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Susceptibility status of *Anopheles gambiae* s.l. to insecticides used for malaria control in Kinshasa, Democratic Republic of the Congo

Statut de la sensibilité des Anopheles gambiae s.l. *aux insecticides utilisés pour le contrôle du paludisme à Kinshasa, République Démocratique du Congo*

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Résumé

Contexte et objectif. Malgré plusieurs années de lutte, le paludisme demeure toujours la première cause de mortalité infantile sous les tropiques. Actuellement, la stratégie de contrôle vise des actions simultanées contre l'agent causal et le vecteur du paludisme. L'objectif de la présente étude était de décrire la distribution de la sensibilité d'*Anopheles gambiae* sl aux insecticides à travers la ville de Kinshasa. *Méthodes*. Des larves d'anophèles ont été collectées, à travers sept sites de Kinshasa, pendant la période allant de septembre 2017 à mai 2018. Des bioessais standard de l'OMS ont été utilisés pour mesurer la sensibilité d'*Anopheles gambiae* s.l. aux insecticides. La distribution des espèces et le profil de résistance ont été évalués en recourant aux tests diagnostiques moléculaires. *Résultats*. Deux espèces du complexe gambiae ont été identifiées : *An. gambiae* (98,3 %) et *An. coluzzii* (1,7 %). Une variabilité du statut de résistance à la deltaméthrine par site a été observée. Cependant, une restauration de la sensibilité a été notée après une pré-exposition au butoxyde de pipéronyle (PBO) dans tous les sites présentant une résistance à la deltaméthrine. *Conclusion*. La présente étude a démontré qu'An. *gambiae* s.l. était résistant à la perméthrine dans tous les sites retenus. Cependant, la résistance à la deltaméthrine était variable. Le profil de résistance indique que les moustiquaires deltaméthrine+PBO devraient être envisagées pour la lutte anti vectorielle.

Mots-clés : *Anopheles gambiae* sl, République démocratique du Congo, Résistance aux insecticides
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Summary

Context and objective. Despite many years of control, malaria remains the leading cause of child mortality in the tropics. Currently, the control strategy aims at simultaneous actions against the causal agent and the vector of malaria. This study aimed to describe the distribution of the susceptibility of *Anopheles gambiae* sl to insecticides across the city of Kinshasa. *Methods*. *Anopheles larvae* were collected from seven sites in Kinshasa during the period from September 2017 to May 2018. Standard WHO bioassays were used to determine the sensitivity of *Anopheles gambiae* s.l. to insecticides. The species distribution and the resistance profile were evaluated by polymerase chain reaction. *Results*. Two species of the gambiae complex were identified: *An. gambiae* (98.3 %) and *An. coluzzii* (1.7 %). Variability of deltamethrin resistance status by site was observed; however, a restoration of susceptibility was noted after pre-exposure to piperonyl butoxide (PBO) in all sites with deltamethrin resistance. *Conclusion*. The present study showed that *An. gambiae* s.l. was resistant to permethrin in all the selected sites. However, resistance to deltamethrin was variable. The resistance profile indicates that deltamethrin+PBO nets should be considered for vector control.

Keywords: *Anopheles gambiae*; Democratic Republic of the Congo; Insecticide resistance.

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Introduction

Despite several years of struggle, morbidity due to malaria remains a global concern (1). More than 3 billion people worldwide live in malaria-endemic areas (2). According to the 2021 World Malaria Report, approximately 241 million cases and 627,000 deaths were linked to malaria in 2020 (2). Almost 80 % of all malaria cases worldwide have occurred in 17 African countries and India. Nearly 30 % of all cases globally were accounted for by Nigeria (19 %) and the Democratic Republic of the Congo (11%) (2).

Current approaches to controlling this morbidity and mortality are based on simultaneous actions against the vector and the parasite (2). Thus, in the DRC, the national strategic plan for malaria control is based on three major strategies, namely: early therapy with artemisinin-based combinations (ACT); intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine for pregnant women; and vector control through the distribution of insecticide-treated mosquito nets (ITN) (3). The latter is an essential element in the control of malaria morbidity because it reduces the transmission of parasites by actions targeting adult Anopheles.

