Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Multiple insecticide resistance target sites in adult field strains of *An. gambiae* s.l. from southeast Senegal.

El hadji Diouf (dioufelhadjied@gmail.com)
UCAD https://orcid.org/0000-0002-4635-1610
El hadji Amadou Niang
Universite Cheikh Anta Diop Faculte des Sciences et Techniques
Badara Samb
Universite Cheikh Anta Diop Faculte des Sciences et Techniques
Cheikh Tidiane Diagne
Institut Pasteur
Mbaye Diouf
Universite Cheikh Anta Diop Faculte des Sciences et Techniques
Abdoulaye Konaté
Universite Cheikh Anta Diop Faculte des Sciences et Techniques
Ibrahima Dia
Institut Pasteur de Dakar
Ousmane Faye
Universite Cheikh Anta Diop Faculte des Sciences et Techniques
Lassana Konaté
Universite Cheikh Anta Diop Faculte des Sciences et Techniques

Research

Keywords: Insecticide resistance, Kdr (Vgsc-1014F, Vgsc-1014S); Ace-1, Rdl (A296S or A296G), An. arabiensis, An. coluzzii, An. gambiae s.s., Southeast, Senegal

Posted Date: September 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-44879/v2

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Version of Record: A version of this preprint was published at Parasites & Vectors on November 11th, 2020. See the published version at https://doi.org/10.1186/s13071-020-04437-z.

Abstract

Background: Malaria prevention strategies are based on the use of long-lasting insecticide-treated mosquito nets (LLINs), indoor residual spraying of insecticides (IRS) and seasonal malaria chemoprevention (SMC). The combination of these strategies with artemisinin-based combination therapy (ACTs) has led to a significant reduction in malaria cases. However, malaria remains a major public health issue in most sub-Saharan African countries. Indeed, the resistance of vectors to most WHO-approved insecticides could jeopardize vector-control strategies. This study examines insecticide resistance and associated genetic mutations among malaria vectors in southeast Senegal.

Methods: The study was conducted in October and November 2014 in two sites in southeast Senegal. *An. gambiae* s.l. populations were sampled from Kedougou (Kedougou district) and Wassadou-Badi (Tambacounda district) and were evaluated for insecticide resistance according to WHO susceptibility tests. Specimens were 3 to 5-day-old adults raised from collected larvae. Eleven insecticides belonging to the four known classes of insecticides were assessed. Mosquito species were identified and mutations associated with insecticide resistance (*ace-1*, *rdl (*A296S or A296G*)*, *Vgsc-1014F* and *Vgsc-1014S)* were determined.

Results: A total of 3,742 *An. gambiae* s.l. were exposed to insecticides (2,439 from Kedougou and 1,303 from Wassadou-Badi). In both sites, mosquitoes showed high levels of resistance to all the five pyrethroids tested (mortality rates ranged from 42.8 to 81.4% in Kedougou and 52.4 to 86.4% in Wassadou-Badi) as well as to dieldrin (67.8 and 83%) and DDT (12.7 and 55%). The mosquitoes were susceptible to pirimiphos-methyl (mortality rate 100%) and malathion (mortality rates 100% and 99% in Kedougou and Wassadou-Badi respectively). *An. gambiae* s.l. populations from Kedougou were also resistant to bendiocarb and fenitrothion.

Of the 745 *An. gambiae* s.l. genotyped *An. gambiae s.s.* (71.6%) was the predominant species, followed by *An. arabiensis* (21.7%), *An. coluzzii* (6.3%) and hybrids (*An. gambiae s.s./An. coluzzii;* 0.4%). The *Vgsc*-1014F mutation was widely distributed and is predominant in *An. gambiae* s.s. and *An. coluzzii* in comparison to *An. arabiensis*. *Vgsc*-1014S was present in *An. gambiae* s.l. populations in Wassadou but not in Kedougou. The ace-1 and rdl mutations were more frequent in *An. gambiae s.s.* compared to An. arabiensis whereas they were detected weakly in *An. coluzzii* populations.

Conclusions: The present study demonstrates the resistance of malaria vectors to pyrethroids and organo chlorines in southeast Senegal as well as the presence of genetic mutations associated with this resistance in *An. gambiae* s.l. No *Vgsc*-1014S mutation was detected in *An. gambiae s.s.* population in Kedougou. These findings are key for monitoring and managing the resistance of vectors to insecticides in this region.

Background

Malaria remains a major public health challenge in endemic countries. It mainly affects vulnerable groups, including pregnant women and children less than five years old. In most endemic countries, the fight against this endemic disease is based on (i) the early detection of *Plasmodium* infection by a biological diagnosis of cases (rapid diagnosis test and blood smear), (ii) treatment with effective drugs (artemisinin-based combination therapy (ACT)) and (iii) prevention (intermittent preventive treatment in pregnancy, seasonal malaria chemoprevention in children under ten years and vector control). Worldwide, the number of malaria cases has decreased from 251 million in 2010 to 228 million in 2018 At the same time, the disease incidence declined from 71 to 57 cases per 1000 in 2010 and 2018, respectively [1].

Despite these advances, malaria incidence in Africa has increased between 2014 and 2016 [51], because of factors including the development and spread of insecticide resistance in the main malaria vectors, such as *An. funestus* and *An. gambiae* s.l. Currently, resistance to at least one of the four major classes of insecticides has been reported in malaria vectors in all African endemic areas [1]. This could be a major obstacle to the efficacy of insecticide-based vector control strategies [2, 3]. Resistance to pyrethroids (the only insecticide class currently approved for long-lasting insecticidal mosquito nets: LLINs) [2, 4, 5] and to DDT (dichlorodiphenyltrichloroethane) [6] has been reported in many endemic settings [7, 8], particularly in Asia and in tropical African countries.

Several insecticide resistance mechanisms have been described in the major malaria vectors. The "knock down" resistance (*kdr*) mutation, which confers resistance to pyrethroids and DDT, is the most common. It occurs at 1014 position of the gene encoding the S6 Trans membrane domain II of para voltage-gated sodium channel (*Vgsc*), the interaction site of insecticides and protein targets.

