Chapter

Effect of the Mass Distribution of ITNs in an Endemic Area with a High Entomological Index, the Case of Bandundu-City, Kwilu, DRC

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Abstract

The bio-efficacy of Yorkol-branded ITNs collected from Bandundu-city was assessed on the Kisumu strain and wild specimens of *Anopheles gambiae*. The susceptibility of the wild *An. gambiae s.l.* was tested to select insecticides. Adult *An. gambiae s.l.* sampled by PSC and HLC were screened for the presence of *Plasmodium falciparum*. Blood samples were diagnosed by microscopy and RDTs. ITN distributed in Bandundu-city were fully effective on the Kisumu strain, but on wild *An. gambiae s.l.* population (22.3 ± 11.5%). *Anopheles gambiae s.l.* was the main vector in Bandundu. No significant difference was observed between the entomological indices before and after the deployment of nets (OR = 0.8; p = 0.39). Wild *An. gambiae s.l.* populations were resistant to pyrethroids and DDT, with the restoration of the susceptibility to pyrethroids post pre-exposure to PBO. *Plasmodium falciparum* was the main parasite species and was found alone or mixed with. *P. malariae* or *P. ovale*. The confirmation rates by microscopy and RDT were respectively 57.9% and 53.6%. Nets deployed in Bandundu-city were not effective on wild *An. gambiae s.l.* populations. This operational failure is likely explained by the observed resistance to pyrethroids. In the future only PBO-net should be deployed Bandundu-city.

Keywords: *Anopheles gambiae* s.l, ITN, entomological indices, Bandundu-city, DRC

1. Introduction

Anopheles gambiae s.l. is a primary vector of *Plasmodium falciparum* across sub-Saharan African countries causing malaria that continues to be a leading cause of

morbidity and mortality across the continent [1]. Malaria is the world's number one parasitic disease, threatening more than 1 billion people [2]. According to the World Health Organisation (WHO), 241 million cases and 62,700 deaths due to malaria were reported in 2020 [3]. Of these cases, 95% occur in sub-Saharan Africa. Globally, nearly 95% of deaths were recorded in 31 countries, of which 3 in Africa accounted for nearly 39% (Nigeria 23%; DRC 11%, Tanzania 5% [2]. More than 200 million cases of malaria are recorded annually in DRC [3]. This high prevalence of malaria reflects a situation of poverty and inadequate health services [4–6]. Moreover, malaria itself and its consequences contribute to keeping populations in a state of poverty. Malaria costs sub-Saharan African countries more than US$ 12 billion each year through lost income, foreign investment and tourism resources [4–6]. International agencies (WHO, UNDP, UNICEF and the World Bank) launched the Roll Back Malaria (RBM) programme, which set out to combat malaria with the aim of reducing malaria mortality by 50% in 2010, 30% in 2015 and 20% in 2025. In its technical strategy for malaria control, WHO has set the goal of eliminating malaria by 2030 [7]. Thus, by the year 2030, malaria should cease to be a major cause of morbidity, mortality, and socio-economic loss [8].

To achieve its objectives, the DRC has developed malaria control strategies based on preventive measures centred on targeted chemoprophylaxis offered to high-risk or vulnerable populations such as infants, pregnant women and migrants; early case management, which implies diagnosis and rapid recourse to appropriate care; and finally, vector control which must remain accessible to malaria-endemic populations [9]. Regarding preventive measures, the WHO gives an important place to vector control. To respond favourably to both international and national strategies, approaches and recommendations aimed at eliminating malaria, the DRC has been committed for at least 7 years through the NMCP and its partners to scale up high-impact malaria control interventions [10]. These interventions include universal distribution of ITNs, chemoprevention of malaria in pregnant women, administration of artemisinin-based combination therapies (ACTs) and strengthening of epidemiological surveillance [11, 12].

Following the evaluation of malaria control interventions, with reference to the implementation framework of the Global Technical Strategy for Malaria Control 2016–2030 in the African Region, WHO has launched the High Burden High Impact (HBHI) approach [13, 14] aiming to reduce malaria morbidity and mortality in countries with a high burden of malaria, including the DRC. It is based on four main pillars: i) political will in favour of the fight against malaria, ii) strategic information that can increase the impact of malaria tenfold, iii) improved support for policies and strategies and iv) coordination of the national response [13, 14].

The significant progress made in malaria control over the past decade in endemic countries is largely attributable to the mass coverage of insecticide-based vector control interventions, such as long-lasting insecticidal nets (ITNs) and indoor residual spraying (IRS) [7, 13, 14].

Long-lasting insecticidal nets are one of the main malaria control tools recommended by WHO and adopted by DRC NMCP through mass and routine distributions channels [15, 16]. Several field studies have demonstrated the effectiveness of its large-scale use [15, 16] showing they can reduce morbidity by 50% and overall mortality by 20–30% in children under 5 years of age [10, 16, 17].

Based on these observations, the promotion of ITNs among the population is an essential component of the NMCP in DRC. Several studies have shown that the combined action of insecticides and the physical barrier is an effective means of controlling

malaria vectors [15–17]. Nevertheless, the disease remains endemic in the country, with a worrying prevalence of 32%, despite this large-scale promotion [3, 10]. Counteracting the effectiveness of ITNs are two major handicaps: the increasingly widespread mosquito resistance to pyrethroids used for impregnation of ITNs; and the low recorded durability of ITNs compared to the expected duration of 3 years [10, 13]. The number of *Anopheles* species resistance to insecticides has increased and affects nearly two-thirds of the countries where transmission persists. Resistance to pyrethroids in *An. gambiae* s.l. was first observed in Côte d'Ivoire in followed by other West African countries (Benin and Burkina Faso) and first reported in the DRC in 2012 [12, 18]. Pyrethroid resistance in *An. gambiae* has now been reported in all the DRC NMCP's sentinel sites, where it has the potential to cause the operational failure of the vector control tools put in place by the malaria control programmes [12, 18].

