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CYTOCHROME P450 MONOOXYGENASE AND PYRETHROID RESISTANCE IN ANOPHELES: A NARRATIVE REVIEW

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ABSTRACT
Malaria remains a major global public health problem, with approximately 282 million cases reported in 2024. Although vector control interventions such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) have significantly reduced malaria transmission, their effectiveness is increasingly compromised by pyrethroid resistance in Anopheles mosquitoes. This review examines the role of cytochrome P450 monooxygenase enzymes in mediating pyrethroid resistance. A literature search was conducted in PubMed, Springer, and Google Scholar for articles published between 2016 and 2026 using the keywords "Cytochrome P450," "P450 monooxygenase," "Insecticide Resistance," "metabolic resistance," "Pyrethroid," "Anopheles," and "malaria vector." Of 10,854 articles identified, 142 were screened, and six relevant studies were included, focusing on resistance mechanisms, P450 characteristics, gene regulation, and vector control implications. The findings indicate that overexpression of specific P450 genes significantly enhances the detoxification of pyrethroids, thereby reducing insecticide susceptibility. Furthermore, interactions between metabolic resistance and target-site mutations contribute to elevated resistance levels. Recent evidence suggests that non-coding RNAs (ncRNAs) regulate detoxification pathways and contribute to adaptive resistance. In conclusion, insecticide resistance in Anopheles is a multifactorial process involving genetic, biochemical, and regulatory mechanisms. Understanding these mechanisms is crucial for developing more effective and sustainable vector control strategies.

Keywords: anopheles; cytochrome P450; insecticide resistance; metabolic resistance; pyrethroids

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INTRODUCTION

Globally, malaria cases reached an estimated 282 million in 2024 across 80 endemic countries, an increase of about 9 million (3%) from 2023. In the Western Pacific region, approximately 2.4 million cases (<1% of global cases) were reported, mainly from Papua New Guinea, Indonesia, and the Solomon Islands (WHO,2023). Despite fluctuations, malaria cases declined by 3.8% between 2000 and 2015, largely due to intensified vector control strategies. Key interventions include insecticide-treated nets (ITNs), particularly long-lasting insecticidal nets (LLINs) effective for up to three years, and conventional ITNs lasting around 12 months (Ingham et al., 2023). Indoor residual spraying (IRS) involves applying insecticides to indoor surfaces, while larval source management (LSM) targets mosquito breeding sites through habitat modification, habitat manipulation, biological control, and larviciding (Hayder et al., 2023). Vector control through insecticide-treated nets (ITNs) and indoor residual spraying (IRS) remains the cornerstone of malaria prevention due to their proven effectiveness in reducing disease burden. WHO reports highlight IRS as a key intervention in global malaria control, with around 106 million people protected in 2015. Meanwhile, high ITN coverage has been consistently associated with significant reductions in malaria incidence, leading WHO to recommend their use not only for pregnant women and young children but for all populations in endemic areas (Tizifa et al., 2018).

There are four main classes of chemical insecticides used in vector control: carbamates, organochlorines, organophosphates, and pyrethroids (Wangrawa et al., 2024). Organophosphate insecticides are commonly used as larvicides, targeting immature stages of mosquitoes. Of the four classes of insecticides, pyrethroids are the primary choice for adult mosquito control, particularly in the agricultural sector, due to their strong knockdown effect, relatively long residual activity, repellent properties, and low toxicity to mammals (Syahrani et al., 2025). However, since 2015, malaria control efforts have tended to stagnate, thought to be due to insecticide resistance in the primary malaria vector (Ingham et al., 2023).

Insecticide resistance has been reported in over 500 insect species worldwide, including more than 50 Anopheles species that transmit human malaria. Resistance generally occurs through two main mechanisms, there are target-site resistance, which reduces insecticide binding, and metabolic resistance, which enhances detoxification and limits the active compound reaching its target. Other mechanisms, such as cuticular resistance and behavioral changes, remain less well understood. Among these, metabolic resistance plays a central role, particularly through cytochrome P450 monooxygenases. In several Anopheles species, overexpression of specific P450 genes increases the capacity to metabolize pyrethroids, thereby reducing their effectiveness in vector control (Corbel & N'Guessan, 2013). Several studies have shown that cytochrome P450 enzymes play a crucial role in this process, but the available information is fragmented and incompletely summarized. Therefore, this literature review aims to summarize and simplify various existing research results, so as to provide a clearer picture of the role of cytochrome P450 in pyrethroid resistance in Anopheles malaria vectors, and help support more effective and sustainable malaria control.

