Impact of sublethal pyrethroid exposure on resistant *Anopheles gambiae* mosquitoes' fitness

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Abstract

Background : There is increasing evidence of insecticide resistance spreading among wild mosquito populations, which is widely believed to compromise vector control once it reaches a threshold that enables mosquitoes to survive exposure to long lasting treated bed-net (LLIN) or indoor residual spraying (IRS). However, very little is known about the long-term impact of insecticide resistance on malaria transmission, which makes the consequence of insecticide resistance spreading difficult to predict.

Methods: To gain more clarity, we have assessed five life-history traits of a resistant *Anopheles gambiae* laboratory strain that was repeatedly exposed to a LLIN and compared with individuals issued from the same strain but exposed to an untreated bed-net.

Results: The non-parametric Kruskal-Wallis test did not show any significant impact of gonotrophic cycle on the five traits. However, the Kolmogorov-Smirnov non-parametric test revealed a significant (i) drop in blood feeding mean rates ($D = 0.800$; $P < 0.0001$), (ii) increase in 24-hours post-exposure ($D = 0.600$; $P < 0.001$) and (iii) end of gonotrophic cycle mortality ($D = 0.611$; $P < 0.006$), and (iv) drop in egg laying rate ($D = 0.730$, $P < 0.0001$) when mosquitoes were exposed. Surprisingly, there was rather an upward trend in the number of larvae/female mosquito for the exposed group comparing to the unexposed one, although the difference was not significant ($D = 0.417$, $P > 0.05$).

Conclusion: Our study shows that in a context of widespread of resistance to insecticides, current pyrethroid-based vector control tools can still confer protection against malaria.

Introduction

Malaria is a parasitic disease that is transmitted to humans by female mosquitoes of the genus *Anopheles* [1]. It is considered as a major obstacle to development in Sub-Sahara African countries [2]. Large and sustained funding in the two last decade has enabled widespread geographical prevention of malaria disease. The most widely utilized strategies are the mass distribution of long lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and artemisin-based combination therapy (ACT). According to Bhatt et al, global malaria incidence has been reduced by 40% between 2000 and 2015 [3]. The authors estimate LLINs to be accountable for 68% of this reduction and ACT and IRS for 19% and 13%, respectively. For the period of 2016 to 2030, new goals and a new benchmark to reduce and eliminate malaria have been established taking into account the current situation [4–5]. The core methods of malaria control remain unchanged and rely on insecticides-based vector control. To date, only few classes of insecticides are approved by the World health organization (WHO) to be used in public health with only one, the class of pyrethroids, being recommended for LLINs [6]. Unfortunately, insecticide resistance is now widespread among malaria vector populations especially in sub-Saharan African regions [7–9]. Even more worrisome are reports from countries such as Côte d'Ivoire where resistance to all four classes of insecticides have been recorded [10] within the same mosquito populations. While much information has been gained on the biological and genetic mechanisms behind insecticide

resistance, its long term impact on vector control methods is poorly known and remains difficult to predict [11]. It is widely assumed that insecticide resistance will compromise vector control, as chemicals will no longer be able to kill upon contact [12–13]. As an evolutionary process, insecticide resistance has an associated fitness cost that can be reduced, for instance, through phenotypic plasticity. This associated cost has previously been assessed in regards to age, multiple exposures and delayed mortality [12, 14–15]. The current study aims to investigate over the course of the mosquito's lifespan whether individuals that have survived insecticide exposure have a reduced fitness and reproductive success. This was assessed through the measurement after exposure to a sub-lethal dose of a public health insecticide of various life-history traits including blood-feeding rate, 24 hour post-exposure mortality rate, oviposition rate, mortality rate at the end of each gonotrophic cycle and the reproductive success. By assessing this, we hoped to gain a better understanding of insecticide resistance implication for malaria vector control.

Material And Methods

Mosquito strain

All experiments were conducted on a strain of *An. gambiae* isolated in 2016 from an *An. gambiae* s.l. strain, originating from the locality of Tiassalé in Côte d'Ivoire and bred without selection at the insectary of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) since 2010. According to WHO criteria [2, 16], Tiassale mosquito population has been described as resistant to at least three of the four classes of insecticides [17]. Both the Kdr and Ace1R point mutations, respectively responsible of resistance to pyrethroids and carbamates and organophosphates have been identified as a resistance mechanism in the Tiassalé strain, as well as metabolic resistance [10, 18]. The strain was a mixed of about 80% *An. coluzzii* and 20% *An. gambiae*. In order to isolate the *An. gambiae* from the original Tiassalé strain, pupae were isolated individually in plastic cups. After emergence, couples composed of a female and a male were formed and left for observation for five days to allow them to mate. A number was assigned to each couple. Following the oviposition, the parent couple was removed, killed and processed for molecular analysis through the Sine PCR [19] for species identification. Only larvae whose both parents were *An. gambiae* were kept and nurtured to adulthood.

