained from the WHO collaboration Centre, Vector Control Research Unit, School of Biological Sciences, Penang, Malaysia. Batches of 25 mosquitoes in four replicates were exposed in test kit tubes with insecticide impregnated papers for all bioassays for one hour against each insecticide and knockdown were recorded at 10, 15, 20, 30, 40, 50, and 60 minutes [44]. After one hour, mosquitoes were transferred into holding tubes and provided with 10% sucrose solution with soaked cotton pads. Mortality was recorded 24 hour post exposure. Mosquitoes, both dead and alive, were individually preserved in Eppendorf tubes over silica-gel for further molecular assays. The same numbers of mosquitoes were exposed to insecticide free papers as controls.

DNA extraction of An. gambiae s.l

Genomic DNA of individual *Anopheles* mosquito was extracted from 75 survived and 11dead mosquitoes (sampled from mosquitoes exposed to alpha-cypermethrin and deltamethrine) using DNAzol reagent (MRCgene, USA) with minor modification of the protocol [45]. Briefly, the whole part of each mosquito was put individually in an Eppendorf tube. A volume of 50µl DNAzol was first added to 1.5ml Eppendorf tube. A volume of 50µl DNAzol was added to grind the mosquitoes and then 150µl to the same tube. Then, the tubes were centrifuged for 10 min at 10000rpm. Then after, the supernatant transferred into new 1.5 Eppendorf tube and 100µl pure ethanol was added to the supernatant. The ethanol allowed to be mixed with the supernatant and centrifuged for 20 min at 13000rpm. Then, the supernatant was discarded and the gDNA pellet in the Eppendorf tube was washed with1000µl 75% ethanol and then centrifuged for 5min at 13000rpm. The supernatant was removed, the DNA pellet dried by air and resuspended in 20µl Elute buffer for further use.

Molecular identification of An. gambiae s.l

Morphologically identified *Anopheles gambiae s.l* female mosquitoes (alpha-cypermethrin and deltamethrine survived and dead mosquitoes) were selected for the molecular identification of members of *An. gambiae* species using species specific polymerase chain reaction (PCR) at Molecular Biology Laboratory, Tropical and Infectious Diseases Research Centre (TIDRC), Sekoru, Jimma University. *An. arabiensis* susceptible colony strain found in Sekoru insectary was used as a positive control. The PCR assay was done adapting the established protocol [46]. In brief, PCR reaction was prepared for 20µl final volume of 10µl master mix, 0.5µl of each primer (Universal primer (5′-GTG TGC CCC TTC CTC GAT GT-3′), *An. arabiensis* (5′-GTG TGC CCC TTC CTC GAT GT-3′), *An. quadrinnulatus* (5′-CTG GTT TGG TCG GCA CGT TT-3′), 7.5µl nuclease free water and1µl template DNA. The PCR program was set for 30 cycles at 94°C, 50°C and 72°C for 30sec each, respectively and a final extension step at 72^0C for 5 min. Finally, the band size of PCR products for each species was visualized on a 2% agarose gel.

Detection of LF14F (Kdr allele)

Allele specific PCR assay was conducted on the same specimens used for the identification of member species of *An. gambiae* s.l. as indicated under 3.5 above. The presence of West Africa *Kdr* (L1014F) mutation was detected adapting the established protocol [23]. The *kdr* PCR was done with 25µl final volume including 2.5µl PCR buffer, 0.5µl dNTP mixture, 0.75µl MgCl, 0.8µl Agd1 (5'-atagattccccgaccatg-3') primer, 0.8µl Agd2 (5'-agacaaggatgaatgaacc-3') primer, 1.5µl Agd3 (5'-aatttgcattacgaca-3') primer, 1.5µl Agd4 (5'-ctgtagtgatgataggaaattta-3') primer, 0.15µl Taq DNA polymerase, 15.5µl nuclease free water and 1µl DNA template to detect L1014F allele. The PCR program was set at 94°C for 3 min, and 94°C for 1min, 52°C for 30 sec, 72°C for 30 sec for 40 cycles and a final extension step at 72°C for 10 min. Finally, the band size of the PCR products for *kdr* allele was visualized on a 2% agarose gel to determine the genotype to homozygous resistant, heterozygous resistant and susceptible or wild type *Kdr* allele.