The DRC, like all the countries of sub-Saharan Africa, pays a heavy price for malaria. However, its geographical location and the diversity of its climates make it unique among its neighbours [2, 10, 19]. Spread over an area of approximately 2,345,000 km^2, this country has almost all the epidemiological facies found in Africa, from the Sahelian savannahs to the equatorial forests [10, 19, 20]. Moreover, 97% of the population lives in the stable malaria zones characterised by the equatorial and tropical facies [13, 14]. Three plasmodial species are present (*Plasmodium falciparium* (*P.f*) 95%, *Plasmodium ovale* (*P.o*) and *Plasmodium malariae* (*P.m*)). Currently, *Plasmodium vivax* infections are present in some areas of the country, but in very small numbers [9, 19, 20]. The most common vectors in the DRC are *An. gambiae*, 92%, *An. funestus*, *An. nili*, *An. moucheti* and *An. paludis* [9, 21, 22]. These *Anopheles* can have highly variable vectorial capacities and behaviours [23, 24].

However, this heterogeneity does not allow us to understand malaria in the DRC in its entirety. Knowledge of the different Congolese geographical areas (territories, districts, etc.) and the description of the local epidemiology of malaria in these different environments are initial conditions for a good understanding of the malaria endemicity in this country [13, 14, 18].

Work carried out in a few sites in the DRC, such as Kingasani, Bolenge, Kimpese and Katana by Watsenga and collaborators, has shown that pyrethroid resistance was associated with the presence of the kdr mutation [25]. However, the problem of *Anopheles* resistance to insecticides seems to be growing in the DRC, according to the PMI entomological surveillance report and the DRC Malaria Control Strategic Plan 2013–2015 [26, 27]. Therefore, it is not only necessary to regularly monitor the susceptibility of vectors to these insecticides and to understand the mechanisms of this resistance, but also to consider the actual impact of this resistance on vector control [27, 28].

Our study focused on Kwilu province (ex-Bandundu) where the prevalence of malaria was 18%, sporozoite index 5.6%. *A. gambiae* s.l. is the major malaria vector with 8.86 anopheles per house, 1.55 bites per man per night and entomological stability index 6.512 [29]. On this basis, it was necessary to assess and determine the response of *An. gambiae* s.l. to insecticides and test the hypothesis that the selection of a resistance gene in malaria vectors is associated with the widespread use of insecticides [12, 15].

The aim of this study was to examine the status of *An. gambiae* s.l. resistance to pyrethroids and to determine the mechanism of this resistance in several locations in Kwilu province, once the country's agricultural heartland where different farming systems were used [2, 30, 31]. In addition, this province has long been the focus of vector control interventions through the distribution of ITNs [32]. The results from

this study will serve as a basis for decision-makers to properly orientate resistance management policy in the DRC particularly considering the recrudescence and outbreak of malaria cases noted in 2019, and the lack of documentation of *Anopheles* resistance in this part of the country.

2. Effect of the mass distribution of ITNs in an endemic area with a high entomological index, the case of Bandundu-city, Kwilu, DRC

2.1 Methodology

2.1.1 Study site

The town of Bandundu was once considered a city in the territory of Bagata, before it was made the capital of the province. It is located between 17°22′43″E longitude, 3°21′05”S latitude and at an altitude of 324 m to the West. This area is bounded to the north by the NIOKI health zone, to the south by the Nkutu health zone in the province of Maï-Ndombe, to the east by the Bagata health zone and to the west by the Kwamouth health zone (province of Maï-Ndombe). It is located 432 km from Kikwit, 200 km from Kenge and 400 km from Kinshasa, the capital of the DRC [19, 20]. Kwilu province is located in a low altitude climate, characterised by a humid tropical climate, constant heat throughout the year and two well-marked seasons. The rainy season is characterised by heavy rainfall, and the dry season lasts 4 months and rainfall drops to zero [23, 24]. The average annual temperature is 26.9°C, annual rainfall ranges from 800 to 1500 mm and the average annual humidity is 77% [24].

Bandundu city experiences a humid tropical climate with two well-marked seasons: a long rainy season and a short (4-month) dry season, as shown [23, 24]. A short dry period is often noted in January-February, followed by a short rainy period in March-April. Climatological data obtained from the meteorological department of Bandundu-City (METELSAT/BDD). The mass distribution campaign of ITNs (Yorkol) was carried out between 17 and 29 December 2018.

The Town has developed in order to promote Pisco-agricultural activities (ponds, flowerbeds, etc.). The plots constitute the traditional dwelling unit for a family with one or more houses with dry earth walls and roofs usually made of corrugated iron and/or straw. Such dwellings are permeable to mosquito ingress and egress. Cattle, sheep, goats, pigs and poultry are well represented. All of these animals roam and spend the night in the plots. These eco-climatic conditions are favourable to the development of Anopheles vectors of malaria and the multiplication of breeding sites [24–32, 33]. Its surface area is 291 km^2 with approximately 285,411 inhabitants, i.e. a population density of 980.79 inhabitants/Km^2 sites [19, 20, 33].

2.1.2 Data collection

ITNs distributed in Bandundu-city in December 2018 were collected to evaluate their effectiveness with *An. gambiae* s.l. strain Kisumu reared in the insectarium of the Bioecology laboratory of the School of Public Health (BIOLAV/ESP) and wild strains collected in the different sites of Bandundu. The WHO sensitivity test was carried out on collected *An. gambiae* specimens and blood samples taken from the inhabitants of the households for the preparation of thick drops and thin smears in

search of plasmodium parasites. Mosquitoes were also captured by morning house spraying with pyrethrum (PSC) and human bait (HLC).

a. Bio-efficacy of ITNs

All ITNs were carefully inspected for physical integrity, date of manufacture and batch number. The ITNs were coded according to the order of analysis: Y1, Y2, Y3... Y30. Five 30 x 30 cm samples were taken from each ITN: a sample from the short side at the bottom (C1), a sample from the long side at 30 cm from the bottom edge (L1), a sample from the short side at 60 cm from the bottom edge (C2), a sample from the long side at its top edge (L2) and a sample from the roof (T). Individual samples were wrapped in aluminium foil and placed inside plastic bags to avoid possible cross-contamination between sub-samples [15].