The breeding conditions were standard insectary conditions of 27 ± 2 °C, $70 \pm 5\%$ relative humidity (RH) and a 12 hours light:12 hours dark photoperiods. Eggs were placed in plastic trays of $15 \times 30 \times 5$ cm and filled with 800 mL of dechlorinated tap water. Hatched larvae were separated into groups of about 200 larvae/tray in order to limit size variation. They were fed on mashed Friskies dry cat food. Starting day ten after hatching, pupae were collected for two days and transferred into $25 \times 25 \times 25$ cm holding cages for emergence. All emerged adults, before and after experimentation, were maintained under standard insectary conditions of 27 ± 2 °C, $70 \pm 5\%$ RH. They were provided with a 10% glucose solution daily.

Experimental design and processing

Prior the experimentation, a sub-lethal exposure time to deltamethrin treated bed-net that procured 20% was determined. The experimental design used has been drafted in order to mimic exposure to

insecticides in natural settings where mosquitoes are coming in contact to the chemical on bed-net when they are trying to reach a person sleeping underneath for blood meal. Thus, a treated bed-net has been used. To increase accuracy, the bed-net was furnished by the National Control Program for Malaria (NCPM) of Côte d'Ivoire and was issued from the pool of Dawa 2.0 LLINs distributed to the population. It is coated with 80 mg/m² of deltamethrin. Net Quality was assessed with a WHO standardized cone-test with the susceptible An. gambiae Kisumu strain maintained at the CSRS laboratory in Abidjan.

During the course of the experiment, four cohorts of 30 to 100 females aged four to five days depending on mosquito availability were separated into two same-sized groups. Each group was either exposed to a LLIN or to an untreated bed-net for 10 minutes, corresponding to the sub-lethal exposure time. For the exposure, mosquitoes were released into a 15 × 15 × 15 cm cage that was covered with either a LLIN or an untreated net. After exposure, all individuals were transferred into a new holding cage according to their respective groups for 30 minutes recovery period whereafter, they were given the opportunity to have a blood meal for 30 minutes. All mosquitoes were allowed to rest and digest for two days before being transferred into individual 125 ml plastic cups. At this point, 24-hour mortality-post exposure and the blood feeding rates were recorded for each group. Each cup was lined at the bottom with damp cotton wool covered with a filter paper to stimulate oviposition. Each blood fed female was allowed to lay eggs for three days or until over 50% of the control group has laid. The egg laying rate or oviposition rate was defined as the number of females having laid at least one egg out of the total number of females that have taken a blood meal for each group. A second mortality was recorded at this point, corresponding to the mortality after a gonotrophic cycle. Afterwards, all females, fed and not fed, were transferred back into their initial cages and the experiment was repeated. Each cohort was exposed four times. Intervals between exposures were adapted to the times needed for egg laying.

Laid eggs were collected and pooled accordingly as exposed or non-exposed group and reared under standard insectary conditions. When larvae from each group grown to stage three (L3), they were counted and recorded. The Reproductive Success was estimated as the mean number of L3 counted per female and per batch that had laid eggs.

Data analysis:

From the exposure to the end of gonotrophic cycle, five life-history traits were measured including the blood-feeding rate, the mortality rate 24-hour post-exposure, the egg laying rate, the mortality rate at the end of gonotrophic cycle, the reproductive success defined as the success of producing viable offspring. Each trait was confronted to two independent variables, "gonotrophic cycle" and "exposure status" as well as their interaction. The non-parametric Kruskal-Wallis test with Bonferroni correction was used to test any significant difference between means of different traits among the variable "gonotrophic cycle", and the Kolmogorov-Smirnov non-parametric test was run to compared the distribution of traits according to the variable "exposure status". For both statistical methods, the level of significance was fixed at 5%. Statistical analysis was performed via XLSTAT version 2019.3.2 [20].

Results

A total of 477 females *An. gambiae* from the TIAS strain split into two groups were either exposed or not to a LLIN and allowed to complete four gonotrophic cycles. Both groups of mosquitoes were well balanced. Details on sample sizes, cohort composition over the different gonotrophic cycles are given in Table 1 as supplementary information. The unexposed mosquitoes consisted of 4 cohorts (or replicates) of 50, 100, 28 and 60 mosquitoes for a total of 238 versus 4 cohorts of 50, 100, 29 and 60 mosquitoes for the exposed group for a total of 239 mosquitoes (Table 1, Additional information).