METHOD

This article employs a literature review methodology. Data were collected from NCBI PubMed, Springer, and Google Scholar, focusing on: (1) insecticide resistance mechanisms, (2) characteristics of cytochrome P450 monooxygenase, (3) its overexpression, (4) genetic and epigenetic regulation, (5) gene and allelic variation, (6) insecticide cross-resistance, and (7) implications for malaria vector control. The search strategy used the keywords: “Cytochrome P450” OR “P450 monooxygenase” AND “Insecticide Resistance” OR “metabolic resistance” AND “Pyrethroid” AND “Anopheles” OR “malaria vector.” A comprehensive article search was conducted for the period 2016 to 2026. Inclusion criteria comprised original research (in vitro, in vivo, or clinical studies), free full-text availability, and publication in English or Indonesian, selected based on scientific relevance. Studies without full text or not relevant to the topic were excluded. A total of 10,854 articles were obtained from the initial search. Title and abstract screening was then conducted to obtain 142 articles. Finally, selection was conducted based on inclusion and exclusion criteria, resulting in six relevant publications.

RESULT

Table 1.
Analisys Article

No	Title	Year	Author	Result
1	*Activity of Cytochrome P450 Monooxygenase (CYPs) Metabolic Enzymes as Markers of Insecticide Resistance in Anopheles vagus Muara Enim Mosquitoes, Indonesia.*	2021	Anwar, C., Ramdja, M., Handayani, D., Ambarita, L. P., Irpan Pahlepi, R., & Ghiffari, A.	There was an increase in cytochrome P450 monooxygenase enzyme levels in *Anopheles vagus* mosquitoes from Muara Emil and Pagar Dewa villages. Most cytochrome P450 monooxygenases are involved in the detoxification process in the endoplasmic reticulum and catalyze the oxidation of xenobiotic compounds or endogenous

No	Title	Year	Author	Result
				compounds in the presence of NADPH cytochrome P450 reductase.
2	*Two highly selected mutations in the tandemly duplicated CYP6P4a and CYP6P4b genes drive pyrethroid resistance in Anopheles funestus in West Africa.*	2024	Tatchou-Nebangwa, N. M. T., Mugenzi, L. M. J., Muhammad, A., Nebangwa, D. N., Kouamo, M. F. M., Tagne, C. S. D., Tekoh, T. A., Tchouakui, M., Ghogomu, S. M., Ibrahim, S. S., & Wondji, C. S.	The CYP6P4a and CYP6P4b alleles cause insecticide resistance in the malaria vector *Anopheles funestus* through mechanisms involving gene duplication, allelic variation, and gene overexpression.
3	*Multi- omics analysis identifies a CYP9K1 haplotype conferring pyrethroid resistance in the malaria vector Anopheles funestus in East Africa*	2022	Hearn, J., Djoko Tagne, C. S., Ibrahim, S. S., Tene-Fossog, B., Mugenzi, L. M. J., Irving, H., Riveron, J. M., Weedall, G. D., & Wondji, C. S.	CYP9K1 is the main determinant of pyrethroid resistance in *Anopheles funestus* populations in East Africa, especially Uganda, which plays a role in the detoxification mechanism of insecticides before they reach the mosquito's nervous system.
4	*Pyrethroid resistance in the New World malaria vector Anopheles albimanus is mediated by cytochrome P450 CYP6P5*	2022	Kusimo, M. O., Mackenzie-Impoinvil, L., Ibrahim, S. S., Muhammad, A., Irving, H., Hearn, J., Lenhart, A. E., & Wondji, C. S.	AaCYP6P5 is able to metabolize pyrethroid insecticides, with higher efficiency against type II pyrethroids than type I pyrethroids, but is not effective enough to induce resistance against type I pyrethroids.
5	*Expression of pyrethroid metabolizing P450 enzymes characterizes highly resistant Anopheles vector species targeted by successful deployment of PBO-treated bednets in Tanzania*	2022	Matowo, J., Weetman, D., Pignatelli, P., Wright, A., Charlwood, J. D., Kaaya, R., Shirima, B., Moshi, O., Lukole, E., Mosha, J., Manjurano, A., Mosha, F., Rowland, M., & Protopopoff, N.	*Anopheles gambiae s.s.* and *Anopheles funestus* in Muleba District showed high resistance to pyrethroid insecticides with the main mechanism being metabolic, particularly through cytochrome P450 enzymes characterized by overexpression of P450 genes.
6	*Rapid selection of a pyrethroid metabolic enzyme CYP9K1 by operational malaria control activities*	2018	Vontas, J., Grigoraki, L., Morgan, J., Tsakireli, D., Fuseini, G., Segura, L., de Carvalho, J. N., Nguema, R., Weetman, D., Slotman, M. A., & Hemingway, J.	Pyrethroid resistance has increased rapidly in *Anopheles coluzzii* following intensive application of pyrethroid-based IRS and LLINs. Resistance is caused by a combination of kdr mutations and metabolic resistance, characterized by overexpression of the CYP9K1 gene, which metabolizes the pyrethroid deltamethrin.