Two types of *kdr* mutation, widely distributed in *An. gambiae* complex species, are reported in many studies in Africa [44-46]. Both mutations due to substitution: the first mutation changes a leucine to a phenylalanine at amino acid position 1014 of voltage gated sodium channel gene (*Vgsc*-1014F).While the second mutation changes a leucine to a serine at amino acid position 1014 of the same gene: (*Vgsc*-1014S) [9, 10].

Other target-site mutations have been described in *An. gambiae* s.l. These include the gene encoding acetyl cholinesterase (*ace-1*) and the gamma-amino butyric acid (GABA) receptor [34]. The *ace-1*^{R} (Acetyl cholinesterase insensible) mutation, caused by a substitution of a glycine to a serine at position 119 (G119S), results in insensitivity of acetyl cholinesterase (AChE1) to organophosphates and carbamates [11]. The GABA receptor mutation results from a nucleotide substitution at amino acid position 296, leading the change of an alanine to a serine in *An. arabiensis* or a glycine in *An. gambiae s.s.* and *An. coluzzii*. It generally confers resistance to dieldrin (*rdl*-A296S or *rdl*-A296G) or can lead to a cross-resistance to cyclodiene organo chlorines and phenyl pyrazole (fipronil) [12].

Previous studies of susceptibility of *An. gambiae* s.l. to insecticides have revealed, often at different levels, a phenotypic resistance to DDT and to pyrethroids in most parts of Senegal, except in the north and extreme southeast [13, 31, 30]. The aims of this study were to update the current status of insecticide

resistance among *An. gambiae* s.l. populations in southeast Senegal and to identify the mechanisms of insecticide resistance, particularly for target-site mutations involved in insecticide resistance.

Methods

Study area

The study was conducted in October and November 2014 in two sites of southeast Senegal: Kedougou (12 ° 33'11.3 "N and 12 ° 10'09.5" W in Kedougou district) and Wassadou-Badi (13°22'22.3"N and 13°22'53.5"W in Tambacounda district) (Figure 1).The area is bordered by the Republics of Mali and Guinea. The climate is a Sudano-Guinean type with a rainy season generally extending from May to October [14]. The average precipitation is between 1, 200 mm and 1,300 mm per year with an average temperatures between 33-42 ° C for maxima and 21- 25 ° C for minima. Agriculture is the main economic activity with a wide production of sorghum, maize, fonio, rice and cotton. The area of Kedougou is also a gold-mining zone, and has a significant potential for mineral resources. With a malaria incidence greater than 25 per 1000 [17], the study area remains the most holo endemic area in Senegal. In 2014, 265,624 clinical cases were recorded; including 12,636 severe cases [18]. Malaria transmission is seasonal, and occurs during the rainy season and the beginning of the dry season. *An. gambiae s.s.*, *An. coluzzii* and *An. arabiensis* are responsible for most malaria transmission, but in some specific settings, *An. funestus* and *An. nili* are involved [15, 16].

Anopheles immature stages collection and mosquito rearing

Larval collections were carried out in Kedougou, Wassadou and Badi. Wassadou and Badi belong to the same area and are just 1.5km apart. The larval sites for *An. gambiae* s.l. consisted of temporary water collections, footprints and hollows associated with human activities. During the study period, immature stages were collected from positive larval sites located in or around villages. All larval collections from Kedougou were pooled to form a sample and those of Wassadou and Badi a sample. After collection, immature stages were transferred to a local insectary for rearing. *Anopheles* larvae were fed with fishmeal (Tetramin Baby®). Pupae were collected daily and introduced into rearing cages. At emergence, mosquito adults were fed using absorbent cotton soaked with 10% sucrose solution.

WHO bioassay tests and morphological identification

WHO susceptibility tests were performed according to the standardized protocol [19] with adults 3 to 5 days post emergence from field collected larvae. Eleven insecticides belonging to four insecticide classes were tested: five pyrethroids (0.05% deltamethrin, 0.75% permethrin, 0.05% lambda cyhalothrin, 0.1% alpha cypermethrin and 0.15% cyfluthrin), two organo chlorines (4% DDT and 4% dieldrin), three organophosphates (1% fenitrothion, 5% malathion and 1% pirimiphos-methyl) and one carbamate (0.1% bendiocarb).For the pyrethroids and DDT, the number of knock down individuals was recorded at 10, 15, 20, 30, 40, 50 and 60 minutes during the exposure period. Mortality rates were determined 24 hours post-exposure. The mortality rates in the tested groups were corrected when needed, using Abbot's formula [20] to validate tests results according to mortality rate in controls.

Finally, tested specimens were identified morphologically under a binocular microscope using a conventional key [21] and then individually stored in Eppendorf tubes containing silica-gel. All surviving specimens and ten for bendiocarb, twenty for organo chlorines, thirty for organophosphates and fifty for pyrethroids randomly-selected dead specimens were individually stored for laboratory analysis.

DNA extraction, molecular identification of species and detection of *kdr*, *ace-1* and *rdl*.

Genomic DNA extraction was carried out by the 2% CTAB (Cetyl trimethyl ammonium bromide) method [50] adapted to animal tissues. Each sample was grounded in an Eppendorf tube containing 200µl of CTAB and incubated at 65 ° C for one hour. Then 200µl of chloroform was added and mixed by inversion. The mixture was centrifuged at 12,000 rpm for 5min, after which the supernatant containing DNA was recovered in a new Eppendorf tube. DNA was then precipitated with isopropanol and the mixture was then centrifuged at 12,000 rpm for 15min and washed with 70° ethanol after a centrifugation of 12.000 rpm for 5min and then brought to speed-vac for drying. The DNA, thus obtained was suspended in molecular biology grade water: DNA/RNA free (*Invitrogen*, 10977 035). One tenth of dilution was carried out before PCR (identification of species of the *An. gambiae* complex and detection of target site mutations). Species were identified using IMP-PCR (intentional mismatch primer-PCR) as described by Wilkins et al. [22]. *Kdr* mutations (*Vgsc*-1014F and *Vgsc*-1014S), G119S (*ace-1*^{R}) and *rdl*-296S (*An. arabiensis*) and *rdl*-296G (*An. gambiae s.s.* and *An. coluzzii*) were determined using the protocols described by Huynh et al. [23] and by Weill et al. [24] and Du et al [12] respectively.

