1. Introduction

Despite the remarkable efforts in controlling malaria during the last 10 years, this disease continues to have a negative impact on people’s health and livelihoods in Africa. The World Health Organization (WHO) estimates that 216 million cases occurred globally in 2016, leading to 445,000 deaths, most of which were in children aged under 5 years in Africa [1]. In the Central African Republic (CAR), malaria is the most important disease responsible for 58% of all hospital consultation and a principal cause of death among children [2]. Malaria prevention mainly relies on insecticide-based interventions notably long lasting insecticidal nets (LLINs) and indoor residual sprays (IRS) [3]. However, the emergence of insecticide resistance in malaria vectors can compromise these control efforts. In Africa, several Anopheles species are implicated in malaria transmission. Among them, species such as *An. gambiae sensu stricto* (s.s.), *An. arabiensis* and *An. coluzzii* belonging to *An. gambiae* complex and *An. funestus* s.s. belonging to *An. funestus* group are implicated as major malaria vectors [4]. All these vectors have been found to be resistant to several insecticides belonging to four main classes (organophosphates, carbamates, organochlorines and pyrethroids) used in public health [5–7]. It has also been shown that several resistance mechanisms are involved in insecticide resistance to malaria vectors such as the target-site resistance and metabolic resistance. The most commonly reported target-site mutation described in *An. gambiae* s.s. and *An. coluzzii*, is the knockdown resistance (*kdr*) mutation which has two variants; L1014F (*kdr-w*) and L1014S (*kdr-e*) and confers resistance to pyrethroids and (dichlorodiphenyltrichloroethane) DDT [8,9]. Another mutation, the Ace*16G119S*, confers resistance to carbamates and organophosphates [10]. In both *An. gambiae* s.s. and *An. coluzzii*, metabolic resistance has been shown to be driven by multiple genes belonging to the cytochrome P450 monooxygenases (P450s) such as CYP6P2 and CYP6P3 [11–13] and glutathione S-transferases (GSTs) [14]. Contrary to *An. gambiae* s.s. or
An. coluzzi, no kdr mutations have been reported in An. funestus s.s. despite the presence of some non-synonymous substitutions [7,15–17]. However, two target-site resistance markers have been described in this species, the N485I Ace-1 in southern African populations [18] and the Rdl A296S mutation mainly in West, Central and East Africa populations [19]. Metabolic resistance has been shown as the main mechanism driving pyrethroid and DDT resistance in this species [20–23]. The cytochrome P450s CYP6P9a, CYP6P9b and CYP6M7 and the glutathione S-transferase GSTe2 are the main resistance genes implicated in An. funestus s.s. [21,23,24]. It was notably demonstrated that the single amino acid change L119F in an up-regulated glutathione S-transferase gene, GSTe2, confers high levels of metabolic resistance to DDT and pyrethroids in the malaria vector An. funestus s.s. [23].

In the Central African Republic, the knowledge of malaria vectors remains limited. A recent study showing a multiple insecticide resistance in An. gambiae s.l. and An. funestus s.l. in Bangui [25,26]. To implement an optimal strategy to prevent malaria and reduce its burden in this country, up-to-date information is required about vector characterization as well as the molecular basis of insecticide resistance. Thus, we present here the characterisation of major malaria vectors in Bangui, the capital city of CAR including their resistance profiles to insecticides and the underlying resistance mechanisms.

2. Material and methods

2.1. Sampling sites and mosquito collection

Mosquitoes were sampled in July 2016 in Bangui (04° 21’ N, 18°33’ E) in two neighbourhood, Gbaniokola and Taoka St Paul. These locations are characterised by the presence of lakes, fishponds and polluted stagnant water. In each neighbourhood, mosquitoes were collected during six days in 30 houses per day from 7 am to 11 am. Houses were selected based on three main criteria: the proximity to the potential breeding sites, the type of building (houses without ceiling) and the physical state of bed-net or non-possession of mosquito nets. Blood-fed mosquitoes resting indoors were collected using electric aspirators and kept in cages. Mosquitoes were identified using morphological criteria described previously [27]. All female mosquitoes identified morphologically as belonging to the An. gambiae complex or to An. funestus group were kept in cardboard cups and fed with 10% sugar solution until they were fully gravid and ready to lay eggs. Females (F₀) were allowed to oviposit individually using the forced-egg laying method as previously described [28]. After oviposition, the eggs (F₁) were brought to the insectary at the Liverpool School of Tropical Medicine (LSTM) research Unit at OCEAC in Yaoundé, Cameroon where they were pooled together and reared to adults (F₂). Larvae were fed with fish food (TetraMin Baby) and female adults were allowed to feed on blood from rabbits to induce eggs-laying. Due to the low number of adult (F₁) obtained, mosquitoes were maintained in the insectary until F₂ adult notably for An. gambiae s.l. All the females (F₀) that had laid eggs were put individually in the Eppendorf tubes containing desiccant (silica gel) and stored in −20 °C for molecular analysis.

