Research Article

Phenotypic Characterization of the Resistance Status of *Anopheles gambiae* s.l. from Oyem, North Gabon to Four Classes of Insecticides

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Abstract | This study attempts to characterise the resistance status of *Anopheles gambiae* s.l., a major vector of malaria to insecticides recommended by World Health Organization in Oyem, north Gabon. Indeed, larval collections were conducted in July 2020 (dry season) in 14 quarters of Oyem town. All anopheline larvae were collected from natural water reservoirs. They were bred to adult stage in the field laboratory. All the adults (n=560) identified belonged to the *An. gambiae* complex. Their susceptibility to insecticides (Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin 0.05%, Cyfluthrin 0.15%, DDT 4%, Bendiocarb 0.1% and Malathion 5%) was assessed using the WHO susceptibility test protocol. Susceptibility test results showed a weak knock-down effect response of Anopheles to these insecticides tested. These results indicates their resistance to 4% DDT and pyrethroids, with mortalities between 0% and 68%. However, these Anopheles shows some reasonable degree of sensitivity to Bendiocarb (mortality = 100%) and Malathion (mortality = 100%). The strong resistance of *An. gambiae* s.l. to Pyrethroids, the only family used in the impregnation of mosquito nets, indicates the need to design efficient malaria vector control strategies for Oyem.

Keywords | *Anopheles gambiae* s.l., Susceptibility, Resistance, Insecticides, Oyem, Gabon.

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INTRODUCTION

Malaria is a parasitic disease caused by *Plasmodium* species through an infected bite of a female Anopheles species (WHO, 2015; Indika et al., 2020). This vector-borne disease still remains an important public health problem in many countries of sub-Saharan Africa where it kills a child every 30 seconds (Tia et al., 2016; WHO, 2019). In Gabon, malaria is hyper-endemic (Maghendji-Nzondo et al., 2016a; Pamba et al., 2020) and its transmission occurs throughout the year (Bouyou-Akotet et al., 2012). It is the leading cause of hospital consultations where 45% of hospitalized people are children and 71% are pregnant women (Mbouloungou et al., 2019). In Gabon, the towns with high infection prevalence with malaria include: Lastourville (79.5%), Fougamou (53.6%), Oyem (44.2%), Koulamoutou (36.1%), Libreville (24.1%) and Franceville (22.00%) (Mawili-Mboumba et al., 2013; Maghendji-Nzondo et al., 2016a, b; Minsanté-SNIS, 2016; Minsanté-SNIS, 2018). According to Mawili-Mboumba et al. (2017), Oyem is the third city highly affected by malaria with prevalence of 44.2%. In Gabon, the fight against malaria is mainly based on the use of insecticide-treated mosquito nets (LLINs). However, the emergence of resistance of Anopheles to insecticides reported in other localities of Gabon (Mourou et al., 2010; Koumba et al., 2018b; 2019), indicates the weakness of this approach for the fight against malaria vectors in the country. The objective of this study is to phenotypically characterise the resistance status of *Anopheles gambiae* s.l. from Oyem to insecticides.

MATERIALS AND METHODS

Study area

This study was conducted in July 2020 (dry season) in the city of Oyem (600 km from Libreville, capital of Gabon) (Figure 1).

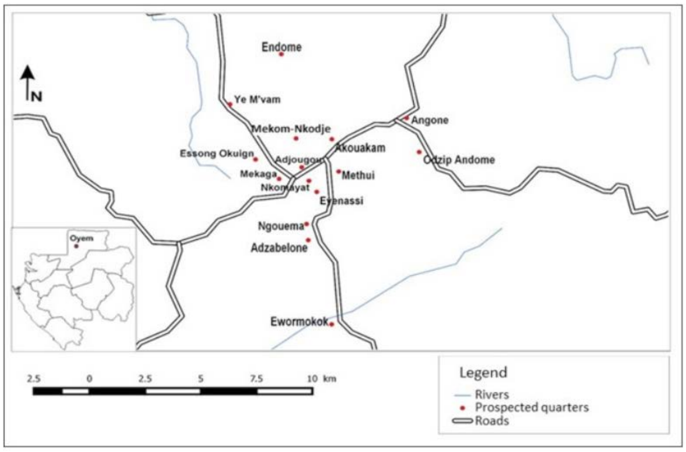

Figure 1: Location of Oyem and sampled quarters (red dots).