Data analysis

Susceptibility status data was analyzed based on the WHO 2016 classification criteria. As per the criteria 24 hour mortality rate 98% and above considered fully susceptible; between 90 and 98%, possible resistance or suspected resistance; and mortality below 90% classified as resistant. When the control mortality was between 5 and 20%, the average observed mortality was corrected using Abbott's formula [47]. When the control mortality was above 20% the test result was discarded and the test was repeated.

Results

Mosquito densities and species composition

In total, 468 mosquitoes were collected using CDC light trap and PSC collection methods from different houses (Table 1). The majority of mosquitoes were *Culex* spp. (80.4%) followed by *Anopheles* mosquitoes. Three species of *Anopheles* mosquitoes such as *An. coustani, An. pharoensis*, and *An.*

gambiae s.l were identified, of which *An. coustani* was dominant among others (8.1%; n = 38) (Table 1). As shown in Table 1 the higher number of mosquito was collected outdoor by CDC light traps.

Table 1
Species composition and abundance of mosquitoes in Itang special woreda, southwestern Ethiopia from September 2020 to February 2021

Mosquito Species	Light trap				PSC		Total	
	Indoor	(%)	Out door	(%)		(%)		(%)
An. coustani	10	5.4	26	11.2	2	3.9	38	8.1
An. pharoensis	8	4.3	24	10.4	3	5.9	35	7.5
An. gambiaes.l	9	4.8	8	3.5	2	3.9	19	4
Culex spp	159	85.5	173	74.9	44	86.3	376	80.4
Total	186	100	231	100	51	100	468	100

Abdominal status of different mosquito species

The abdominal conditions of all collected mosquito samples were classified into unfed, freshly fed, half gravid, and gravid. Out of 468 mosquitoes collected, 294 were fed while 46 half-gravid and gravid (Table 2).

Table 2
Fed to gravid ratio of different mosquitoes collected from Itang special woreda, southwestern Ethiopia, from September 2020 to February 2021

Species	Blood fed	Unfed	Half gravid	Gravid	Total (%)
An. coustani	9 (3%)	24 (18.8%)	4 (12.9%)	1 (6.7%)	38 (8.1%)
An. pharoensis	9 (3%)	19 (14.8%)	5 (16.1%)	2 (13.3%)	35 (7.5%)
An. gambiae s.l	4 (1.4%)	11 (8.6%)	3 (9.7%)	1 (6.7%)	19 (4.1%)
Culex spp	272 (92.6%)	74 (57.8%)	19 (61.3%)	11 (73.3%)	376 (80.3%)
Total	294 (100%)	128 (100%)	31 (100%)	15 (100%)	468 (100%)

Insecticide resistance status of An. gambiae s.l **against different insecticides**

The results of the susceptibility status of populations of *An. gambiae* s.l. are presented in Table 3. Mortalities in control groups were zero and no correction of mortality data was required. As per WHO criterion, the local mosquito populations of *An. gambiae* s.l. were resistant to two groups of pyrethroid insecticides (deltamethrin and alpha-cypermethrin) tested. Mortality rates of *An. gambiae* s.l. against deltamethrin and alpha-cypermethrin was 58% and 42%, respectively. However, the populations of *An. gambiae* s.l. were completely susceptible to pirimiphos-methyl, propoxur and bendiocarb, where 100% mortality rate was recorded for the three insecticides (Table 3).