2.2. Insecticide susceptibility assay

Due to the low number of An. funestus s.l. collected, bioassays were carried out only with An. gambiae s.l. according to WHO procedures [29]. Impregnated papers with insecticides were provided by LSTM. F₂ adults aged between two-five days with four replicates of 20 mosquitoes were exposed for one hour to insecticide impregnated papers. After exposure, mosquitoes were transferred into holding tubes (and allowed to feed on a 10% sugar solution) and mortality recorded 24 h later. Assays were performed in controlled conditions (at 28 ± 2 °C and a relative humidity of 80 ± 10%). Two insecticide classes used in public health were assessed: the pyrethroids (0.75% permethrin and 0.05% deltamethrin) and organochlorine (4% DDT). Each experiment included control mosquitoes exposed to untreated papers. Bioassay tests were concomitantly performed with the Ngousso susceptible reference strain An. coluzzi.

2.3. Molecular identification and Plasmodium infection rate

Genomic DNA was extracted in whole bodies of oviposited mosquitoes (F₀) using the Livak protocol as previously described [30]. Different species within the An. gambiae complex and An. funestus group were determined as previously described [31,32]. DNA extracted in whole mosquitoes were used to screen the Plasmodium infection using the TaqMan assay as described by Bass and collaborators [33]. One µl of DNA sample was used as template in a 3-step PCR program with a denaturation at 95 °C for 10 min followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The primer sequences used are: Falcip+ _ TCTGAATACGAAATGTC; OVM+ _ CTGAAATCAAAATGCC; Plas-F_ GCTTAGTTACGATTAATAGGAGTAGCTTG and Plas_R_ GAAATCTAAGAATTTCACCTCTGACA. These primers were used together with two probes labelled with fluorophores: FAM to detect Plasmodium falciparum, and HEX to detect Plasmodium ovale, Plasmodium vivax and Plasmodium malariae. Plasmodium falciparum samples and a mix of P.
ovale, P. vivax and P. malariae were used as positive controls.

2.4. Genotyping kdr and of Ace1R G119S mutations in An. gambiae s.s. and An. coluzzii

The presence of the target-site mutations Ace-1, L1014F (kdr-w) and L1014S (kdr-e) was determined in An. gambiae s.s. and An. coluzzii using TaqMan assay according to the protocols previously described [34,35]. Sequences of primers used are presented in Table S1. Ten μl volume containing 1 x Sensimix (Bioline), 80 x primer/probe mix and 1 μl template DNA were used. Probes were labelled with two specific fluorophores FAM and HEX, FAM to detect the resistant allele, HEX to detect the susceptible allele. The assay was performed on an Agilent MX3005 real-time PCR machine with cycling conditions of 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

2.5. Transcriptional profiling of two major resistance genes in An. coluzzii

Several P450 genes have been detected associated to insecticide resistance in Anopheles, but in this study we focused our analyses on CYP6M2 and CYP6P3 previously found associated to pyrethroid resistance in An. coluzzii and An. gambiae s.s [11,12], including populations from Central Africa [14]. The expression profiles of these genes was assessed by quantitative real time (qRT)-polymerase chain reaction (PCR) as described previously [36]. Total RNA was extracted from three pools of ten F2 female mosquitoes for three conditions: unexposed to insecticides, permethrin-resistant, and full susceptible laboratory strain Ngousso (An. coluzzii). RNA extraction, cDNA synthesis and qRT-PCR reactions and analysis were performed as previously described [36] in three biological replicates. Primer sequences used are presented in Table S1. The relative expression and fold-change of the two target genes were calculated as previously. The relative expression and fold-change of the two target genes were calculated according to 2−ΔΔCT method, after normalization with reference genes Actin (AGAP00651_RA) and 40S ribosomal protein S7 (AGAP010592_RA) [14]. Differences in expression were statistically analysed using unpaired Student’s t-test.

