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# Distribution of *Anopheles gambiae* s.l siblings, insecticide resistance and prevalence of markers of resistance (Kdr & Ace-1) in Edea and Buea: forest region of Cameroon

Pilate N. Kwi[1,4], Jubilatio E. Dze[1], Raymond N. Tabue[2], Jude D. Bigoga[3], Alfred Amambua-Ngwa[4*] and Tobias O. Apinjoh[1,5*]

## Abstract

**Background** The complexity and heterogeneity of malaria transmission in Cameroon is enhanced by the different eco-systems and topology which enhance the proliferation of Anopheline mosquitoes. Though long-lasting insecticide-treated nets have been massively distributed across the country, malaria still remains a significant public health concern, with innate and adaptive resistance mechanisms exploited by malaria vectors against different insecticides; the micro-ecological variations in Cameroon could be playing a viral role in the expression of essential insecticide resistance genes in malaria vectors. Thus, this study sought to assess and compare phenotypic resistance to commonly used insecticides and the prevalence of markers of insecticide resistance to *Anopheles gambiae* s.l from two localities within the forest ecological zone of Cameroon.

**Material and methods** Three to five days mosquitoes obtained from larva collected at eight breeding sites in Buea and Edea were morphologically identified and phenotypic resistance to pyrethroid, organophosphate and carbamate insecticides assessed using the WHO bioassay protocol. Molecular speciation of *An. gambiae* s.l and the prevalence of L1014F and *Ace-1R* G119S mutations was assessed using PCR.

**Results** *Anopheles coluzzii* and *An. gambiae* were the only siblings species identified in both communities, with *An. coluzzii* being the dominant sibling in Edea and the latter in Buea. *Anopheles gambiae* s.l was resistance to diagnostic concentrations of all insecticides in Buea but susceptible to 1X bendiocarb and 1X pirimiphosmethyl in Edea. In both communities, mortality increased with increasing concentration of alphacypermethrin, permethrin, pirimiphosmethyl and bendiocarb while PBO had a synergistic effect on all pyrethroid insecticides tested. There was a significant difference in the mortality to 1X permethrin ($p=0.014$),1X permethrin + PBO ($p=0.001$), 5X permethrin ($p<0.001$), 1X alphacypermethrin + PBO ($p<0.001$), 1X pirimiphosmethyl ($p<0.001$) and 1X bendiocarb ($p<0.001$) in Buea compared to Edea.

*Correspondence:
Alfred Amambua-Ngwa
alfred.ngwa@lshtm.ac.uk
Tobias O. Apinjoh
apinjoh.tobias@ubuea.cm
Full list of author information is available at the end of the article

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**Conclusion** *Anopheles gambiae* and *An. coluzzii* were the major malaria vectors in both communities.Though these vectors were resistant to the diagnostic dose of all insecticides in Buea, they were susceptible to 1X bendiocarb and 1X pirimiphosmethyl in Edea.

**Keywords** Anopheles *gambiae s.l*, Insecticide resistance, Molecular markers, Control

## Background

Malaria remains a persistent life-threatening disease, responsible for significant morbidity and mortality, especially in children below the age of 5 years in sub-Saharan Africa. In Cameroon, the transmission of *Plasmodium* spp. is heterogenous and complex as a result of the different ecological zones [1] that provide a diverse landscape and conducive environment for a variety of Anopheles mosquito species.

*Plasmodium falciparum* has been incriminated as the main parasite responsible for most malaria cases in all ecological zones of Cameroon [2–4] and *Anopheles gambiae* s.l and *An. funestus* group are the main malaria vectors as across sSA. However, the siblings of *An. gambiae* s.l have been reported to be unevenly distributed across the eco-climatic zones of the country, with *An. Arabiensis* mostly abundant in the Sahelian and Savannah zones with seasonal malaria parasite transmission [5] while *An. gambiae* and *An. coluzzii* are more prominent in the forested zones [6–8]. These vectors are highly anthropophilic, and highly adaptable to different environmental settings, evolving different mechanisms for survival under different environmental conditions [9]. *Anopheles nili, An. moucheti* and *An. ziemanni* are thought to contribute, to a lesser extent, to the transmission of the parasites in Cameroon [8, 10, 11].

Vector control is of paramount importance in the fight against malaria, with the use of long-lasting insecticidal nets (LLINs) widely exploited in Cameroon [4]. The successful implementation of control interventions is thought to have resulted in the decline in malaria cases from 2010 till 2016 [12]. However, the efficacy of vector control strategies is being threatened by the emergence and spread of insecticide resistance to almost all insecticide classes (pyrethroid and carbamate subclasses) widely used for control interventions in Cameroon. Previous studies around the slope of Mount Cameroon revealed that all anophelines tested were susceptible to malathion but resistant to varying concentrations of deltamethrin and permethrin [13]. In a recent study in the Southern region of Cameroon, *An. coluzzii* was shown to be susceptible to carbamate insecticides (1 X propoxur and bendiocarb), organophosphates insecticides (1 X fenitrothion and pyrimiphosmethyl) but resistant to the 1X, 5 X and 10 X pyrethroids (permethrin, deltamethrin and alphacypermethrin) [14]. *Anopheles gambiae* s.l in Cameroon have also been shown to be resistant to deltamethrin, permethrin, alphacypethrin and etofenprox [15] across other ecological zones.