DISCUSSION

Insecticide resistance

Insecticide resistance is the ability of insects to survive standard insecticide exposure due to physiological or behavioral adaptations. It involves four main mechanisms, metabolic resistance, target-site changes, cuticle thickening, and behavioral avoidance. Among these, metabolic resistance is the most dominant, driven by an increased capacity of detoxification enzymes that metabolize insecticides. This system evolved from long term plant insect interactions, enabling enzymes to neutralize diverse xenobiotics. Resistance arises through overexpression of detoxification enzymes or amino acid changes that enhance enzyme affinity for insecticides. In mosquitoes, this is often caused by gene amplification or regulatory changes that increase gene expression, leading to faster detoxification before toxic effects occur. Key enzymes involved include esterases, cytochrome P450s, and glutathione-S-transferases. These enzyme families have broad and overlapping substrate specificities, allowing detoxification of multiple insecticides. As a result, metabolic resistance can range from low to very high levels and has been documented

against all major insecticide classes used in vector control, including organochlorines, organophosphates, carbamates, and pyrethroids (Corbel & N'Guessan, 2013; WHO,2018).

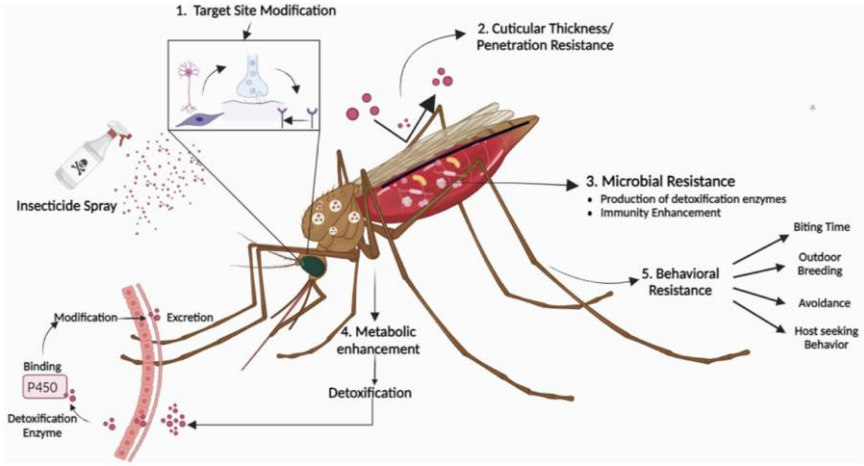

Figure 1.Mechanism of Mosquito Resistance to Insecticides (Mushtaq *et al*., 2024).