Unfortunately, the occurrence of insecticide resistance in Anopheles vectors is increasingly common. This is probably due to the limited number of insecticide classes and the near-exclusive use of pyrethroids in mosquito nets as well as the extensive use of these molecules in agriculture.

Resistance to pyrethroids is often associated with genetic mutations (Kdr mutations) (4). The Kdr mutation of the L1014F type is frequently found in *Anopheles gambiae* s.l. from West Africa, while in populations of East Africa, the L1014S mutation is more common (5). *Anopheles gambiae* s.l. from Okyerekoare, Ghana were resistant to pyrethroids, organochlorines, and carbamates, as well as to organophosphates used by the National Malaria Control Program (6). In Rwanda, a progressive increase in resistance to pyrethroids (Lambda-cyhalothrin, deltamethrin, permethrin) and organochlorines (DDT) was noted over the course of three years (7). In Mali, the same trend was observed through *Anopheles gambiae* s.l. remained susceptible to pyrethroids in one of the thirteen sites involved in the study (8). In Benin, *Anopheles gambiae* s.l. had widespread resistance to permethrin in the south of the country with a significant increase in kdr frequency accompanied by a low frequency of Ace-1 (9). In the DRC, the few studies carried out show the existence of anopheles resistance to conventional insecticides (10-11).

The use of insecticide-treated mosquito nets is the main strategy for preventing malaria in the DRC. Its implementation in the country during the last decade has likely contributed to increasing the vectors' resistance to common insecticides. Entomological monitoring of vector susceptibility to standard insecticides is one of the pillars of insecticide resistance management (12). Resistance to permethrin was noted in *Anopheles gambiae* s.s. from Kinshasa in 2010 (13), but two mass campaigns of mosquito net distribution have been conducted since then (2013, 2016). Resistance to permethrin and deltamethrin was found in *Anopheles gambiae* s.l. and *An. funestus s.s.* collected in Ndjili-Brasserie in 2015 (10).

The transmission of malaria in Kinshasa is heterogeneous (14), as is ITN use, so insecticide resistance should be monitored in multiple sites. The present study was conducted to describe the current status of distribution of the susceptibility of *Anopheles gambiae* s.l. in 7 sites in the city of Kinshasa.

Methods

Design, period, and study sites

The present cross-sectional study was carried out in the city of Kinshasa, from September 15th, 2017, to May 15th, 2018. This large city is divided into 4 districts with 24 municipalities. The climate is tropical humid, with a rainy season lasting from October to May (15).

According to the classification of Pain (16), Kinshasa's vegetation is of three types, namely: the secondary subequatorial semi-deciduous forest degraded, semi-deciduous forest and forest regrowth, and active fields. Today, human activity has significantly disrupted the environment of the city; specifically, the agricultural activities scattered throughout the city, the accelerated and uncontrolled urbanization as well as the presence of localized industrial activities.

In this present study, seven sites were selected by considering the representativeness of the four districts of Kinshasa, their ecological diversity, the presence of industrial and agro-pastoral activities as well as the accessibility of the breeding sites (Figure 1).

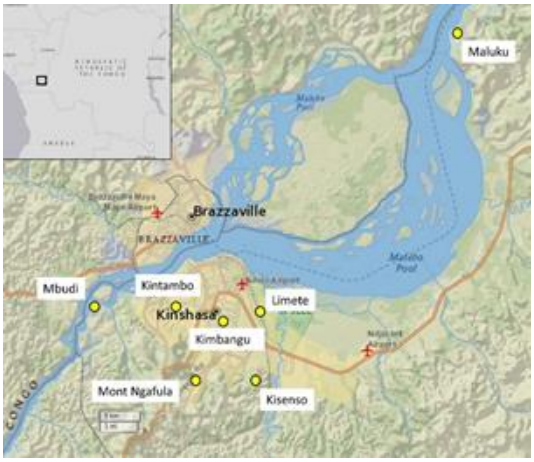

Figure 1. Sites where *Anopheles larvae* were collected for susceptibility tests in Kinshasa