Table 3 Insecticide susceptibility status of An. gambiae s.l **populations from Itang special woreda, southwestern Ethiopia, from September 2020 to February 2021**

S.N	Insecticide	*An. gambiae* s.l tested			*An. gambiae* s.l control	
		No. tested	No. dead	% mortality	No. test	% mortality
1	Alpha-cypermethrin (0.05%)	100	42	42%	50	0
2	Deltamethrin (0.05%)	100	58	58%	50	0
3	Propoxur (0.1%)	100	100	100%	50	0
4	Bendiocarb (0.1%)	100	100	100%	50	0
5	Pirimiphos-methyl (0.25%)	100	100	100%	50	0

Avaliblity of LLINs

The data taken from thirteen households where CDC light traps and PSC were done revealed that a total of 61.5% of the households had at least one LLIN (Table 4) where in average four people are living in one house.

Table 4
Number of LLINs found per household in Itang special woreda, southwestern Ethiopia, from September 2020 to February 2021

Number of LLINs	Number of family	Number of house holds	Percentage
1	1–2	5	38.5
1	3	3	23
2	4–6	5	38.5
Total	41	13	100

Malaria suspected, laboratory examined and confirmed cases in Itang health center

In Itang health center a total of 3183 request was sent to laboratory from September 2020-Feburary 2021 to test and identify *Plasmodium* parasite. Among the specimens examined *P. falciparum* identified in 1784 which accounts 99.7% of the whole positive result whilst, *P. vivax* comprised 0.3% (Table 5).

Table 5
Rate of *Plasmodium* parasite among patients visited Itang special woreda health center, from September 2020- February 2021

Months	Request	Positive for PF	Positive for PV	Positive for mixed	Total	Negative
September	347	166	1	0	167	180
October	565	254	2	0	256	309
November	784	449	1	0	450	334
December	444	269	0	0	269	175
January	650	425	1	0	426	224
February	393	221	0	0	221	172
Total	3183	1784	5	0	1789	1394

Molecular identification of An. gambiae s.l **and detection of L1014F** Kdr **allele**

Out of the total 86 *An. gambiae* s.l specimen assayed using species specific PCR, 79 (92%) of the specimens were successfully amplified and all were identified as *An. arabiensis.* The allele specific PCR revealed the presence of the knock-down resistance *(kdr*) L1014F allele with 92.7% (n = 51) homozygous and 7.3% (n = 4) heterozygous *kdr* resistance allele respectively with the *Kdr* allele frequency ranging from 67–88% (Table 6).

Table 6
Genotypic and *kd r*allele frequency in populations of *An. arabiensis* from Itang special woreda, southwestern Ethiopia, from September 2020 to February 2021

Insecticide	Number assayed	Bioassay phenotype	Genotype	Allele frequency
			SS RS RR	R S
Deltamethrin	34	Survived	5 4 16	72 28
	6	Dead	1 - 2	67 33
Alpha-cypermethrin	41	Survived	4 - 30	88 12
	5	Dead	1 - 3	75 25
Note: RR-Homozygous resistant, RS-Heterozygous resistant and SS-homozygous susceptible				

Discussion

Anopheles coustani, An. pharoensis and *An. gambiae* s.l. were the three species identified from Itang, southwestern Ethiopia. Of all *Anopheles* mosquitoes, *An. coustani* was the most prominent species followed by *An. pharoensis* and *An. gambiae* s.l. In the study site the abundance of mosquito species collected by CDC light trap was higher outdoor than indoor. Unlike many other localities in Ethiopia [48–50], the abundance of *An. custani* dominated *An. gambiae* s.l. A study from Lare, Gambella documented higher density of *An. gambiae s.l* than *An. coustani* [49] contrasting the result of the current study. This difference might be due to sampling period difference which leads to variation or shifts in density for various species of mosquitoes. Taye and his coworkers sampled mosquitoes from May to September 2017 whereas in our study sampling time was between September and February. Moreover, the difference might also be due to the type of breeding habitat. The breeding site where the *Anopheles* mosquitoes sampled was shore to the Baro River and due to this reason the breeding sites were covered by plants and shaded the water which might be favorable for *An. coustani* [51]. Very recently, higher density of *An. coustani* than *An. gambiae* s.l populations were reported from non-irrigated swampy and river edges in Arjo Didessa, South west Ethiopia