Another key mechanism is target-site resistance, where genetic mutations alter the insecticide's binding site, reducing its effectiveness. In organophosphates and carbamates, mutations in the ace-1 gene (e.g., G119S) modify acetylcholinesterase (AChE), decreasing sensitivity to inhibition. In pyrethroids, mutations in the voltage gated sodium channel (VGSC), such as L1014F or L1014S, cause knockdown resistance (kdr). Additional mechanisms include cuticular resistance, where thickened or less permeable cuticles limit insecticide penetration, and behavioral resistance, where mosquitoes avoid contact or quickly leave treated surfaces. Together, these mechanisms reduce insecticide efficacy in vector control (Corbel & N'Guessan, 2013; Ranson *et al*., 2011).

Characteristics of Cytochrome P450 Monooxygenase Enzymes

Cytochrome P450 monooxygenases (P450) are enzymes involved in both detoxification and bioactivation of insecticides and endogenous compounds. They are hemoproteins characterized by a conserved heme-binding sequence and a 450 nm absorbance peak when bound to carbon monoxide. In eukaryotes, P450 enzymes are membrane-bound (40–70 kDa) and located in the endoplasmic reticulum and mitochondria (Hardstone, 2009). Functionally, P450 enzymes catalyze oxidation reactions by incorporating one oxygen atom into a substrate while reducing the other to water. In insecticide detoxification, they mediate reactions such as oxidative ester cleavage and O-dealkylation. In contrast, during bioactivation, they can convert compounds into more toxic forms, such as desulfuration of organophosphates (P=S to P=O). This monooxygenase activity requires oxygen, NADPH, cytochrome P450 reductase, phospholipids, and the substrate (Nelson, 2004).

Cytochrome P450 is a widespread enzyme found in all aerobic organisms, both prokaryotes and eukaryotes, and also in viruses. It is estimated that at least 100 P450 genes evolved from a single gene, making the P450 gene one of the largest gene superfamilies (Oliver, 2015). Cytochrome P450 plays a role in insect growth, development, and reproduction through its involvement in the biosynthesis of ecdysteroids and juvenile hormones. In addition, this enzyme plays a role in the degradation of odorants, pheromones, defense compounds, and plays a role in xenobiotic metabolism. These enzymes are capable of catalyzing up to 60 different reactions, which is why P450s are often referred to as diversozymes (Coon *et al*., 1996 ; Feyereisen, 1999). Cytochrome P450 enzymes are key drivers of insecticide resistance, particularly against pyrethroids, and are frequently overactive in resistant African malaria vectors (Ranson *et al*., 2011; David *et al.*, 2013). Their role is evidenced by their ability to metabolize insecticides through oxidation reactions in the endoplasmic reticulum, mediated by NADPH-cytochrome P450 reductase (Anwar *et al*., 2021). Resistance emerges under sustained insecticide pressure, which enhances detoxification enzyme activity to neutralize toxic compounds, including synthetic pyrethroids. This metabolic mechanism can act independently or alongside target-site resistance. In this study, increased cytochrome P450

monooxygenase activity was observed in *Anopheles vagus*, consistent with earlier findings from the same region that reported no resistance linked to the VGSC gene (Haryanto *et al.*, 2019).

Overexpression of Cytochrome P450 Monooxygenase Enzyme

Elevated cytochrome P450 activity is closely linked to the development of insecticide resistance and is often indicated by increased enzyme expression. This may result from the evolution of new genes, mutations in promoter regions that alter transcription, amino acid substitutions within coding sequences, or post-translational modifications such as changes in phosphorylation. These adaptations generally arise under the selective pressure exerted by prolonged insecticide exposure (Ye *et al*., 2022; Lucas *et al*., 2019). Increased insecticide detoxification may result from increased expression of one or more P450s or possibly from mutations that alter the P450 protein. These findings are consistent with previous studies, including comparative transcriptomic analyses of insecticide-resistant and susceptible *Anopheles gambiae*, using methods such as quantitative PCR, microarray, and next generation sequencing, which consistently demonstrated overexpression of detoxification genes in resistant mosquitoes. Among these, cytochrome P450 enzymes are most commonly linked to pyrethroid resistance, primarily through hydroxylation of insecticide molecules, thereby reducing their toxicity and facilitating excretion (Ingham *et al*., 2023; Lucas *et al*., 2019).

