RESEARCH ARTICLE

High malaria transmission sustained by *Anopheles gambiae* s.l. occurring both indoors and outdoors in the city of Yaoundé, Cameroon [version 1; referees: awaiting peer review]

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Abstract

**Background:** Malaria remains a major public health problem in Cameroon; however, despite reports on the adaptation of anopheline species to urban habitats, there is still not enough information on malaria transmission pattern in urban settings. In the frame of a larval control trial in the city of Yaoundé, we conducted baseline surveys to assess malaria transmission dynamics in this city.

**Methods:** Adult mosquitoes were collected indoors and outdoors using CDC light traps and human landing catches from March 2017 to March 2018 in 30 districts of Yaoundé, Cameroon. Mosquitoes were sorted by genus and identified to the species level using PCR. The TaqMan method and ELISA were used to determine mosquito infection status to *Plasmodium*. Bioassays were conducted to assess female *Anopheles gambiae* susceptibility to insecticides.

**Results:** A total of 218,991 mosquitoes were collected. The main malaria vectors were *An. gambiae* s.l. (*n*=6154) and *An. funestus* s.l. (*n*=229). Of the 1476 *An. gambiae* s.l. processed by PCR, 92.19% were *An. coluzzii* and 7.81% *An. gambiae*. *An. funestus* s.l. was composed of 93.01% (*173/186*) *An. funestus* and 4.84% (*13/186*) *An. leesoni*. The average biting rate of anopheline was significantly high outdoor than indoor (*P*=0.013). Seasonal variation in mosquito abundance and biting rate was recorded. The infection rate by *Plasmodium falciparum* was 2.13% (*104/4893* mosquitoes processed). The annual entomological inoculation rate was found to vary from 0 to 92 infective bites/man/year (ib/m/y). Malaria transmission risk was high outdoor (66.65
ib/m/y) compared to indoor (31.14 ib/m/y). An. gambiae s.l. was found highly resistant to DDT, permethrin and deltamethrin. High prevalence of the West Africa kdr allele 1014F was recorded and this was not found to influence An. gambiae s.l. infection status.

**Conclusion:** The study suggests high malaria transmission occurring in the city of Yaoundé and call for immediate actions to improve control strategies.

**Keywords**
Malaria, urbanization, Anopheles, transmission, Yaoundé, Cameroon
Introduction
The world population has registered unprecedented growth during the last decades with Africa and Asia displaying the most important rates. The fast demographic growth registered so far has increased the path of urbanization across Africa. There are more and more cities with over a million inhabitants and megacities with 5 to 10 million inhabitants. Because in most parts of Africa the rapid development of cities is often associated with unplanned urbanization, this could have important public health implications for vector-borne diseases such as malaria. Urban malaria is now considered as an emerging health problem in Africa and is receiving further consideration in many countries. In Cameroon the population is estimated at 25 million inhabitants and over ¼ of this population is considered to live in the two main cities of the country, Yaoundé and Douala. Yaoundé, the capital city of Cameroon has seen its population multiplied by 6 over the two last decades and now has a population estimated at about 3 million inhabitants. The city is situated at the heart of the equatorial forest region and irrigated by numerous rivers. In the 1950s, indoor spraying campaigns conducted in Yaoundé and its surroundings, resulted in a decrease transmission level and Plasmodium prevalence in the population to near to zero. At 30 years after interruption of these campaigns, very limited transmission level was still recorded in the city centre. In the 1990s, in the districts of Obili and Essos, Manga et al. reported no infected mosquitoes, whereas moderate level of transmission varying from 14 to 30 infected bites per man per year was recorded in the districts of Nkolbikok and Nkobissou. Similar transmission levels (3–33 infected bites per man per year) were recorded few years later in the central district of Dakar. Contrasting pattern was, however, recorded at the city periphery or nearby rural settings where transmission levels varying from 277 to 350 infected bites per man per year were recorded.

Bionomic studies conducted from 2000 onwards indicated important changes on vector populations. In the cities of Douala and Yaoundé, increased distribution of mosquito in polluted habitats and artificial breeding sites was reported. Moreover, mosquitoes breeding in polluted areas or cultivated sites were found to be more resistant to insecticides compared to those breeding in unpolluted sites. Rapid evolution of insecticide resistance was also recorded in vector populations and an increase in the prevalence of resistance genes such as kdr was observed. The exposition of mosquito to xenobiotics was found to shape the distribution of Anopheles gambiae and Anopheles coluzzii in the cities of Douala and Yaoundé with Anopheles coluzzii more tolerant to pollutants being predominant in the urban centre whereas Anopheles gambiae less tolerant to pollutants being prevalent in rural settings. Laboratory experiments provided evidence supporting a possible influence of this adaptation on the vectorial competence of these two species, with An. coluzzii being more competent to transmit malaria parasite compare to An. gambiae.

