Evaluation of resistance to pyrethroid and organophosphate adulticides and \textit{kdr} genotyping in \textit{Aedes aegypti} populations from Roraima, the northernmost Brazilian State

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Abstract

\textbf{Background:} Roraima, the northernmost State in Brazil, borders Venezuela and Guyana. Although mostly covered by the tropical forests, the urban centers of this state are highly infested with \textit{Ae. aegypti} and are endemic for dengue, Zika and chikungunya. We accessed the insecticide resistance status of \textit{Ae. aegypti} populations from the capital Boa Vista, two cities on international borders (Pacaraima and Bonfim) and Rorainópolis bordering Amazonas State, in order to evaluate the chemical control efficacy in these localities.

\textbf{Methods:} Tests with World Health Organization (WHO)-like tubes impregnated with the pyrethroid deltamethrin (0.05\% and 0.12\%) and the organophosphate malathion (0.7\%) were conducted with \textit{Ae. aegypti} from Boa Vista, Pacaraima, Bonfim and Rorainópolis, collected in 2016 and 2018. Genotyping of \textit{kdr} mutations, related to resistance to pyrethroids, was performed for the SNP variations at sites 1016 and 1534 of the voltage gated sodium channel gene (\textit{NaV}) with a TaqMan qPCR approach.

\textbf{Results:} \textit{Aedes albopictus} was absent in our collections, and therefore only \textit{Ae. aegypti} was tested. All \textit{Ae. aegypti} populations were susceptible to 0.7\% malathion in 2016; however, mortality dropped to under 90\% in Bonfim and Pacaraima populations in 2018. All populations were resistant to 0.05\% deltamethrin in both years. The time that 50\% of females suffered knockdown (\textit{KdT}_{50}) under exposure to 0.05\% deltamethrin was 3.3–5.9-fold longer in mosquitoes from the natural populations compared to the susceptible Rockefeller strain. Only the Pacaraima population (2018) remained resistant to 0.12\% deltamethrin. \textit{kdr} genotyping revealed the absence of the wild-type \textit{NaS} haplotype (1016Val + 1534Phe) in the populations from Roraima, indicating that all tested insects had a genetic background for pyrethroid resistance. The double \textit{kdr NaR2} haplotype (1016Ile + 1543Cys) was present in higher frequencies in all populations except for Rorainópolis, where this haplotype seems to have arrived recently.

\textbf{Conclusions:} These results are important for the knowledge about insecticide resistance status of \textit{Ae. aegypti} populations from Roraima and will help improve vector control strategies that may be applied to diverse localities under similar geographical and urban conditions.

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Background
The globalization and rapid adaptation of *Aedes aegypti* to urban conditions favor its dispersion around the globe, posing a serious threat to human public health since this mosquito is a vector of many arboviruses such as dengue, Zika, chikungunya and urban yellow fever, among others [1]. Roraima is a state in the extreme North of Brazil bordering Venezuela and Guyana and is therefore an important Brazilian doorway for the entrance of emergent and re-emergent arbovirus, given the circulation of wildlife, people and goods between the borders. In addition, population genetic studies have suggested that the re-infestation of *Ae. aegypti* in Brazil likely occurred from Venezuela during the 1970's [2], evidencing the need of constant entomological surveillance in Roraima State.

In 2010, the re-emergence of DENV-4 in Brazil, with a hypothesized origin in Venezuela, was first recorded in Boa Vista, the capital of Roraima, rapidly spreading to other states in the country; Amazonas and Pará (North); Bahia, Pernambuco and Piauí (Northeast); and Rio de Janeiro and São Paulo (Southeast) [3]. Attempting to block DENV-4 circulation, efforts targeted the elimination of larval breeding sites and the intensification of chemical control with employment of the insect growth regulator diflubenzuron and the pyrethroid adulticides deltamethrin. However, this strategy did not significantly reduce *Ae. aegypti* infestation and rapidly increased the levels of insecticide resistance to the pyrethroid [4].