Five female *An. gambiae* s.l., not gorged from 2 to 5 days old, were collected using the mouth aspirator and introduced into each cone. After 3 minutes, the mosquitoes were sucked out of the cones with a mouth glass aspirator and moved to the disposable cups covered with the net, fitted with cotton wool soaked in 10% glucose solution according to the sample code ITN. Each subsample was tested once, giving a total of 20 mosquitoes tested per subsample, or 100 mosquitoes tested per ITN. The behaviour of the tested mosquitoes was observed for 1 hour. The number of mosquitoes shocked after 3′, 5′, 10′, 15′, 20′, 25′, 30′, 35′, 40′, 45′, 50′, 55′ and 60′ of observation was recorded to calculate the percentage of mosquitoes shocked after 60 minutes of observation, the Kdt50 and Kdt95, which represent the times after which 50% and 95% of mosquitoes were stunned, respectively. The final mortality rate was determined at 24 hours post-exposure [34, 35]. These tests were conducted at room temperature and relative humidity of 25–27°C and 78–80%, respectively. Cone bioassays were also conducted on the *An. gambiae* s.l. population of the Kisumu strain maintained at the BIO-LAV laboratory. Cone bioassays were also conducted with control mosquitoes that were exposed to non-insecticide-impregnated tissue as a control for the test.

The test results were deliberated according to WHO criteria [36]. An ITN is considered effective if it results in a Kd rate of 95% or greater and/or a mortality rate of 80% or greater [36].

b. Susceptibility testing

Larvae and pupae of *A. gambiae* s.l. were collected at different sites identified in each study site (Bandundu-city). The collected specimens were kept in tanks containing water in CDC cages and reared in the laboratory.

The larvae were fed daily with fish food at a rate of 4 g per day per tank. The temperature and humidity of the laboratory were taken daily by thermo-hygrometer. Pupae were harvested daily and placed in CDC/Atlanta cages and adults were fed with 10% glucose solution [36].

Females aged 2–5 days were selected and tested for susceptibility according to the WHO protocol [36]. The tests were carried out with insecticide-impregnated papers, WHO Kit composed of 4 types of insecticides and the synergist PBO in the following concentrations: Deltamethrin 0.05%, Permethrin 0.75%, Bendiocarb 0.1%, DDT 4% and PBO 5% [36].

The first susceptibility test was performed only for the 4 insecticides. The behaviour of *An. gambiae* s.l. subjected to the insecticide was observed for 60 minutes. The second test was carried out with anopheles pre-exposed to PBO for 60 minutes and then tested with insecticides [36–38]. PBO was used only for pyrethroids

(Deltamethrin and Permethrin), because of its action on the cytochrome P450 mono-oxygenase, which plays an important role in the resistance of anopheles to its insecticides [36, 38]. These two pyrethroid insecticides are authorised for use in the impregnation of mosquito nets used in DR Congo [31, 35].

The behaviour of *An. gambiae* s.l. in contact with insecticides was observed for 1 hour; the number of *An. gambiae* s.l. shocked at each, 3′ 5′ 10′ 15′ 20′ 25′ 30′ 35′ 40′ 45′ 50′55′ and 60′ contact with the insecticide was recorded 20–25 *An. gambiae* s.l were used per trial (replication), in total 4 trials were conducted and one control by each type of insecticide following the WHO protocol [36, 37]. This contact time was used to calculate the knockdown time (KDT) and mortality of *An. gambiae* s.l. observed 24 hours later [36]. The KDT has two components, KDT_{50} and KDT_{95} represent the knockdown times after which 50% and 95% of *An. gambiae* s.l are stunned. The two KDTs were determined according to the type of insecticide [28, 36]. The tests were carried out under conditions of temperature ranging from 25 to 27°C and relative humidity from 78 to 80%. The mortality observed 24 hours after contact time, according to WHO, is used to elaborate the criteria for deliberation of susceptibility test results; Susceptible (S), all *An. gambiae* s.l. with a mortality of 98–100% 24 hours after contact with insecticides; Resistant (R) if the mortality of *An. gambiae* s.l. is less than 90%; Probable Resistant (PR), if the mortality of *An. gambiae* s.l. is between 90 and 97% [36].

c. Mosquito collection

A total of 108 houses were visited during the study from 15 July 2018 to 15 June 2019. All sampled houses had mud walls and had tin or thatched roofs. Each month, nine houses were selected at random for mosquito sampling, with different houses selected for each monthly sampling event. Adult mosquitoes were collected between 06:00 and 10:00 am using pyrethrum spray catches [39, 40]. All openings of the house were closed and white sheets were laid on the floor. A commercially available pyrethroid spray (Baygon, Bayer) was sprayed in the house and doors were closed for 15 minutes. The sheets were carefully removed from the house and inspected for mosquitoes, which were collected and placed individually into labelled tubes [39, 40].

Human landing catches (HLCs) were done primarily to determine malaria vector species composition, the location of biting (indoors or outdoors) and times of biting. Mosquitoes were collected monthly from nine houses by two volunteers in six-hour shifts, from 18:00 h to 0:00 h and from 0:00 h to 6:00 h. Two collectors were posted inside the house in the living room and two outside the house, less than five meters from the front door. Different houses were used for each night. Each collector sat on a stool with his lower legs and feet exposed for mosquitoes to land on. The collector monitored mosquitoes as they landed and captured them with small glass tubes that were sealed with cotton wool. The latter were then placed in a sealed bag and labelled according to the hour of collection. These data were used to calculate the nightly human biting rate (HBR) based on eight person-nights of collection indoors and outdoors for each sampling period. The mosquito collectors for HLCs were recruited from the community and provided with requisite training. Collectors showing any signs of illness up to 3 weeks following collections were screened for malaria at a local health centre. There were no positive cases.

A. gambiae s.l. mosquito larvae were collected from breeding sites and transferred to pans containing water from the site and were reared until adult stage in a field insectary. Larvae were not fed and survived from the nutrients in the site water. Adults were identified to species according to morphological identification keys [41, 42].

d. Entomological inoculation rates

Entomological inoculation rates (EIR) are used to estimate the risk of transmission by looking at the number of infectious bites people can be exposed to if prevention methods are not used. EIR pre- and post-ITN distribution were calculated by multiplying the proportion of mosquitoes found to be infective (sporozoite rate) by the average number of females collected by HLCs.