Insecticide resistance is thought to accrue to several major mechanisms including knockdown resistance (kdr), increased expression of detoxification enzymes, cuticular resistance [16] and behavioural resistance. Knockdown resistance associated with mutations in the voltage-gated sodium channel gene is well-established and has been exploited by anophelines in Cameroon and across Africa [17–19], with the Kdr West (L1014F) mutation and Kdr East (L1014S) in *An. gambiae* s.l associated with resistance against a range of pyrethroids and organochloride insecticides [13, 14, 20, 21]. Reduced sensitivity of organophosphates and carbamates insecticides in *An. gambiae* have also been shown to be associated to increased prevalence of the Ace-1 (G119S) mutation in West and East Africa [22–24] as well as in Cameroon [25, 26] which is a central African country. However, WHO recommends regular monitoring of the resistance profiles of malaria vectors in relation to other intrinsic factors like season, altitude and climatic changes to inform control intervention strategies. Up to date data on insecticide resistance profile of *An. gambiae* s.l across all ecological zones in Cameroon remains paramount for malaria elimination goals. The present study sought to assess and compare the diversity and resistance profile of A*n. gambiae* s.l siblings in Edea (industrial zone) and Buea (agricultural zone) of Cameroon.

## Materials and methods

### Study design and study area

This was a cross-sectional study carried out in two major towns (Buea and Edea) in the forest ecological zone of Cameroon which are 106 km apart (Fig. 1).

These two towns were selected because of their difference in altitude and ecological characteristics. Edea (3.7953° N, 10.1367° E) is situated along the River Sanaga at an average altitude of 35 m above sea level. It covers 180 square km, with an annual temperature range of 20–25 °C, and precipitation and humidity of 2131 mm/79–88%. Buea (4.1560° N, 9.2632° E), situated on the slope of Mount Cameroon has an annual temperature range of 20–34 °C, an altitude of 560–1200 m above sea level, and precipitation and humidity of 2850 mm and 82–87%, respectively. These two towns have an annual