Insecticide resistance (IR) is a threat to the control of arboviruses at a global scale [5]. In Brazil, a nationwide IR monitoring programme coordinated by the Ministry of Health (MoH) has been screening the status of susceptibility to recommended chemicals in *Ae. aegypti* populations of strategic localities since 1999 [6]. In previous surveys, pyrethroid resistance was detected in *Ae. aegypti* from Boa Vista and Pacaraima [7, 8], probably due to selection of *kdr* mutations. These mutations are single nucleotide polymorphisms (SNPs) in the voltage gated sodium channel gene (*NaV*), which encodes a neuronal transmembrane protein that is the target of pyrethroids and DDT. In *Ae. aegypti* populations from Brazil, at least four principal SNPs in the *NaV* gene (V410L, I1011M, V1016I and F1534C) have been identified [8–10], whereas the latter two were more related to resistance to the pyrethroid knockdown effect (*kdr*) [10]. All *Ae. aegypti* samples evaluated from Boa Vista and Pacaraima in 2011 harbored *kdr* mutations, in either *NaV*R1 (1016Val+1534Cys) or *NaV*R2 (1016Ile+1534Cys) haplotypes [8]. In addition to the presence of *kdr* mutations, alterations in the activity of enzymes related to metabolic resistance, such as glutathione S-transferases (GSTs) and esterases, were also observed in these populations [7]. Given resistance to pyrethroids detected in *Ae. aegypti* populations throughout the country, the MoH has replaced these compounds with the organophosphate malathion [7], which in Roraima State was introduced in 2015 by governmental campaigns. Pyrethroids however habitually continued being applied through household sprays vastly available in market stores and by governmental programmes against malaria vectors in endemic regions.

Herein, we evaluate the profile of resistance to pyrethroid and organophosphate adulticides in *Ae. aegypti* populations of four important cities of Roraima State, Brazil, in 2016 and 2018, extending the analyses to localities not previously assessed. *Kdr* haplotype frequency was also investigated.

Methods

Collections and laboratory rearing conditions
Roraima State has a territory of 224,273.83 km² and is the least populous Brazilian state with only 0.29% of the total population in the country (605,761 inhabitants as stipulated by the Brazilian Institute of Geography and Statistics, in 2019) [11]. Roraima has limits with the Amazon in the South and borders Venezuela and Guyana (Fig. 1). The capital, Boa Vista, is 741.1 km from Manaus (the capital of Amazonas State) by the BR-174 road. This same highway connects Boa Vista and Pacaraima on the border of Venezuela, 2013.5 km to the North.

Collections of mosquito eggs were performed in four of the most important cities of Roraima State, including the capital, Boa Vista (02° 49′ 11″ N, 60° 40′ 24″ W), Bonfim (02° 45′ 22.25″ N, 60° 7′ 6.53″ W) in the Northeast on the border with Guyana, Pacaraima (04° 25′ 01″ N, 61° 08′ 27″ W) in the North on the border with Venezuela and Rorainópolis in the South on the Amazonas State limits (00° 56′ 22.62″ N, 60° 26′ 21.91″ W). Ovitraps consisted of 800 ml black plastic cups, containing 300 ml of a 0.04% yeast extract solution to attract gravid females which would lay their eggs in a wood paddle immersed in this solution for 5–7 days. The total of 150 traps were installed in Boa Vista and 50 in each of the other cities, following standard recommendations [12]. Paddles were shipped to the laboratory Núcleo de Pesquisa Observatório da Saúde at the Federal University of Roraima.
State (UFRR), eggs were stimulated to hatch and larvae reared under laboratory conditions as described elsewhere [12]. Adult mosquitoes were screened for species identification and maintained in cardboard cylindrical cages (17 × 18 cm) with a 10% sucrose solution w/v offered ad libitum. Anesthetized Wistar rats (Rattus norvegicus) were offered to blood feed females in order to produce eggs of an F1 generation, following procedures as recommended in the license # 12/2015 approved by the ethical committee on animal use of UFRR (CEUA-UFRR). The reference lineage Rockefeller Ae. aegypti mosquitoes, maintained at LAFICAVE/Fiocruz since 1999 [12], were raised in parallel and adopted in all assays as a control of insecticide susceptibility and vigor under test conditions.