Bioassay and molecular analyses indicate that pyrethroid resistance in *Anopheles coluzzii* is driven by both target-site and metabolic mechanisms. The kdr L1014F mutation approached fixation by 2015, lowering neuronal sensitivity to pyrethroids. Subsequently, resistance intensified with the overexpression of CYP9K1, which increased following the expansion of IRS and LLINs and showed a strong association with rising resistance levels (Vontas *et al*., 2018). Experimental evidence demonstrates that CYP9K1 is capable of metabolizing deltamethrin. Although its catalytic efficiency is lower than some other P450 enzymes, its high expression and interaction with other detoxification systems amplify its contribution. Overall, resistance reflects the combined action of kdr-mediated target modification and CYP9K1-based metabolic detoxification, resulting in greater resistance than either mechanism alone (Chandour-Proust *et al*., 2013).

Genetic and Epigenetic Regulation of Cytochrome P450 Monooxygenase in Pyrethroid Resistance

Studies indicate that *Anopheles funestus* lacks the kdr mutation, highlighting the importance of metabolic resistance via increased detoxification gene expression. Differential expression analysis identified 20 key non-coding RNAs (ncRNAs) between resistant and unexposed populations, suggesting their regulatory role in pyrethroid resistance (Mei *et al*., 2022). Although the precise mechanisms remain unclear, these ncRNAs likely modulate genes involved in insecticide detoxification. ncRNAs including miRNAs, piRNAs, circRNAs, and lncRNAs are widely distributed in insect genomes and regulate gene expression at transcriptional and post-transcriptional levels. They are involved in essential processes such as development, reproduction, immunity, and insecticide resistance. Additionally, ncRNAs may influence signaling pathways and contribute to epigenetic regulation, including histone modification and DNA methylation, thereby affecting adaptive responses under insecticide pressure (Debrah *et al*., 2025)

In general, the biological role of ncRNAs in the detoxification pathway and insecticide resistance remains incompletely understood. However, several studies have shown that ncRNAs, particularly microRNAs, can influence the expression of insecticide detoxification enzymes. For example, miR-2b-3p is thought to suppress the transcriptional activity of the cytochrome P450 9f2 (CYP9F2) gene, which may inhibit the detoxification process in *Plutella xylostella* larvae (Debrah *et al*., 2025). Furthermore, increased miR-13,664 is known to decrease the mRNA expression of the cytochrome P450 314A1 (CpCYP314A1) gene, thereby increasing the susceptibility of Culex pipiens pallens to deltamethrin. In this study, some metabolic genes such as cytochrome P450, GST,

UGT, and others were detected to be moderately overexpressed, while ncRNAs showed high overexpression, indicating an important regulatory role by ncRNAs. Various studies have shown that genes from the cytochrome P450 family, esterases, GSTs, UGTs, cuticular proteins, and ABC transporters play a role in insecticide resistance. Therefore, ncRNAs have the potential to be utilized in the development of RNA interference (RNAi)-based control systems. However, this still requires a deeper understanding of the molecular mechanisms of RNAi in mosquitoes, given the ongoing knowledge gap regarding its application to other insects (Sandeu *et al*., 2020).

Cytochrome P450 Monooxygenase Gene and Allelic Variation Causes Resistance

Microarray studies have identified several cytochrome P450 enzymes associated with pyrethroid resistance in *Anopheles gambiae*, including CYP6Z1, CYP6Z2, CYP6M2, CYP6P3, and CYP325A3. Among these, CYP6P3 and CYP6M2 are consistently overexpressed and are capable of metabolizing permethrin and deltamethrin, making them reliable resistance markers. Similar mechanisms occur in other vectors. In *Anopheles funestus*, QTL analyses highlight CYP6P9, CYP6P4, CYP6Z1, CYP6Z3, and CYP6M7, with CYP6P9 frequently overexpressed and linked to cross-resistance (David et al., 2013). Duplication of CYP6P9 into CYP6P9a and CYP6P9b further enhances metabolic capacity. In *Anopheles minimus*, CYP6P7 and CYP6AA3 metabolize multiple pyrethroids, although enzyme specificity indicates that cross-resistance is not always predictable (Wondji et al., 2007). Additionally, tandem duplication of CYP6P4a and CYP6P4b contributes significantly to pyrethroid resistance in *Anopheles funestus*. Increased copy number, particularly of CYP6P4a, elevates enzyme production and detoxification efficiency. Specific allelic variants, such as CYP6P4a-M220I and CYP6P4b-D284E, show strong directional selection and enhance metabolic activity by altering substrate recognition and catalytic efficiency (Mugenzi et al., 2020). Resistance is therefore driven not only by gene overexpression but also by adaptive allelic variation, where resistant alleles significantly improve survival compared to susceptible ones (Tatchou-Nebangwa et al., 2024; Mugenzi et al., 2022).