- Maluku (04°27'41 "S, 16°04'43 "E), located in the Tshangu district. This site borders the city with the province of ex-Bandundu. The area was highly industrialized in the 1990s and 2000s. It also has an intensification of agro-pastoral and market gardening activities.
- Mont Ngafula (04°25'37"S, 15°17'29 "E), located in the district of Mont Amba. It is located in the southwestern part of the city of Kinshasa. It borders the city with the province of Central Kongo. A peripheral and recent city, with uncontrolled urbanization, Mont Ngafula is characterized by a very capricious relief with the possibility of water retention after the rain.
- Mbudi (04°21'42"S, 15°13'06 "E), located in the Lukunga district. It has the particularity of being a new residential site. Unfortunately, the urbanization policy is very limited. The absence of gutters favors the presence of multitudes of water collections.
- Limete (04°20'59"S, 15°20'17 "E), located in the district of Funa. Old residential city with respected urbanization measures. Unfortunately, the poor sanitation management policy limits the drainage of wastewater.
- Kintambo (04°19'37"S, 15°16'22 "E), located in Lukunga District. This site has the same environmental characteristics as the old residential city of Kinshasa.
- Kisenso (04°24'47"S, 15°20'47 "E), located in the district of Mont Amba. This site has the particularity of being a landlocked area with a clear absence of sanitation management policy. Agricultural activities are very intense.
- Kimbangu (04°20'51"S, 15°19'12 "E), located in the district of Funa. Site with a high level of culinary nuisance. It is a perfect example of the old urbanized areas of Kinshasa with very pronounced waste management failures.

The study areas were explored on foot to locate breeding sites. Samples were collected from a variety of breeding sites including gutters, ponds, small pools of standing water, muddy water, agricultural sites, and run-off from houses.

Mosquito collection

The larvae and pupae of Anopheles were collected in the seven sampling sites described above (Fig. 1). The collected larvae were reared until adult emergence in a room with controlled temperature varying between 25 and 27 °C, with a relative humidity of 80±10%. Pupae were harvested daily and were placed in cages in the insectary. After emergence, adult Anopheles were fed with a 10 % glucose solution. Adult *Anopheles gambiae* s.l. were identified, following the morphological key of Gillies & Coetzee (17).

Insecticide susceptibility tests

WHO susceptibility tests were performed by selecting and subjecting 4-5-day old females which emerged from larvae and pupae collected from the breeding sites, according to the WHO protocol (18). Impregnated papers of three approved insecticide classes were used at diagnostic dose: pyrethroids (deltamethrin 0.05 %, permethrin 0.75 %), carbamate (bendiocarb 0.1%) and organochlorine (DDT 4 %). Approximately 100 mosquitoes (four replicates of 25 mosquitoes) were used per test. Control mosquitoes were exposed to untreated papers. The number of *An. gambiae* s.l knocked down at, 3'5'10'15'20'25'30'35'40'45'50'55'60' was recorded during the exposure time while mortality was recorded after 24h (18). Kdt50 and Kdt95, which represent the shock times after which 50 % and 95 % of *An. gambiae* s.l. were

paralyzed, were determined only for the pyrethrinoid (18). The efficacy of these compounds was compared with that of DDT and bendiocarb to determine whether or not there was cross-resistance between the three chemical insecticide families. Results were interpreted according to WHO criteria: susceptible (S), all *An. gambiae* s.l. whose mortality 24 hours after insecticide contact was 98-100%; resistant (R) if the mortality of *An. gambiae* s.l. is less than 90 %; probable resistant (RP), if the mortality of *An. gambiae* s.l. is between 90 and 97% (18).

WHO synergist papers: *An. gambiae* s.l. were pre-exposed to 5% PBO-impregnated paper. WHO´s susceptibility bioassays with synergist PBO (an inhibitor of monooxygenases) were carried out to assess the implication of detoxifying enzymes in the production of resistant phenotypes. Adult female mosquitoes were exposed for 1 h to 5% PBO impregnated papers in batches of 20-25 mosquitoes. PBO was used only for pyrethroids (deltamethrin and permethrin) because of its role on cytochrome P450 monooxygenase, implicated in the resistance of anopheles to these insecticides (18). WHO susceptibility tests were carried out at the laboratory of bio ecology and vector control of the school of public health in Kinshasa.