The rate corresponds to the number of infected bites at a location per unit time. It is the only way to truly assess malaria transmission. EIR = entomological inoculation rate per night. This rate is referred to as the number of infectious bites, per person, per night;

- BR: daily number of bites (human biting rate) referred to as the number of bites per person per night. The rate corresponds to the number of bites per man per night (p/h/n). It was calculated in two ways depending on the method of capture. By PSC the number of bitten females captured in a neigh hour hood or commune, divided by the total number of people who spent the night in these houses on the day of capture (indirect aggression). And by HLC the number of anopheles captured per man per night, i.e. the number of mosquitoes captured divided by the number of captors and divided by the number of hours of capture (direct aggression).

- SI = Sporozoite index, refers to the percentage of anephelines carrying Plasmodium sp. Sporozoïtes. After morphological identification, mosquitoes captured by PSC were dissected into head-thorax-abdomen complexes and legs and wings under an AmScope entomological microscope. These head-thorax complexes of *An. gambiae* s.l. females were ground up for analysis by CSP Pf ELISA following the protocol of Wirtz et al. 1991 [43] for the determination of the sporozoite index (SI).

e. Determination of human infectivity by microscopy.

Simultaneously with the capture of the mosquitoes, blood samples were taken from the inhabitants of the houses where the vectors were captured. A peripheral drop of blood from the fingertip was taken on a slide to make thick drops and blood smears for microscopic diagnosis of *Plasmodium* sp. As well as the SD Bioline RDT was performed for the rapid diagnosis of malaria [44–46].

Parasite data collection was carried out by laboratory technicians who were part of the study team. They obtained informed consent, completed a questionnaire, prepared blood slides and performed RDTs, under the supervision of the researchers. The purpose of this supervision was to ensure that the study procedures were followed and to verify the interpretation of the RDTs. A one-day training workshop was held on the study's standard operating procedures (SOPs). The expert microscopists were senior laboratory technicians from the university clinics in Kinshasa.

Blood for thick/thin smears and RDTs was collected from the same finger prick and prepared on the same slide with the patient identification code. Approximately 5 μl of blood was collected by the study team using a loop provided with the RDT device. Test preparation and interpretation were performed according to the manufacturer's instructions. Tests were considered positive when the antigen and control lines were visible in their respective windows, negative when only the control band was visible and invalid when the control band was not visible. In case of an invalid result, the RDT was repeated.

Blood smears were stained with 10% Giemsa for 10 minutes. Thin smears were fixed with methanol before staining. The slides prepared by the study team were first examined by field laboratory technicians, blinded to the RDT results and using the WHO semi-quantitative method [28]. Their results were recorded on cards. All slides were stored in secure slide boxes and read by two expert microscopists from the university clinics in Kinshasa. The expert microscopists were blinded to the results of the field microscopy and RDT. In case of discrepancies >15%, the judgement of a senior laboratory technician was required. And the final result was the mean parasite density. The thin smear was used for species identification.

The results of these slides were used to calculate the parasitological indices, the incidences and the relative risk of malaria. The RDT (SD Bioline) was evaluated on the basis of a contingency table to calculate the sensitivity (Se), specificity (Sp), negative (NPV) and positive (PPV) predictive value, as well as the overall value (AG) of the test.

The sample was taken from the digital pulp to make the Blood for thick and the Blood smears on the same slide. Staining was done with 10% Giemsa working solution for 10 minutes, according to WHO instructions [44–46]. The Blood smears were fixed with methanol prior to staining. The reading was carried out at the Bandundu General Referral Hospital and at the parasitology department of the University clinics in Kinshasa.

At the Bandundu General Referral Hospital, only the microscopy was read and the technicians used the semi-quantitative cross method [44–46]. The reading was performed using an ordinary Olympus CX21 microscope at 1000X magnification (objective 100 and eyepiece 10).

The results of these analyses were used to estimate the prevalence of malaria by calculating the parasite or plasmodium index. This index corresponds to the proportion of subjects carrying plasmodium in a location. This cross-sectional indicator remains the most widely used to quantify and classify malaria endemicity.

2.2 Statistical analysis

The times required in minutes to obtain 50% and 95% of knockdown mosquitoes (KdT_{50} and KdT_{95}) were calculated according to WHO criteria, using log probit with the Polo Plus software version 1.0 [35, 36]. The Chi-square test was used to compare the mortality of A. gambiae sl between the insecticide-only trials and after pre-exposure to the synergist (PBO) at the 0.05 significance level. The effect of synergists was calculated with effective values above 10% [34–36, 38]. Thick drop, thin smear and RDT were performed on 190 individuals for the determination of the plasmodium index. The prevalence of malaria, the sensitivity and specificity of each test, and their positive and negative predictive values were calculated [44, 45].

The odds ratio (OR) was used to determine the risk of exposure to malaria parasite infectivity. The 95% confidence interval or Chi-square test at a significance level of 0.05 was used to measure the association between presumptive diagnosis based on fever history and microscopic diagnosis. The sensitivity, specificity (s), positive predictive value (PPV) and negative predictive value (NPV) of SD Bioline were calculated on the basis of contingency tables.

2.3 Results

Sensitivity tests carried out on populations of *A. gambiae* s.l. from Kisumu at the BIOLAV/ESP laboratory and on wild populations collected in Bandundu in DRC,

have allowed us to better understand the levels of sensitivity of this vector to the two pyrethroids (Permethrin and Deltamethrin) commonly used in public health for impregnating nets. The efficacy of these compounds was compared to that of DDT (organochlorine) and Bendiocarb (carbamate), to detect the existence or not of cross-resistance between the three chemical families.

And this insecticide efficacy was measured by their knock down effect and the mortality they caused after 24 hours of observation. This analysis determined the KDT50 and KDT95, the value of these 2 parameters KDT (knock-down time) corresponds to the time after which respectively 50% and 95% of *An. gambiae* s.l. were knocked down. The results are presented in **Table 1**.

