Multiple-Insecticide Resistance in Anopheles gambiae Mosquitoes, Southern Côte d’Ivoire

Constant V.A. Edi, Benjamin G. Koudou, Christopher M. Jones, David Weetman, and Hilary Ranson

Malaria control depends on mosquito susceptibility to insecticides. We tested Anopheles gambiae mosquitoes from Côte d’Ivoire for resistance and screened a subset for target site mutations. Mosquitoes were resistant to insecticides of all approved classes. Such complete resistance, which includes exceptionally strong phenotypes, presents a major threat to malaria control.

Targeting the mosquito vector is the most effective way to prevent malaria transmission; worldwide, this method accounts for more than half of malaria control expenditures (1,2). During the past decade, increased use of insecticide-treated bed nets and indoor residual spraying have made a pivotal contribution toward decreasing the number of malaria cases (1). However, these gains are threatened by the rapid development and spread of insecticide resistance among major malaria vectors in Africa (3). To keep vector resistance from undermining control programs, insecticide-resistance management strategies must reduce the current overreliance on pyrethroids. These compounds are used widely for indoor residual spraying and uniquely for insecticide-treated bed nets. However, having a limited number of insecticides available for malaria vector control restricts options for effective insecticide resistance management. Only 4 classes of insecticide, which share 2 modes of action, are approved by the World Health Organization (WHO).

A mutation at a single target site can result in mosquito resistance to DDT and pyrethroids or to organophosphates and carbamates. Furthermore, mosquitoes can express multiple insecticide-resistance mechanisms (4). For example, in several populations of the major malaria vector in Africa, Anopheles gambiae s.l. mosquitoes, mutations in the DDT/pyrethroid target site, known as knockdown resistance (kdr) alleles, have been found in conjunction with resistance alleles of the acetylcholinesterase gene (Ace-1S), the target site of organophosphates and carbamates (5). To date, however, these cases of multiple-insecticide resistance have been restricted by the relatively low prevalence of organophosphate/carbamate resistance and the limited effect that kdr mutations alone have on pyrethroid-based interventions (6). We report a population of An. gambiae mosquitoes from a rice-growing area of southern Côte d’Ivoire that have high frequencies of kdr and Ace-1S alleles and unprecedentedly high levels of phenotypic resistance to all insecticide classes available for malaria control.

The Study

During May–September 2011, mosquito larvae were collected in irrigated rice fields surrounding Tiassalé, southern Côte d’Ivoire (5°52′47″N; 4°49′48″W) and reared to adults in insectaries on a diet of MikroMin (Tetra, Melle, Germany) fish food. A total of 1,571 adult female An. gambiae s.l. mosquitoes, 3–5 days of age, were exposed to 1 of 5 insecticides (0.1% bendiocarb, 1.0% fenitrothion, 0.75% permethrin, 0.05% deltamethrin, 4% DDT) or a control paper for 1 hour, according to standard WHO procedures (7). Mosquito deaths were recorded 24 hours later. DNA was extracted from individual mosquitoes according to the LIVAK method (8), and a subsample of 500 mosquitoes were all found to be the M molecular form of An. gambiae s.s. by using the SINE-PCR method (9). The target site mutation G119S in the insecticide resistance Ace-1R allele was found in 250 mosquitoes. The target site mutation L1014F and L1014S in the DDT/pyrethroid target site, known as knockdown resistance (kdr) alleles, have been found in conjunction with resistance alleles of the acetylcholinesterase gene (Ace-1S), the target site of organophosphates and carbamates (5). To date, however, these cases of multiple-insecticide resistance have been restricted by the relatively low prevalence of organophosphate/carbamate resistance and the limited effect that kdr mutations alone have on pyrethroid-based interventions (6). We report a population of An. gambiae mosquitoes from a rice-growing area of southern Côte d’Ivoire that have high frequencies of kdr and Ace-1S alleles and unprecedentedly high levels of phenotypic resistance to all insecticide classes available for malaria control.