Molecular work to identify species and resistance mutations

PCR was performed to identify members of *Anopheles gambiae* complex. The samples for this analysis were randomly selected. Anopheles samples were individually placed in 1.5 ml Eppendorf tubes containing RNAlater and sent to the Noguchi Memorial Institute Laboratory for Medical Research Vector Labs (Accra, Ghana) for molecular analysis. The genomic DNA of each mosquito was extracted and amplified according to the protocol of Fanello *et al*. (19). The PCR for the detection of kdr mutations was carried out according to the protocol described by Martinez-Torres *et al* (20). PCR-RFLP was used to detect the presence of the G119S mutation in the ace-1 gene as described by Weill *et al*. (21).

The allelic frequency of kdr and Ace1 genes was calculated based on the Hardy-Weinberg genetic formula: F (kdr) = 2NRR + NRS / 2 (NSS + NRS + NRR) (22).

Data analysis

The data was entered using the EPI DATA 3.1 software and then exported to the Statistical Package for the Social Sciences 23 (SPSS 23) software for analysis. The 24-hour mortality rate of Anopheles was obtained by dividing the number of dead mosquitoes by the number of mosquitoes exposed. The knockdown time of mosquitoes measured during the test was calculated using Polo Plus 1.1 (LeOra Software, Parma, MO, USA) for the log probit analysis of bioassay allowing the determination KDT50 and KDT95. The susceptibility status of Anopheles to each insecticide was determined according to WHO criteria (18):

- A mortality rate between 98 and 100% is an indication of the susceptibility of Anopheles;
- A mortality rate between 90 and 97% indicates possible resistance and further investigation is needed (either molecular detection of resistance genes or additional bioassays);
- A mortality rate of less than 90% indicates resistance

Results

Anopheles fauna

From *Anopheles larvae* collected, *Anopheles gambiae* s.l. was the predominant species, occupying about 99.1% (3815/3850) of the Anopheles fauna and 0.9 % was from the Anopheles funestus group. Molecular identification of 60 female *Anopheles gambiae* s.l. randomly selected, in all sites, revealed the presence of two species: *Anopheles gambiae* (98.3%) and Anopheles coluzzii (1.7%).

Anopheles gambiae s.l. susceptibility to insecticides

Mortality was 100% for bendiocarb and malathion at all sites, whereas *An. gambiae* s.l. exposed to permethrin and DDT exhibited proven resistance at all sites. For deltamethrin, resistance was observed at all sites except Mont-

Ngafula and Kimbangu sites where *An. gambiae* s.l. were susceptible with a mortality of 99 %.

Deltamethrin-tested Anopheles Kdt50 from the Limete, Kintambo, Kimbangu, and Mont Ngafula sites were approximately 30 minutes of exposure (CI 95 %: 28.5-31.1 minutes) compared with Anopheles from the Maluku, Mbudi, and Kisenso sites (CI 95 %: 32.7-38.8 minutes). PBO pre-exposure restored susceptibility to deltamethrin in all sites. PBO restored susceptibility to permethrin in some, but not all sites.

The knockdown during exposure to permethrin (0.75 %) did not result in over 50% knockdown within the required 60 minutes of observation, with the exception of the Kimbangu, Mbudi, and Kisenso sites. After addition of PBO, the Kdt50 decreased in all sites. A similar reduction in kdt50 after exposure to PBO was found deltamethrin, with the exception of Limete (Table 1).