[52]. Unlike *An. coustani*, *An. gambiae* s.l. typically breeds in small, clear, sunlit temporary water pools [53] where vegetative cover is low [51, 54]. Even though the role of *An. coustani* is not yet clearly studied in Ethiopia, the high abundance of the species in Itang might call attention to further study as the species has big role in malaria transmission in some other African countries [55, 56]

The molecular identification of member species of *An. gambiae (s.l.)* confirmed that *An. arabiensis* is the only member species found in the study area. This finding is similar to previously reported studies from other parts of Ethiopia [9, 16, 18, 19]. *Anopheles arabiensis* populations from the study area were found highly resistant to deltamethrin like other populations of *An. arabiensis* from different localities [9, 18, 19] Sudan [57] and Uganda [58]. The current study also showed that *An. arabiensis populations* were resistance to alpha-cypermethrin. Similarly, studies from South-West Ethiopia [59] and Congo [60] reported very high resistance to pyrethroids but susceptible to bendiocarb, propoxur and pirimiphos-methyl. In contrary, populations of *An. arabinesis* form Malawi were found susceptible to alpha-cypermethrin [61].

The population of *An. arabiensis* collected from Itang found fully susceptible to bendiocarb and propoxur. This report is in agreement with other studies from different regions in Ethiopia [10, 18], Sudan [57], Yemen [62], Burkina Faso and Chad [63]. However, unlike the current finding resistance against bendiocarb has been developed by *An. arabiensis* populations in some other parts of Ethiopia [16, 19]. The current result is similar to previously reported studies from different parts of Ethiopia where propoxur fully killed *An. gambiae* s.l [10, 64, 65] Sudan [57]. However, in some other parts of Ethiopia resistance against propoxur has been observed [18, 19]. Similar to propoxur and bendiocarb the *An. arabiensis* populations from the study area were found susceptible to pirimiphos-methyl. This is similar with studies from different parts of Ethiopia [10, 18], Uganda [66] and Congo [60].

In this study, the population of *An. arabiensis* were screened for West African *kdr* allele *(*L1014F*)*. A very high frequency of the West African *kd r*allele *(*L1014F*),* was observed with *kdr* allele frequency ranging from 67–88% indicating that the target site resistance mechanism *(Kdr)* might contributed for the observed high level of pyrethriod *(*deltamethrine and alpha-cypermethrin*)* resistance in the population. This is similar with populations of *An. arabiensis* in some other areas of Ethiopia [10, 16, 18, 19]. The mutation, L1014F, is widespread in West and West Central Africa [67, 68] also becoming common in East African countries including Sudan [69]; Tanzania [70, 71] and Kenya [72, 73]. The L1014F *kdr* mutation in the VGSC, a target point mutation against pyrethroids and DDT [24, 74] might be resulted by natural selection by the use long and extensive use of DDT and pyrethroids for ITNs.

LLINs and IRS are the two most widely implemented malaria vector control interventions, and have resulted in a significant reduction of malaria-related mortality and morbidity in Ethiopia [8, 38, 75]. Universal access and coverage of LLINs by all household members regardless of age or gender [76] is not yet achieved in Ethiopia [76–78].

Plasmodium falciparum and *P. vivax* are the two dominant parasite species causing malaria in Ethiopia. In Itang, *P. falciparum* constituted the most predominant malaria infections. *Plasmodium falciparum* is the dominant parasite species in most malaria epidemic regions which causes severe and complicated manifestations and almost all malaria deaths in different parts of the country [6, 79–81]. However, in some other malarious regions of the country for example Wolkite, Halaba and Arsi Negelle the situation vary and the most prevalent species is *P. vivax* [82–84]

Conclusion And Recommendation

Overall, this study demonstrated that *An. coustani* was the predominant *Anopheles* species recorded among adult *Anopheles* mosquito collected during the study period in the study area. The high abundance of the species in the study area might call attention to further study the species its potential role in malaria transmission. It also revealed that *An. arabiensis*, a member of *An. gambiae* complex, has developed high level of resistance against pyrethroids. Moreover, target site mutation L1014F *kdr* allele with high frequency was detected in the populations.