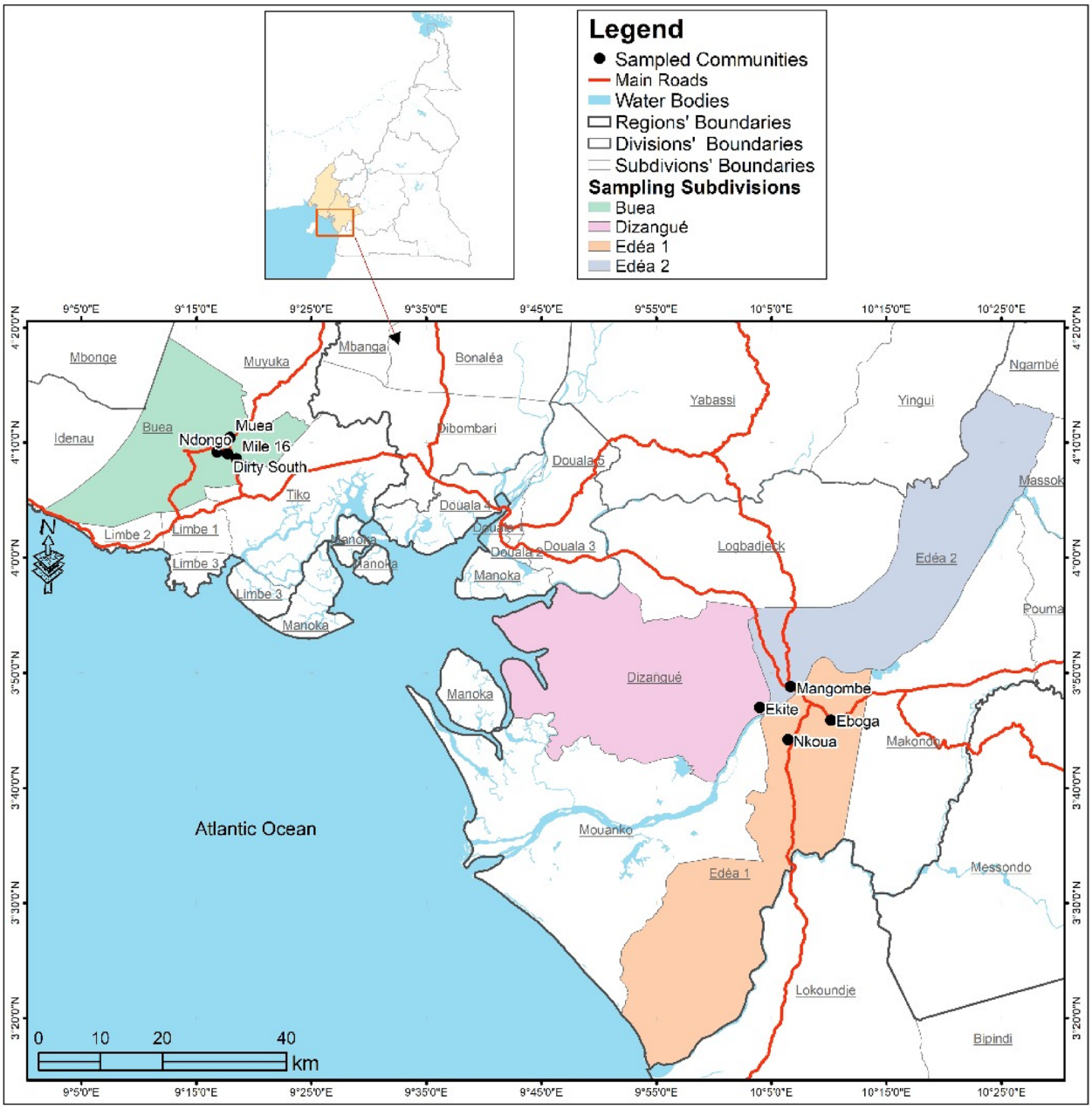


**Fig. 1** Map of the mosquito larval collection sites in Buea (Southwest) and Edea (South), within the forest regions of Cameroon

alternation of a rainy season (May to October) and a dry season (November to April), characterized by high and perennial malaria parasite transmission. Farming is the main activity for the locals, with pesticides widely exploited by farmers. Indoor Residual Spraying is not been exploited in these areas but locals use LLINs as barriers for house widows and to an extent as garden shields from domestic animals.

### Sampling sites and larva rearing

Mosquito larvae were collected across 8 communities; 4 in Buea (Checkpoint Molyko, Dirty South, Mile 16 and Muea) and 4 in Edea (Eboga, Ekite, Mangombe and Nkoua) during the periods of June 2022 to March 2023. Samples were then transported to the Infectious Disease Laboratory of the Faculty of Health Sciences of the University of Buea and reared till emergence (Fig. 2). Larvae were placed in open bowls, fed with tetramine (30g/66ml of fresh water) [27] till they developed in to pupae. The

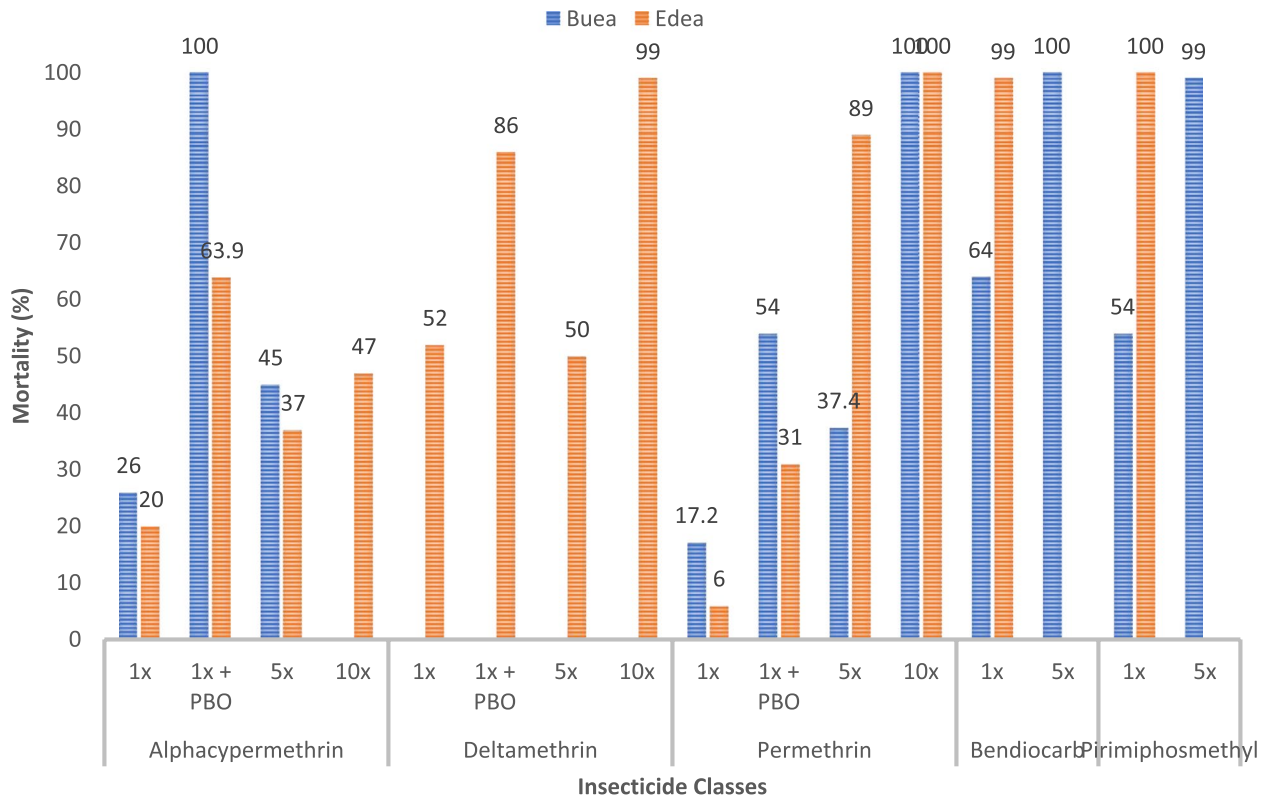


**Fig. 2** Mortality of *An. gambiae* s.l populations from Buea and Edea following exposure to bendiocarb, pirimiphosmethyl, alphacypermethrin and Permethrin. Mortality rates were recorded 24 h post-exposure to insecticides based on the WHO 2022 recommendation

vessel containing the pupae were transported into a net-caged vessel under controlled conditions: temperature and humidity of 27 ± 2 °C and 77–85% respectively with 12 h day/night cycles. Upon emergence, mosquitoes were fed with 10% sucrose and monitored continuously till they became fully mature (3–5 days old) before conducting insecticide bioassay test.

