

Insecticide Resistance in Mosquito Populations: Mechanisms, Distribution, Public Health Impact, and Management Strategies

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Abstract

Mosquito-borne diseases continue to represent a major global health burden, particularly in tropical and subtropical regions where environmental conditions favor mosquito breeding and survival. Vector species such as *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* are responsible for the transmission of dengue, malaria, chikungunya, Zika virus infection, Japanese encephalitis, and lymphatic filariasis. Chemical insecticides remain the most widely used tools for vector control through indoor residual spraying, insecticide-treated bed nets, larviciding, and space spraying. However, the continuous and often indiscriminate use of insecticides has resulted in the rapid development of resistance in mosquito populations across the world.

This research project investigates the occurrence and intensity of insecticide resistance in selected mosquito populations and examines the biochemical and molecular mechanisms underlying resistance development. Standard WHO susceptibility bioassays, enzyme activity assays, and molecular diagnostic techniques were employed to determine resistance status and identify genetic mutations associated with reduced insecticide sensitivity. The results revealed high levels of resistance to pyrethroids and organochlorines, moderate resistance to organophosphates, and comparatively lower resistance to carbamates. Elevated detoxification enzyme activity and

the presence of knockdown resistance (kdr) mutations were identified as major mechanisms contributing to resistance. The findings emphasize the urgent need for continuous resistance monitoring and integrated vector management strategies to ensure sustainable mosquito control and disease prevention.

Keywords: Insecticide resistance, Mosquito vectors, Pyrethroids, kdr mutation, Detoxification enzymes, Vector control, Public health

Introduction

Mosquitoes are among the most medically important insects due to their ability to transmit a variety of pathogens that cause severe human diseases. Malaria, transmitted primarily by species of the genus *Anopheles*, remains a leading cause of morbidity and mortality in many developing countries. Similarly, dengue and chikungunya, transmitted mainly by *Aedes* mosquitoes, have shown dramatic increases in incidence over recent decades. Rapid urbanization, climate change, increased international travel, and poor sanitation have further contributed to the expansion of mosquito habitats and disease transmission cycles.

Vector control remains the most effective preventive measure against mosquito-borne

diseases. Since the discovery of the insecticidal properties of DDT in the 1940s, chemical control has played a dominant role in reducing vector populations. Subsequent development of organophosphates, carbamates, and synthetic pyrethroids provided safer and more effective alternatives. Pyrethroids, in particular, became widely adopted due to their strong knockdown effect, residual activity, and relative safety to humans.

Despite these advantages, prolonged insecticide use has imposed intense selection pressure on mosquito populations, leading to the evolution of resistance. Insecticide resistance is an inherited trait that allows mosquitoes to survive exposure to doses of insecticides that would normally be lethal. The emergence and spread of resistance undermine vector control programs and threaten disease elimination goals. Resistance can arise through multiple mechanisms, including metabolic detoxification, target-site mutations, reduced insecticide penetration, and behavioral avoidance.

Understanding the mechanisms and distribution of insecticide resistance is crucial for designing effective resistance management strategies. Without proper monitoring and strategic interventions, resistance can rapidly spread and render existing control tools ineffective. Therefore, this study aims to assess the current status of insecticide resistance in mosquito populations, analyze the underlying mechanisms, and discuss implications for public health and vector control programs.

Materials and Methods

Mosquito specimens were collected from multiple locations representing urban, peri-urban, and rural ecological settings. Larvae were obtained from stagnant water sources such as drains, containers, overhead tanks, and ponds using standard dipping methods. Adult mosquitoes were collected using CDC light traps and mouth aspirators during peak activity periods. The collected specimens were transported to the laboratory in ventilated containers to prevent mortality during transit. Species identification was performed using morphological keys based on diagnostic characteristics such as wing patterns, palpi length, thoracic markings, and abdominal banding. Identified specimens were maintained under insectary conditions at $27 \pm$

2°C temperature, 75–80% relative humidity, and a 12:12 hour light-dark photoperiod. Larvae were fed standardized diets, and adults were provided with 10% sucrose solution. Only healthy, non-blood-fed female mosquitoes aged two to five days were used for susceptibility testing.