For the success of malaria elimination from Ethiopia by 2030 [6], it is important to control the malaria vectors and focus on successful treatment. Therefore, understanding the type and density of malaria vector species in specific localities, their feeding behavior and interaction with humans is crucial for effective malaria vector control strategies. Moreover, evidence based insecticide susceptibility status of the malaria vector populations and understanding mechanisms of resistance operating in the populations is also crucial for effective insecticide resistance management.

List Of Acronyms And Abbreviations

CCEs: Carboxylcholinesterases

CDC: Center for Diseases Control and Prevention

CSA : Central Statistics Authority

DDT: Dichlorodiphenyltrichloroethane

DNA: Deoxyribonucleic Acid

FMoH: Federal Minister of Health

GSH: Glutathione

GSTs: Glutathione S-transferases

ITNs: insecticide treated nets

Kdr: Knock down resistance

KM: kilo meter

LLINs: Long-Lasting Insecticide Treated Nets

MSF: Medicines sans frontieres

OCs: Organochlorines

Ops: Organophosphates

P450s: Cytochrome-P450 monooxygenase

PSC: Pyrethrum Spray Catches

PY: Pyrethroids

SPSS: Statistical package for the social sciences

VGSC: Voltage Gate Sodium Channel

WHO: World health organization

Declarations

Ethics approval and consent to participate

Ethical approval letter (Ref. No. RPG/ 056/2020) was obtained from research and ethical review board of College of Natural Sciences, Jimma University, Ethiopia.

Consent for publication

Not applicable

Availability of data and materials

Data used for this study are included in the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TC, DY and EA conceived and designed the study. TC performed the field and laboratory experiments, analyzed data and drafted the manuscript. DY and EA analyzed and interpreted the data and wrote the paper.

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Figures

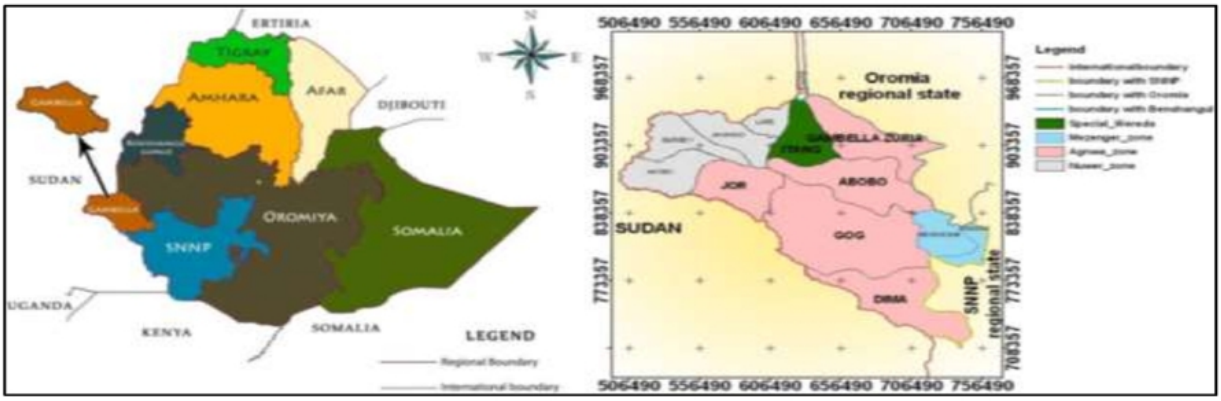

Figure 1

Map of the study area (Riek, 2016).

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