#### WHO insecticide and synergistic bioassay tests

Insecticide Susceptibility testing was undertaken on 3–5 days old non blood fed female *An. gambiae s.l* mosquitoes according to the WHO protocol [28]. These mosquitoes were exposed to different doses of insecticide-impregnated papers. Classes of insecticides mostly used in LLINs/ITNs and IRS were tested at recommended concentration (1X): alphacypermethrin (0.05%) [with or without 5% piperonylbutoxide (PBO)], permethrin (0.75%), pirimiphosmethyl (170mg/$m^2$), and Bendiocarb (0.1%). Higher concentrations (5 X and 10 X) were used to determine the intensity of insecticide resistance in these populations and their ability to tolerate insecticide doses higher than the diagnostic dose. Four replicates of the WHO tube lined with insecticide-impregnated paper and containing 20–25 adults female's mosquitoes each were runned per insecticide. At the same time, two tubes lined with non-impregnated paper and containing the same number of mosquitoes per tube, were used as control. The number of mosquitoes that were inactive was reported at 10, 20, 30, 40, 50, and 60 min intervals and used to determine knockdown resistance (Kdr). At the end of the 60 mins exposure period, test mosquitoes were gently transferred to the holding tubes, which contained nonimpregnated filter papers, and fed with a 10% sucrose solution. Mortality was determined 24 h post-exposure to insecticides according to the WHO insecticide bioassay protocol (WHO 2022). The mosquitoes were then placed on cotton and stored in Eppendorf tubes containing silica gel for downstream analysis.

#### Molecular Identification of *Anopheles gambiae* s.l

After exposure, dead and alive mosquitoes were identified by PCR. Genomic DNA was extracted from 198 *An. gambiae* s.l (99 from Edea and 99 from Buea) using the Qiagen DNA Mini Kit (Qiagen, Germany) according to manufacturer's instruction. The mosquitoes were identified by multiplex PCR using predesigned ribosomal DNA specific primers as described previously [27]. PCR amplicons were subsequently separated on a 2%

agarose gel containing ethidium bromide (10 mg/ml) and visualized on a UV illuminator (TOYOBO Trans Modele TM-20) against a 100bp ladder. Furthermore, amplicons were later incubated in a water bath at 38 °C for 3 h with the H*hal* restriction enzyme to assess the presence of the M form (*An. coluzzii*) and S form (*An. gambiae*). The resulting products were analyzed on a 2% agarose gel stained with ethidium bromide at 85V. The species were then ascertained based on estimated band sizes after electrophoresis.

#### Detection of the kdr west (1014F) mutation

Genomic DNA from 1360 previously extracted and identified *An. gambiae* s.l samples (68 from each site) were randomly selected and the kdr L1014F allele genotyped by PCR as previously described [29]. The PCR reaction conditions included initial denaturation at 94℃ for 2 mins, 35 cycles of denaturation, annealing and extension at 94℃ for 30s, 94℃ for 30s and 72℃ for 30s respectively, final extension at 72℃ for 5 mins. The reaction was set up in a total volume 15µl, consisting of 7µl GoTaq green master mix, 1µl each of Agd1 (5′-ATA GATTCCCCGACCATG-3'), Agd2 (5′-AGACAAGGA TGATGAACC-3'), Agd3 (5′-AATTTGCATTACTTA CGACA-3') and Agd4 (5′-CTGTAGTGATAGGAAATT TA-3) primers, 3µl nuclease-free water and 1µl of a 1 in 5 dilution of the mosquito DNA sample. The amplicons were separated on a 2% agarose gel stained with ethidium bromide and visualized using a Gel Doc (version 2.0). *Anopheles gambiae* s.l carrying the wild type or mutant allele were ascertained based on the fragment lengths of 293bp and 195bp or 137bp respectively.

#### Detection of the Ace-1 gene (G119S) mutation

The G119S mutation in the *Ace-1* gene was identified by Restriction Fragment Length Polymorphism following PCR as described previously [30]. For a total of 106 *An. gambiae s.l* genomic DNA previously extracted (53 from each town), the 541-bp DNA fragment was amplified using the PCR GoTaq® Flexi DNA polymerase kit (Promega, USA) in a total volume of 25 µl containing 10 µM of each primer [Ex3AGdir (5′-GATCGTGGACAC CGTGTTCG-3′) and Ex3AGrev (5′-AGGATGGCC CGCTGGAACAG-3′)]. The PCR reaction conditions included; initial denaturation at 94℃ for 3 mins, 35 cycles of denaturation, annealing and extension at 94℃ for 30s, 60℃ for 30s and 72℃ for 30s respectively, and final extension at 72℃ for 5 mins. To identify the haplotypes present, 7.5 µl of PCR product was mixed with 5U of *Alu*I enzyme (New England Biolab) in a final volume of 25 µl and incubated at 37 °C for three hours. Digestion products were analyzed by electrophoresis on a 2% agarose gel. Samples with 403 bp and 153 bp long fragments were considered homozygous suceptible (SS), two fragments of 253 bp and 150 bp considered homozygous resistant (RR) while the heterozygous (RS) showed a combination (403bp, 253bp and 150bp) of susceptible and homozygous resistant bands [31].