The shock time of *An. gambiae* s.l. was variable depending on the type of insecticide used in the test. The reaction of anopheles after contact with deltamethrin was very rapid compared to other insecticides. This insecticide was very active against anopheles compared to permethrin and DDT. The shock effect of anopheles was very low compared to permethrin and DDT. Only deltamethrin reached the Kdt_{50}. No effect of Kdt_{50} and Kdt_{95} was observed in anopheles tested with permethrin and DDT. These anopheles showed a very high resistance to these two insecticides with low mortality. Wild An. gambiae were resistant to deltamethrin, permethrin and DDT. Pre-exposure to PBO fully restored the efficacy of deltamethrin and partially of permethrin. Bendiocarb still remains effective.

Kdt50 and Kdt95 were too early for *An. gambiae* s.l. Kisumu, tested with deltamethrin, 8.45 minutes with 95% CI (7.09–9.79) and 30.34 (25.41–38) minutes. The shock effect of *An. gambiae* s.l. Kisumu tested with DDT did not reach Kdt_{95}.

These *An. gambiae* s.l. Kisumu were sensitive to all insecticides used (deltamethrin and permethrin 100% and DDT 99%) in **Table 2**.

Anopheles gambiae s.l.	**Insecticides**	**N**	**KdT_{50} (minute)**	**KdT_{95} (minute)**	**Mortality 24 h**	**Statut**
wild Souches Bandundu-city	Deltaméthrine 0.05%	100	43.8 (39.9–47.9)	n/a	52	R
	Deltaméthrine 0.05% + PBO 5%	100	16.5 (15–17.8)	38.5 (35–43)	98	S
	Permethrine 0.75%	100	n/a	n/a	17	R
	Permethrine 0.75% + PBO 5%	100	n/a	n/a	88	R
	Bendiocarb 0.1%	100	—	—	100	S
	DDT 4%	100	n/a	n/a	2	R
Kisumu	Deltaméthrine 0.05%	100	8.45 (7.09–9.79)	30.34 (25.41–38)	100	S
	Permethrine 0.75%	100	17.6 (13.58–21.49)	60.5 (45.57–96.37)	100	S
	DDT 4%	100	32.5 (25.68–41.19)	n/a	99	S

KdT50: Knockdown time (min) 50%; KdT95: Knockdown time (min) 95%; 95% CI: confidence interval; S: susceptible, R: resistant; n/a = no available "Knockdown" (< 15% of mosquitoes killed after 1 h exposure).

Table 1.
Insecticide susceptibility, expressed as KDT50, KDT95, and 24 hour mortality, of an. Gambiae s.l.

ITN	*n An. gambiae* s.l. wild strain					*Anopheles gambiae* s.l. Kisumu				
		Kdt50	Kdt95	Mortality(%)	Statut	n	Kdt50	Kdt95	Mortality(%)	*Status
ITN	100	n/a	n/a	20	R	100	5.4(4,1–6.8)	44.4(35,6–59.2)	100	S
ITN	100	n/a	n/a	27	R	100	5.9(4,7–7.0)	35.9(29,8–45.2)	100	S
ITN	100	n/a	n/a	30	R	100	5.6(4,6–6,6)	35.5(30,0–43.6)	100	S
ITN	100	n/a	n/a	27	R	100	6.1(5,3–7.0)	39.8(34,6–47.0)	100	S
MILD5	100	n/a	n/a	32	R	100	6.1(5,1–7.1)	39.3(33,5-47.7)	100	S

**Status: S (Sensitivity) = 24 hr mortality ≥ 80%; R (Resistance) = 24 hr mortality < 80%.*

Table 2.
Efficacy of LLINs against Anopheles gambiae s.l. *wild strain and Kisumu.*

From **Table 2**, it can be seen that low mortality of *A. gambiae* s.l. wild strain was observed in relation to ITNs shot in Bandundu-city, with an average of 22.3 ± 11.5% mortality 24 hours later. The Kdt_{50} and Kdt_{95} were not reached with *A. gambiae* s.l. wild strain. These wild anopheles unanimously showed a very strong resistance to the insecticide used for ITN impregnation. Concerning the sensitive strain, the Kdt_{50} was reached early at 5.8 ± 0.3 minutes and Kdt_{95} was observed early at 39.0 ± 3.6 minutes and mortality was 100 ± 0% 24 hours later. This indicates a high sensitivity to the insecticide.

From **Table 3**, Kdt_{50} and Kdt_{95} were highly variable depending on the type of insecticide and the test period.

Bendiocarb and Deltamethrin+ PBO were effective in both periods. *A. gambiae* s.l. tested to this insecticide were sensitive (100%). The Kdt_{50} and Kdt_{95} were too early in the second period of May-August 2019, i.e. 18.6 minutes with 95% CI (17.3–19.8) and 31.5 (28.9–35.4) minutes. No effect of Kdt_{50} and Kdt_{95} was observed in anopheles tested with permethrin and DDT during both periods. These anopheles showed very high resistance to these two insecticides with low mortality before and after the ITN mass distribution campaign. The mortality of anopheles exposed to permethrin was 31% in the first period and 36% in the second period, no significant difference was observed.

From **Table 4**, it can be seen that no significant association was observed between the dry and rainy seasons prior to the ITN mass distribution campaign. Microscopy

Période	Insecticides	n	Kdt_{50} (min)	Kdt_{95} (min)	Mortality 24 h(%)	Statut*
Before ITN distribution (September-November 2018)	Deltamethrin 0.05%	100	42.6 (40.7–44.8)	n/a	52	R
	Deltamethrin 0.05% + PBO 5%	100	22.7 (21.5–23.7)	39.7 (37–43.3)	99	S
	Permethrin 0.75%	100	n/a	n/a	31	R
	Permethrin 0.75% + PBO 5%	100	66.6 (61.7–74.2)	n/a	84	R
	Bendiocarb 0.1%	100	27.7 (25.5–29.8)	43.2 (39.1–50.3)	100	S
	DDT 4%	100	n/a	n/a	4	R
After ITN distribution (May-August 2019)	Deltamethrin 0.05%	100	31.5 (30.4–32.5)	51.7 (49.2–54.9)	34	R
	Deltamethrin 0.05% + PBO 5%	100	30.9 (29.5–32.3)	52.1 (48.6–56.8)	100	S
	Permethrin 0.75%	100	n/a	n/a	36	R
	Permethrin 0.75% + PBO 5%	100	67.9 (61.9–77.2)	n/a	97	RP
	Bendiocarb 0.1%	100	18.6 (17.3–19.8)	31.5 (28.9–35.4)	100	S
	DDT 4%	100	n/a	n/a	16	R

