Permethrin and Dichlorodiphenyltrichloroethane (DDT) resistant *Anopheles gambia* and *Culex quinquefasciatus* in Southern Ghana

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Insecticides play a major role in controlling the spread of malaria. However, this means of protection is losing its efficacy due to the development of resistance to commonly used insecticides. Pyrethroids are often used in vector control projects due to lower human toxicity, high insecticidal potency and rapid knockdown (KD) effects. *Anopheles gambia* is a major vector of malaria in West Africa and has shown pyrethroid resistance in several countries. The other species tested, *Culex quinquefasciatus*, is not a vector of malaria but causes important inconvenience to the locals. In this study *A. gambia* and *C. quinquefasciatus* larvae were collected from five separate locations in Central and Western Ghana: Amamoma, Duakor, North O.L.A., Okyereko and Sekondi. Adult mosquitoes were exposed to permethrin (0.75%) and DDT (4%) according to WHO protocol. Knockdown, mortality and resistance were recorded over a 24-hour period. Six experiments were conducted under WHO protocol resulting in an 4.65% average mortality for *A. gambia* and an 80.53% for *C. quinquefasciatus*. These results support the hypothesis that resistance continues to rise in *Anopheles* mosquitoes despite new insecticide combinations. Recording and understanding resistance is essential for effective vector control and disease prevention using different insecticides combinations in impregnated nets.

**INTRODUCTION**

Malaria eradication in the U.S.A., U.S.S.R., Southern Europe and most Caribbean islands was due to successful vector control using insecticides (Macdonald 1957, Bruce-Chwatt 1985, Spielman et al. 2001, Curtis 2002). Insecticide resistance in African malaria vector mosquitoes is an increasing problem for programs aimed at fighting against this issue with the use of insecticide treated nets. Vectors’ resistance status must be assessed in specific areas before deciding on strategies to control malaria.

Currently, pyrethroids are the only class of insecticide recommended by the World Health Organization (WHO) for the treatment of bed nets. However, resistance has emerged and is rapidly spreading, especially in *Anopheles gambia* (Hemingway et al. 2002, Etang et al. 2004, WHO, 2005). *Anopheles gambia* shows pyrethroid/DDT resistance due to the knockdown resistance mutation (*kdr*) of the sodium channel gene that leads to the substitution of leucine for phenylalanine (Adasi and Hemingway, 2008).

In West Africa *C. quinquefasciatus* exploits the conditions created by tropical urbanization. This species thrives in polluted waters common in urban slum areas due to inadequate sanitation and waste disposal facilities. This mosquito has migrated into a number of unplanned urbanized areas and is the main vector of bancroftian filariasis (Lehane, 1991).

Oddly enough, it has been observed that it does not spread this disease in West Africa; *A. gambiae*, on the other hand, transmits both malaria and filariasis. Despite the lack of vector function for *C. quinquefasciatus* in West Africa, understanding the resistance development is crucial for the control of maladies in other locations (Lehane, 1991).

While extensive research has been conducted on the mechanisms, the purpose of this research is to test the level of resistance present in *Anopheles* and *Culex* mosquitoes at the five collection sites. It is hypothesized that despite different combinations of insecticides used in WHO distributed nets, resistance has continued to increase.

The importance of studies on the development of resistance to pyrethroids resides in the use of insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs). The WHO has initiatives to cover people at risk of malaria with LLINs and believes they should be considered a public good for populations living in endemic areas. These pyrethroid-impregnated bed nets are the first and most effective defense against the spread of malaria. However, if the vectors become resistant, this method will lose its efficacy, forcing the WHO to develop new strategies for controlling the disease (WHO, 2007). Although Ghana is a country where pyrethroid-impregnated materials are the main component in malaria prevention, studies on insecticides and the development of resistance in *A. gambiae* are still rare.