In addition to the rapid demographic growth of the city, important physical changes have occurred during the last decade with the construction of new roads and buildings, creation of parks and the drainage of rivers. Yet it is still unknown whether the current adaptation of malaria vectors to the urban environment and physical changes occurring in the city are affecting malaria transmission pattern and vector biting behavior. The present study was conducted in 30 districts of the city of Yaoundé to assess the trend of malaria transmission and to capture spatial and temporal variations.

Methods
Study site
The study was conducted in Yaoundé, the capital city of Cameroon (3° 52’ 12 N; 11° 31’ 12 E), Yaoundé is situated 726 meters above sea level and receives over 1700 mm of rainfall annually. Yaoundé features an equatorial climate with two raining seasons extending from March to June and from September to November lasting 7 to 8 months. Although Yaoundé is situated in the equatorial forest domain, the extension of settlements has significantly reduced the forest cover which is now restricted to the nearby rural area. The city extends on 20 km wide and about 25 km long. Yaoundé landscape comprises highland and lowland areas, which are irrigated by several rivers. Lowland areas are exploited during the dry season for agriculture. Main rivers present in the city are rivers Mfounid, Ekozoa, Biyeme and Mefou, Adult mosquitoes’ collections were performed in thirty districts (Figure 1).

Mosquitoes sampling
Adult mosquitoes were sampled using Human Landing Catches (HLC) and the Centres for Disease Control and Prevention Light Traps (CDC-LTs) both indoors and outdoors. Collections were performed once every 2 months from March 2017 to March 2018.

There were four HLC volunteers trained to collect mosquitoes landing on their legs. Collections were performed from 19:00 to 06:00 AM indoors and outdoors. During each night two teams of collectors were used to avoid bias due to sleep and tiredness.

A total of 16 to 20 CDC-LTs were placed indoors and outdoors in 10 to 15 houses per district. Collections were undertaken from 19:00 to 06:00 during 3 consecutive days per district per month.

The study was conducted under the ethical clearance No. 2018/06/1039/CE/CNERSH/SP delivered by the Cameroon National Ethics (CNE) Committee for Research on Human Health Ref N°D30-172/L/MINSANTE/SG/DROS/TMC of 4 April 2017. All volunteers participating to human landing catches signed a written inform consent form indicating their willingness to take part to the study and received free malaria prophylaxis.

Mosquito processing
Once collected, anopheles were separated from culicines using the morphological identification keys of Edwards et al. Anophele species were identified using morphological identification keys of Gillies and De Meillon and Gillies and Coetzee. Mosquitoes belonging to the Anopheles gambiae...
complex were further processed by PCR to identify between *Anopheles coluzzii* and *Anopheles gambiae*, the two members of the complex found in Yaoundé. Molecular identification of members of the *Anopheles funestus* was conducted using the protocol of Koekemoer *et al.* DNA extracted from the wings and legs according to the Livak method was used for the analysis. Each anopheline specimen was stored individually in a numbered Eppendorf tube containing dessicant, archived and kept in a freezer at −20°C. The heads and thoraxes of female anophelines were tested for the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* by ELISA, as described by Wirtz *et al.* or using the Taqman method.

**Insecticide susceptibility tests**

Susceptibility of *An. gambiae* s.l. to 0.75% permethrin, 0.05% deltamethrin, 0.1% bendiocarb and 4% DDT was assessed using the WHO guidelines at temperatures of 25±2°C and 70–80% relative humidity. Insecticide susceptibility tests were performed with 2- to 4-day-old unfed females. Batches of 20 to 25 mosquitoes per tube were exposed to impregnated papers for 1 hour. The number of mosquitoes knocked down by the insecticide was recorded every 10 minutes during exposure. After exposure, mosquitoes were fed with a 10% glucose solution and the number of dead mosquitoes was recorded 24 hours post-exposure. Tests using untreated papers were conducted as well (controls).
The mortality rates were corrected using the Abbot formula whenever the mortality rate of the controls was between 5 and 20%. Susceptibility and resistance levels were assessed according to the World Health Organization criteria. Mosquitoes were classified into three groups: insecticide resistant (<90%), possible insecticide resistant (90–97%) and insecticide susceptible (>98%).