**Status: R = resistant S = susceptible PR = probable resistance n/a = no available.*

Table 3.
Mortality of Anopheles gambiae *s.l 24 hours after 60 minutes exposure to insecticides before and after distribution.*

Before ITN distribution						
Seasons	**N**	**%**	**Microscopy**		**OR**	**p-value**
			n (positif)	%		
Dry	62	53.4	35	56.4	1.4(0.6–3,2)	0.36
Rainy	54	46.6	35	64.8		
After ITN distribution						
Dry	44	59.5	28	63.4	0.4(0.1–0.9)	**0.04**
Rainy	30	40.5	12	40		
Total (throughout the period)						
Before	116	61.0	70	60.3	0.8(0.4–1,5)	0.39
After	74	39.0	40	54.0		

Table 4.
Variation in plasmodial indices before and after ITN distribution.

(thick drop) positive cases were in a tie (35 positive cases). The ITN mass distribution campaign had a beneficial effect on reducing the prevalence of malaria cases (malaria index), although no significant association was observed before and after the ITN distribution. After the ITN mass distribution campaign, a significant association was observed between thick drop and the rainy season with p-value = 0.04. Many cases were recorded during the dry season.

During, the second phase (July to December 2018), a very highly significant difference was observed between aggressivity and entomological inoculation rate during the dry season and the rainy season with p‹0.001. A significant difference was observed between density and sporozoite indices in both seasons (p = 0.01).

Comparing the two capture phases, no significant difference was observed between aggressivity, sporozoite index and entomological inoculation rate, respectively with p (0.098 and 0.896) before and after ITN distribution. During the second phase, the season influenced the entomological transmission indices. A highly significant difference was then observed between aggressivity and entomological inoculation rate during the dry and rainy seasons with p<0.001.

2.4 Discussion

2.4.1 Evaluation of the bioefficacy of ITN

Vector control is one of the important components of the global malaria control strategy. It is the main pillar of malaria control aiming to interrupt the transmission of malaria parasites through indoor residual spraying (IRS) or the use of pyrethroid-impregnated fabrics (nets and/or curtains) [7, 30].

Worldwide, pyrethroids are the insecticides of choice for impregnation, as they are highly effective and fast acting, with an irritating effect on mosquitoes and less toxicity to humans [1, 30]. Besides the less effectiveness of insecticides treated ITNs observed in Bandundu, its use as a physical and chemical barriers against mosquito vectors, constituted a repellent with a killing effect on mosquitoes [7, 30]. Resistance of *An. gambiae* s.l. to insecticides is increasingly widespread and reported in the

sub-Saharan region. This resistance now affects nearly two-thirds of the countries where transmission persists. It affects all major vector species and all insecticide classes [1–7, 36]. However, evaluations based on the lab reared *An.gambiae* s.l. Kisumu strain show efficacy.

While both interventions are used in the DRC, IRS is carried out on a limited scale by mining companies and a few non-governmental organisation [1–31, 36]. The National Malaria Control Programme (NMCP) adopted mass distribution of ITNs as a malaria control strategy in 2004, as a tool to interrupt malaria transmission [1–19, 20]. Since then, the NMCP has distributed millions of pyrethroid-treated ITNs. However, little data is available on insecticide resistance in Anopheles mosquitoes in the DRC, while the emergence of insecticide resistance may have an impact on the effectiveness of vector control interventions [19, 38]. The aim of this study was therefore to assess the insecticide resistance of mosquitoes and the main entomological indicators associated with malaria transmission before and after ITN distribution in the city of Bandundu.

In our study, *An. gambiae* s.l. susceptible strain Kisumu showed high susceptibility with 98.1 ± 1.2% of mosquitoes shocked after 60 minutes of observation and 24 hours mortality of 100 ± 0% was recorded. The Kdt_{50} and Kdt_{95} were reached very early at 5.9 ± 0.3 minutes and 39.3 ± 4.4 minutes of observation. This indicates the efficacy of ITNs on the susceptible *An. gambiae* s.l. strain according to the WHO deliberation criteria. These observations corroborate those found by Mansiangi and colleagues on the durability of ITNs [47]. Similarly, several studies conducted in the tropics on the evaluation of ITNs with *An. gambiae* s.l. strain Kisumu have shown very good efficacy [26, 27, 48]. However, observations done on wild A. gambiae mosquitoes showed resistance to pyrethroids. Thus, *An. gambiae* s.l. wild strain, 7.9 ± 5.7% were shocked after 60 minutes of observation and the observed mortality was 22.3 ± 11.5% after 24 hours, thus showing a strong resistance. And the Kdt50 and 95 were not reached. In the DRC, several studies report the emergence of Anopheles resistance to common insecticides [12–27, 48]. Anopheles resistance can impact on the efficacy of ITNs [30–37, 49]. This work focused first on the bio-efficacy and compliance with WHO specifications of ITNs distributed by the DRC NMCP in high malaria prevalence areas in Bandundu-city. We compared the bio-efficacy of *An. gambiae* s.l. wild and Kisumu strains.

From this quality control, it appears that none of these nets demonstrated efficacy on local *An. gambiae* s.l. strains according to WHO deliberation criteria (24 hour mortality greater than or equal to 80% of *An. gambiae* s.l. tested and 95% of mosquitoes shocked after 60 minutes of observation). These results are similar to those found by Bamou and colleagues in Yaoundé who, after exposing wild *An. gambiae* s.l., found high resistance [50]. Riveron and colleagues in Kinshasa on the evaluation of the efficacy of newly purchased nets: OlysetNet and Permanet 2.0 with local strains of *An. gambiae* s.l. had revealed low efficacy [27].