**MATERIALS AND METHODS**

**Larvae Collection:**

*A. gambiae* and *C. quinquefasciatus* larvae were collected from Southern Ghana in May and June 2011. Five collection sites were sampled: Amamoma, Duakor, North O.L.A.; all found in...
Cape Coast, Okyereko, which is adjacent to a rice irrigation scheme in the Central region and Sekondi located in Western Ghana. Each collection site provided roughly 50 – 100 larvae, depending on the amount of rain each area received prior to collection. The larvae turned into adults in two days and were transferred to 30 cm x 30 cm netted boxes.

**Pyrethroid and DDT Susceptibility Tests:**

*Anopheles* and *Culex* mosquitoes were morphologically identified and extracted from the netted box using an aspirator, then transferred to insecticide kits containing WHO issued paper treated with either permethrin (0.75%), DDT (4%) or an untreated control. Experiments were carried out in accordance with WHO protocols: *A. gambiae* were exposed to permethrin (0.75%) and DDT (4%) for one hour; *C. quinquefasciatus* were exposed to permethrin (0.75%) for two hours and DDT (4%) for four hours. Knockdown, mortality and resistance were recorded immediately following the experiments and 24 hours afterwards. Mosquitoes unable to cling to the sides of insecticide kits were considered knockdown, which comes in agreement with WHO protocol. The experiments were replicated six times, using 15 – 25 mosquitoes per experiment.

**RESULTS**

Mosquito surveys carried out in May and June 2011 showed the presence of two species in the five collection sites: *Anopheles gambiae* and *Culex quinquefasciatus*. The former was present in Amamoma, Sekondi and Duakor while the later was present in Amamoma, Okyereko and North OLA. Resistance, by the standard WHO susceptibility tests, to DDT and permethrin occurred with *A. gambiae* at all three collection sites. In contrast, *C. quinquefasciatus* showed resistance solely to DDT at North OLA.

Six experiments were conducted under WHO protocol, resulting in a 4.65% average mortality for *A. gambiae* and 80.53% for *C. quinquefasciatus*. Overall, less than 7% of *A. gambiae* were killed with WHO recommended doses of DDT and less than 6% were killed with the respective permethrin dosage. In contrast, *C. quinquefasciatus* showed great susceptibility to DDT (>90%) and permethrin (100%) with the exception of those tested from North OLA, with 0% mortality when exposed to DDT. WHO criteria for susceptibility are: 98 – 100% mortality – susceptible; 80 – 97% mortality – resistance suspected and more investigation required; 0 – 79% mortality – resistance confirmed (WHO, 1998). Therefore, *A. gambiae*’s resistance is confirmed to both permethrin and DDT at the Sekondi, Amamoma, and Duakor collection sites. In contrast, *C. quinquefasciatus* is resistance suspected (90.47%) to DDT and susceptible (100%) to permethrin at the Amamoma collection site. It resistance suspected to DDT (92.68%) and susceptible (100%) to permethrin at the Okyereko collection site. However, at the North OLA collection site, the *Culex* mosquitoes showed confirmed resistance (0%) to DDT, but susceptibility (100%) to permethrin.

**DISCUSSION**

The results supported the hypothesis that resistance to permethrin and DDT is increasing both in *A. gambiae* and *C. quinquefasciatus*. There was a large difference in ratios for *C. quinquefasciatus* and *A. gambiae* due to the rainfall during collection time. *C. quinquefasciatus* was in abundant numbers, due to its ability to breed in polluted, swampy waters. *A. gambiae*, dependent on temporary sunlit pools of rainwater, was expected to be found in large numbers in rain puddles and wheel ruts. Despite those were common in many of the sampling areas, the high frequency of rainfall caused larvae to be washed away before collection. This contrasts with Coetzee et al. (2005), who found this species abundantly during April and June 2004 collections in Ghana.