To detect the presence of the kdr alleles (L1014F and L1014S) conferring resistance to DDT and pyrethroids, DNA extracted from a sub sample of *An. gambiae* s.l. females was screened using the TaqMan assay.

Data analysis
Mosquito densities were compared between seasons, collection sites and districts. Biting rate (number of bites per person per night, b/p/n) was calculated as the number of mosquitoes caught in one night divided by the number of collectors. The infection rate was calculated as the number of infected mosquitoes divided by the total number of *Anopheles* processed. The entomological inoculation rate (EIR) (the number of infected bites per person per night, ib/p/n) was calculated by multiplying the infection rate by the biting rate/night. To assess linear correlations between the two collection methods, the Pearson correlation coefficient was used to calculate the average number of mosquitoes collected nightly by the CDC-LT and HLC methods. Prior to analysis, the average number for each catch (x) was transformed to $Y = \log(x+1)$.

To compare methods and determine if mosquito abundance was affected by the sampling efficiency of each method used, the ratio of the number of mosquitoes in LT to the number of mosquitoes in HLC $\frac{\log(HLC+1)-\log(LT+1)}{\log(HLC+1)+\log(LT+1)}$ was plotted against the average abundance $\frac{\log(HLC+1)+\log(LT+1)}{2}$ as described by Overgaard et al.

The knock down time for 50% of mosquitoes ($K_{d_{50}}$) and 95% of mosquitoes ($K_{d_{95}}$) representing the time when 50% or 95% of the mosquitoes exposed are knocked down, was calculated using the WINDL32 version 2.0 software. Chi-square test was used to compare proportions, student tests and one way ANOVA were used to compare averages. All these tests were performed using SPSS software (SPSS version 20.0) and R software version 3.4.0. The level of significance of each test was set at $\alpha < 0.05$. The coefficient r of Pearson was used to assess correlation between CDC-LT and HLC.

**Results**
All raw data associated with this study are available on OSF.

**Mosquito collection**

**Mosquito diversity and abundance.** Among the 218,991 specimens identified morphologically, *Culex* spp. was the most abundant (96.48%; n=211,993) followed by *An. gambiae* s.l. (2.81%; n=6154) and *Mansonia* spp. (0.47%; n=1026). *An. funestus* s.l. was less abundant (0.10%; n=229) (Table 1). Overall, 187, 404 (85.58%) mosquitoes were collected using a total of 9968 CDC-LTs placed per night per house and 31,587 (14.42%) were collected using a total of 348 human-night collectors. The average density of mosquitoes collected using CDC-LTs, was high indoors than outdoors (t = 8.28, d=29.6, P< 0.0001) while the trend was not significantly different between outdoor and indoor catches using HLC (t = -0.68, df =58, P=0.2508).

Correlation analysis between CDC-LT and HLC catches using the Pearson correlation coefficient, indicated that when all mosquitoes were considered, CDC-LTs catches were positively and significantly correlated with those of HLC (r = 0.408, P = 0.025). When mosquitoes were separated into the two most common genera (*Culex* and *Anopheles*), the correlation was positive but not significant for *Culex* (r = 0.324, P = 0.081) whereas it was negative for *Anopheles* (r = −0.013, P = 0.94).

Comparing the relative sampling efficiencies of the two methods against mosquito abundance (Figure 2), it appears that there was a significant tendency for increased mosquito abundance with the ratio of LT to HLC (P < 2.2×10^-10).