Table 1. Mortality rates and status of *An. gambiae* s.l. population exposed to insecticides (Maluku, Limete, Mbudi and Kitambo sites)

Site	Insecticides	N	KdT50 (CI)	KdT95 (CI)	Mortality 24 h	Statut
Maluku	deltamethrin 0,05 %	100	36.9 (36.1-38.8)	n/m	64	Re
	deltamethrin 0,05 % + PBO 5%	100	25.6 (24.5-27.1)	49.8 (46.1-54.7)	98	Se
	permethrin 0,75 %	100	n/m	n/m	21	Re
	permethrin 0,75 % + PBO 5 %	100	n/m	n/m	41	Re
	bendiocarb 0,1 %	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4 %	100	n/m	n/m	9	Re
Limete	deltamethrin 0,05 %	100	29.9 (28.8-31.1)	56.4 (52.8-61.1)	62	Re
	deltamethrin 0,05 % + PBO 5%	100	30.1 (27.3-32.6)	54.6 (47.9-67.3)	99	Se
	permethrin 0,75 %	100	n/m	n/m	36	Re
	permethrin 0,75 % + PBO 5 %	100	n/m	n/m	86	Re
	bendiocarb 0,1 %	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4 %	100	n/m	n/m	12	Re
Mbudi	deltamethrin 0,05 %	100	35.7 (33.7-37.7)	74.1 (66.4-86.2)	52	Re
	deltamethrin 0,05 % + PBO 5%	100	30.1 (28.3-31.8)	46 (42.3-52.1)	98	Se
	permethrin 0,75 %	100	55.2 (52.7-58.6)	n/m	31	Re
	permethrin 0,75 % + PBO 5 %	100	40.6 (39.3-41.9)	58.3 (55.1-62.7)	100	Se
	bendiocarb 0,1 %	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4 %	100	n/m	n/m	16	Re
Kintambo	deltamethrin 0,05 %	100	29.8 (28.7-31.0)	55.2 (40.6-53.1)	62	Re
	deltamethrin 0,05 % + PBO 5%	100	25.5 (23.5-27.5)	45.3 (40.6-53.1)	100	Se
	permethrin 0,75 %	100	n/m	n/m	36	Re
	permethrin 0,75 % + PBO 5 %	100	n/m	n/m	96	Re
	bendiocarb 0,1 %	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4 %	100	n/m	n/m	7	Re

Key: KDT, knock-down time; N, number of mosquitoes exposed; Se, susceptibility; Re, Resistance; n/m, no manifested

Mortality was 100 % for bendiocarb and malathion at all sites, whereas *An. gambiae* s.l. exposed to permethrin and DDT exhibited proven resistance at all sites; similarly, for deltamethrin, except Mont Ngafula and Kimbangu sites where *An. gambiae* s.l. were susceptible with a mortality of 99 % (Table 2).

Table 2. Mortality rates and status of *An. gambiae* s.l. population exposed to insecticides (Mont Ngafula, Kimbangu and Kisenso sites)

Site	Insecticides	N	KdT50 (CI)	KdT95 (CI)	Mortality 24 h	Statut
Mont Ngafula	deltamethrin 0,05%	100	29.2 (28.1-30.1)	54.9 (51.1-59.1)	99	Se
	permethrin 0,75%	100	n/m	n/m	45	Re
	permethrin 0,75% + PBO 5%	100	n/m	n/m	100	Se
	bendiocarb 0,1%	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4%	100	n/m	n/m	2	Re
Kimbangu	deltamethrin 0,05%	100	29.0 (27.5-30.5)	52.7 (48.8-58.1)	99	Se
	permethrin 0,75%	100	46.1 (44.8-47.3)	n/m	79	Re
	permethrin 0,75% + PBO 5%	100	40.9 (39.5-42.3)	n/m	98	Se
	bendiocarb 0,1%	100	-	-	100	Se
	malathion 5%	100	-	-	100	Se
	DDT 4%	100	n/m	n/m	10	Re
Kisenso	deltamethrin 0,05%	100	34.9 (32.7-37.0)	52.3 (47.8-59.4)	65	Re
	deltamethrin 0,05% + PBO	100	31.8 (27.8-35.8)	48.6 (41.8-66.2)	99	Se
	Permethrine 0,75%	100	59 (56.3-62.7)	n/m	24	Re
	Permethrine 0,75% + PBO 5%	100	48.5 (46.2-51.2)	n/m	68	Re
	Bendiocarb 0,1%	100	-	-	100	Se
	Malathion 5%	100	-	-	100	Se
	DDT 4%	100	n/m	n/m	9	Re

Key: KDT, knock-down time; N, number of mosquitoes exposed; Se, susceptibility; Re, Resistance; n/m, no manifested

With regards to the mortality at 24 hours, PBO restored susceptibility to deltamethrin in all sites tested. However, PBO only restored susceptibility to permethrin in 3 of 7 sites.