#### Data analysis

The *An. gambiae* populations were classified as susceptible, suspected resistant or resistant if mortality rate recorded after 24 h following exposure were ≥98%, 90 and 97% or <90% respectively [28].

Data were analyzed using the statistical software, SPSS (SPSS Inc. Released 2009). Association between mortality and allele frequency in *An. gambiae* s.l populations between sites were compared using the Pearson's $\chi 2$-test. For each $P$-value <0.05, the difference were considered significant.

## Results

#### Phenotypic insecticide resistance of *Anopheles gambiae* s.l

A total of 3108 female *An. gambiae* s.l mosquitoes collected in Edea and Buea were exposed to insecticide-impregnated papers, with a total of 1419 and 1689 mosquitoes from Buea and Edea communities respectively. No mortality was recorded for a total of 250 female Anopheles mosquitoes that were exposed to the unimpregnated papers used as controls, thus the mortality rates for the mosquitoes were not corrected using Abbott's formula. Varying resistance of the female *An. gambiae* s.l to pyrethroid, carbamate and organophosphates insecticides subclasses were recorded in Buea and Edea, though a few *An. gambiae* s.l were completely susceptible (Fig. 2). In Buea, for a total 398 *An. gambiae* s.l exposed to 1X (diagnostic dose), 1X + PBO, 5X and 10X permethrin, mortality rate increased from 17.2%, 54.0%, 37.4% to 100% respectively (Fig. 2). Similarly, the mortality rate of *An. gambiae* s.l were exposed to alphacypermethrin was 26%, 100%, 45% for 1X, 1X + PBO and 5X dosage respectively (Fig. 2). In Edea, for a total 400 *An. gambiae* s.l exposed to 1X (diagnostic dose), 1X + PBO, 5X and 10X permethrin, mortality rates increased from 6.0%, 31.0%, 89.0% to 100% respectively (Fig. 2). As for the mortality rate when Edea sampled *An. gambiae s.l* were exposed to alphacypermethrin, 20%, 64%, 37% and 47% mortality rate for 1X, 1X + PBO, 5X and 10X dosage respectively (Fig. 2).

Furthermore, the same batch of F1 generation of *An. gambiae* s.l from Buea also recorded increasing mortality rate of 54% and 99% for 1X and 5X pirimiphosmethyl respectively while *An. gambiae* from Edea were completely succeptible to 1X pirimiphiphosmethyl (Fig. 2). The mosquitoes that were exposed to 1X and

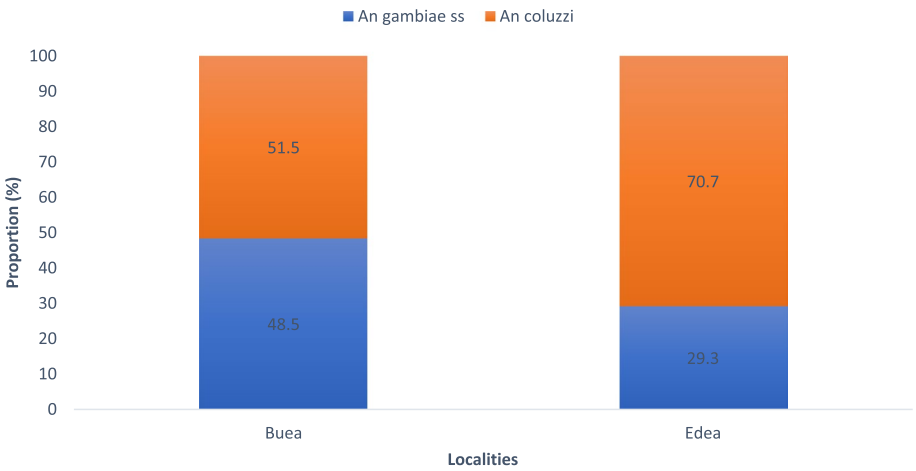


**Fig. 3** *Anopheles gambiae* s.l sibling forms identified in Buea and Edea

5X bendiocarb insecticide dosages after 24 h registered a mortality rate of 64% and 100%. Generally, based on the mortality rates of these mosquitoes to the different classes of insecticides and similar insecticide concentrations, these vectors were increasingly more susceptible to bendiocarb and pirimiphosmethyl compared to permethrin and alphacypermethrin (Fig. 2).