**Insecticide resistance related assays**

**Bioassays**

Bioassays were performed with F1 generation Ae. aegypti females 3–5 days post-adult emergence with World Health Organization (WHO) tube tests [13]. We employed the pyrethroid deltamethrin (technical grade; Sigma-Aldrich, St. Louis, USA) dissolved in acetone to a 1% stock solution, this was then diluted to a concentration of 0.05% and 0.12% in silicone oil (Dow Corning, Midland, USA) as the solvent carrier. Malathion (Sangroce Agroquímica, Curitiba, Brazil) emulsion concentrate was diluted in the silicone oil to a concentration of 0.7% and subsequently applied over a 12 × 15 cm filter paper (Whatman Grade 1) as previously described [14]. It is noteworthy that olive oil is the solvent/carrier recommended for paper impregnation with organophosphates. However, we employed silicone oil since we did not have a source of olive oil (chemical grade) with a certificate of analyses available in the country. Mosquitoes were exposed for 1 h in the tube chamber with insecticide and then gently transferred to the resting chamber until mortality was registered 24 h later. In addition, the knockdown rate was evaluated every 5 min (or 2 min for the Rockefeller strain) during exposure to 0.05% deltamethrin. A probit analysis [15] was incorporated in order to calculate the time when 50% of females were knocked down (KdT<sub>50</sub>). The knockdown resistance ratios (RR KdT<sub>50</sub>) of the populations were obtained by the quotient between the KdT<sub>50</sub> of the populations with the Rockefeller strain, the control strain.

**kdr genotyping**

DNA was extracted from male mosquitoes and genotyped for the single nucleotide polymorphisms (SNPs) at the 1016 (Val<sup>+</sup> or Ile<sup>kdr</sup>) and 1534 (Phe<sup>+</sup> or Cys<sup>kdr</sup>) sites of the voltage gated sodium channel gene (Na<sub>V</sub>) using exactly the same procedures and reagents as described by Macoris et al. [16]. As these sites are linked in the same gene, both SNPs were considered in order to define the genotype frequencies for a single locus [8].
The haplotypes usually found in Ae. aegypti populations from Brazil are the wild-type Na,v S (1016 Val + 1534 Phe) and the kdr Na,v R1 (1016Val + 1534Cys) and Na,v R2 (1016Ile + 1534Cys), whereas the possible genotypes are SS, SR1, SR2, R1R1, R1R2 and R2R2. As kdr mutations act as a recessive trait, insects resistant to pyrethroids based on this target site mechanism present one of the genotypes R1R1, R1R2 or R2R2. The Na,v R2 haplotype generates higher levels of resistance [14].

Results

Bioassays

We evaluated the susceptibility status of Ae. aegypti from four Roraima municipalities to the pyrethroid deltamethrin (0.05% and 0.12%) and the organophosphate malathion (0.7%) adulticides, in 2016 and 2018.

In 2016, populations exhibited some survival to malathion 0.7%, however with mortality rates greater than 90%, except that from Rorainópolis, attaining a mortality rate of 100% (Fig. 2). There was a reduction in the mortality levels in 2018 in all localities, ranging from 84.8% in Pacaraima to 94.2% in Rorainópolis (Table 1). Therefore, Pacaraima and Boa Vista were classified as resistant to malathion in 2018.