Resistance gene (kdr and ace 1R) frequencies by site

Forty-eight *Anopheles gambiae* s.l., with resistant status after WHO susceptibility tests, from Mont Ngafula, Kintambo, Limete and Maluku were randomly selected. The kdr-west mutation (L1014 F) was the only kdr-mutation detected. All *Anopheles gambiae* s.l. from the Kintambo and Maluku sites were homozygous (RR), whereas those from the Mont Ngafula site were homozygous (SS). Only two Anopheles from the Limete site showed a heterozygous genotype (SR) (Table 3). In addition, no Ace-1R mutation was observed in any of the tested samples.

Table 3: Genotypes of *An. gambiae* s.l at Kinshasa

Sites	N	(kdr-west)			
		RR	RS	SS	Fr (kdr)
Limete	12	10	2	0	0.95
Mont Ngafula	12	0	0	12	1.00
Kitambo	12	12	0	0	1.00
Maluku	12	12	0	0	1.00

Key: kdr, knock-down resistance gene; n, quantity of mosquitoes analysed; Fr, allelic frequency of kdr

Discussion

Knowledge of the susceptibility profile of Anopheles to insecticides used in public health is a major asset in malaria control (23). To do this, seven sites representative of the city of Kinshasa were selected to assess the susceptibility of the malaria vector.

In terms of the Anopheles mosquitoes collected, our collections revealed a high proportion of *Anopheles gambiae* s.l. This is in large part due to the fact that our larval sampling methodology specifically targeted the preferred sites of this species. *Anopheles gambiae* s.l. can develop in a number of different site types but particularly favor shallow sunlit ponds or pools (24, 25), of which there are many in Kinshasa during the rainy season. This corroborates previous observations in DRC (26). When *An. gambiae* s.l. was identified to species, the vast majority (98.3%) were found to be *An. gambiae*, followed with *An. coluzzii*, consistent with other recent work in Kinshasa (10).

The knockdown time after insecticide exposure (Kdt) was variable according to the sites of origin of *An. gambiae* s.l. and the types of insecticides used. The knockdown occurred later for the *An. gambiae* s.l. exposed to deltamethrin (0.5 %) in the Maluku sites compared to other sites (Table 1).

This is probably due to the inherent characteristics of each site. Maluku is a long-term industrial and agropastoral site where many pesticides are used. The knockdown effect was not achieved in all sites with DDT. *An. gambiae* s.l. tested with permethrin (0.75 %) showed a variable susceptibility by site. Apart from the Mbudi and Kisenso site, no available knockdown time effect was observed during the 60 minutes of the test in the Kitambo, Mont Ngafula, Kimbangu, Limete, and Maluku sites. This absence of knockdown reveals an adaptation of the vector to the action of insecticides. Basilua *et al*. in 2012 did not calculate the Kdt50 for DDT in Kinshasa (Kingasani site) due to the low knockdown during the 60-minute bioassay (26). However, in the same study, permethrin showed rapid knockdown effects. Our different observations, with regard to permethrin (Maluku) might be explained by the progressive development of resistance to pyrethroids or by a difference of bioecological characteristics of these two points in Tshangu District. These differences may be justified by very variable microenvironments depending on the study sites, but they may also be affected by the inherent variability in bioassays, and this complicates the understanding of the variability of resistance in mosquito populations.

Our study revealed that the population of *An. gambiae* s.l from Kinshasa was resistant to DDT and pyrethroids (with the exception of the Kimbangu and Mont Ngafula sites for deltamethrin) (Table 2) with a high prevalence of the kdr-west mutation.