Data entry and statistical analysis

Data was recorded in a Microsoft Excel 2010 spreadsheet. Homogeneity tests of percentages and averages were performed using the standard chi-square tests with a 5% significance level threshold. The level of insecticide susceptibility of mosquitoes was evaluated following WHO criteria [19] and validated by considering mortality rates of control mosquitoes. If the control mortality was less than 5%, no correction of test results was necessary whereas mortality of ≥ 5% required Abbott's correction [19]. KDT_{50} and KDT_{95} times with 95% confidence intervals were determined using a log-probit regression model. The mortality rates, the genotypes and allelic frequencies were estimated for each studied population. All statistical analyses and graphs were made using R software version 3.0.3 [43].

Results

Susceptibility tests

A total of 3,742 specimens of *An. gambiae* complex (between 109 to 240 per insecticide per site) were exposed to the WHO recommended diagnostic doses (2,439 from Kedougou and 1,303 fromWassadou-Badi). In both sites, a high number of mosquitoes were resistant to all five tested pyrethroids (mortality ranged from 42.8 to 86.4%) as well as to the organo chlorines (mortality rates ranged from 67.8 and 83% for dieldrin and 12.8 and 55.8% for DDT in Kedougou and Wassadou-Badi respectively) (Figure 2 and Table 1). In the group of organophosphates, the populations of *An. gambiae* s.l. tested in both areas were susceptible to 5% malathion and 1% pirimiphos-methyl. Fenitrothion resistance (89% mortality rate, 95% CI 85-95) was detected in Kedougou, where *An. gambiae* s.l. populations were also resistant to bendiocarb 0.1% (Figure 2).

Knockdown times/knockdown effect

In Kedougou, KDT_{50} greater than 60 min were recorded for DDT, permethrin and deltamethrin. In Wassadou-Badi a KDT_{50} greater than 60 min were noted with DDT and lambda cyhalothrin. The KDT_{50} value for permethrin was 3.5 time higher in Kedougou compared to Wassadou-Badi (χ^2=10.029, df=1, P= 0.0015). However, KDT_{50} value for deltamethrin in Kedougou was 2.7 higher than KDT_{50} value of Wassadou-Badi (χ^2=3.0083, df= 1, P= 0.08284), no significant different was observed between these two sites. Conversely, for cyfluthrin and lambda cyhalothrin, KDT_{50} were respectively 1.8 and 1.18 times higher in Wassadou-Badi (χ^2= 19.3177, df= 1, P<0.0001; χ^2= 15.2239, df= 1, P<0.0001). Cyfluthrin and alpha cypermethrin had the lowest KDT50 compared to other pyrethroids tested (Table 1).

Vgsc*-1014F, *Vgsc*-1014S, ace-1 (G119S), rdl-A296S and *rdl*-A296G mutation frequencies in *An. arabiensis*, *An. coluzzii* and An. *gambiae s.s.

The frequency of *kdr (Vgsc)* gene mutations was different among the three different members of the *An. gambiae* complex. The wild-type allele dominated in both Kedougou and Wassadou-Badi in *An. arabiensis*. In *An. gambiae* s.s. and *An. coluzzii* population, a predominance of FF homozygotes was noted in both sites for the *Vgsc*-1014F mutation. The results revealed two homozygous hybrids resistant to the *Vgsc*-1014F mutation. The *Vgsc*-1014S mutation was not found any member of the *An. gambaie* complex in Kedougou but was predominant in the *An. arabienesis* in Wassadou-Badi.

The allelic frequencies of the *Vgsc*-1014F mutation (Kedougou: Fisher's exact test: P<0.001, Wassadou-Badi: χ^2 = 455.3289, df = 2, P<0.001) and *Vgsc*-1014S (Wassadou-Badi: Fisher's exact test: P<0.001) were significantly higher in *An. gambiae s.s.* compared to *An. coluzzii* and *An. arabiensis* (Table 2).

The wild-type allele was the most frequent allele for the *ace-1*^{R} for all species of the *An. gambiae* complex in both sites. The frequency of the *ace-1*^{R} (G119S) mutation was low in both sites and heterozygotes genotypes (GS) were predominant for carriers of an 119S allele.

In Wassadou-Badi, a relatively higher allelic frequency was noted in *An. gambiae* s.s., the only species in which all SS homozygotes were found (Table 2). As with a*ce-1*^{R}, the predominant allele for *rdl* gene was the wild type allele. The mean allelic frequencies of A296S or A296G were significantly different among species of the *An. gambiae* complex in Kedougou (Fisher's exact test: P= 0.0147), but not in Wassadou-Badi (Fisher's exact test: P= 0.1236). However, only *An. gambiae s.s.* population has homozygous (GG) for A296G *rdl* allele (Table 2).

Allelic frequencies at the *Vgsc*-1014F, *Vgsc*-1014S, ace-1^R (G119S) and *rdl*-A296S or *rdl*-A296G locus according to the phenotype after insecticide exposure

Table 3 shows the allelic frequencies of the *Vgsc*-1014F, *Vgsc*-1014S, G119S and *rdl*-A296G or *rdl*-A296S mutations in the selected specimens that survived or died after exposure to insecticides.

In both study areas, *An. gambiae s.s.* was the predominant species among surviving specimens (96.6% in Kedougou; 64.1% in Wassadou-Badi). The percentage of *An. gambiae s.s.* was higher in surviving compared to the dead specimens (χ^2= 32.4, df = 1, P < 0.0001) while *An. arabiensis* (82.7%, n =52) predominated only in dead specimens in Wassadou-Badi.

In *An. gambiae* s.s., the frequencies of resistant allele in surviving versus dead specimens after exposition to DDT and pyrethroids were comparable for the 1014F allele (Fisher's exact test: P ≥0.058) and significantly different between those specimens exposed to bendiocarb for the *ace-1*^{R} (G119S) allele (Fisher's exact test: P ≤0.001) in both sites (Table 3).