F1 female *An. gambiae* s.l from Edea were exposed to varying concentrations of pyrethroids, carbamates and organophosphates as in Buea, yielding similar mortality rate in some cases and different mortality rates in others. Of the 400 mosquitoes exposed to deltamethrin, only 113 (27.9%) were resistant, with mortality rate increasing from 52.0%, 86.0%, 50.0% and 99.0% when 1X, 1X + PBO, 5X and 10X concentrations were used respectively (Fig. 2). Even though the mortality rates were different with alphacypermethrin and permethrin, all the F1 generation exposed to 10X permethrin didnot survive as opposed to only 47% that did not survive when exposed to 10X alphacypermethrin insecticide dosage (Fig. 2). Interestingly, the mortality rates of all F1 generations that were exposed to 1X pirimiphosmethyl and 1X bendiocarb insecticides were 100% and 99% respectively (Fig. 2).

Comparatively, there were significant differences in the mortality rates for samples from Buea and Edea exposed to 1X permethrin ($p = 0.014$),1X permethrin + PBO ($p = 0.001$), 5X permethrin ($p < 0.001$), 1X alphacypermethrin + PBO ($p < 0.001$), 1X pirimiphosmethyl ($p < 0.001$) and 1X bendiocarb ($p < 0.001$). However, there was no significant different between the mortality rates recorded between Buea and Edea communities' samples when the F1 generation of *An. gambiae* s.l were exposed to 1X alphacypermethrin ($p = 0.313$) and 5X alphacypermethrin + PBO ($p = 0.250$).

### Molecular speciation of *Anopheles gambiae* s.l

A group of 198 *An. gambiae* s.l., including 50 susceptible (25 permethrin and 25 deltamethrin) and 148 resistant (74 permethrin and 74 deltamethrin) specimens, were randomly selected for molecular speciation after insecticide bioassay susceptibility testing. Among a total of 198 *An. gambiae s.l* sampled from Buea and Edea communities, analyses showed that *An. coluzzii (M)* and *An. gambiae (S)* were the two most prevalence siblings of the *An. gambiae* s.l identified during the surveyed across the localities (Fig. 3). *Anopheles melas, An. merus, An. quadrannulatus* were not found. *Anopheles coluzzii* (121, 61.1%) was the most common species across both study communities. There was a significant association ($p = 0.006$) between the sibling type and the locality of collection, with higher proportion of *An.gambiae* in Buea (45, 48.5%) and *An. coluzzii* in Edea (70, 70.5%) (Fig. 3).

### Prevalence of kdr L1014F and *Ace-1* G119S alleles in *An. gambiae* s.l

One hundred and thirty-five and 106 *An. gambiae* s.l mosquitoes were successfully genotyped for the kdr and Ace-1 gene respectively. There were no significant associations between the distribution of both genetic variants in *An. gambiae* s.l siblings between Buea and Edea (Table 1). However, in both communities, the kdr L1014F (107, 79.3%) and the Ace-1 RS genotype (87, 82.1%) was more prevalent than the wild type kdr and Ace-1 SS genotype respectively.

Furthermore, the kdr RS and Ace-1 RS genotypes were more prevalent compared to the SS genotypes in both *An. gambiae* s.s and *An. coluzzii*. However, there was an association between the Ace-1 genotype ($p = 0.002$) in *An. gambiae s.s* as well as kdr allele in *An. coluzzii* ($p = 0.036$) and the locality of sampling (Table 2).

**Table 1** Prevalence of *Ace-1* and Kdr variants in *An. gambiae* s.l from Edea and Buea

| Gene | Variant | Proportion [% (*n*)] | | | *P* value |
|---|---|---|---|---|---|
| | | Total | Buea | Edea | |
| Kdr | RR | 79.3 (107) | 78.8 (63) | 80.0 (44) | 0.860 |
| | SS | 20.7 (28) | 21.3 (17) | 20.0 (11) | |
| *Ace-1* | SS | 17.9 (19) | 20.0 (10) | 16.1 (9) | 0.599 |
| | RS | 82.1 (87) | 80.0 (40) | 83.9 (47) | |

Across Buea and Edea communities, 50 (82.0%) of mosquitoes phenotypically resistant to 1X permethrin had the kdr homozygous resistance allele while 11 (18.0%) had the wild allele (Table 3). However, there was no significant association ($p = 0.729$) between the kdr allele and the phenotypic resistance status of the *An. gambiae* s.l exposed to 1X permethrin. There was a significant association ($p < 0.001$) between the kdr allele and the phenotypic resistance status of mosquitoes exposed to 1X deltamethrin, with most susceptible (9, 90%) and most resistant (36, 97.3%) mosquitoes harboring the S and R allele respectively (Table 3). Similarly, there was a significant association ($p < 0.001$) between the Ace-1 genotype and the phenotypic resistance status of mosquitoes exposed to 1X permethrin, with most susceptible (9, 52.9%) and most resistant (32, 97.0%) mosquitoes harboring the SS and RS genotype respectively.