In East African populations of Anopheles funestus, particularly in Uganda, pyrethroid resistance is predominantly associated with CYP9K1 rather than CYP6P4, which is more common in West Africa. This enzyme functions by detoxifying insecticides before they reach neural targets. Evidence of reduced genetic variation in CYP9K1 suggests a selective sweep driven by intense insecticide pressure. Its metabolic efficiency against deltamethrin is comparable to other resistance-related P450 enzymes such as CYP6P9a, CYP6P9b, CYP6M7, CYP9J11, and CYP6AA1 (Riveron et al., 2017). However, CYP9K1 does not metabolize type I pyrethroids like permethrin, indicating specificity toward type II compounds. It may still indirectly contribute to type I resistance through subsequent metabolism by other highly expressed P450 enzymes (e.g., CYP6P9a/b and CYP6P5), as well as through potential sequestration mechanisms (Hearn et al., 2022; Vontas et al., 2018).

Experimental studies show that CYP6P5 is capable of metabolizing type II pyrethroids, likely due to better compatibility of their α-cyano group with the enzyme's active site, which enhances catalytic efficiency. In contrast, its activity toward type I pyrethroids is limited, possibly due to constraints in substrate recognition. Compared to major resistance-associated P450s such as CYP6P9a/b and CYP6P4, CYP6P5 plays a more supportive role, acting synergistically with other detoxification enzymes rather than as a primary determinant of resistance (Mackenzie-Impoinvil et al., 2019). It may also aid in detoxifying intermediate metabolites, thereby reducing toxic accumulation. Furthermore, the roles of CYP6M2 and CYP6P3 in Anopheles gambiae have been confirmed in vivo, where their overexpression decreases susceptibility to pyrethroids, reinforcing their contribution to resistance (Ingham et al., 2023).

Insecticide Cross-Resistance

The broad substrate range of cytochrome P450 enzymes means that metabolic resistance often contributes to cross-resistance across different insecticide classes. In *Anopheles funestus* from South

Africa, CYP6AA1 has been shown to metabolize multiple pyrethroids and dioxacarb, while elevated expression of CYP6Z1 is linked to resistance against both pyrethroids and carbamates. In *Anopheles gambiae*, overexpression of CYP6P3 has been implicated in cross-resistance to pyrethroids and carbamates, whereas CYP6M2 is associated with resistance to pyrethroids and organophosphates. Moreover, pyrethroid-resistant strains of *Anopheles gambiae* are capable of metabolizing several complex type I inhibitors, including fenazaquin, pyridaben, tolfenpyrad, and fenpyroximate. CYP9K1 has likewise been reported to metabolize deltamethrin and pyriproxyfen, although no such activity has been observed for bendiocarb (Kusimo *et al*., 2022).