With regard to the pyrethroid, all populations were considered resistant both in 2016 and 2018 when evaluated with 0.05% deltamethrin. There was an increase from 2016 to 2018 in the mortality in Boa Vista (64.0 to 86.6%) and Bonfim (31.2 to 78.5%) and a decrease in Rorainópolis (89.9 to 74.3%) and Pacaraima (78.3 to 61.7%). A higher dosage of deltamethrin (0.12%) was tested in parallel, and as expected, this higher concentration increased the mortality in all cases; however, mortality did not reach 100% in any population (Fig. 3a). We also evaluated the knockdown rate during exposure to deltamethrin 0.05% for 1h (Additional file 1: Figure S1). According to a probit analysis, 50% of the Rockefeller strain females were knocked down (kDT 50) within 11.9 min, compared to 29.6 and 69.9 min in Boa Vista (2018) and Bonfim (2016) populations, respectively (Table 2). Nevertheless, the kDT 50 values of all populations were similar if we consider the 95% confidence (CI) interval, except for Bonfim population in 2016, which showed a higher kDT 50 (Fig. 3b). The knockdown-resistance ratio (kDT-RR 95) of the populations ranged from 2.5 to 5.9 (Table 2).

kdr genotyping

We successfully genotyped 282 Ae. aegypti mosquitoes from Roraima State for both 1016 (Val + or Ile kdr) and 1534 (Phe + or Cys kdr) sites. All tested insects demonstrated a kdr mutation in at least one site, presenting genotypes: homozygotes for the 1534 kdr (R1R1) and the double kdr 1016 + 1534 (R2R2) in addition to the heterozygote (R1R2). Therefore, there was no evidence of the wild-type haplotype S (1016Val + 1534Phe) in Ae. aegypti populations from Roraima (Fig. 4a).

The double kdr haplotype R2 (1016 Ile + 1534Cys) had a higher frequency than R1 (1016 Val + 1534Cys) in Boa Vista (2018), Bonfim (2016 and 2018) and Pacaraima (2016 and 2018) (Table 3). In Rorainópolis, R2 showed low frequency (8 and 11% in 2016 and 2018, respectively), only appearing in heterozygotes R1R2 (Fig. 4b). In all cases, the genotypic frequencies did not deviate from the Hardy-Weinberg equilibrium assumption (Table 3).

When comparing the genotypic frequencies between the 2016 and 2018 collections, the R2R2 genotype decreased in Boa Vista (25 to 18.6%), Bonfim (45 to 36.7%) and particularly in Pacaraima (45.5 to 20.7%). Indeed, as aforementioned, the levels of mortality to 0.05% deltamethrin increased in both Boa Vista and Bonfim, however decreasing in Pacaraima (Fig. 3b). We also genotyped some dead (susceptible) and survived (resistant) mosquitoes from Boa Vista and Bonfim 24 h after 1 h of exposure to 0.05% deltamethrin. Although the number of samples was small (41 live and 14 dead), the R1R1 genotype was absent in the resistant group while R2R2 was more representative (Fig. 4c).

Discussion

Here, we showed that Ae. aegypti populations from Roraima State, Brazil collected in 2016 and 2018 were resistant to the pyrethroid deltamethrin and under the process of becoming resistant to the organophosphate malathion. Remarkably, only Ae. aegypti was present in these collections, regardless of a recent register of Ae. albopictus in a rural area of Rorainópolis.

Pyrethroid resistance in Ae. aegypti from Roraima State had already been high in previous evaluations. The first registers were in 2007 and 2010 when populations of Ae. aegypti from the capital Boa Vista were detected as pyrethroid-resistant [4, 17]. In 2011, the population from Pacaraima presented the second highest resistance ratio (RR95 = 60.3) in the whole country [7]. Herein we demonstrate that even two years after pyrethroid governmental application was substituted by malathion, pyrethroid resistance has been maintained in Ae. aegypti populations from the four localities evaluated. It is noteworthy that a new diagnostic dose was established for deltamethrin (0.03%) in WHO paper tests [13]. In this study we adopted a higher dosage (0.05%), signifying that the rate of mortality would probably be even lower if tested with 0.03% deltamethrin papers.

The Brazilian Ministry of Health started replacing pyrethroids with the organophosphate malathion in ultra-low volume-based applications against Ae. aegypti in 2010...
Although pyrethroids are still used in campaigns against other vectors such as anophelines, phlebotomines and triatomines. In Roraima State, the first stock of malathion was received in December 2015. Herein, we showed that all four *Ae. aegypti* populations collected in 2016, although not 100% killed by malathion 0.7%, had mortality above 90%, and were not classified as resistant. The rate of mortality decreased to under 90% in Bonfim and Pacaraima two years later, the mosquitoes therefore being classified as resistant to malathion. The diagnostic dose for malathion indicated by the WHO is a bit higher, 0.8% [13]. Had we used 0.8% instead of 0.7%, the decrease in the mortality levels from 2016 to 2018 might have also been noticeable.