For both the unexposed and exposed groups, none of the five traits studied appear to be significantly affected by the gonotrophic cycle. However, these parameters were negatively affected once the mosquitoes were exposed to the treated net.

Regarding the blood-feeding, its rate was relatively stable between the different gonotrophic cycles in the unexposed group, and varied between 66.8% and 82.7%, the difference was not significant ($P > 0.05$; Fig. 1a). In the exposed group, this parameter varied between 32.2% and 51.3% within the gonotrophic cycles. As in the unexposed group, the difference however was not significant ($P > 0.05$; Fig. 1a). But when compared the unexposed to the exposed group, a significant drop in blood feeding mean rates was observed ($D = 0.800$; $P < 0.0001$; Fig. 1b).

With regards to the mortality 24 hours post-exposure, we did not observed any significant variation between the gonotrophic cycles either in the unexposed group (5.9 to 11.9%; $P > 0.05$; Fig. 2a) or in the exposed group (11.1 to 38.9%; $P > 0.05$; Fig. 2a) despite that the trend was upward in the exposed group (Fig. 2a). However, when the two groups are compared together, the increase in mean mortality rate in the exposed group is significant ($D = 0.600$; $P < 0.001$; Fig. 2b).

Concerning the mortality at the end of the gonotrophic cycle or delayed mortality, we noted a non-significant variation in the unexposed group between the gonotrophic cycles (19.2 to 35%; $P > 0.05$; Fig. 3a) as well as in the exposed group (31.7 to 74.5%; $P > 0.05$; Fig. 3a), despite that it was increasing over the gonotrophic cycles in the latter. However, when the two groups were compared together, the increase in the mortality at the end of gonotrophic cycle was significant ($D = 0.611$; $P < 0.006$; Fig. 3b).

Like the blood-feeding rate or the mortality, the oviposition rate did not vary significantly between the gonotrophic cycles in the unexposed (33.3 to 21.4%; $P > 0.05$) nor in exposed group (15.0 to 9.6%; $P > 0.05$), although there was a slight downward trend in both groups (Fig. 4a). As for the other above traits, the egg laying rate was significantly affected by exposure to the treated net ($D = 0.730$, $P < 0.0001$, Fig. 4b).

As all for the other traits, we did not find any significant variation in the reproductive success between the different gonotrophic cycles in both the unexposed and the exposed groups (Fig. 5a). Contrary to what might have been expected in view of the results obtained on the other traits, the reproductive success did not decrease following exposure. Although it was not significantly different between the two groups ($D =$

0.417, $P > 0.05$, Fig. 5b), there was rather an upward trend in the exposed group comparing to the unexposed one.

Discussion

Resistance to insecticide has a certain fitness cost on the short and long-term that can be observed on a phenotypic level [21–25]. Here we could show that at least four life-history traits implicated in transmission were affected by a sublethal exposure to insecticide on resistant mosquitoes.

The first examined trait being the blood-feeding rate. Females that were exposed to deltamethrin fed significantly less than females that were not exposed. As, insecticide exposure can have an effect on chemo-sensitivity, exposed female may be less likely to detect physical and chemical cues from their prey. The interaction between insecticide resistance, exposure and behavior remains however poorly understood and needs more investigation [26]. Recent studies showed that reduction in blood-feeding in female mosquitoes post-exposed to LLINs returns to the same rates as control after a few hours [27]. We here argue that while that might be true, it would also mean that in natural settings mosquitoes would not be re-exposed to insecticides after the effect wears off. The success of blood feeding is doubly important as it affects not only pathogen transmission but also reproduction and thus population density and dynamic. In our setting we could show that, the oviposition or egg-laying rate was significantly lower in females that were exposed. This effect can be explained through a resources based trade-off between fecundity and survival [28]. Indeed, the over-production of detoxifying enzymes triggered upon exposure to insecticide is probably associated with energetic cost, which impacts the resource available for egg laying. Such a regulation would indicate a high adaptative cost related to insecticide resistance [29], which have been already demonstrated in previous studies [30–34]. Pyrethroids are known to be very effective at reducing transmission as they act both on the density and the longevity of the vectors [35]. In our setting, those two parameters were significantly affected by exposure to LLIN over the gonotrophic cycles. Explanations for an increase in mortality and a decrease in longevity can be diverse. They could be related to variation in resistance capacity between individual within a population, a resource-based trade-offs or an oxidative-stress [26]. Hence, delayed mortality or longevity is likely to have been increased/reduced by oxidative-stress and/or resource based trade-off as enhanced detoxification activity due to exposure has been attributed to an increased oxidative-stress [36]. Moreover, oxidative-stress has been identified as an accelerant of ageing processes in various insect models including mosquitoes [37–40]. Oxidative-stress may also explain why older mosquitoes were more susceptible than their younger counterparts, to insecticide [15, 41–43]. Regarding the reproductive success, although the number of offspring per female was not significantly different between the two groups, it was higher in the exposed group comparing to the unexposed group. Maybe this is a result of a resource-based trade-off for species perpetuation as final investment. In this study, no evidence of differed impacts of insecticide exposure on the offspring viability was observed at larval stage. These effects were not further analyzed as we consider the variance acting on those parameters to be too high to draw conclusions.