Table 1. Prevalence of insecticide resistance in Anopheles gambiae mosquitoes, M form, from Tiassalé, Côte d’Ivoire, 2011

| Insecticide | No. tested | No. dead | % Dead (95% CI) |
|-------------|------------|----------|-----------------|
| Permethrin  | 288        | 69       | 24.0 (19.1–29.3) |
| Deltamethrin| 282        | 90       | 31.9 (26.5–37.7) |
| DDT         | 306        | 25       | 8.2 (5.4–11.8)  |
| Fenitrothion| 296        | 219      | 74.0 (68.6–78.9) |
| Bendiocarb  | 299        | 37       | 12.4 (8.9–16.6) |

*Measured by death within 24 h, after 1h exposure to each insecticide. All mosquitoes were resistant according to World Health Organization classification (<80% dead) (7).
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We found an unexpectedly strong resistance phenotype to the 2 insecticides (Figures 1, 2). For deltamethrin, 4 hours of exposure were required to kill 50% (median lethal time, [LT50]); in comparison, the LT50 for the susceptible Kisumu strain was <2 minutes (resistance ratio = 138) (online Technical Appendix). Similarly, the LT50 for bendiocarb was nearly 5 hours for the Tiassalé strain yet <12 minutes for the susceptible strain (resistance ratio = 24) (online Technical Appendix).

To investigate the causes of this resistance, we screened a subset of mosquitoes for the target site mutations, kdr1014F and 1014S. Only the 1014F kdr mutation was detected, and this resistance allele was found at high frequency (83%). There was a significant association between presence of the 1014F kdr allele and ability to survive exposure to DDT but not to either pyrethroid (Table 2). In contrast, the Ace-1R allele was strongly associated with survival after exposure to bendiocarb and fenitrothion (Table 2).

Conclusions

Pyrethroid resistance in An. gambiae mosquitoes was first reported from Côte d’Ivoire in 1993 (12); carbamate resistance was detected in the 1990s (13). Nevertheless, ≈2 decades later, it is surprising and worrying to find complete resistance to all insecticides tested, particularly for deltamethrin and bendiocarb—at such high levels. Resistance mechanisms seem to be varied. Ace-1R is strongly associated with organophosphate and carbamate resistance, and the absence of 119S homozygotes might be attributable to the high fitness cost of the Ace-1R allele in the absence of insecticide (14). Presence of the 1014F kdr allele alone does not confer the ability to survive diagnostic doses of pyrethroids; thus, alternative mechanisms must be responsible for the high-level pyrethroid resistance in this population.

The selective pressures responsible for this intense multiple-insecticide resistance in Tiassalé mosquitoes are unclear. There is a high coverage of insecticide-treated bed nets, but this coverage does not differ from that in other parts of the continent, and indoor residual spraying has not been conducted in this region. Use of insecticides in agriculture has been linked to resistance in malaria vectors. This use is perhaps the most likely explanation in this district of intense commercial production of rice, cocoa, and coffee.

Whatever the cause, the implications of this resistance scenario for malaria control are severe. With no new classes of insecticides for malaria control anticipated until 2020 at the earliest (15), program managers have few options available when confronted with multiple-insecticide resistance. Assessing the effect of pyrethroid resistance on the efficacy of insecticide-treated bed nets is complex because of the poorly understood associations between net integrity, insecticide content, net usage, and net efficacy. Nevertheless, resistance levels, such as those reported here, combined with continual selection pressure will inevitably lead to suboptimal mosquito control by use of insecticide-treated bed nets and indoor residual spraying. If unchecked, this resistance could spread rapidly and threaten the fragile gains that have been made in reducing malaria across Africa.
Table 2. Association between genotype and mosquito survival after insecticide exposure*

| Insecticide | No. tested | Status | No. | LL | LF | FF | Frequency† | Odds ratio§ | p value |
|-------------|-----------|--------|-----|----|----|----|------------|-------------|---------|
| DDT         | 73        | Alive  | 48  | 2  | 7  | 39 | 88.5       | 4           | 0.02    |
|             |           | Dead   | 25  | 2  | 10 | 13 | 72         |             |         |
| Permethrin  | 88        | Alive  | 44  | 1  | 12 | 31 | 84.1       | 1.23        | 0.82    |
|             |           | Dead   | 44  | 3  | 12 | 29 | 79.5       |             |         |
| Deltamethrin| 89        | Alive  | 45  | 1  | 12 | 32 | 84.4       | 0.82        | 0.86    |
|             |           | Dead   | 44  | 2  | 9  | 33 | 85.2       |             |         |
|              |            | GG     | 50  | 48 | 2  | 0  | 50         | 100         | 0.40 × 10^-12 |
|              |            | GS     | 48  | 48 | 2  | 0  | 50         |             |         |
|              |            | SS     | 50  | 50 | 2  | 0  | 50         | 1,176       | 0       |