Oyem is a town in north Gabon and capital of the Woleu-Ntem province. It is the fourth largest city in the country in terms of demography with a total population estimated at 60,685 inhabitants (DGS-RGPL, 2015). Geographically, it is located between latitude 1° 37′ 00″ north and longitude 11° 35′ 00″ east and covers an area of 38,465 km^2. Oyem has an equatorial climate, marked by an alternation of two rainy seasons and two dry seasons (UNDP, 2009). The average rainfall fluctuates between 1600 and 2100 mm per year (MDDEPIP, 2015). The average temperature is 23 to 24 ° C with maximum recorded in the months of March and April and minimum (18° C) in July. Relative humidity is high all year round and does not drop until the dry season (MDDEPIP, 2015).

Collection and rearing of mosquito larvae

The population of Anopheles for the bioassay were collected from natural water reservoirs (ponds, swamps, etc.). The collection of mosquito larvae was made using the "dipping" technique (Talipouo et al., 2017). These larvae were transferred to trays using transfer pipettes and then transported to the field laboratory for rearing following the protocol of Egbuche et al. (2016). They were reared in natural water and fed daily with ornamental fish feed (sera Vipagran ©) (Kone et al., 2013). Adults were fed with 10% sugar solution *ad libitum*. The identification of adult mosquitoes was conducted using the Central African Culicidae identification key made by Baldacchino and Paupy (2010).

Susceptibility testing of *Anopheles gambiae* s.l. to insecticides

Susceptibility testing was performed according to the WHO protocol (2017). Female *Anopheles gambiae* (2 to 4 days old) were tested. In the test tube, 20 mosquitoes were exposed to insecticide impregnated papers for 60 minutes. In the control tube, 20 mosquitoes were exposed to paper impregnated only with olive oil. The insecticides and their concentrations used in this bioassay are presented in Table 1.

Table 1: Insecticides used for the susceptibility bioasay

Classes	Chemical name	Concentration (%)
Organochlorines	DDT	4
Pyrethroids	Deltamethrin	0.05
	Permethrin	0.75
	Lambda-cyhalothrin	0.05
	Cyfluthrin	0.15
Carbamates	Bendiocarb	0.1
Organophosphates	Malathion	5

The knock-down effect was checked every 5 minutes for the first 20 minutes of exposure and then every 10 minutes intervals till 60 minutes. At the same time, those in the control group were also checked. After 1 h of exposure, the tested mosquitoes were transferred to observation tubes containing untreated papers. They were fed with 10% sugar solution. In addition, all the insecticide-impregnated papers were pre-tested with adults of the sensitive reference strain (Kisumu) in order to confirm their efficacy (Djogbenou et al., 2011). Sensitivity tests were performed under the following laboratory conditions: 26-29 ° C and 74-82% relative humidity.

Data analysis

Knock-down time fifty and Ninety five (KdT50 and KdT95), defined as the time required for 50% (KdT50) and 95% (KdT95) of mosquitoes to be knocked-down after 1 hour of exposure to insecticide, were evaluated using the Polo Plus 1.0 sofware. On the other hand, the mortality rates observed after 24 hours were calculated by dividing the number of mosquitoes killed by the total number of mosquitoes tested for each insecticide.

The resistance and sensitivity status of the tested mosquitoes were determined according to the criteria defined by WHO (2017) as follows:
Resistant if mortality <90%;
Sensitive if mortality> 98%;
Resistance is probable (to be confirmed) if mortality occur between 90% and 97%.