Our results are also similar to those found by Ahogni, in Benin [51], Loonen and colleagues in Baraka, DRC [13], Abilio and colleagues in Mozambique [15], and Darriet and colleagues in Côte d'Ivoire [12], who proved the ineffectiveness of new ITNs on wild strains. The presence in the DRC of mosquito populations capable of resisting diagnostic doses of insecticides may account for the reduced efficacy of ITNs.

The results of this study contrast with those found by Kweka and colleagues in Tanzania on the evaluation of PermaNet®3.0 ITNs on An. gambiae s.l. wild strain populations, which showed 98–100% effectiveness [48].

This difference is believed to be due to the mosquito colony used in this study being tolerant to pyrethroids and Permanet 3.0 being impregnated with pyrethroids combined with PBO.

Similarly Bobanga and colleagues in Kinshasa on the bioefficacy of PermaNet 3.0 ITNs on the wild population of vectors, which revealed a 100% mortality [52].

The results obtained by Bobanga et al. with the wild strain of *An. gambiae* s.l. showed low efficacy of treated nets, with mortality ranging from 11 to 66%. This difference in results with the susceptible strain is thought to be due to the different brand of ITNs, Olyset being made of permethrin-impregnated polyethylene [52].

2.4.2 Evaluation of entomological transmission indices

Entomological inoculation rates during ITN distribution in Bandundu town in December 2018 resulted in a decrease in the number of anopheles mosquitoes collected from households by both the pyrethroid spray capture and human landing capture techniques, although the difference was not statically significant. Similarly, no significant difference was found between sporozoite indices, bite rates or entomological inoculation rates (EIR) between the two periods. The risk of infectious bites before net distribution was approximately 0.13 infectious bites per person per night, or 47.2 infectious bites per person per year, compared to 0.08 infectious bites per person per night, or 27.6 infectious bites per person per year after ITN distribution. However, this decrease was not significant.

ITN distribution resulted in a decrease in the number of Anopheles mosquitoes. However, it is difficult to attribute this decrease to ITNs. There was a clear seasonal influence on entomological transmission indices, which were high during the rainy season and low during the dry season. In addition, there is the distribution of two seasons, the rainy season (September-December and April-May) and the dry season (June-August and January-March) which makes it difficult to assess the decrease in entomological indices. A high rate of mosquito bites was recorded outside houses in Bandundu town during the collection campaign, suggesting changes in the behaviour of *An. gambiae* s.l. as reported elsewhere [25, 29]. However, it is difficult to attribute the change in behaviour to the repellent effect of deltamethrin-treated ITNs alone [25, 29]. A habit was observed among the population of staying out late at night to watch TV programmes. This may explain why the peak of anopheline activity was reached between 2 and 3 am.

2.4.3 Evaluation of the parasitological indices of transmission of malaria

The improvement in the epidemiological situation of malaria remains unstable and could even worsen due to the emergence of parasite resistance to the usual and affordable antimalarial drugs.

The deterioration of primary health services, the emergence of insecticide-resistant strains in mosquitoes and the misuse of antimalarial drugs are believed to have contributed to the selection of resistant strains [13–35, 38]. Diagnostic confirmation is crucial because clinical diagnosis is at the root of thousands of erroneous treatments and this leads to economic consequences as well as increased morbidity and mortality due to the delay of specific treatment [6–35, 38]. Reliable diagnosis could thus serve to avoid unnecessary exposure of patients to antimalarial drugs (risk of parasite resistance and economic loss) and allow for timely exploration of other possible pathologies. Thus, the success of the challenge of containing the spread of resistance absolutely implies an efficient diagnosis.

Currently, two modalities are used in the field for the biological diagnosis of malaria: microscopy and rapid tests (RDT). The NMCP has chosen the SD-Bioline test with HRP2, which is distributed free of charge in the country's health facilities via the central office [9, 44, 45].

In this study, the sensitivity (Se) of the SD-Bioline test evaluated in the field was 77%, specificity (Sp) 78%, positive predictive value (PPV) 82%, negative predictive value (NPV) 72% and overall test value (VG) 77%. This can be justified by the fact that Bandundu-ville is an endemic area and the presence of other non-falciparium plasmodial species could be detected in the area. These observations are similar to those made by Muhindo and colleagues in Kinshasa [53].

Similar results were found by Laurent and colleagues in Tanzania in an area of intense malaria transmission. These results varied by age group and disease prevalence [54] and are in contrast to those found by Abeku and colleagues in East Africa, where the difference was related to prevalence [55]. The prevalence of malaria by parasite index was 57.9% by microscopy and 53.6% by RDT. This is justified by the possibility of diagnosing non-falciparum species by microscopy. In Tanzania, the prevalence by microscopy was low (34.3%) and slightly higher by RDT (57.2%) [53].

Pf was the dominant species in 94% of mono-infections and 4.7% of co-infections. This dominant presence of Pf may justify this high prevalence. This plasmodial species is at the root of the severity and complications of malaria infection [56]. These results are consistent with those found in Kano State, Nigeria, with high malaria prevalence (60%) and this prevalence was strongly related to age [57].

Malaria infection was seasonally influenced; thus there was a significant association between malaria infection and the long rainy season (September-October) with a risk of 2.8. Similarly, during the short dry season (January-March), a significant association was observed with a risk of 2.6 [55, 56, 58].

We find that age and fever during collection were strongly correlated with the presence of parasites. Children <5 years of age were at higher risk of infection, with a 6.0-fold risk than adults, followed by children aged 6–12 years with a 3.2-fold risk.

The presence of fever at the time of sampling was 6.4 times more associated with the presence of malaria infection, reflecting the fact that in endemic areas fever is the main sign of malaria. These results corroborate those found by Mokoso and colleagues in Bandundu-city, of the aetiologies associated with fever in this area, 80% are due to malaria [59]. Our results corroborate those found by several authors in endemic areas [54–57, 58].

The inhabitants of the commune of Mayoyo in Bandundu-city were more exposed to infections with a risk of 2.3. This commune is urban-rural and has biotopes favourable to anopheles, as well as high entomological parameters.