In Wassadou-Badi, the frequencies of the 1014S allele in *An. arabiensis* (0.34 vs 0.06, Fisher's exact test: P<0.001) as well as that of the *rdl*-296G allele in *An. gambiae s.s.* (0.21 vs 0.0, P= 0.014) were higher in surviving compared to the dead specimens after exposure to dieldrin. On the other hand, there was no significant difference between the frequencies of the 1014F allele in dead and surviving specimens in both *An. gambiae s.s.* (0.98 vs 0.88, Fisher's exact test, P= 0.07931) and *An. arabiensis* (0.068 vs 0.0, Fisher's exact test: P= 0.01764).

Discussion

This study aimed to update data relating to insecticide susceptibility and to determine the frequencies of mutations of *kdr* (*Vgsc*-1014F and *Vgsc*-1014S), *ace-1*^{R} and *rdl* alleles associated with the resistance of *An. gambiae* s.l. populations to insecticides in southeastern Senegal.

The results of WHO susceptibility tests showed vector resistance to pyrethroids organo chlorines (DDT and dieldrin) and carbamates insecticides that are recommended by PQT-VC (Prequalification Team: Vector Control Products). These insecticides are the only ones currently approved for LLIN treatment [25, 26], and are offered by nongovernmental organizations such as the United States President's Malaria Initiative (PMI) and Senegal River Basin Development Organization (OMVS). The use of LLINs over several years could have led to the increase of resistance genes in vectors of *An. gambiae* species complex, through selection pressure [27, 35]. The resistance of *An. gambiae* s.l. to pyrethroids has been shown to be strongly associated with their excessive use in agriculture especially in cotton growing areas [28].

Moreover, *An. gambiae* s.l. populations in the area were also resistant to organo chlorines (DDT and dieldrin). Since the first malaria eradication attempt, DDT and dieldrin resistance phenotypes have been reported in many African countries by Hamon [29]. Despite several decades of non-use, DDT may persist in the environment due to lack of microbial degradation system [49].

Previous studies have reported resistance only to DDT and pyrethroids in southeastern and central Senegal where LLIN use is high, [30, 31]. However, unlike previous studies conducted in Senegal, this study shows that vectors are resistant to almost all tested pyrethroids and bendiocarb.

Bioassays likewise showed resistance to bendiocarb in Kedougou. This resistance could come from selection pressure in larval from insecticide residues (bendiocarb) used on cotton crops by SODEFITEX [47].This phenotypic resistance to bendiocarb should be closely monitored as there is cross- resistance to carbamates and organophosphates.

The search for mutations involved in the phenotypic resistance of *An. gambiae* s.l. population to insecticides showed the presence of *Vgsc*-1014F, *Vgsc*-1014S, *ace-1* (G119S) and *rdl*-A296S or *rdl*-A296G mutations. The *Vgsc*-1014S mutation was not found in *An. gambiae* s.l. from Kedougou, where the *Vgsc*-1014F was at 0.99 *in An. gambiae s.s.* Although not yet fixed in Wassadou-Badi, the allelic frequency of *Vgsc*-1014F mutation was more than 0.50.

The frequency of the *Vgsc*-1014F mutation was higher both in the surviving and dead phenotypes in the *An. gambiae s.s.* populations from Kedougou. The frequency of the *Vgsc*-1014S mutation in *An. arabiensis* populations from Wassadou-Badi was higher in the surviving than the dead specimens whereas no correlations were detected between the *Vgsc*-1014F mutation and the resistance phenotype in *An. gambiae s.s.* and *An. coluzzii species*. It is therefore likely that mechanisms other than *Vgsc*-1014F mutation are involved in the insecticide-resistance of these species. This hypothesis should be investigated in the future. These results are in line with those of Thiaw et *al.* [31] and Ahoua et *al.* [32], who found no correlation between the *kdr* mutation and the phenotypic alive or dead phenotypic respectively in *An. arabiensis* and *An. coluzzii*. Only the *Vgsc*-1014F mutation was noted in *An. coluzzii*. This finding could be explained by introgression from *An. gambiae s.s.*to *An. coluzzii* [36, 37]. Furthermore, our results show an absence of the *Vgsc*-1014S mutation in *An. coluzzii* obtained. This finding is similar to results obtained in Benin [40], but not those obtained in Cameroon [41] and in Equatorial Guinea Republic [42].The occurrence of the *Vgsc*-1014F mutation was detected in 2 hybrids (*An. gambiae s.s./An. coluzzii*) and were homozygote resistant genotype (FF). This is the first report of this mutation in hybrids from *An. gambiae s.s.* and *An. coluzzii* in Senegal. Other mutations could be involved in resistance of *An. gambiae* s.l. to insecticides, including the *Vgsc*-1575Y mutation [48] that was not investigated in this study.

With a significantly higher frequency in surviving specimens after exposure, the study shows that the *ace-1*^{R} mutation was implicated in phenotypic resistance of *An. gambiae s.s.* to bendiocarb. The involvement of the *ace-1*^{R} mutation in the phenotypic resistance to bendiocarb has been reported in *An. gambiae s.s.* populations from Côte Ivoire [32] and Ghana [33]. However, it was not present in surviving *An. arabienesis*.

The presence of heterozygotes in surviving specimens may explain the resistance of *An. gambiae* s.l. population to carbamates (bendiocarb) from Kedougou and Wassadou-Badi and organophosphates (fenitrothion) from Kedougou area.

Often associated with *rdl* mutation (*rdl*-A246S or *rdl*-A296G), the phenotypic resistance to dieldrin was found in *An. gambiae* s.l. population in both localities. A similar result was obtained in Benin [39]. The allelic frequencies obtained in our study are quite similar to those described by Corbel [39]. The phenotypic resistance to dieldrin could be explained by the long use of dieldrin in the past or other insecticides belonging to different families (such as fipronil or lindane) with the same mode of action as dieldrin on one hand and by the presence of *rdl*- A296G mutation, which is associated with a 2La chromosomal polymorphic on the other hand [37]. This is a very stable polymorphic inversion that limits crossover and would help preserve this mutation in a given population. The occurrence of multiple-resistance locus in *An. gambiae s.s.*, the main malaria vector in the study area, is indicative of the genes involved in resistance to the insecticides used in this area.