RESULTS

Determination of the knock-down time of *Anopheles gambiae* s.l. upon exposure to insecticides

In the presence of DDT, no Kd effect was observed in *An. gambiae* s.l. (Table 2). In contrast, in the presence of all pyrethroids, KdT95 remained above 60 mins for adult females of *An. gambiae* s.l. The KdT50 remained greater than 60 mins in the presence of Permethrin 0.75%, Lambda-cyhalothrin 0.05% and Cyfluthrin 0.15%. On the other hand, a knock-down effect of less than 44 mins with 0.05% deltamethrin (Table 2) was observed. Regarding the knock-down times, it should be noted that deltamethrin 0.05% is the only insecticide that knocked-down 50% of the population of *An. gambiae* s.l. in less than 1 hour of exposure. It was noticed that all the mosquitoes in the control group remained alive throughout the observation period.

Table 2: Determination of KdT50 and KdT95 knock-down times

Insecticide	KdT50 (CI)	KdT95(CI)
DDT 4%	No Kd	No Kd
Deltamethrin 0.05%	43.291 (39.042 – 48.938)	169.587 (130.068 -246.013)
Permethrin 0.75%	742.368 (191.864 – 539982.419)	42005.659 (2218.506 – 0.000)
Lambdacyalothrin 0.05%	117.115 (84.942 – 203.344)	968.763 (447.681 – 3878.299)
Cyfluthrin 0.15%	129.293 (74.335 – 838.504)	992.470 (282.293 – 98325.017)

No Kd: No knock-down effect (less than 10% of mosquitoes knocked-down after 60 minutes of exposure); CI: 95% Confidence Interval; KdT50 and KdT95.

Determination of the resistance and sensitivity status of *Anopheles gambiae* s.l. tested

The results of the insecticide test indicates that the mortality rates of *An. gambiae* s.l. was 0% with DDT, 68% with deltamethrin 0.05%, 25% with Lambda-cyhalothrin 0.05%, 28% with Cyfluthrin 0.15% and 56% with Permethrin 0.75% (Table 3). However, we recorded 100% mortality of *An. gambiae* s.l. in the presence of Bendiocarb 0.1% and Malathion 5% (Table 3). Regarding the KdTs and the recorded mortality rates, it should be noted that populations of *An. gambiae* s.l from the town of Oyem are resistant to DDT (Organochlorine) and Pyrethroids (Deltamethrin 0.05%, Permethrin 0.75%, Lambda-cyhalothrin 0.05% and Cyfluthrin 0.15%). The highest resistance status was observed with DDT. However, *Anopheles gambiae* s.l from our prospection sites still remain sensitive to Bendiocarb (Carbamate) and Malathion (Organophosphate) (Table 3).

Table 3: Mortality of *Anopheles gambiae* s.l. obtained after 24 hours of observation

Insecticides	N	Mortality rate (%) after 24 hrs observation	Status
DDT4%	80	0	Resistant
Deltamethrin 0.05%	80	68	Resistant
Permethrin 0.75%	80	56	Resistant
Lambda-cyhalothrin 0.05%	80	25	Resistant
Cyfluthrin 0.15%	80	28	Resistant
Bendiocarb 0.1%	80	100	Sensitive
Malathion 5%	80	100	Sensitive