This mutation is widespread in Africa and is no longer observed only in West Africa. It is also present in East and Central Africa, thereby allowing significant gene flow between different Anopheles populations (27). Although DDT resistance has been known for decades, the proven resistance to permethrin in all the sites selected for our study can be explained by the widespread use of these molecules in the city of Kinshasa as a preventive measure against malaria in impregnated mosquito nets and by commercial spray-insecticide. The susceptibility of *An. gambiae* s.l. to deltamethrin in the Mont Ngafula and Kimbangu sites, shows the environmental diversity of this megacity on the susceptibility of Anopheles to commonly-used insecticides. Indeed, Mont Ngafula is a recently developed site marked by an almost total absence of clogged gutters that can encourage nuisance Culex, suggesting a low use of insecticides. Regarding Kimbangu, the susceptibility of *An. gambiae* to deltamethrin may be explained by the absence of industrial activities or intense market gardening in this part of the city of Kinshasa. The influence of field and industrial activities in the Kisenso, Kintambo, Mbudi, Limete, and Maluku sites on the occurrence of insecticide resistance corroborates data from the Central African Republic (RCA) where Lidwine *et al*. (28)

revealed resistance to insecticides used in public health in predominantly agricultural and industrial areas.

Although the sample size was small, the frequencies of the resistance genes per site provided very interesting information for further investigations. The resistance mechanism of *An. gambiae* observed in this study was the kdr L1014F mutation. With the exception of Mont Ngafula samples, all mosquitoes presented with RR genotype (20.9 % at Limete and 25% at Maluku and Kitambo) or RS (2.1% at Limete for *An. gambiae*). This indicates a great influence of the inherent aspects of each site, explaining the heterogeneity of the sensitivity of *Anopheles gambiae* s.l. to the usual insecticides. The studies conducted by Bobanga *et al*. in 2010 in Kinshasa (Kindele and Kimbangu), showed that *An. gambiae* s.s. was resistant to permethrin and the kdr mutation gene (L1014F) was responsible for this resistance. Similar situations have been highlighted by Basilua *et al*., in DRC, also Kerah-Hinzoumbé *et al*., in Africa (26, 29). The dynamics of occurrence of resistance associated with the not available of knockdown effect on DDT and permethrin suggest the presence of the high-frequency kdr mutation throughout the city of Kinshasa province. Indeed, Corbel *et al*. state that the absence of knockdown effect coupled with low mortality rates with DDT and permethrin suggest the presence of the high-frequency kdr mutation (30). No Ace-1R mutation was observed in all samples tested. This may be explained by the small size of the anopheles specimens submitted to the molecular analysis.

Pre-exposure to PBO has improved the response of pyrethroids in a variety of ways. This indicates the likely involvement of oxidases. The presence of kdr genes and the fact that the sensitivity of *An. gambiae* has been improved after pre-exposure to PBO suggests that both metabolic and target site mechanisms contribute to insecticide resistance.

Conclusion

The present study provides interesting information on the resistance status of the *Anopheles gambiae* s.l. in Kinshasa. A clear predominance of *An. gambiae* compared to Anopheles coluzzii is observed in this city. The heterogeneity of the insecticide resistance status of *Anopheles gambiae* can be explained by the variability of environmental conditions in the city of Kinshasa and the large-scale use of ITNs for a decade. Our data show that ITNs treated with a mixture of deltamethrin with a synergist (PBO) might be more effective than the presently deployed pyrethroid-only nets. The national malaria control program should be guided in its choice of insecticides for use in malaria control.

Conflict of interest

The authors declare no competing interest.

Authors' contributions

Josue Zanga, Emery Metelo, and Paul Mansiangi designed and implemented the study. Josue Zanga, Kennedy Mbanzulu and Emery Metelo were responsible for collecting the data. Josue Zanga, Emery Metelo, and Paul Mansiangi performed the statistical analysis and prepared the manuscript for publication. All the authors helped write the manuscript. Seth Irish, Basimike Mulenda, Roger Wumba, and Paul Mansiangi read and edited the manuscript before submission.

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