Conclusion

The study demonstrates phenotypic resistance in *An. gambiae* s.l. population to DDT, pyrethroids, benbiocarb and fenitrothion in southeastern Senegal. The relatively higher frequency in specimens surviving insecticide exposure demonstrates the role of target site modifications, including *Vgsc*-1014F and *Vgsc*-1014S, *ace-1*^{R} and *rdl*-A296S or *rdl*-A296G. Though they are one of the main factors, investigation of other mechanisms involved remains necessary for better management of the resistance to *An. gambiae* s.l. populations. Resistance to insecticides may jeopardize the effectiveness of the main strategies (indoor residual spraying (IRS) of persistent insecticides and the use LLIN mosquito nets) to reduce malaria transmission in the area.

Abbreviations

CTAB: cetyl trimethyl ammonium bromide, PCR :polymerase chain reaction, *kdr*: knockdown resistance, *rdl*: resistance to dieldrin (*rdl*), OMVS: Senegal River Basin Development Organization, IMP-PCR: intentional mismatches primer-PCR, DDT : dichloro diphenyltrichloroethane, *ace-1*:target-site resistance gene for carbamate and organophosphate insecticides conferring insensitive acetyl cholinesterase, *Vgsc*: voltage-gated sodium channel, WHO : world health organization, LLINs: long-lasting insecticide-treated nets, *ace1*^{R} : Acetyl cholinesterase insensible (G119S), *Vgsc*-1575Y : polymorphism non synonym, PMI: United States President's Malaria Initiative, KDT_{50} and KDT_{95} : knock down 50% and 95%, min: minutes, DNA : dexocy ribonucleic acid. RNA: ribonucleic acid, GABA: gamma-amino butyric acid, IRS: indoor residual spraying of insecticides, PQT-VC: Prequalification Team: Vector Control Products.

Declarations

Author's contribution

ED and EAN were involved field data collections. ED and AK have done laboratory analyses. ED, DI and KL have done the data analyses. ED, EAN, MD, LK, ID, OF and BS reviewed and edited manuscript.

Acknowledgments

We sincerely thank the PMI (President's Malaria Initiative) and the National Malaria Control Programin Senegal. We thank Dr. Ellen M. Dotson and Mr Omar Thiaw (thiawomar185@yahoo.com) for his valuable contribution.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The data used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by PMI (President's Malaria Initiative) monitoring activities in Senegal.

References

1. WHO. World malaria report 2019. https://www.who.int/publications/i/item/world-malaria-report-2019.
2. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z and Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasito* 2011, 27:91-98.
3. Dabiré RK, Namountougou M, Diabaté A, Soma DD, Bado J, Toé HK, et al. Distribution and frequency of *kdr* mutations within *Anopheles gambiae s.l.* populations and first report of the ace.1 G119S mutation in *Anopheles arabiensis* from Burkina Faso (West Africa). *PloS One*. 2014; 31:9(7)
4. Sangba MLO, Deketramete T, Wango SP, Kazanji M , Martin Akogbeto and Mamadou O Ndiath. Insecticide resistance status of the *Anopheles funestus* population in Central African Republic: a challenge in the war. *Parasit Vectors*. 2016 ; 9:230
5. Soromane Camara, Koffi AA, Ahoua LP Alou, Koffi K, Kabran JP K, Koné A,et al. Mapping insecticide resistance in *Anopheles gambiae* (s.l.) from Côte d'Ivoire. *Parasit Vectors*. 2018; 11:19
6. Fossog Tene B, Poupardin R, Costantini C, Awono-Ambene P, Wondji CS, Ranson H. Resistance to DDT in an urban setting: common mechanisms implicated in both M and S forms of *Anopheles gambiae* in the city of Yaoundé Cameroon. *PloS One*. 2013; 8: e61408
7. Awolola TS, Oduola O A, Strode C, Koekemoer LL. Evidence of multiple pyrethrinoid resistance mechanism in malaria vector *Anopheles gambiae s.s*. from Nigeria. *Am J Trop Med Hyg*. 2008; 103, 1139-1145.
8. Wondji CS, Coleman M, Kleinschmidt I, Mzilahowa T, Irving H, Ndula M, et al. Impact of pyrethroid resistance on operational malaria control in Malawi. *Proc Natl Acad Sci U S A*. 2012; 20; 109 (47):19063-70.
9. Martinez-Torres D, Fabrice Chandre, Williamson M S, Darriet F, Berge JB, Devonshire A L, et al. Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae s.s. Insect Mol Biol*. 1998; 7: 179-184
10. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Mol Biol*. 2000; 9(5):491-7.
11. Weill M, Lutfalla G, Mogensen K, Chandre F, Berthomieu A, Berticat C, et al . Comparative genomics: Insecticide resistance in mosquito vectors. *Nature*. 2003; 423: 136-137
12. Du W, Awolola TS, Howell P, Koekemoer LL, Brooke B D, Benedict MQ, et al. Independent mutations in the *Rdl* locus confer dieldrin resistance to *Anopheles gambiae* and *An. arabiensis*. *Insect Mol Biol*. 2005 ; 14(2), 179–183
13. Ousmane Faye, Lassana Konate & Abdoulaye Diop .Profil entomologique du paludisme au Sénégal. Ministère de la Santé et de la Prévention Médicale. 2011 ; 39
14. Food and Agriculture Organization. Animal Production and Health paper 41, Integrating crops and livestock in West Africa. 1983. http://www.fao.org/docrep/004/x6543e/x6543e01.htm. Accessed 25 Mar 2014
15. Ibrahima Dia, Takhy Diop, Ignace Rakotoarivony, Pierre Kengne, Didier Fontenille. Bionomics of *Anopheles gambiae* Giles, *An. arabiensis* Patton, *An. funestus* Giles and *An. nili* (Theobald) (Diptera: Culicidae) and transmission of *Plasmodium falciparum* in a Sudano-Guinean zone (Ngari, Senegal). *J Med Ento*.2003; 40: 279-283.
16. Ndiath MO, Mazenot C, Ablaye Gaye, Lassana Konate, Charles Bouganali, Ousmane Faye,et al. Methods to collect *Anopheles* mosquitoes and evaluate malaria transmission: A comparative study in two villages in Senegal, *Malar J*. 2011; 10:270