Mortality rate for both groups was high over the course of the experiment. By the end of gonotrophic cycle 4, mosquitoes were aged 25 to 28 days. At this point, 85% of the control population was eliminated and 97% of the treated group. Such an overall mortality rate approximates what can be observed in the field where only about 20% of the female live up to day 20 [44]. Those 13% difference in population survival are important for malaria transmission as only old female transmit the parasite. Reduction of longevity is thus the target of current vector control methods and insecticide resistance management strategies [33, 45–46]. While longevity remains one of the most sensitive parameters in vectorial capacity, we were able to show that the life traits that are negatively affected by exposure to insecticide even for a sublethal time can reduce overall transmission potential. And also, when combined together, the overall impact on transmission can be much higher. Thus, according to our results, it seemed that exposure to a sub-lethal dose of deltamethrin through LLIN remains efficient in controlling transmission in presence of pyrethroids resistance. It may partly explain why LLINs efficacy does not seem to have been curtailed despite the widespread of pyrethroids resistance in African malaria vectors [11, 27]. Vectors and insects in general have great adaptability potential. Nowadays, it appears that the cost of resistance is still sufficient to be exploited for resistance management strategy, but cross-resistance, adaptation and reduction of resistance cost are expected to evolve sooner or later [11]. Worrisome synergetic effects between alleles involved in resistance to different insecticide class have already been reported [47]. Thus, these findings need to be considered with cautious as there is no evident prediction if the still observed efficacy of the current vector control methods will remain so or if the insecticide resistance will affect existing vector control effort to the level of operational failures.

Conclusion

This study puts in evidence that in a context of widespread of resistance to insecticides, especially to pyrethroids, the current limited toolbox for vector control can still confer protection against malaria, however, this efficacy can be limited in time. Furthermore, the observed complexity and multiple effects of exposure on resistant females also highlighted the need for a greater understanding of the link between resistance, cost and adaptation to a changing environment.

Abbreviations

ACT

Artemisinin Combined Therapy, CSRS:Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, GC:Gonotrophic Cycle, IRS:Indoor Residual Spraying, L3:larvae stage three, LLINs:Long Lasting Insecticidal Nets, NMCP:National Malaria Control Program, RH:Relative Humidity, WHO:World Health Organization. BF:blood-feeding, M24:mortality 24-hour post-exposure, EggL:egg laying, MGC:mortality at the end of gonotrophic cycle, RS:reproductive success, E:Exposed, NE:Non exposed.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AK carried out the laboratory study and drafted the manuscript. BKF, GSC, AKFP and ZGM helped with the laboratory experiments. CSM conceived and initiated the study, ran data analysis and improved the manuscript. All authors have seen and approved that last draft.

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Figures

Figure 1

Blood-feeding of *Anopheles gambiae* after 30 minutes post-exposure at a sublethal time to a deltamethrin-treated long lasting bed-net. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant drop in blood-feeding in the exposed group (b).

Figure 2

Mortality of *Anopheles gambiae* after 24 hours post-exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant increase in mortality in the exposed group (b).

Figure 3

Mortality of *Anopheles gambiae* at the end of gonotrophic cycle (delayed mortality) following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 4 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant increase in mortality in the exposed group (b).

Figure 4

Egg laying rate of *Anopheles gambiae* following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 3 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, the non-parametric Kolmogorov-Smirnov test revealed a significant decrease in the rate of egg laying in the exposed group (b).

Figure 5

Reproductive success of *Anopheles gambiae* following exposure at a sublethal time to deltamethrin-treated long lasting bed-net. The non-parametric Kruskal-Wallis test (a) did not show any significant difference between successive gonotrophic cycles (GC) 1 to 3 either in non-exposed (NE) mosquitoes (blue color) or in exposed (E) one (red color). However, there was an increase in the rate of egg laying in the exposed group (b).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalinformation.Table1.Cohortcomposition.xlsx](#)