2.6. Genotyping of resistance markers L119F-GSTe2 and A296S-RDL in An. funestus s.s

To assess the potential role of the L119F-GSTe2 mutation in conferring DDT resistance in An. funestus s.s. in CAR, an allelic specific (AS)-PCR assay (Tchouakui et al., unpublished) was used to genotype 51 F1 mosquitoes. The primers employed for the genotyping are presented in Table S1. Amplification was carried out using PCR parameters of 95 °C for 5 min; 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 45 s, final extension at 72 °C for 10 min.

The role of A296S-RDL mutation in dieldrin resistance was also assessed with a newly designed TaqMan assay and used to genotype 51 F1 female mosquitoes as previously described [6]. Experiments were performed in 10 μl volume containing 1 x Sensimix (Bioline), 80 x primer/probe mix and 1 μl genomic DNA. The probes were labelled with two distinct fluorophores FAM and HEX, FAM to detect the resistant allele and HEX to detect the susceptible one. The assay was performed on an Agilent MX3005 real-time PCR machine with cycling conditions of 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

2.7. Sequencing of resistance markers

To detect potential signatures of selection acting on key resistance genes, the polymorphism of a fragment of voltage-gated sodium channel gene (VGSC), spanning intron 19 and exon 20 was assessed in ten F0 An. gambiae s.s. and ten F0 An. coluzzii as previously described [28]. The sequences of primers employed are presented in Table S1. PCR products were purified using the Exo-SAP protocol and directly sequenced. Similarly, the full-length of GSTe2 and a portion of the GABA receptor gene spanning the A296S RDL mutation were amplified in ten F0 An. funestus s.s. as previously described [19,23] and directly sequenced after purification.

2.8. Sequence data analysis

Sequences were corrected manually using BioEdit software version 7.2.1 and aligned with ClustalW [37]. The number of haplotypes (h), the number of polymorphism sites (S), haplotype diversity (Hd), nucleotide diversity (n), synonymous mutations (syn) and nonsynonymous mutations (nonsyn) were computed with DnaSP. Similarly, the full-length of GSTe2 and a portion of the GABA receptor gene spanning the A296S RDL mutation were amplified in ten F0 An. funestus s.s. as previously described [19,23] and directly sequenced after purification.

3. Results

3.1. Anopheles composition and Plasmodium infection rate

In total 61 An. gambiae s.l. and 13 An. funestus s.l. were collected in Bangui after intense screening of the houses in the selected neighbourhoods in around 180 houses during 6 days. Polymerase chain reaction
Summary statistics for polymorphism of some insecticide resistance markers in key malaria vectors from Bangui.

| Species       | N    | Pf positive | Kdr w-RR | Kdr w-RS | Kdr w-SS | Kdr e-RR | Kdr e-RS | kdre-SS | Ace 1-SS |
|---------------|------|-------------|----------|----------|----------|----------|----------|---------|----------|
| An. coluzzii  | 21   | 7 (33.3%)   | 2 (9.5%) | 18 (85.7%) | 1 (4.8%) | 0 (0.0%) | 0 (0.0%) | 20 (100%) | 20 (100%) |
| An. gambiae   | 16   | 4 (25.0%)   | 6 (40.0%) | 9 (60.0%) | 0 (0.0%) | 0 (0.0%) | 6 (37.5%) | 10 (62.5%) | 10 (100%) |

N, number of specimens; Pf positive, Plasmodium falciparum infection rate; RS, heterozygote resistant; RR, homozygote resistant; SS, homozygote sensitive.

Table 1. Summary of molecular characterization of An. coluzzii and An. gambiae s.s. from Bangui.

Table 2. Summary statistics for polymorphism of some insecticide resistance markers in key malaria vectors from Bangui.

| Species       | Gene   | 2n | S | h | hd | Syn | Nonsyn | n(k) | D | D* | F* |
|---------------|--------|----|---|---|----|-----|--------|-----|---|----|----|
| An. funestus s.s. | RDL    | 18 | 6 | 7 | 0.771 | 0    | 1      | 0.0021(2.94) | -0.849** | -1.467** | -1.493** |
| An. gambiae s.s. | GSTe2 | 18 | 14 | 11 | 0.882 | 4    | 7      | 0.0073(3.46) | -0.865** | -0.125** | -0.389** |
| An. coluzzii | Exon 20 | 20 | 5 | 6 | 0.637 | 0    | 0      | 0.0031(3.357) | -0.112** | 1.186** | 0.950** |

N = number of sequences (2n); S, number of polymorphic sites; h, haplotype; Hd, haplotype diversity; Syn, Synonymous mutations; Nonsyn, Nonsynonymous mutations; n, nucleotide diversity (k = mean number of nucleotide differences); Tajima’s D and Fu and Li’s D* and F* statistics, *s, not significant.