17. Bulletin épidémiologique annuel du paludisme au SENEGAL ; 2016.
18. Bulletin épidémiologique annuel du paludisme au SENEGAL ; 2014.
19. WHO. 2013. Test procedures for insecticide resistance monitoring in malaria vectors mosquitoes Geneva.
20. Abbott WS. A method of computing the effectiveness of an insecticide. *J Eco Ento*.1925; 18:265-267
21. Gillies MT, De Meillon B. The *Anophelinae* of Africa South of the Sahara (Ethiopian zoogeographical region). Johannesburg: *Publ South Afri Inst for Med Res*. 1068; 54: 343 pages
22. Wilkins EE, Howell PI, Benedict MQ. IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis*. *Malar J*. 2006; 5:125 .
23. https://www.beiresources.org. Methods in *Anopheles* Research Manual Full Version (MR4). 2014p: 250-251
24. Weill H, Hughes JM, Churg AM. Changing trends in US mesothelioma incidence. *Occup Env Med*. 2004; 61: 438-41
25. Hakizimana E, Karema C, Munyakanage D, Iranzi G, Githure J, Tongren JE, et al. Susceptibility of *Anopheles gambiae* to insecticides used for malaria vector control in Rwanda. *Malar J*. 2016; 15(1):582
26. Dadzie S, Appalu MA, Kerah-Hinzoumbé C, Akogbeto MC, Adimazoya M, Israël DK, et al. Species composition and insecticide resistance status of *Anopheles gambiae* (*s.l.*) (Culicidae) in Kome, southern Chad and the implications for malaria control. *Parasit Vectors*. 2016 ; 9(1) : 465.
27. Zoh DD, Ahoua Alou LP, Toure M, Pennetier C, Camara S, Traore DF, et al. The current insecticide resistance status of *Anopheles gambiae* (s.l.) (Culicidae) in rural and urban areas of Bouaké, Côte d'Ivoire. *Parasit Vectors*. 2018; 11:118.
28. Diabate A, Baldet T, Fabrice C, Akogbeto Martin, Guiguemde TR, Darriet Frédéric, et al. the role of agricultural use of insecticides in resistance to pyrethroids in *anopheles gambiae* s.l. in Burkina faso. *Am J Trop Med Hyg*. 2002 ; 67(6), 2002, p. 617–622
29. Hamon J and Garrett-Jones C. La résistance aux insecticides chez des vecteurs majeurs du paludisme et son importance opérationnelle. *Bulletin OMS*. 1963; 28(1):1-24 **(in French)**.
30. El hadji A Niang, Lassana Konaté, Diallo Mawlouth, Ousmane Faye and Ibrahima Dia. Patterns of insecticide resistance and knock down resistance (*kdr*) in malaria vectors *An.arabiensis*, *An. coluzzii* and *An. gambiae* from sympatric areas in Senegal. *Parasit Vectors*. 2016 ; 9:71
31. Omar Thiaw, Souleymane Doucouré, Seynabou Sougoufara, Charles Bouganali, Lassana Konaté, Nafi Diagne, et al. Investigating insecticide resistance and knock-down resistance (*kdr*) mutation in Dielmo, Senegal, an area under long lasting insecticidal-treated nets universal coverage for 10 years. *Malar J*. 2018; 17:123.
32. 32.Ahoua AL, Koffi AA, Adja MA, Assi SB, Kouassi PK, N'Guessan R. Status of pyrethroid resistance in *Anopheles gambiaes.s.* M form prior to the scaling up of Long Lasting Insecticidal Nets (LLINs) in Adzopé, Eastern Côte d'Ivoire. *Parasit Vectors*. 2012; 5:289.
33. John Essandoh, Alexander E Yawsonand David Weetman. Acetylcholinesterase (Ace-1) target site mutation 119S is strongly diagnostic of carbamate and organophosphate resistance in *Anopheles gambiaes.s.* and *Anopheles coluzzii* across southern Ghana. *Malar J*. 2013; 12:404
34. Brooke BD, Hunt RH, Maurine Coetzee. Resistance to dieldrin + fipronil assorts with chromosome inversion 2La in the malaria vector *Anopheles gambiae*. *Med. Vet. Entomol*. 2000; 14:190–194
35. Thwing JI, Perry, RT, Townes DA, Diouf, MB, Ndiaye S, &Thior M. Success of Senegal's first nationwide distribution of long-lasting insecticide-treated nets to children under five - contribution toward universal coverage. *Malar J*. 2011 ; 10 (1), 86.
36. Weill M, Fabrice Chandre, Brengues Cécile, Manguin S,Akogbeto Martin,Pasteur N, et al. The *kdr* mutation occurs in the mopti form of *Anopheles gambiae s.s.* through introgression. *Insect Mol Biol*. 2000; 9, 451–455.
37. Diabate A, Brengues C, Baldet T, Dabire KR, Hougard JM, Martin Akogbeto, et al. The spread of the Leu-Phe kdr mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and de novo phenomena. *Trop Med Int Health*.2004; 9:1267–73.
38. Akogbéto MC, Djouaka FR, Kinde-Gazard DA.Screening of pesticide residues in soil and water samples from agricultural settings. *Malar J*. 2006 ; 5, p. 22
39. Vincent Corbel, N'Guessan R, Brengues Cécile, Fabrice Chandre, Djogbenou L, Martin T, et al. Multiple insecticide resistance mechanisms in A*nopheles gambiae* and Culex quinquefasciatus from Benin, West Africa. *Act Trop*. 2007; 01.005.
40. Innocent Djègbè, Olayidé Boussari, Aboubakar Sidick, Thibaud Martin, Hilary Ranson, Fabrice Chandre, et al. Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S *kdr* mutation in *Anopheles gambiae* from West Africa. *Malar J*. 2011; 10: 261.
41. Reimer L, Fondjo E, Patchoké S, Diallo B, Lee Y, Ng A, et al. Relationship between *kdr* mutation and resistance to pyrethroid and DDT insecticides in natural populations of *Anopheles gambiae*. *J Med Ento*. 2008; 45(2):260-6.
42. Ridl FC, Bass C, Torrez M, Govender D, Ramdeen V, Yellot L, et al. A pre-intervention study of malaria vector abundance in Rio Muni, Equatorial Guinea: their role in malaria transmission and the incidence of insecticide resistance alleles. *Malar J*. 2008; 7:194.
43. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL http: //www.R-project.org/ R Core Team (2014).
44. Geraldine Marie Foster, Michael Coleman, Edward Thomsen, Hilary Ranson, Elise Yangalbé-Kalnone, Tchomfienet Moundai et al. Spatial and Temporal Trends in Insecticide Resistance among Malaria Vectors in Chad Highlight the Importance of Continual Monitoring. *PloS One* 2016.
45. Keita K, Camara D, Barry Y, Osse R, Wang L, SyllaM, et al. Species Identification and Resistance Status of *Anopheles gambiae* s.l. (Diptera: Culicidae) Mosquitoes in Guinea *J Med Ento* 2017; 54(3), 677–681.