In Dielmo, Senegal, an area where pyrethroids were the main insecticides used for malaria control, a rebound in malaria cases was recorded, followed by the development of resistance after multiple distributions of ITNs (**Table 5**) [60]. In another study in Benin, a reduction in the efficacy of ITNs was observed, leading to an increase in malaria cases in an area where An.gambiae is resistant to pyrethroids and where nets are treated with deltamethrin [15, 51].

As shown in **Table 1**, a considerable number of unfed mosquitoes were collected from the houses, which may have played a role in malaria transmission by later feeding. These results therefore probably underestimate the number of bites per person and may have been biased when new ITNs were deployed, resulting in more mosquitoes exiting before they could be collected. In addition, blood-fed mosquitoes were not tested for human blood, although it was assumed that most blood-fed An.

Parameters (Index)	Phase 1 (July-December 2018) before distribution of ITNs								
	Dry seasons				Rainy seasons				p-value
	Index	Median	P25	P75	Index	Median	P25	P75	
Density	7.67	0.04	0.02	0.22	15.8	0.15	0.027	0.35	**0.01**
SI	6.03	0	0	9	11.7	0.1	0	22	**0.01**
BR	2.8	0.52	0.52	1.55	9.1	1.81	1.55	2.72	**<0.001**
EIR	0.16	0	0	0	1.22	0.18	0	0.58	**<0.001**
Phase 2 (January-June 2019) after distribution of ITNs									
Density	3.3	0.07	0.03	0.1	2.2	0.16	0.04	0.11	**0.24**
SI	8.8	0	0	0.22	11.3	0.07	0	0.25	**0.04**
BR	2.4	1.55	0.52	2.6	7.6	9.5	6.2	10.4	**<0.001**
EIR	0.2	0	0	0.4	0.9	0.1	0	2.2	**0.001**

d = Relative density (mean numbers of An. gambiae s.l. collected) (Anopheles per house) SI=Sporozoite index (percentage of mosquitoes positive for CSP) BR = Biting rate (A. gambiae s.l. per person per night) EIR = Entomological Inoculation Rate (Number of infectious bites per person per night).

Table 5.
Distribution of entomological transmission indices according to periods.

gambiae s.l. resting indoors would have fed on humans. Thirdly, as mosquito collections for pre- and post-net distribution were carried out in different months of the dry and rainy seasons, seasonal variations cannot be excluded from the interpretation of the collection and bioassay data.

Metelo et al. reported seasonal differences in An. gambiae s.l. populations in Bandundu between the dry and rainy seasons [49]. It should be noted that in many settings, nets are not used immediately upon receipt, but rather after the old nets have been torn and are no longer usable. The use of newly distributed nets in homes was not quantified, although the presence of Dawa Plus nets was observed in most households during the implementation of CHPs and HLCs. Large-scale use of DHS data has shown that ITN use is always associated with reduced malaria transmission, especially when community use is high, however, insecticide resistance may reduce this effect, Ferrari et al. found that sleeping under an ITN the previous night was associated with a reduced risk of Plasmodium infection [61]. In the present study, however, no significant impact on entomological measures of transmission was observed immediately after ITN distribution. In an area where insecticide resistance levels are already high, the distribution of new ITNs no longer has an immediate or strong effect on key entomological measures of malaria transmission. This may be due to increased resistance in the study area, compromising both new nets with a full dose of insecticide, and old nets, which will have lost some of their insecticide. This may also mean that the old nets remained effective for the full 3 years of the net's life expectancy, and therefore the distribution of new nets did not improve control. However, the presence of sporozoite-positive mosquitoes in both periods indicates that a better control measure is needed to reduce transmission in this area.

Resistance to pyrethroids and DDT: With the exception of bendiocarb which caused 100% mortality of Anopheles mosquitoes, the other insecticides tested were ineffective against An. gambiae s.l. collected before and after ITN distribution. An. gambiae s.l. was resistant to pyrethroids (deltamethrin and permethrin) and DDT in both periods. Mortality of Anopheles to insecticides varied according to the period (before and after mass ITN distribution). Mortality was limited to deltamethrin, 52% before the mass distribution and was reduced to 34% afterwards, which reduced the effectiveness of this product. After pre-exposure to PBO, the efficacy of deltamethrin was fully restored during both study periods. For permethrin (31–36%) and DDT (4–16%), Anopheles mosquitoes were also resistant to varying degrees depending on the period (pre-post). It can be observed that after the mass ITN distribution, permethrin and DDT increased their efficacy somewhat. These two molecules have not been used for a decade. This could be explained by the fact that the distributed ITNs were impregnated with deltamethrin, which increased the selective pressure and served as a basis for the emergence of resistance in *An. gambiae* s.l. this resistance poses fundamental and operational problems as the behaviour of the Anopheles is altered, resulting in a significant decrease in the efficacy of these products. The endemicity of malaria and the high number of infected An. gambiae in the city of Bandundu are of concern and must be taken into account.

3. Conclusions

The ITNs deployed in Bandundu-city in 2018 are still effective on A. gambiae sl strain Kisumu but are ineffective on the wild *An. gambiae* s.l. wild strain found in the city. The wild *An. gambiae* s.l. were resistant to both pyrethroids and DDT.

An increase in the sensitivity of *An. gambiae* s.l to pyrethroids was observed after pre-exposure to 5% PBO suggesting the resistance was partly of metabolic origin, i.e. related to P450 mono-oxygenases. The high entological indices in Bandundu-city throughout the year indicate intense malaria transmission. This reflects the ineffectiveness of the vector control strategy which has been based solely on the mass distribution of ITNs for several years. We find that mass distribution of ITNs in Bandundu has not had a significant effect on malaria transmission. Given the intensity of transmission and the levels of resistance observed, it is necessary to consider a new alternative to curb the emergence of resistance and maintain the gains of mass distribution.

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Contributions of the authors

E.MM, JZ: drafting of the study protocol.

EMM, JZ, BM, VN: coordination of field activities (larval survey and sensitivity testing). EMM, JZ, VN, SN, AM: Coordination of laboratory activities. EMM, JZ, VN, SN database and statistical analysis. E.MM, JZ, JMNK, FA, EHMN, BM: writing of the article and correction. BM editing of the paper.

Conflict of interest

The authors declare no conflict of interest.

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