Different from what occurs with pyrethroids, the active ingredient of household sprays and other governmental campaigns, the only probable source of organophosphate pressure was the governmental campaigns with malathion at that time. The larvicide temephos has in theory not been applied in Roraima since 2013, when it was definitively substituted by IGRs [7]. The most recent data about temephos resistance in Roraima indicated resistance ratios (RR<sub>50</sub>) of 2.0 in Boa Vista (2010) and 4.3 in Pacaraima (2011), which were not considered high levels of resistance [4, 17]. However, we cannot reject the possibility that mechanisms prior selected by temephos and pyrethroids are inducing cross-resistance to malathion, as reported in some classical studies. For instance, a laboratory strain of *Culex quinquefasciatus* selected for temephos resistance in larvae developed cross-resistance to several organophosphate adulticides, including malathion [18]. On the other hand, in Guadeloupe and Saint Martin Caribbean islands, *Ae. aegypti* populations developed high levels of resistance to temephos (8.9–33.1-fold) but low levels to malathion (1.7–4.4-fold) [19].

Concerning the possible mechanisms selected for insecticide resistance, alterations in the activity of GST and esterase enzymes were detected in Boa Vista (2007) and Pacaraima (2011). Reduced activity of the acetylcholinesterase enzyme was also observed in Pacaraima (2011) [7]. Lineages of *Ae. aegypti* Brazilian populations that acquired resistance to malathion through selection pressure in the laboratory exhibited increased activity of GST, multi-function oxidases (MFO P450) and esterases, as determined by biochemical analyses [20]. Overexpression of genes related to metabolic resistance was detected in *Ae. aegypti* populations from the Caribbean, such as French Guyana and French West Indies islands [19, 21]. On the other hand, the high levels of resistance to pyrethroids in Roraima might be partially justified by the absence of the wild-type Na<sub>V</sub>S haplotype, already reported in 2010 and 2011 in Boa Vista and Pacaraima [8].

**Table 1** Bioassays with the organophosphate malathion (0.7%) in *Aedes aegypti* populations from Roraima State, Brazil (2016 and 2018)

| Population     | 2016      | 2018      |
|----------------|-----------|-----------|
|                | n (mean mortality±SD (%)) | n (mean mortality±SD (%)) |
| Boa Vista      | 175 97.8±2.48 | 197 100±0  |
| Bonfim         | 171 97.1±4.97 | 181 85.6±16.77 |
| Rorainópolis   | 185 95.9±2.37 | 184 94.2±7.51 |
| Pacaraima      | 188 93.6±8.37 | 178 84.8±25.01 |

**Fig. 2** Evaluation of mortality of *Ae. aegypti* from Roraima caused by the organophosphate malathion. Bars indicate the mean percent mortality (± standard error of the mean) registered 24 h after exposure to malathion 0.7% for 1 h. Populations with mortality > 90% (red dotted line) are classified as resistant to the insecticide
missing with predominance of the double \textit{kdr Na}_vR2 (1016Ile+1534Cys), except in Rorainópolis, where the \textit{Na}_vR1 (1016Val+1534Cys) predominates. We corroborated that \textit{Na}_vR2 leads to higher levels of resistance to pyrethroids [14] once homozygote R2R2 insects were only present among the survivors in the bioassays with Boa Vista and Bonfim. The remaining high levels of resistance to pyrethroids even after the substitution by malathion in Roraima may be associated with the high prevalence of domestic use of insecticides, all composed of pyrethroids and easily acquired in local markets, as reported in other Brazilian states [16, 22]. In addition,