*F and L represent mutant resistant alleles (phenylalanine) and wild-type alleles (leucine), respectively; S and G represent mutant resistant alleles (serine) and wild-type alleles (glycine), respectively. No resistant homozygotes GG were found among the 186 mosquitoes genotyped for Ace-1R by restriction fragment length polymorphism (a subset of 48 was further screened by using the TaqMan assay; congruence between the 2 methods was 100%).
†The frequencies were calculated for each insecticide and mosquito status (alive/dead) after exposure.
‡1014F represent the kdr frequencies.
§Genotypic odds ratios (ORs) are shown because these exceed allelic ORs for DDT (recessive model), bendiocarb, and fenitrothion (both overdominant models), and are similar for permethrin and deltamethrin. For bendiocarb and fenitrothion absence of GG genotypes in the “Alive” group means that ORs are infinity, therefore ORs are shown if one GG was present. F and L represent mutant resistant alleles (phenylalanine) and wild-type alleles (leucine), respectively; S and G represent mutant resistant alleles (serine) and wild-type alleles.

Mr Edi is a PhD student at the Liverpool School of Tropical Medicine. His research interests are the causes and consequences of insecticide resistance in malaria vectors.

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Address for correspondence: Hilary Ranson, Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK; email: hranson@liverpool.ac.uk

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Anopheles

[ə-nofˈe-lēz]

From the Greek an (“not”) + ophelos (“benefit”), a genus of mosquitoes, many species of which are vectors of malaria. Anopheles was first described by German entomologist Johann Wilhelm Meigen in 1818. Although some sources translate Anopheles as “harmful,” it would be decades before Ronald Ross showed in 1897 that these mosquitoes transmit malaria parasites, and Meigen was most likely using Anopheles in a more literal interpretation as “useless.” That said, the connotation of “harmful” was prophetic in describing a mosquito that, even today, is indirectly responsible for ≈1 million deaths per year.

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Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30333, USA; email: boq3@cdc.gov

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**Technical Appendix**

Table. Time–death data for adult female *Anopheles gambiae* s.s. Tiassalé strain and standard susceptible colony Kisumu 24 hours after exposure to bendiocarb or deltamethrin

| Strain  | Insecticides | Parameters | 0.5 | 1   | 2   | 3   | 5   | 10  | 15  | 25  | 30  | 60  | 120 | 240 | 260 | 280 | 300 | 330 | 340 | 360 | 480 | 720 |
|---------|--------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tiassalé| Bendiocarb (0.1%) | No. tested | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |       |     |     |     |     |     |     |     |     |     |     |     |     |
|         |              | No. dead   | 12  | 28  | 45  | 62  | 67  | 78  | 80  |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | Mortality (%) | 12  | 28  | 45  | 62  | 67  | 78  | 80  |     |     |       |     |     |     |     |     |     |     |     |     |     |
|         | Deltamethrin (0.05%) | No. tested | 100 | 100 | 100 | 100 |     |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | No. dead   | 32  | 34  | 49  | 87  |     |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | Mortality (%) | 32  | 34  | 49  | 87  |     |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
| Kisumu  | Bendiocarb (0.1%) | No. tested | 50  | 50  | 50  | 50  | 50  |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | No. dead   | 2   | 13  | 43  | 48  | 50  |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | Mortality (%) | 4   | 26  | 86  | 96  | 100 |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |     |
|         | Deltamethrin (0.05%) | No. tested | 50  | 50  | 50  | 50  | 50  |     |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |     |
|         |              | No. dead   | 11  | 23  | 28  | 46  | 49  | 49  |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |
|         |              | Mortality (%) | 22  | 46  | 56  | 92  | 98  | 98  |     |     |     |       |     |     |     |     |     |     |     |     |     |     |     |     |
Figure. Time-death curve for Anopheles gambiae mosquitoes, Kisumu strain, exposed to (top) deltamethrin (median time to death = 1.8 minutes, $R^2= 0.93$) and (bottom) bendiocarb (median time to death = 12 minutes, $R^2= 0.99$).