46. Patricia N, Okorie, George OAdemowo, Helen Irving, Louise A Kelly-Hope, and Charles S Wondji. Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* mosquitoes in Ibadan, South-West Nigeria. *Med Vet Entomol*. 2015; 29(1): 44–50
.https://www.sodefitex.sn/images/2014_appels/decembre_2013_dao_39_fournitures_peoduits_insecticides.pdf

48. Jones CM, Liyanapathirana M, Agossa FR, Weetman D, Ranson H, Donnelly MJ, et al. Footprints of positive selection associated with a mutation (N1575Y) in the voltage-gated sodium channel of *Anopheles gambiae. Proc Natl Acad Sci U S A.2012; 109:*6614–9.

49. Samir SR, Leo MLN. Pesticides: evaluation of environmental pollution. Boca Raton: CRC Press; 2012.

50. Murray MG and Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*; 1980.

51. WHO. World malaria report 2017. https://www.who.int/publications/i/item/world-malaria-report-2017.

Tables

Table 1. Mortality rates following insecticides (pyrethroids, DDT and dieldrin) exposure of *Anopheles gambiae* s.l. populations from Kedougou and Wassadou-Badi in October and November 2014.

Localities	Insecticides	Mortality rate (n)	KDT_{50} [95% IC] min	KDT_{95} [95% IC] min
Kedougou	DDT	12.8 (211)	161 [119.26-267.1]	784.95 [420.5-2287.86]
	Permethrin	53.2 (220)	164.89 [122.6-263.2]	1234.25 [634-3563.8]
	Deltamethrin	67.9 (240)	161 [128-222.83]	784.95 [490.65-1556.5]
	Lambda cyhalothrin	57.1 (231)	53.92 [49.2-60.5]	149.27 [118.71-208.3]
	Cyfluthrin	81.4 (200)	22.72 [20.7-24.8]	65.63 [56.39-80.15]
	Alpha cypermethrin	42.8 (217)	28.35 [26.8-30]	84 [74.87-96.66]
	dieldrin	67.8 (239)	97.56 [73.32-324.7]	223.3 [123.2-3120.3]
Wassadou-Badi				
	DDT	55.8 (116)	161 [119.26-267.14]	784.95 [420-2287.86]
	Permethrin	58 (119)	47.47 [43.40-52.87]	153.23 [121.9-210]
	Deltamethrin	68 (122)	58.8 [50.03-68.01]	113.38 [97.73-139.29]
	Lambda cyhalothrin	53.4 (118)	63.56 [56.5-75.5]	174.65 [130.28-280.74]
	Cyfluthrin	86.4 (109)	41.7 [49.79-105.55]	87.45 [87.45-139.6]
	Alpha cypermethrin	86 (110)	27.63 [25.64-29.72]	62.94 [55.76-73.56]
	dieldrin	83 (124)	0.00 -	0.00 -

Abbreviations: (), Number of mosquitoes tested; (95% IC), 95% confidence interval; KDT_{50} and KDT_{95}, Knock down 50% and 95%; Min, minutes

Table 2. Genotypes and allelic frequencies of mutations *Vgsc*-1014F, *Vgsc*-1014S, *Ace-1(G119S)*, *rdl*-A296S, and *rdl*-A296G in *An. arabiensis, An. coluzzii* and *An. gambiae s.s.*in Kedougou and Wassadou-Badi in October and November 2014.

Localities	Species	*Vgsc*-1014F				P	*Vgsc*-1014S				P	*Ace-1* (G119S)				P	*rdl*-A296S or *rdl*-A296G			
		LL	LF	FF	(freq R)		LL	LS	SS	(freq R)		GG	GS	SS	(freq R)		AA	AG	GG	(fre R)
Kedougou	*An.arabiensis*	23	1	3	0.129		14	0	0	0		1	0	0	0		25	1	0	0.01
	An. coluzzii	0	1	18	0.973		0	0	0	0		19	1	0	0.025		11	1	0	0.04
	An.gambiae s.s.	0	1	298	0.998	0.001	2	0	0	0	*n/a*	101	28	7	0.154	0.33	145	49	2	0.13
Wassadou-Badi	*An.arabiensis*	114	3	3	0.037		55	11	12	0.22		38	3	0	0.036		32	0	0	0
	An. coluzzii	10	5	10	0.500	< 0.001	8	0	0	0.00	< 0.001	21	0	0	0 .00	0.043	4	0	0	0
	An.gambiae s.s.	3	0	134	0.978		0	0	10	1		57	11	6	0.155		44	4	1	0.06

Abbreviations: **P**- probability of significant difference for each mutation among species within each site; **L** - Leucine; **F** -phenylalanine; **S** - Serine; **G** - Glycine; **A**,-Alanine; Freq R - Frequency of resistant allele; *n/a - not applicable*, **FF**- phenyl alanine- phenyl alanine.

Table 3. Numbers of specimens and frequencies of G119S and *rdl* A296G or *rdl* A296S mutations by surviving or dead phenotypes in*An. arabiensis*, *An.coluzzii* and *An. gambiae s.s.* of Kedougou and Wassadou-Badi in Octoberand November 2014.