## Discussion

Although the implementation of vector management strategies has curbed malaria morbidity across many malaria endemic countries over the years, the development and spread of insecticide presents a major impediment to control efforts. Although there have been several reports in ssA of anopheline resistance to pyrethroids, the main insecticides for the impregnation of LLINs/ITNs and also, to organophosphates, organochlorides, carbamates used for IRS, studies in Cameroon remain limited. Unfortunately, there has been no routine monitoring of insecticide resistance in the Southwest and Litoral regions of Cameroon since 2018. Data on insecticide resistance profile of malaria vectors across all ecological zones in Cameroon remains paramount for malaria elimination goals. The present study assessed and compared the diversity and resistance profile of *An. gambiae* s.l siblings in Edea, an industrial zone and Buea an agricultural zone of the country, with implications for the control of the disease. *Anopheles gambiae* s.l showed either high or moderate levels of resistance to diagnostic

**Table 2** Prevalence of *Ace-1* and Kdr variants in *An. gambiae* and *An. coluzzii* from Edea and Buea

| *An. gambiae* s.l sibling | Gene | Variant | Proportion [% (*n*)] | | | *P* value |
|---|---|---|---|---|---|---|
| | | | Total | Buea | Edea | |
| *An. gambiae* s.s | Kdr | RS | 63.3 (31) | 72.4 (21) | 50.0 (10) | 0.110 |
| | | SS | 36.7 (18) | 27.6 (8) | 50.0 (10) | |
| | Ace-1 | SS | 16.3 (7) | 35.0 (7) | 0 (0) | 0.002 |
| | | RS | 83.7 (36) | 65.0 (13) | 100 (23) | |
| *An. coluzzii* | Kdr | RS | 88.4 (76) | 17.6 (9) | 2.9 (1) | 0.036 |
| | | SS | 11.6 (10) | 82.4 (42) | 97.1 (34) | |
| | Ace-1 | SS | 19.0 (12) | 10.0 (3) | 27.3 (9) | 0.081 |
| | | RS | 81.0 (51) | 90.0 (27) | 72.7 (24) | |

**Table 3** Distribution of Ace-1 and kdr genetic variants in *An. gambiae* s.l susceptible and resistant to permethrin and deltamethrin

| Gene | Variant | Proportion [% (n)] | | *P* value | Proportion [% (n)] | | *P* value |
|---|---|---|---|---|---|---|---|
| | | 1X permethrin | | | 1X deltamethrin | | |
| | | Resistant | Susceptible | | Resistant | Susceptible | |
| Kdr | RS | 82.0 (50) | 76.5 (13) | 0.729 | 97.3 (36) | 10.0 (1) | < 0.001 |
| | SS | 18.0 (11) | 23.5 (4) | | 2.7 (1) | 90.0 (9) | |
| Ace-1 | SS | 3.0 (1) | 52.9 (9) | < 0.001 | 15.6 (5) | 0.0 (0) | 0.315 |
| | RS | 97.0 (32) | 47.1 (8) | | 84.4 (27) | 100.0 (10) | |

concentrations of all insecticides except to pirimiphosmethyl and bendiocarb in Edea and alphacypermtherin + PBO in Buea. This is in line with previous reports of *An. gambiae* s.l resistance to the diagnostic concentration of bendiocarb and pirimiphosmethyl in Edea and across Cameroon [17, 25]. However, although there have been no previous reports of resistance to alphacypermethrin in both communities, resistance to the diagnostic dosage and 5X of the insecticide has been reported previously in mosquitoes from different communities within the same ecological zone [14]. Generally, there is increasing resistance to the *An. gambiae* s.l to commonly used insecticides within the sampled communities based on the WHO 2022 guidelines. There were quite contrasting results to varying concentrations of pirimiphosmethyl and bendiocarb, with *An. gambiae* s.l from Edea susceptible to diagnostic concentration of both insecticides, but only to 5X concentrations of these insecticides in Buea. Studies within Africa corroborate the complete susceptibility of *An. gambiae* s.l to 1X bendiocarb [17, 25, 32]. The recorded resistance to 1X bendiocarb in Buea in this study is in line with a recent study in Cameroon [33]. Further studies are required to confirm this finding as well as the role of PBO in moderating the interaction of this insecticide.

Across both communities, the mortality rate of the Anophelines increased almost proportionately when 1X, 5X and 10X permethrin were used, with complete mortality observed with 10X permethrin. As expected, susceptibility to the insecticide increased in this study when diagnostic dosage was used with PBO. The ineffectiveness of the diagnostic dosage of permethrin on *An. gambiae* s.l in this study contradicts previous studies in Cameroon in 2011 [17] and reflects the development and spread of resistance against the insecticide or metabolic resistance over the years. Although this study found no correlation between the phenotypic resistance *of An. gambiae* s.l to permethrin (a pyrethroid) and the kdr mutation, the association of resistance to this insecticide with the Ace-1 genotype may imply the involvement of detoxifying enzymes in the phenotypic resistance of *An. gambiae* s.l to pyrethroids [34].

In spite of observed resistance to the diagnostic dosage of alphacypermethrin in both communities, *An. gambiae* s.l from Buea were susceptible to 1X alphacypermethrin + PBO. This phenomenon could be as a result of changes in environmental factors due to the continued use of pesticides in the area, resulting in varying expression of metabolic enzymes effective in the detoxification of these insecticides [35]. Thus, the resistance against alphacypermethrin in Buea accrue to metabolic enzymes, which were then inhibited by PBO. The increasing trend in the mortality with 1X, 1X + PBO, 5X and the complete susceptibility with 10X deltamethrin mirrors previous studies in other parts of Cameroon [14]. Nevertheless, mosquitoes from a different ecological setting in Cameroon have been shown to be resistance 1X, 5X and 10X deltamethrin [33]. Based on this report, monitoring the expression levels of of cytochrome P450s genes, chemosensory genes such as the SAP1 and cuticular genes in Anophelines might provide insights into the increased resistance to permethrin and deltamethrin. It is plausible that gene flow across ecological zones and local selection pressure from intensive use of pesticides for agricultural purposes could escalated resistance to these insecticides [36]. The fact that local inhabitants have developed a habit of fencing their gardens with insecticide treated bed nets (ITBNs) in these communities will increase exposure of these vectors to insecticides and risk of increase resistance to these insecticides and their derivatives with time.