Sources of error include mechanical damage to mosquitoes and lack of specimens to test. As stated previously, the mosquitoes were transferred from the netted box to the insecticide kit via an aspirator. It is possible, that a small percent are injured during this procedure, leading to deaths not caused by the insecticides. It was also mentioned that due to heavy rains, the collection of *C. quinquefasciatus* was much larger than that of the *A. gambiae*. A higher resistance was found in the *Anopheles* mosquito, as previously explained, but this may be partially due to the smaller amount of test organisms. The WHO recommends testing about 25 adults per experiment; therefore, the smaller test groups could be sources of error as well. However, the information gained from the six experiments was enough to draw conclusions about the level of resistance in the five testing sites. The results of this study can be used to estimate the level of resistance in the Western and Central regions of Ghana. The insecticide-treated nets must be as effective as possible in the prevention of the spread of malaria and other vector-born diseases. This research can assist in the trial of new insecticide combinations for impregnated nets in order to effectively reduce the disease spread. The results strengthen the hypothesis that mosquito larvae are already resistant due to the mating and reproducing of mosquitoes in areas where insecticide is present in the runoff from farms or other agricultural activities. This needs to be further tested but it might lead to the conclusion that the effort for stopping disease spread must be targeted towards both larvae and adults.

Molecular analysis of mosquitoes in these specific test areas would be needed to understand the genetic’s role in resistance levels. Past chromosomal analysis on *A. gambiae* using nucleotide sequencing revealed an M and S form on the X chromosome. The two forms are believed to locate in different geographic areas, but can also overlap, creating hybrid progeny. Pyrethroid kdr, resulting from a single point mutation, was previously confined to the S form but has now been observed at low frequencies in the M form in countries bordering Ghana.
Given to the genetic component of resistance, it would be beneficial to target mosquito larvae in addition to adults, before the genetic component can evade preventative measures.

Coetzee et al. (2005) studied resistance of A. gambiae to DDT in Obuasi, Ghana with collections from April and June 2004. Anopheles gambiae S form was tested for resistance against DDT, resulting in 30.8% mortality. Coetzee compared the study to that of Kristan et al. (2003) that found >94% resistance at the same collection area. Discrepancies can be credited to locality and usage of insecticides. Okoye et al. (2008) that performed resistance studies in Obuasi, supports Coetzee’s data. However, Okoye claims that metabolic sequestration and detoxification are the main cause of resistance. This conclusion was reached based on the finding that A. funestus lacks the kdr mutation but remains resistant to pyrethroids and DDT. Resistance to DDT in A. gambiae from areas sampled in this study showed much higher levels than of those found in Coetzee and Okoye.

Yawson et al. (2004) studied the correlation of the kdr mutation in A. gambiae collected from Okyereko, Ghana and found 95% frequency of the kdr mutation in the S form and 3.83% in the M form. A brief investigation into the practices at the rice and vegetable farms in Okyereko revealed heavy usage of pyrethroids during the growing season to control a wide range of pest,. Thus, heavy exposure of mosquito larvae to pyrethroids from agricultural use could be the main factor contributing to the high level of resistance observed. A. gambiae were not collected from Okyereko in this study, but C. quinquefasciatus showed high susceptibility to DDT (92.68% mortality) and Permethrin (100% mortality) at this collection site.

Insecticide-treated bed nets are known to reduce child mortality by up to 60% or more in malaria endemic countries in sub-Saharan Africa (Alonso et al. 1991, D’Alessandro et al. 1995, Neville et al. 1996, Binka et al. 1998). However, resistance to pyrethroids would reduce their utility. High kdr frequencies ( > 90%) have been detected in A. gambiae in Cote d’Ivoire, Burkina Faso and Ghana (Adasi et al. 2008). Therefore, it is essential that resistance be documented and prevented, to control the spread of malaria and other diseases.

**CONCLUSIONS**

The results presented here can be used to assist programs in controlling the spread of malaria through vector control. Resistance to permethrin and DDT is an issue that should be further studied by the WHO World Malaria Programme to allow for invention of new strategies to eradicate and prevent malaria.

**REFERENCES**

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