**Table 1. Mosquitoes sampled in Yaoundé using CDC-LTs and HLC from March 2017 to March 2018.**

<table>
<thead>
<tr>
<th>Species</th>
<th>CDC-LT</th>
<th>HLC</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indoor N %</td>
<td>2017-2018</td>
<td>Indoor N %</td>
</tr>
<tr>
<td>An. funestus</td>
<td>199 0.13</td>
<td>15 0.05</td>
<td>5 0.30</td>
</tr>
<tr>
<td>An. gambiae s.l.</td>
<td>3,542 2.29</td>
<td>414 1.26</td>
<td>772 5.08</td>
</tr>
<tr>
<td>An. ziemanni</td>
<td>4 0.00</td>
<td>8 0.02</td>
<td>0 0.00</td>
</tr>
<tr>
<td>Total anopheine</td>
<td>3,745 2.42</td>
<td>437 1.33</td>
<td>777 5.11</td>
</tr>
<tr>
<td>Aedes spp.</td>
<td>182 0.12</td>
<td>52 0.16</td>
<td>14 0.09</td>
</tr>
<tr>
<td>Culex spp.</td>
<td>150,020 97.09</td>
<td>32,054 97.46</td>
<td>14,381 94.56</td>
</tr>
<tr>
<td>Coquillettidia</td>
<td>6 0.00</td>
<td>8 0.02</td>
<td>0 0</td>
</tr>
<tr>
<td>Mansonia spp.</td>
<td>563 0.36</td>
<td>337 1.02</td>
<td>36 0.24</td>
</tr>
<tr>
<td>Total culicines</td>
<td>150,771 97.58</td>
<td>32,451 98.67</td>
<td>14,431 94.89</td>
</tr>
<tr>
<td>Overall</td>
<td>154,516 70.56</td>
<td>32,888 15.02</td>
<td>15,208 6.94</td>
</tr>
</tbody>
</table>
Figure 2. Relationship between CDC-LT catches, human landing catches (HLC) and mosquito abundance.

Figure 3. Spatial distribution of Anopheles species in the city of Yaoundé. Source: National Institute of Cartography, Cameroon.

Distribution of Anopheles species. Anopheline species recorded includes An. ziemanni mainly found in Nkolbisson, An. gambiae s.l. and An. funestus s.l. Out of 1476 An. gambiae s.l. analysed, 92.19 % (n=1351) were An. coluzzii and 7.81% (n=125) An. gambiae. An. coluzzii was highly predominant across the city whereas An. gambiae was found in a sizeable density at Nkolbisson, Emia and Etoug ebe (Figure 3). Amongst the 186 An. funestus s.l. tested, 4.84% (n=8) were An. leesoni and 93.01% (n=173) An. funestus s.s. The small proportion of An. leesoni observed was mainly recorded from Ekounou palais, Etam Bafia and NVR Nkolbisson. An. funestus was found in 21 sites out of 30 districts. Significant variation in species densities was recorded between districts (P=0.008).

Seasonal variation of anopheline species densities collected with CDC-LTs
Seasonal variation of anopheles densities was recorded both indoors and outdoors. Our results indicated high densities indoor than outdoor whatever the species and the sampling period. The indoor density of An. gambiae s.l. estimated by CDC-LTs varied from 1.41 mosquitoes/trap/night in May–June period (short raining season) to 0.09 mosquitoes/trap/night in September–October period (long raining season). The same trend was observed with mosquitoes sampled outdoor during the same period with densities varying from 0.23 mosquitoes/trap/night to 0.04 mosquitoes/trap/night in May–June and 0.02 mosquitoes/trap/night in September–October period (Figure 4). An. funestus densities were found to increase, at the onset of the short rainy season in March–April 17 (0.06 mosquitoes/trap/night (indoor) and 0.02 mosquitoes/trap/night (outdoor)). Low densities were recorded during the short rainy season of March 2018 (0.01 mosquitoes/trap/night both in and outdoor) (Figure 4).
Seasonal variation of anopheline species densities collected using HLC

High *An. gambiae* s.l. biting densities were recorded outdoor in May-June (21.53 b/m/n) during the short rainy season then decrease to 2.37 b/m/n in September-October at the onset of the long rainy season. Indoor collections declined from 10.43 b/m/n in May-June during the short rainy season to 1.67 b/m/n in July-August period during the short dry season (Figure 5).

*An. funestus* was more abundant at the onset of the short rainy season with a 0.2 b/m/n outdoors and 0.1 b/m/n indoors, then its outdoor biting rate fell during the short dry season to 0.033 b/m/n while the indoor biting rate was reduced to 0 b/m/n during the same period (Figure 5).

The average biting rate for *An. gambiae* s.l. varied from 4.44 b/m/n indoor to 8.20 b/m/n outdoor and the difference was significant (P = 0.013). Concerning *An. funestus* its biting rate was 0.03 b/m/n and 0.06 b/m/n for indoor and outdoor respectively; the difference was not significant (P = 0.733).