**Table 2** Time of knockdown to the pyrethroid deltamethrin (0.05%) in \textit{Aedes aegypti} populations from Roraima State, Brazil (2016 and 2018)

| Populations          | 2016 | 2018 | 2018 |
|----------------------|------|------|------|
|                      | n    | \(KdT_{50}\) min | 95% CI | \(KdT-RR_{50}\) | n       | \(KdT_{50}\) min | 95% CI | \(KdT-RR_{50}\) |
| Boa Vista            | 320  | 40.6 | 34.7–47.5 | 3.4 | 178  | 29.6 | 24.9–35.3 | 2.5 |
| Bonfim               | 146  | 69.9 | 52.9–92.4 | 5.9 | 172  | 34.4 | 28.8–41.2 | 2.9 |
| Rorainópolis         | 187  | 40.6 | 34.6–47.8 | 3.4 | 177  | 42.7 | 38.1–47.9 | 3.6 |
| Pacaraima            | 182  | 39.8 | 35.4–44.7 | 3.3 | 193  | 42.1 | 35.1–50.6 | 3.5 |
| Rockefeller strain   | 182  | 11.9 | 8.6–16.5  | \(b\) | \(b\) | \(b\) | \(b\) | \(b\) |

**Abbreviations:** n, total number of insects used/ population; 95% CI, 95% confidence interval

**Fig. 3** Evaluation of mortality of \textit{Ae. aegypti} from Roraima caused by the pyrethroid deltamethrin. \textbf{a} Bars indicate the mean percent mortality (± standard error of mean) registered 24 h after 1 h exposure to deltamethrin. Populations with mortality beyond 90% (red dotted line) are classified as resistant to the insecticide. \textbf{b} \(KdT_{50}\) with 95% CI. Bars with identical letters indicate similar times (overlapped 95% CI)
we cannot neglect the migration of *Ae. aegypti* resistant populations from the neighboring countries and measures adopted against other vector-borne diseases. On the border between Pacaraima (Brazil) and Santa Helena (Venezuela) there is an intense control of malaria where pyrethroids are employed against *Anopheles* even in the urban area, thus also submitting *Ae. aegypti* to this selection pressure. In Bonfim, it is common to find pyrethroid
sprays acquired in Lethen City, on the Guyanese side of the border.

We speculate that the kdr Na,R2 haplotype in Ae. aegypti populations in this region must have migrated mostly from Venezuela as it has been detected in Roraima State at least since 2010, when it was either absent or at low frequencies in the neighboring states Amazonas and Pará [8]. Interestingly, Rorainópolis is the only municipality in Roraima with Ae. albopictus colonization; however it is encountered in areas on the border with Amazonas State where the species has been recorded since 2015 (CGVS-SESAU-RR). Therefore, it seems that the transition between the biomes, Amazon Forest in the south and the savannah-like “Lavrado” in the north, has been limiting the dispersion of Ae. aegypti from Roraima downwards to the Amazonas State. On the other hand, Ae. albopictus from Amazonas has not yet invaded the capital Boa Vista in the Lavrado zone.

Besides new promising tools of Ae. aegypti control currently being tested in Brazil, such as Wolbachia, release of insects carrying a dominant lethal gene (RIDL) and pyriproxyfen autodissemination stations [23–26], insecticides continue to play an important role on a long-term basis in order to rapidly decrease the densities of a target population and consequently, mitigate the cycle of the arboviruses. Therefore, it is of prime importance that a constant surveillance of insecticide resistance be inherent to the chemical control strategy so as to guide authorities about product efficacy.

**Conclusions**

We provided evidence that Ae. aegypti populations from Roraima were resistant to the pyrethroid deltamethrin in 2016 and 2018, probably due to selection pressure exerted by household use of insecticide sprays. The kdr mutations were present under high frequencies and were probably the main mechanism acting in favor of pyrethroid resistance in these populations. Mortality under 90% in bioassays with malathion was observed in some populations in 2018, evidencing that resistance has also been selected to this chemical. *Aedes albopictus* were absent from our collections. These results are important for our understanding of the status of insecticide resistance of *Ae. aegypti* populations from Roraima, and will help improve vector control strategies that may be applied to diverse localities under similar geographical and urban conditions.