Figure 1. Differential expression of two resistance genes by qRT-PCR between permethrin-resistant An. coluzzii mosquitoes from Bangui and susceptible laboratory strain Ngousso. Error bars represent standard deviation (n = 3).
was detected carrying kdr-e mutation. Three haplotypes (H1, H4 and H5) were detected with 1014F mutation and two haplotypes (H2 and H3) carrying the wild susceptible allele (L1014) (Figure 2).

Similar analysis from ten *An. gambiae* s.s., evidenced also a low genetic diversity with a major haplotype (H1) carrying the 1014F resistant allele at 60% (12/20) (Figure 3). The genetic parameters are summarised in Table 2. In six haplotypes detected in *An. gambiae* s.s., kdr-w mutation (1014F) was detected in two haplotypes (H1 and H5) and *kdr-e* mutation (1014S) in four haplotypes (H2, H3, H4 and H6) (Figure 3, Table 2). Neutrality test of Tajima’s D had a negative value in both species but it was not significant in either species.

![Figure 2](image2.png)

**Figure 2.** Genetic diversity pattern of fragment of VGSC spanning exon 20 in *An. coluzzii* from Bangui. a) Haplotype diversity patterns of the 512 bp fragment in Bangui. b) TCS and tcsBU haplotype network showing a low polymorphism of the exon 20 fragment with low number of mutational steps between haplotypes. c) Molecular phylogenetic analysis by maximum likelihood method based on the Tamura 3-parameter model. In green represent the haplotype detected in this study.

![Figure 3](image3.png)

**Figure 3.** Genetic diversity pattern of fragment of VGSC spanning exon 20 in *An. gambiae* s.s. from Bangui. a) Haplotype diversity patterns of the 512 bp fragment in Bangui. b) TCS and tcsBU haplotype network showing a high polymorphism of the exon 20 fragment with high number of mutational steps between haplotypes. c) Molecular Phylogenetic analysis by maximum likelihood method based on the Tamura 3-parameter model. In green represent the haplotype detected in this study.
Phylogenetic analysis of the six haplotypes found in *An. gambiae* s.s. was performed using previous sequences published in GenBank originating from Cameroon, Nigeria, Equatorial Guinea and Gabon (Table S2) using the Maximum Likelihood method (Figure 3(c)). The results indicated that the dominant haplotype (H1) matched with that found across Africa suggesting extensive gene flow in *An. gambiae* s.s. populations across the continent. Overall, this analysis detected two distinct haplotype groups: the haplotypes carrying 1014F resistance mutation and the haplotypes carrying 1014S mutation.

Similar phylogenetic analysis in *An. coluzzii* also revealed the existence of two distinct groups: one including all the haplotypes carrying the resistant mutation 1014F and the other made up of haplotypes of the susceptible allele 1014L.

Genealogical relationships between haplotypes are presented in Figures 2 and 3. In *An. gambiae* s.s., haplotype H2-1014S and H5-1014F derived from single mutational steps from the dominant haplotype H1-1014F. H6-1014S and H3-1014S resulted from three mutational steps and H5-1014S from two mutational steps (Figure 3(b)). In *An. coluzzii*, three haplotypes H1, H4 and H5 carrying the 1014F mutation were separated by one mutational step. Haplotype H2-1014L is the most isolated to the dominant haplotype H1-1014F with three mutational steps (Figure 2(b)).

3.6. Genotyping of GSTe2 and Rdl in *An. funestus* s.s

The genotyping of L119F-GSTe2 and A296S-Rdl with TaqMan in 51 non-exposed F1 revealed a contrasting result (Figure 3) with a higher frequency of the 119F resistance allele for GSTe2 but very low frequency for the resistant 296S allele for the Rdl mutation. The distribution of the genotypes for the L119F-GSTe2 marker was 30.23% homozygotes (13 of 43 mosquitoes) and 48.84% heterozygotes (21 of 43 mosquitoes) of (L119F). In contrast, the analysis of frequency of A296S-Rdl mutation revealed no homozygote resistant allele and only one heterozygote allele (1 of 51 mosquitoes; 1.90%) was detected (Figure 4).