Indoor and outdoor biting behaviour of *An. gambiae* and *An. funestus*

*An. gambiae* s.l. densities collected during the second part of the night (24:00-06:00) were high compare to those of the first part of the night both indoors and outdoors (Figure 6). The difference was not significant for both indoor and outdoor catches (P > 0.26). For *An. funestus*, densities collected outdoor from 24:00 to 06:00 were not significantly different from those of the first part of the night (t=-0.42, df=8.33, P=0.68). Similarly, no significant difference was recorded between indoor and outdoor collections (t=0.47, df= 9.9811, P=0.65).

Plasmodium infection in mosquitoes

Of the 4893 anopheline (223 *An. funestus* s.l., 4661 *An. gambiae* s.l. and 09 *An. ziemanni*) screened for *Plasmodium falciparum* presence, 104 specimens were positive (103 *An. gambiae* s.l. and 01 *An. funestus* s.l.). Of the 103 *An. gambiae* s.l. detected positive 91% (n=94) were *An. coluzzii* and 9% (n=9) *An. gambiae*. The overall *P. falciparum* infection rate was 2.13%. Out of the infected mosquito, 66 were collected using...
CDC-LTs while the remaining (n=38) was collected using HLC. The circumsporozoite rates between indoor and outdoor collections are presented in Table 2. An. gambiae s.l. infection rate was 2.3% indoor and 2% outdoor. The circumsporozoite rate for An. funestus was 0.4%.

Relationship between infection and kdr allele presence
Analyses were conducted to compare the prevalence of the West Africa kdr allele in infected and non-infected mosquitoes. A total of 143 An. gambiae s.l. including 73 positive for Plasmodium CSP and 70 non infected were randomly selected

![Figure 5. Seasonal variation of An. gambiae s.l. and An. funestus densities after human landing catches.](image)

**Table 2.** Sporozoite rates for Anopheles mosquitoes from indoor and outdoor collections in Yaoundé.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tested</th>
<th>Inf</th>
<th>% (95% CI)</th>
<th>Tested</th>
<th>Inf</th>
<th>% (95% CI)</th>
<th>Tested</th>
<th>Inf</th>
<th>% (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>An. gambiae s.l.</td>
<td>3,278</td>
<td>76</td>
<td>2.3 (2-3)</td>
<td>1,383</td>
<td>27</td>
<td>1.3-3</td>
<td>4,661</td>
<td>103</td>
<td>2.1 (1.8-2.7)</td>
</tr>
<tr>
<td>An. funestus s.l.</td>
<td>199</td>
<td>1</td>
<td>0.5 (0.0133)</td>
<td>24</td>
<td>0</td>
<td>0 (0-15)</td>
<td>223</td>
<td>1</td>
<td>0.4 (0.01-2.5)</td>
</tr>
<tr>
<td>An. ziemanni</td>
<td>3</td>
<td>0</td>
<td>0 (0-123)</td>
<td>6</td>
<td>0</td>
<td>0 (0-61.5)</td>
<td>9</td>
<td>0</td>
<td>0 (0-41)</td>
</tr>
<tr>
<td>Total</td>
<td>3,480</td>
<td>77</td>
<td>2.2 (1.7-3)</td>
<td>1,413</td>
<td>27</td>
<td>1.9 (1.3-3)</td>
<td>4,893</td>
<td>104</td>
<td>2.1 (1.7-2.6)</td>
</tr>
</tbody>
</table>

Inf, infected; %, infection rate; 95% CI, 95% confidence interval.
and genotyped for kdr-West (L1014F) mutation. The L1014F mutation was detected in both groups (Table 3). There was no significant difference between genotypes to kdr gene and mosquito infectious status (P=0.30).

Table 3. Relationship between An. gambiae s.l. infection status to Plasmodium falciparum and kdr allele frequencies.