**Supplementary information**

Supplementary information accompanies this paper at [https://doi.org/10.1186/s13071-020-04127-w](https://doi.org/10.1186/s13071-020-04127-w)

Additional file 1: Figure S1. Knockdown curve to 0.05% deltamethrin. *Aedes aegypti* populations from Boa Vista, Bonfim, Rorainópolis and Pacaraima, collected in 2016 and 2018, additional to the reference lineage Rockefeller. Red dotted line indicates the kGT_{50}.

**Abbreviations**

DDT: Dichlorodiphenyltrichloroethane; Na,R: Voltage-gated sodium channel gene; WHO: World Health Organization; MoH: Ministry of Health; LIRAa: Quick survey of *Aedes aegypti* indexes; IGR: Insect growth regulator; KdR: Knockdown resistance; RR: Resistance ratio; kGT: Time of knockdown; SNP: Single nucleotide polymorphism; 95% CI: 95% Confidence interval.

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**Authors’ contributions**

RLNH and AJM conceptualized the study, curated the data and performed the formal analysis. RLNH, JBPL and AJM acquired the funding and RLNH, AJM, JML and NCVA performed the investigation. LC, JBPL and AJM carried out the methodology. AJM provided project administration and JBPL and AJM provided resources. AJM provided supervision and validated the study. All authors assisted with visualization of the study. RLNH and AJM wrote the original draft of the manuscript, and all authors reviewed and edited the manuscript. All authors read and approved the final manuscript.
References

1. Kraemer MUG, Reiner RC Jr, Brady OJ, Messina JP, Gilbert M, Pigott DM, et al. Past and future spread of the arbovirus vectors Aedes aegypti and Aedes albopictus. Nat Microbiol. 2019;4:854–63.

2. Kotsakiozi P, Gloria-Soria A, Caccone A, Evans B, Schama R, Martins AJ, et al. Tracking the return of Aedes aegypti to Brazil, the major vector of the dengue, chikungunya and Zika viruses. PLoS Negl Trop Dis. 2017;11:e0005653.

3. de Souza RP, Rocco IM, Maeda AV, Spenasassato C, Bisordi I, Suzuki A, et al. Dengue virus type 4 phylogenetics in Brazil 2011: looking beyond the veil. PLoS Negl Trop Dis. 2011;5:e1439.

4. Maciel-de-Freitas R, Avendanho FC, Santos R, Sylvestre G, Araujo SC, Lima JB, et al. Undesirable consequences of insecticide resistance following Aedes aegypti control activities due to a dengue outbreak. PLoS One. 2014;9:e92424.

5. Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, et al. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS Negl Trop Dis. 2017;11:e0005625.

6. Braga IA, Valle D. Aedes aegypti: vigilância, monitoramento da resistência e alternativas de controle no Brasil. Epidemiol Serv Saúde. 2007;16:295–302.

7. Valle D, Bellinati DF, Viana-Medeiros PF, Lima JBP, Martins AJ, Junior A. Resistance to temephos and deltamethrin in Aedes aegypti from Brazil between 1985 and 2017. Mem Inst Oswaldo Cruz. 2019;114:e180544.

8. Linss JG, Brito LP, Garcia GA, Araki AS, Bruno RV, Lima JB, et al. Distribution and dissemination of the Val10166e and Phe1534Cys kdr mutations in Aedes aegypti Brazilian natural populations. Parasit Vectors. 2014;7:25.

9. Martins AJ, Brito LP, Linss JG, Rivas GB, Machado R, Bruno RV, et al. Evidence for gene duplication in the voltage-gated sodium channel gene of Aedes aegypti. Evol Med Public Health. 2013;2013:148–60.

10. Haddi K, Tomé HV, Duf Y, Valbon WR, Nomura Y, Martins GF, et al. Detection of a new pyrethroid resistance mutation (Y410L) in the sodium channel of Aedes aegypti: a potential challenge for mosquito control. Sci Rep. 2017;7:46549.