Previous studies have demonstrated cross-resistance between carbamates and pyrethroids mediated by cytochrome P450 enzymes. Multiple lines of evidence suggest that CYP6Z1 plays a central role in this phenomenon, as this gene consistently shows the highest level of overexpression among detoxification genes in resistant mosquito populations. In addition, functional analyses have confirmed that CYP6Z1 is capable of binding and metabolizing bendiocarb, with a greater affinity for both carbamate and pyrethroid insecticides compared with other tested P450 enzymes. This enzyme also converts bendiocarb into highly polar metabolites that elute early during chromatographic analysis. Similar P450 mediated cross resistance has been reported in other resistant insect populations for instance, CYP6P3 and CYP6M2 have been implicated in cross-resistance between pyrethroids and carbamates in *Anopheles gambiae* populations from Ivory Coast (Ibrahim *et al*., 2016). The role of CYP6Z1 in cross-resistance is further supported by inhibition assays demonstrating that both pyrethroids and carbamates compete for binding to its recombinant protein. The low IC50 value against DDT also indicates that CYP6Z1 can bind organochlorine insecticides. These findings are consistent with previous studies in *Anopheles gambiae*, where its ortholog exhibits broad substrate specificity and is capable of metabolizing both carbaryl and DDT (Chiu *et al*., 2008).

Implications for Malaria Vector Control Interventions

Synergist assays using piperonyl butoxide (PBO) show increased mosquito mortality when PBO is applied prior to insecticide exposure, confirming the role of cytochrome P450 enzymes in pyrethroid resistance. PBO inhibits P450 activity by forming a stable complex at the enzyme's heme site, thereby blocking detoxification and enhancing insecticide efficacy (Hardstone, 2009). Gene expression studies further support this mechanism, showing overexpression of key P450 genes such as CYP6M2, CYP6P3, CYP6P4, CYP6Z3, CYP6AA1, and CYP9K1 in *Anopheles gambiae*. Similarly, in *Anopheles funestus*, elevated expression of CYP6N1, CYP6M7, CYP6M1, and CYP6Z1 indicates that metabolic resistance alone can drive high resistance levels. Comparable overexpression patterns across African regions suggest a widespread mechanism driven by prolonged pyrethroid use, explaining reduced effectiveness of standard nets and supporting the use of PBO-based LLINs as a more effective control strategy (Matowo *et al*., 2022).

Based on existing findings, ncRNA-based insect control strategies can also be developed, focusing on two main approaches: the use of biodegradable ncRNA-based insecticides and the application of metabolic engineering to target ncRNA-controlled signaling pathways. However, understanding the molecular mechanisms of ncRNA in the detoxification process and insecticide resistance is still limited, given that research in this field is still in its early stages. Technological advances such as CRISPR-Cas9 genome editing also open up opportunities to explore ncRNA functions in more depth and develop more specific and effective vector control strategies. Furthermore, approaches using inhibitors that target specific ncRNAs have the potential to disrupt gene expression regulation, thereby reducing insecticide resistance. Another innovative alternative is the use of synthetic DNA oligomers that target rRNA, which are more stable and abundant than mRNA, potentially providing a more lasting effect in overcoming resistance. *Anopheles* is the primary vector of malaria and plays a crucial role in disease transmission. Therefore, a thorough understanding of the molecular mechanisms of resistance, particularly those involving ncRNAs, is

crucial to support the development of more effective and sustainable vector control strategies in the future (Debrah *et al*., 2025).

CONCLUSION

Insecticide resistance in Anopheles mosquitoes, particularly to pyrethroids used in insecticide treated nets (ITNs) and indoor residual spraying (IRS), represents a major challenge to global malaria control. Evidence from the literature indicates that the dominant mechanism underlying this resistance is metabolic resistance mediated by cytochrome P450 monooxygenase enzymes. These enzymes enhance the detoxification of insecticides by metabolizing active compounds before they reach their target sites. Overexpression of key P450 genes, including CYP6P3, CYP6M2, CYP6P9, CYP6P4, and CYP9K1, significantly contributes to increased detoxification capacity and resistance levels in various Anopheles species. Resistance is multifactorial, involving interactions between metabolic mechanisms and other processes such as target-site mutations (kdr), as well as genetic variations like mutations and gene duplications. Additionally, genetic and epigenetic regulation, particularly via non-coding RNA (ncRNA), plays an important role in modulating detoxification gene expression. The broad substrate specificity of P450 enzymes also results in cross-resistance to multiple insecticide classes, complicating vector control strategies. These findings highlight the need for adaptive, molecular-based interventions, including the use of synergists like piperonyl butoxide (PBO), combination-based ITNs, and emerging approaches such as ncRNA targeting and gene editing to achieve sustainable malaria control.

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