DISCUSSION

This study shows strong resistance of *An. gambiae* s.l. from the city of Oyem (northern Gabon) to DDT and Pyrethroids. This result is similar to that of Koumba et al. (2018b; 2019) who reported the resistance of *An. gambiae* s.l. to DDT 4% and low concentrations of pyrethroids. Similar findings have been obtained by authors from Cameroon (Akono et al., 2018; Bamou et al., 2019), Benin (Djouaka et al., 2017), Senegal (Diouf et al., 2020), Nigeria (Chukwuekezie et al., 2020), Gambia (Ochieng'Opondo et al., 2019) and Central African Republic (Kamgang et al., 2018). The strong resistance to DDT observed in *Anopheles gambiae* s.l. populations from Oyem could be linked to the frequent use of DDT through indoor spraying and for impregnating mosquito nets during vector control campaigns launched in sub-Saharan Africa in the 1950s and 1980s (Carnevale & Mouchet, 2001). Indeed, *Anopheles gambiae* s.l., the major malaria vector in Africa, appeared resistant to insecticides for the first time in 1954 in Nigeria, after intra-domiciliary spraying with Dieldrin; then the phenomenon rapidly spread to the entire African continent (Etang and Simard, 2002). In addition, this strong resistance to DDT could also be explained by the repeated use of this insecticide during programs to protect cocoa and coffee crops against pests in the 1990s (Petithuguenin, 1994). Moreover, according to some authors, excessive usage of insecticides in agricultural and industrial areas (such as Oyem) favors the selection of resistant vector populations (Hien et al., 2017; Chabi et al., 2018). Additionally, in some countries, resistance to DDT and Pyrethroids emerged in 1967 in Burkina Faso and 1993 in Côte d'Ivoire respectively. Also, this resistance seems to be linked to the massive use of these insecticides in those countries for the treatment of cotton.

The resistance of Anopheles to pyrethroids observed in this study could be caused by the use of insecticides of this class to impregnate mosquito nets since 2006 in some households in most localities of Gabon. According to the evaluation report of the Global Fund for Malaria Round 4 of the WHO (2008), between 2006 and 2008, 323,586 insecticide-treated mosquito nets (ITNs) were distributed in Gabon. In the province of Woleu-Ntem where the town of Oyem is located, 68% of households in rural areas and 52% of households in urban areas benefited from these ITNs. Despite the efforts put in place to reduce malaria vectors burden in Oyem, malaria cases keeps increasing. This increment could be explained partly by the occurrence of resistant strains of the major malaria vector *Anopheles gambiae* s.l. Futhermore, this resistance could also be explained by the proximity of Oyem to Cameroon where resistance tests in the city of Douala, its ecconomic capital revealed mosquito resistance to Pyrethroids in the 1950s, and in the cotton region of Garoua (Etang & Simard, 2002).

Regarding the resistance and sensitivity status of *An. gambiae* s.l, we observed a reasonable degree of sensitivity of the tested population to 0.1 % Bendiocarb and 5 % Malathion (mortality = 100%). Molecular genotyping of the resistant individuals is necessary to detect the resistance genes involved. The sensitivity of *An. gambiae* s.l. from Oyem to these two insecticides could be linked to the fact that Organophosphates and Carbamates are rarely used in this area. This observation was also made by Koumba et al. (2018a) in the agricultural areas of Mouila in Gabon where many classes of insecticides are used for plant protection. Because *An. gambiae* s.l. from Oyem were sensitive to Organophosphates and Carbamates, they could be carefully used in order to avoid the emergence of resistance.

CONCLUSION

Populations of *Anopheles gambiae* s.l. from Oyem are resistant to insecticides commonly used in public health, except Bendiocarb and Malathion which are still effective. These results could serve as baseline information for malaria vector control in this part of the country.

However, further analyses are needed to highlight the mechanisms involved in insecticide resistance in these *An. gambiae* s.l. populations in order to guide the choice of insecticide molecules to be used according to the eco-entomological context of this study area.

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CONFLICT OF INTEREST

Authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS CONTRIBUTION

This study was designed by Jacques F. Mavoungou. Pyazzi Obame OK, CR Zinga-Koumba, Aubin A. Koumba, Audrey P. Melodie Ovono and Rodrigue Mintsa N. performed the field works and collected data. El Hadji Malick Ngom and Seynabou Mocote Diedhiou helped with data analysis. Boris Kevin Makanga helped with identification of mosquito collected on field. Aubin A. Koumba, Silas L. Sevidzem, Pyazzi Obame OK wrote the manuscript. Ousmane Faye, Jacques F. Mavoungou, Prospere Abessolo Mengue and CR Zinga Koumba revised the manuscript. All authors reviewed the final version of the manuscript.

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