11. IBGE: IBGE Cidades. 2019. https://cidades.ibge.gov.br/brasil/rr/panorama. Accessed 12 Jan 2019.

12. Lima JB, Da-Cunha MP, Da Silva RC, Galardo AK, Soares Sda S, Braga IA, et al. Resistance of Aedes aegypti to organophosphates in several municipalities in the State of Rio de Janeiro and Espirito Santo, Brazil. Am J Trop Med Hyg. 2003;68:329–33.

13. WHO. Monitoring and managing insecticide resistance in Aedes mosquito populations-intern guidance for entomologists, vol. WHO/ZIKV/V/C. 16/1. Geneva: World Health Organization, 2016.

14. Brito LP, Carrara L, Freitas RMd, Lima JBP, Valle D. Frequency of pyrethroid resistance persists after ten years without usage against Aedes aegypti in governmental campaigns: lessons from Sao Paulo State, Brazil. PLoS Negl Trop Dis. 2018;12:e0006590.

15. Macoris ML, Martins AJ, Andringhetti MMT, Lima JBP, Valle D. Fitness evaluation of two Brazilian Aedes aegypti field populations with distinct levels of resistance to the organophosphate temephos. Mem Inst Oswaldo Cruz. 2012;7:916–22.

16. Peiris HTR, Hemingway J. Temephos resistance and the associated cross-resistance spectrum in a strain of Culex quinquefasciatus Say (Diptera: Culicidae) from Pelliagoda, Sri Lanka. Bull Entomol Res. 1990;80:49–55.

17. Goidin D, Delannay C, Gelairse A, Ramdini C, Gaude T, Faucon F, et al. Levels of insecticide resistance to deltamethrin, malathion, and temephos, and associated mechanisms in Aedes aegypti mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). Infect Dis Poverty. 2017;6:38.

18. Viana-Medeiros PF, Bellinati DF, Valle D. Laboratory selection of Aedes aegypti field populations with the organophosphate malathion: negative impacts on resistance to deltamethrin and to the organophosphate temephos. PLoS Negl Trop Dis. 2018;12:e0006734.

19. Raymond M. Presentación d’une programme d’analyse logprobit pour microcoordonnée. Cah ORSTOM Sér Ent Med Parasitol. 1985;22:117–21.

20. Macoris ML, Martins AJ, Andringhetti MMT, Lima JBP, Valle D. Fitness evaluation of two Brazilian Aedes aegypti field populations with distinct levels of resistance to the organophosphate temephos. Mem Inst Oswaldo Cruz. 2012;7:916–22.

21. Dusfour I, Zorrilla P, Guidez A, Issaly J, Giord R, Guillamont L, et al. Deltamethrin resistance mechanisms in Aedes aegypti populations from three French overseas territories worldwide. PLoS Negl Trop Dis. 2015;9:e0004226.

22. Garcia GA, Sylvestre G, Aquiar R, Costa GB, Martins AJ, Lima JBP. Matching the genetics of released and local Aedes aegypti populations is critical to assure Wolbachia invasion. PLoS Negl Trop Dis. 2019;13(1):e0007023.

23. Achee NL, Grieco JP, Vatandoost H, Seixas G, Pinto J, Ching-Ng L. Alternatives for mosquito-borne arbovirus control. PLoS Negl Trop Dis. 2019;13(1):e0006822.

24. O'Neill SL, Ryan PA, Turley AR, Wilson G, Retzki K, Iturbe-Ormaetxe I, et al. Tracking the return of Aedes aegypti to Brazil, the major vector of the dengue, chikungunya and Zika viruses. PLoS Negl Trop Dis. 2015;9:e0004226.

25. Evans BR, Kotsakiozi P, Costa-da-Silva AL, Ioshino RS, Garziera L, Pedrosa MC, et al. Transgenic mosquitoes transfer genes into a population of mosquitoes from the Guadeloupe and Saint Martin islands (French West Indies). Infect Dis Poverty. 2017;6:38.

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