<table>
<thead>
<tr>
<th>Infection status</th>
<th>N</th>
<th>L1014F kdr genotypes</th>
<th>F(kdr)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected*</td>
<td>73</td>
<td>45 26 2</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Non-infected*</td>
<td>70</td>
<td>57 13 0</td>
<td>0.91</td>
<td>0.30</td>
</tr>
<tr>
<td>Overall</td>
<td>143</td>
<td>102 39 2</td>
<td>0.79</td>
<td></td>
</tr>
</tbody>
</table>

**RR, homozygote resistant; RS, heterozygote; SS, susceptible; F(kdr), frequency of the kdr allele.**

**Malaria transmission and EIRs**
Malaria transmission was found to occur continuously through the city but with different intensities levels according to seasons and districts. The EIR was estimated at 31.14 infected bites/man/year (ib/m/yr) indoor and 66.65 ib/m/yr outdoor. An estimation of malaria transmission risk throughout the city of Yaoundé was conducted and significant differences were recorded between districts (Figure 7). Very low transmission risk with EIR varying from 0 to 5 ib/m/yr was recorded in nine districts (Tam-tam, Biyemassi Carrefour, Efoulan, Etoug Ebe, Ngousso, Obobogo, Labogenie, Santa Barbara, Obili). Low transmission risk with transmission level varying from 5 to 15 ib/m/yr was recorded in eight districts (Nkolndongo, Ambassade de France, Biyemassi Somatel, Cité des nations, Etam-bafia, Melen, Oyomabang, Emia) while moderate transmission risk (EIR 15 – 40 ib/m/yr) was recorded in seven districts (Biyemassi lycée, Biyemassi lac, Ekounou palais, Nkolbikok, Nkolbisson, NVR Nkolbisson, Oledza). High transmission risk with EIR exceeding 40 ib/m/yr was recorded in six districts (Essos, Ekounou Ekié, Bastos, Mvog-Ada, Tongolo, Tsinga) with Ekounou Ekié scoring the highest EIR (92 ib/m/yr). Malaria transmission in the city of Yaoundé was recorded all year long with the intensity varying from one season to the other. The short rainy season of April-June 2017 was the period where most transmission cases were recorded (Figure 8).
Insecticide susceptibility bioassay
Preliminary tests were conducted with An. gambiae s.s. Kisumu strain to assess the quality of the impregnated papers. A total of 100 mosquitoes were tested for each insecticide. A mortality rate ranging from 95% for DDT to 100% for permethrin deltamethrin and bendiocarb was recorded. Susceptibility tests were subsequently undertaken with field females raised from larval collection. The mortality rate ranged from 2.85% for DDT to 96.31% (n= 911) for bendiocarb (n = 1709) (Table 4).

Genotyping of the West kdr mutation
Of the 194 anopheline females (including susceptible, resistant and control) screened for the presence of the West Africa kdr allele (L1014F), 187 were detected carrying the allele 39 as homozygotes, 148 as heterozygotes and 7 were homozygote for the wild allele (Table 5). The frequency of the West Africa kdr allele (L1014F) was 0.59. The kdr allele frequency was not significantly different between resistant and susceptible samples ($\chi^2 = 0.039, P= 0.84$).
Figure 8. Seasonal variation of the entomological inoculation rate in Yaoundé, from March 2017 to March 2018.

Table 4. Susceptibility level to deltamethrin, permethrin, bendiocarb and DDT for An. gambiae s.l. populations in Yaoundé.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Kisumu colony</th>
<th>Field populations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mortality% (95%CI)</td>
</tr>
<tr>
<td>Deltamethrin 0.05%</td>
<td>100</td>
<td>100 (81.36-121.63)</td>
</tr>
<tr>
<td>Permethrin 0.75%</td>
<td>100</td>
<td>100 (81.36-121.63)</td>
</tr>
<tr>
<td>DDT 4%</td>
<td>100</td>
<td>95 (81.36-121.63)</td>
</tr>
<tr>
<td>Bendiocarb 0.1%</td>
<td>100</td>
<td>100 (81.36-121.63)</td>
</tr>
</tbody>
</table>

*N: number tested, 95%CI : 95% confidence interval.

Table 5. Frequency of the kdr alleles in An. gambiae s.l. samples from Yaoundé.

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>L1014F kdr genotype</th>
<th>F(kdr)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>RS</td>
<td>SS</td>
<td></td>
</tr>
<tr>
<td>Survival population*</td>
<td>97</td>
<td>22</td>
<td>70</td>
<td>5</td>
</tr>
<tr>
<td>Susceptible specimen*</td>
<td>58</td>
<td>10</td>
<td>46</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>39</td>
<td>7</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Overall</td>
<td>194</td>
<td>39</td>
<td>148</td>
<td>7</td>
</tr>
</tbody>
</table>

*N: number tested; RR, homozygote resistant; RS, heterozygote; SS, susceptible; F(kdr), frequency of the kdr 1014F allele.