3.7. Genetic variability of GSTe2 and Rdl in *An. funestus* s.s

Polymorphism analysis of 444 bp of GSTe2 and 574 bp of GABA receptor gene was performed for nine specimens of F0 *An. funestus* s.s. successfully amplified. The GABA receptor gene presented six substitution sites, seven haplotypes, no synonymous mutations and only one nonsynonymous mutation (A296S) resulting in high haplotype diversity (hd = 0.771) (Table 2). The haplotype H7 was the only resistant haplotype (1 of 18) which is in accord with genotyping assay.

Similar analysis with the GSTe2 gene indicated a high level of polymorphism compared to RDL gene with 14 polymorphic sites, 11 haplotypes, and four synonymous and seven nonsynonymous sites. As with RDL gene, one haplotype (H10) was found resistant but with the difference that the resistant haplotype is the dominant (6/18) (Figure 5).

Neutrality tests revealed negative values of Tajima’s D and Fu and Li’s D* and F* statistics for both genes but none of these values was statistically significant (Table 2).

Phylogenetic analysis of 18 DNA sequences from Rdl gene in *An. funestus* s.s. collected in Bangui detected three distinct groups (Figure 5(c)). Genealogical
relationships between haplotypes using TCS software revealed that haplotypes H2, H6, H7 and H3 are separated from a common ancestor, haplotype H1, by one mutational step. Haplotype H5 is the most isolated to ancestor haplotype with three mutational steps (Figure 5(b)).

A similar analyses was performed with GSTe2 sequences including previously detected haplotypes across Africa [23]. The dominant haplotype (H10R) carrying 119F mutation matched with the resistant haplotype found in Benin, Ghana, and Cameroon (Figure 6(c)). TCS network showed that haplotypes H10 and H11 are mostly related to the ancestor haplotype and are separated by two mutational steps. Haplotype H2 is the most isolated from the core group and separated by seven mutational steps (Figure 6(b)).

4. Discussion

4.1. Malaria vector composition and their contribution in Plasmodium transmission

This study has revealed the presence of three malaria vectors, An. gambiae s.s., An. coluzzii and An. funestus s.s., in two neighbourhoods of Bangui in July 2016 with An. coluzzii being the most abundant species. This is in contrast with the previous work done at Bangui where An. coluzzii was not collected from seven districts in Bangui [25]. However, our findings are consistent with previous results in Central Africa notably in Cameroon showing that An. coluzzii is the most abundant Anopheles species in the urban area due to the greater tolerance of this species to pollution [44–46].

Figure 5. Genetic diversity pattern of rdl gene in An. funestus from Bangui. a) Haplotype diversity patterns of the 574 bp fragment in Bangui. b) TCS and tcsBU haplotype network showing a low polymorphism of the rdl gene fragment with low number of mutational steps between haplotypes. c) Molecular phylogenetic analysis by maximum likelihood method based on the Hasegawa-Kishino-Yano model.
High *P. falciparum* infection rate was observed in all these vectors examined. This indicates the high burden of malaria at Bangui. Meanwhile, it is important to highlight that high infection rate found in this study may be due to the fact that the parasite DNA was extracted from whole mosquitoes and not just from head and thorax. Nevertheless, other studies performed with whole mosquitoes have detected significantly lower infection rate such as in Malawi in southern Africa where Riveron et al. [47] only found an infection rate of 4.8% or across Uganda and Kenya where the rate varied from 4.2 to 10.4% [48]. Therefore, it is likely that the high infection rates in the three species reflect a high level of malaria transmission in the area although further studies across the city with larger samples will help to confirm it.

4.2. *Anopheles gambiae* s.s. and *An. culuzii* are highly resistant to pyrethroids and DDT

Both species were fully resistant to both types of pyrethroid: deltamethrin (type II) and permethrin (type I), and highly resistant to DDT. These findings are in contrast with the results of previous study performed at Bangui in 2014 indicating the moderate level of resistance to deltamethrin with mortality rate varying from 68.1% to 74.6% [25]. The high pyrethroid resistance reported in this study in Bangui *An. gambiae* s.l. populations could be of great concern for malaria prevention since this insecticide class is the only one used for bed net impregnation. A high level of resistance to DDT has been repeatedly reported in Central Africa [7,14] even though this insecticide is no longer used for malaria control in the region. It is possible that this higher level of resistance is linked to over-expression of other detoxification genes including other cytochrome P450 genes not tested here or even ο gluthione-S transferases such as GSTe2 as seen in *An. funestus*. Furthermore, it is also possible that presence of additional kdr mutations such as the N1575Y could be enhancing the resistance phenotype as this mutation has been shown to provide an additive resistance in combination with the L1014F mutation [49] as also recently shown in *An. gambiae* from Mali [50]. More surveys