Localities	Phenotypes	*n*	*An. arabiensis* (freq R)	P	*n*	*An. coluzzii* (freq R)	P	*n*	*An. gambiae s.s* (freq R)	P
Kedougou										
Vgsc-1014F	Surviving	11	0.31		18	0.97		281	1.0	
	Dead	16	0.06	0.031	0	0.0	*n/a*	18	0.97	0.058
Vgsc-1014S	Surviving	2	0.0		1	0.0		42	0.0	
	Dead	12	0.0	*n/a*	0	0.0		16	0.0	*n/a*
Wassadou-Badi										
Vgsc-1014F	Surviving	51	0.068		11	0.6		100	0.98	
	Dead	41	0.0	0.017	8	0.37	0.44	9	0.88	1
Vgsc-1014S	Surviving	24	0.48		3	0.0		51	1.0	
	Dead	33	0.07	<0.001	7	0.0	*n/a*	6	0.0	*n/a*
Kedougou										
Ace-1 G119S	Surviving	21	0.0		0	0.0		31	0.46	
	Dead	33	0.0	*n/a*	2	0.0	*n/a*	22	0.11	<0.001
Rdl-A296S or *Rdl*-A296G	Surviving	2	0.0		2	0.0		34	0.21	
	Dead	0	0.0	*n/a*	0	0.0	*n/a*	15	0.0	0.014
Wassadou-Badi										
Ace-1 G119S	Surviving	0	0.0		1	0.0	*n/a*	11	0.36	0.034
	Dead	11	0.0	*n/a*	1	0.0		6	0.0	
Rdl-296S or *Rdl*- A296G	Surviving	2	0.0		-	-	-	-	-	-
	Dead	6	0.0	*n/a*	-	-	-	-	-	-

Figures

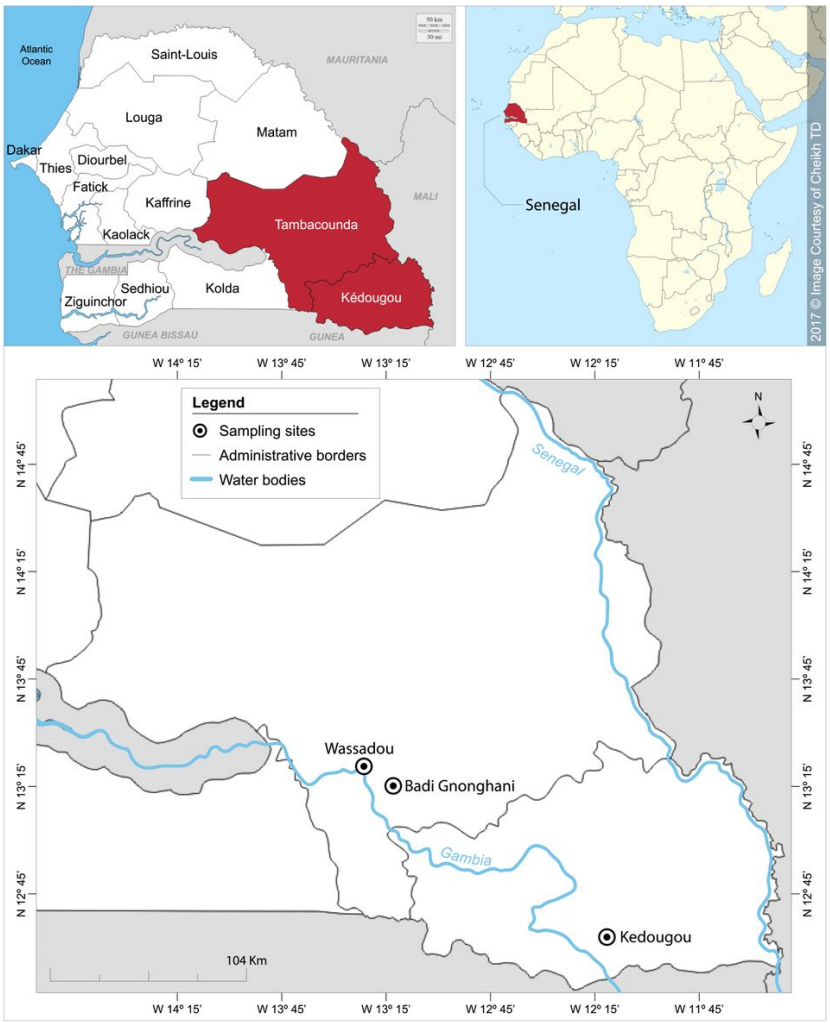

Figure 1

Map showing mosquito sampling areas in southeast Senegal.

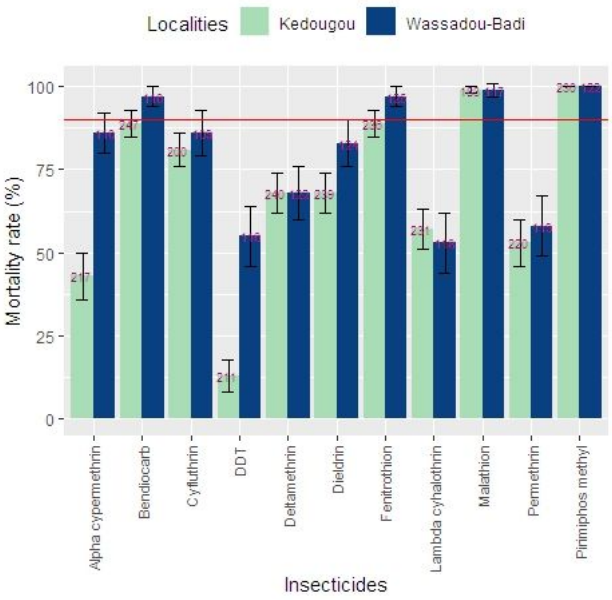

Figure 2

Mortality rate of Anopheles gambiae s.l. 24h populations after exposure to WHO recommended insecticide doses in October and November 2014. Abbreviations: Red line, 90% threshold; Black bars, 95% Confidence Interval; Numbers, sample size for each insecticide tested