Discussion

High and permanent malaria transmission was recorded in the city of Yaoundé and was consistent with studies conducted so far suggesting urban malaria as a public health threat in most sub-Saharan Africa cities. The spatial design of the study, covering up to 30 districts, permitted to capture the heterogeneous pattern of malaria transmission risk in the city of Yaoundé varying from very low to high risk zones. This pattern was in accordance with the presence of hotspots areas across the city. According to Robert et al., malaria transmission in sub-Saharan Africa could be less intense in the city centre with mean annual entomological inoculation rate estimated at 7.1 ib/man/y, whereas it is more intense in the periurban and rural settings, where it could be estimated at 45.8 and 167.7 ib/man/y, respectively. The complexity of malaria situation in Yaoundé could derive from the combination of different factors, including the peculiarity of the city landscape. The city of Yaoundé is composed of an alternation of hills and lowland areas and is irrigated by several permanent rivers. During the rainy season, inundation of floodplains resulting from the overloading of river systems in lowlands areas create suitable habitats for mosquitoes, whereas during the dry season, river channels and/or the exploitation of floodplains for urban agriculture, provide suitable mosquitoes breeding habitats. In addition to these factors the rapid unplanned urbanisation affecting almost all districts characterized by the creation of slums, the absence of drainage system for surface water, the presence of large open space

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and the development of several activities, such as car washing, urban farming, public and private constructions, create numerous breeding opportunities for mosquito of the An. gambiae complex in the urban environment as reported elsewhere. Similar contrasting pattern of malaria transmission was reported for the city of Libreville with high transmission occurring at the city centre and low transmission at the periphery. According to the authors, this situation could have resulted from poor housing, poor waste management and slum-like conditions in the urban centre compare to the city periphery.

Compared to previous studies in Yaoundé which indicated transmission intensity varying between 0 to 33 infected bites/man/yr, transmission rate reaching up to 92 ib/man/yr was detected in the present study, highlighting the changing pattern of malaria transmission dynamic and vector bionomic in the city of Yaoundé. Indeed, three of the most efficient malaria vectors in Africa An. gambiae, An. coluzzii and An. funestus were recorded in Yaoundé. However, An. coluzzii was by far the most abundant species. Its distribution was in conformity with previous studies conducted so far in Yaoundé suggesting high capacity of adaptation of this species to urban environments and polluted habitats. An. funestus was scarce in the city centre; its presence could be associated with the development of new adaptation capacities or the development of suitable breeding habitats in urban districts. An. funestus breed in permanent water collection with emerging vegetation and could have benefited from the practice of urban agriculture in swamps to extend its distribution range. The adaptation of An. funestus to the urban environment alongside An. gambiae and An. coluzzii and the changing transmission pattern in Yaoundé all suggest the need for in-depth assessment of main determinants of transmission in order to address sustainably challenges impeding malaria control efforts in this city.

A high EIR was recorded outdoors, which supports an increased transmission risk outdoors. Similar findings were recorded in several cities across sub-Saharan Africa. An. funestus was scarce in the city centre; its presence could be associated with the development of new adaptation capacities or the development of suitable breeding habitats in urban districts. An. funestus breed in permanent water collection with emerging vegetation and could have benefited from the practice of urban agriculture in swamps to extend its distribution range. The adaptation of An. funestus to the urban environment alongside An. gambiae and An. coluzzii and the changing transmission pattern in Yaoundé all suggest the need for in-depth assessment of main determinants of transmission in order to address sustainably challenges impeding malaria control efforts in this city.

Conclusions
The study suggests heterogeneous pattern of malaria transmission in the city of Yaoundé. Several factors including outdoor transmission and increased expansion of insecticide resistance were found to affect control measures the following call for concerted efforts in order to improve malaria control interventions in the city of Yaoundé. The use of a larvicial strategy, as is planned for the city of Yaoundé, could be well adapted for controlling outdoor and indoor biting mosquitoes and insecticide resistant vectors. As the study indicated there are focal sites in the city more affected than others so increasing control efforts in these sites could be indicated for the sustainable control of malaria in Yaoundé.

Data availability
The raw data underlying the findings reported in this study are available on OSF. DOI: https://doi.org/10.17605/OSF.IO/KYXTN.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Grant information
This work received financial support from Wellcome Trust senior Fellowship in Public Health and Tropical Medicine (202687/Z/16/Z) to CAN.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We are grateful to mosquito collectors and the population of Yaoundé for their participation to the study.
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