Susceptibility tests were conducted following WHO guidelines. Adult mosquitoes were exposed to insecticide-impregnated papers containing diagnostic doses of deltamethrin, permethrin, malathion, bendiocarb, and DDT for one hour. After exposure, mosquitoes were transferred to holding tubes and supplied with glucose solution. Mortality was recorded after 24 hours. Resistance status was interpreted according to WHO criteria, where mortality below 90% indicated confirmed resistance.

For biochemical analysis, individual mosquitoes were homogenized in phosphate buffer solution, and enzyme assays were conducted spectrophotometrically. Cytochrome P450 monooxygenase activity was measured to assess oxidative detoxification potential. Glutathione S-transferase activity was evaluated to determine conjugation-based detoxification, and esterase activity was measured to analyze hydrolytic detoxification mechanisms. Enzyme activity levels were compared with a known susceptible reference strain.

For molecular analysis, genomic DNA was extracted from resistant specimens. Polymerase chain reaction (PCR) amplification was performed to detect mutations in the voltage-gated sodium channel gene associated with knockdown resistance. Amplified products were analyzed using gel electrophoresis, and selected samples were sequenced to confirm mutation types. Statistical analyses were performed to correlate phenotypic resistance with biochemical and genetic findings.

Results

The susceptibility bioassays demonstrated widespread resistance to pyrethroid insecticides across the studied mosquito populations. Mortality rates following exposure to deltamethrin and permethrin were significantly below the WHO susceptibility threshold, confirming resistance. Resistance intensity was highest in urban populations, possibly due to frequent exposure to household insecticide sprays and municipal fogging programs. Moderate resistance to malathion was

observed, while bendiocarb showed comparatively higher mortality rates, indicating partial susceptibility. Resistance to DDT was nearly universal.

Biochemical assays revealed significantly elevated levels of detoxification enzymes in resistant populations compared to susceptible controls. Cytochrome P450 monooxygenase activity was markedly increased, suggesting enhanced oxidative metabolism of insecticides. Glutathione S-transferase activity was also elevated, indicating increased detoxification capacity for organochlorines and pyrethroids. Esterase activity was higher in populations exposed to organophosphates, supporting its role in hydrolytic detoxification. Molecular diagnostics confirmed the presence of knockdown resistance mutations in a large proportion of resistant mosquitoes. These mutations alter the structure of the voltage-gated sodium channel protein, reducing insecticide binding efficiency and preventing neural paralysis. The coexistence of metabolic resistance and target-site mutations suggests a multifactorial resistance mechanism that enhances survival under insecticide exposure. Statistical analysis showed a strong association between enzyme overexpression, mutation frequency, and reduced mortality rates.

Discussion

The findings of this study confirm that insecticide resistance in mosquito populations is widespread and driven by multiple mechanisms. The high level of pyrethroid resistance is particularly concerning because pyrethroids are extensively used in insecticide-treated bed nets, which are a key component of malaria control programs. The presence of knockdown resistance mutations reduces the sensitivity of nerve cells to insecticides, thereby diminishing the knockdown effect. Metabolic resistance further strengthens survival by enhancing detoxification processes. Overexpression of cytochrome P450 enzymes allows mosquitoes to metabolize insecticides before they reach their neural targets. Similarly, glutathione S-transferases and esterases contribute to detoxification through conjugation and hydrolysis reactions. The coexistence of these mechanisms increases resistance stability and may lead to cross-resistance between insecticide classes.

Environmental factors such as agricultural pesticide use and improper insecticide application practices accelerate resistance selection. Therefore, resistance management should involve rotational use of insecticides with different modes of action, biological control strategies, environmental sanitation, and community awareness programs. Continuous resistance monitoring is essential for early detection and timely intervention.

Conclusion

Insecticide resistance poses a serious threat to global vector control efforts. The present study demonstrates that resistance in mosquito populations is mediated by both metabolic detoxification mechanisms and target-site genetic mutations. The widespread occurrence of resistance reduces the effectiveness of commonly used insecticides and increases the risk of disease transmission. Sustainable mosquito control requires integrated vector management, rational insecticide use, and regular resistance surveillance. Future research should focus on alternative control strategies, including biological agents and genetic approaches, to overcome the growing challenge of resistance.

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