Figure 6. Genetic diversity pattern of GSTe2 gene in *An. funestus* from Bangui. a) Haplotype diversity patterns of the 444 bp fragment in Bangui. b) TCS and tcsBU haplotype network showing a low polymorphism of the GSTe2 gene fragment with low number of mutational steps between haplotypes. c) Molecular phylogenetic analysis by maximum likelihood method. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. In green represent the haplotype detected in this study. BN, Benin; CAM, Cameroon; GH, Ghana; MAL, Malawi; MOZ, Mozambique; UG, Uganda.
with greater sample sizes are needed to establish the extent and distribution of this resistance in Bangui and across CAR.

4.3. Molecular mechanism involved to insecticide resistance

The full resistance to deltamethrin and permethrin and the high resistance to DDT in An. gambiae s.l. correlated with high frequency of the L1014F kdr mutation. Indeed, both An. coluzzii and An. gambiae s.s. present a high frequency of the L1014F kdr mutation whereas, the L1014S kdr mutation was detected only in An. gambiae s.s. The presence of both type of kdr mutations have been previously reported in An. coluzzii and An. gambiae s.s. in Central Africa [51,52]. The higher frequency of heterozygotes is contrary to the previous results from Bangui indicating rather higher frequency of resistant homozygous allele of kdr-w, in Gbanikolla (the same location we sampled) with 100% of homozygotes resistant [25]. None of the specimens tested had the Ace-1 mutation. This is in accordance with previous results obtained in Central Africa notably in CAR [25].

The exploration of the metabolic resistance mechanisms performed through the transcription profiling of the common major resistance genes \(\text{CYP6M2}\) and \(\text{CYP6P3}\) surprisingly revealed that both genes are not up regulated in An. coluzzii suggesting that they play no role in the observed resistance in Bangui. This result contrasts with previous reports in Central Africa notably in Cameroon showing that these genes are the main drivers of metabolic resistance to pyrethroid and DDT in An. gambiae s.s. and An. coluzzii [14,52]. However, other P450 genes like \(\text{CYP9K1, CYP6Z1}\) and \(\text{CYP325A}[53,54]\) have been also detected associated with insecticide resistance in Anopheles but these genes were not tested in this study. Further studies are needed to elucidate the molecular basis of pyrethroid/DDT resistance in CAR.

Genotyping of L119F-GSTe2 and A296S-Rdl mutations revealed a contrasting result with a high frequency of the resistant allele of GSTe2 and only one heterozygote allele of Rdl. This finding matches with sequencing data which detected only one resistant haplotype (1/18) for Rdl whereas the predominant GSTe2 haplotype was found in resistant mosquitoes. This observation suggests higher resistant of An. funestus s.s. from Bangui to DDT and possibly to pyrethroids. Indeed, up-regulation for GSTe2 gene combined with the presence of the L119F-GSTe2 mutation have been strongly associated with DDT resistance but also to pyrethroid resistance [23]. The haplotype H10 carrying 119F-GSTe2 mutation, which has been shown to play a significant role in DDT resistance in West-Central Africa [7,23], was found in high frequency. The 119F resistant allele at Bangui was probably selected during the intense DDT spraying between 1950s-1960s as part of the malaria elimination campaign as suggested previously [55]. Another possibility is that the 119F allele could also have been further selected by pyrethroid-based interventions as it was demonstrated that GSTe2 can metabolize permethrin as previously suggested [23].

5. Conclusion

This study shows that three Anopheles species: An. gambiae s.s., An. coluzzii and An. funestus s.s. are present in Bangui and can contribute to malaria transmission in this city. Full resistance to deltamethrin and permethrin, which are used for impregnating bed net, could pose a serious threat for malaria control in Bangui. Further study is required to assess the vector composition and their distribution in entire CAR as well as the resistance status and the resistance mechanisms involved. This will be needed to better manage the insecticide resistance and develop alternative malaria control strategies in Central Africa.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethics approval

Verbal consents were obtained from the house owners for the mosquito collection.

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