Molecular speciation of *An. gambiae* s.l, in both communities, revealed the presence of only *An. gambiae* and *An. Coluzzii*, in line with previous studies, suggesting that these two siblings mostly occur in sympatry in forest areas of Cameroon [7, 8, 13, 25]. The absence of *An. arabiensis*, one of the major members of the complex responsible for malaria transmission in the country in both communities, further affirms the fact that, the vector only survives and reproduces in the Sahelian ecosystem with less humid, warm and dry lands [36, 37]. The difference in the abundance of siblings in the two communities could be as a result of the breeding site preference or their ecological niches. Edea has more permanent or larger water bodies with vegetation compared Buea; which support observed discrepancies as *An. Coluzzii* prefer permanent or larger water bodies with vegetation as opposed to *An. gambiae* which prefers smaller or temporal water collections.

The high prevalence of the kdr West allele and Ace-1 RS genotype in phenotypically resistant *An. gambiae* s.l populations in Buea and Edea is quite concerning. The emergence and spread of resistant variants has been attributed to the continuous and intensive use of insecticides such as cypermethrin, deltamethrin, chlorpyrifos ethyl, lambdacyhalothrin, carbofuran, Dimethoate, Diazinon, and Endosulfan by farmers against pests [38] or ITNs to shield their crops in gardens from domestic animals, leading to high selection pressure on mosquitoes in these localities. The short-term reproductive cycles of the vectors lead to increased gene flow and consequently resistance.

The high prevalence of the Ace-1 (G119S) mutation in both communities mirrors previous reports in the humid rain forested region of the country [25] and indicates possible resistance to pyrethroid and carbamate insecticide

in this ecological setting. The mutation was quite high in both *An. gambiae* and *An. coluzzii* from both communities, in line with previous reports in the country [39] and neighboring Nigeria [40] and this is suggestive of a complete introgression of the different vectors within the population. All samples from both communities were either homozygous susceptible (SS) or heterozygous (RS) as reported previously in Cameroon [26]. This is not surpring as previous studies from west Africa has incriminated *ace-1* duplicated allele in *An gambiae* M and S forms for increased resistance against commonly used insecticide [38, 40]. Indeed, the Ace-1 mutation was absent in *An. coluzzii* in a recent study in Cameroon [25] while the variant was previously observed only at low frequency in *An. gambiae* populations in the country [26]. The evolving ability and patterns of these vectors in evading insecticide action poses a serious problem to vector control and intervention strategies against malaria. Rational measures like rotational use of insecticides, use of insecticide synergists and combination products, area-wide management and spatial mosaic application, community engagement and resistance monitoring are imperative for better management of the disease.

## Conclusion

The study highlights the importance of continuous mentoring *An. gambiae* s.l diversity and insecticide resistance profiles within ecological settings. Only *An. gambiae* and *An. coluzzii* were identified as the major malaria vectors in Buea and Edea, with both sympatric *An. gambiae* s.l siblings responding similarly to almost all classes of insecticides within both communities. *Anopheles gambiae* s.l from Buea were more resistant to diagnostic insecticide dosages except for 1X alphacypermethrin while those in Edea that were only susceptible to 1X bendiocarb and 1X pirimiphosmethyl. This provides baseline data for a larger scale study for continuous monitoring and implementation nationwide.

### Abbreviations

| | |
|---|---|
| PCR | Polymerase chain reaction |
| ACE-1 | Acetylcholinerase gene-1 |
| PBO | Piperonyl butoxide |
| An | Anopheles |
| LLINs | Long Lasting Insecticidal Nets |
| ITNs | Insecticide Treated Nets |
| Kdr | Knockdown Resistance |

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### Clinical trial

Not applicable.

### Author's contributions

PNK: coordinated the study, performed field and laboratory experiments and drafted the manuscript. JDE: performed field survey and laboratory experiment. RNT: performed field survey. JDB contributed reagents and materials AA: Coordinated the study, provided reagents and substantial improvement information in manuscript. TOA conceived, designed and coordinated the study, performed the statistical analysis and restructured the manuscript. All authors read and approved the final manuscript.

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### Data availability

All Data is provided within the manuscript.

## Declarations

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon. [2]National Malaria Control Programme, Yaoundé, Cameroon. [3]Biotechnology Centre, University of Yaoundé 1, Yaoundé, Cameroon. [4]Medical Research Council Unit at London School of Hygiene and Tropical Medicine, Fajara, The Gambia. [5]Department of Chemical and Biological Engineering, National Higher Polytechnic Institute, The University of Bamenda, Bambili, Cameroon.

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