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## Review Article

# Impact of Omics Studies on Understanding Insecticide Resistance Mechanisms in Sub-Saharan Malaria Vectors: A Systematic Review

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## Abstract

**Background**: Malaria remains a major global health challenge, disproportionately affecting sub-Saharan Africa. The growing threat of resistance to insecticides in *Anopheles* vector populations poses a major challenge to the efficacy of core interventions such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). This systematic review aimed to evaluate the contribution of omics approaches, particularly genomics, transcriptomics, and multi-omics, to understanding the resistance mechanisms in malaria vectors in sub-Saharan Africa and their impact on current and future vector control strategies.

**Methods:** A comprehensive search was conducted using PubMed, Web of Science, and Google Scholar for eligible studies published between January 2016 and April 2025. Studies using at least one omics approach to investigate resistance in *Anopheles* species were included. We extracted and analyzed data on study location, vector species, omics methods, insecticide classes, resistance mechanisms, and key findings according to PRISMA guidelines.

**Results**: Twenty-two studies met the inclusion criteria. Genomic and transcriptomic approaches revealed key resistance mechanisms, notably involving metabolic resistance, target-site mutations, and cuticular changes. Multi-omics studies uncovered novel resistance markers such as CYP450 reductase (CPR), UDP-glycosyltransferases (UGTs), and salivary gland proteins. Multi-country collaborations were common, reflecting the cross-border nature of insecticide resistance, while species-specific responses highlighted localized adaptation.

**Conclusion**: Omics studies have significantly enhanced the understanding of resistance to insecticides among malaria vectors, offering valuable insights for molecular diagnostics and region-specific vector control. Integrating these approaches into routine surveillance is crucial to inform sustainable malaria control and elimination strategies.

**Keywords:** Malaria; *Anopheles*; Omics approaches; Insecticide resistance; Vector control

## Introduction

Malaria is a leading vector-borne disease worldwide, with an estimated 263 million cases and approximately 597,000 deaths reported in 2023 (1). The burden is disproportionately concentrated in sub-Saharan Africa, with countries like Nigeria, the Democratic Republic of the Congo, Uganda, Mozambique and Ethiopia collectively accounting for nearly half of the global malaria incidence (1, 2). Malaria transmission is closely linked to the presence and efficiency of *Anopheles* mosquitoes, which serve as the primary vectors of *Plasmodium* parasites (3, 4). Vector control strategies have played a critical role in reducing transmission, particularly the use of insecticide-based interventions such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) (5). These interventions rely primarily on insecticides, including pyrethroids, carbamates, organophosphates, and organochlorines (6–8).


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However, the prolonged use of insecticides has driven the emergence and rapid spread of resistance among *Anopheles* populations. Resistance to all major insecticide classes has been documented, compromising intervention efficacy and threatening progress toward malaria elimination (9–11). *Anopheles* mosquitoes have evolved various resistance mechanisms, which can be broadly classified into metabolic resistance, target-site resistance, reduced cuticular penetration and behavioral avoidance (12–14). Of these, metabolic resistance is the most prevalent, characterized by the overproduction of detoxification enzymes, such as Cytochrome P450 monooxygenases (CYP450), esterases, and glutathione-S-transferases (GSTs), which neutralize insecticides before they can exert toxic effects (15–17).

Target-site resistance involves mutations that alter insecticide binding sites (14, 18). Notably, knockdown resistance (kdr) mutations in the voltage-gated sodium channel gene (VGSC), such as L1014F and L1014S (19, 20). Reduced cuticular penetration limits insecticide absorption through cuticle thickening or biochemical modifications, often involving genes such as CYP4G16 (21–23). Behavioral resistance, though less understood, involves altered mosquito behaviors that reduce contact with insecticide-treated surfaces (24, 25).

The widespread rise of insecticide resistance undermines current malaria control strategies, especially in regions with high pyrethroid resistance. It diminishes the impact of key interventions, while also affecting mosquito fitness, survival and transmission potential (12). Thus, understanding the molecular basis of resistance is essential to the development of sustainable vector control measures. While traditional bioassays can detect resistance phenotypes, they cannot identify the underlying molecular mechanisms. Several biochemical and molecular studies have also investigated insecticide resistance in *Anopheles* populations in Africa, including Nigeria (26, 27). While such studies provide valuable insights, this review focuses specifically on omics-based approaches, which provide deeper resolution into the genetic and transcriptomic drivers of resistance. Recent advances in omics technologies, particularly genomics and transcriptomics, have provided powerful tools for exploring the complexity of insecticide resistance. In addition to confirming well-established mechanisms, omics studies have uncovered novel resistance pathways involving salivary gland proteins, structural and behavioral genes and epigenetic regulators (24, 28). This systematic review synthesizes current evidence from omics-based studies investigating insecticide resistance in *Anopheles* mosquitoes across sub-Saharan Africa. It highlights the specific omics tools applied, key resistance mechanisms identified, along with their significant impact for advancing vector control strategies and resistance surveillance.

## Materials and Methods

### Literature search strategy

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Fig. 1). A comprehensive search was performed across three major databases: Google Scholar, PubMed and Web of Science, targeting peer-reviewed articles published between January 2016 and April 2025 to ensure inclusion of the most recent studies available at the time of the review. The literature search was conducted in May 2025 and the retrieved records were exported for screening. As databases are continuously updated, the search output may differ if repeated at a later date. Search terms were developed using combinations of relevant keywords such as "*Anopheles*", "insecticide resistance", "resistance mechanism", "Genomics", "whole genome sequencing", "Transcriptomics", "RNA sequencing" and "Multi omics". Boolean operators "OR" and "AND" were applied appropriately to refine search results. The full PubMed search string is provided in Supplementary File 1 for


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reproducibility. Although the search was global, studies conducted in sub-Saharan Africa were prioritized during the selection due to the region's high malaria burden. Additionally, reference lists of relevant studies were manually reviewed to identify potentially missed studies. Only articles that applied at least one omics technique to investigate insecticide resistance in *Anopheles* mosquitoes were included in the review.

### Eligibility criteria

Eligibility was guided by the PICO framework (Population, Intervention, Comparison, and Outcome) as presented in Table 1. Studies not conducted in sub-Saharan Africa, those not involving *Anopheles* species, or lacking omics methodology were excluded.

### Data extraction

Two reviewers independently screened all retrieved records. Duplicate entries were removed using Mendeley Reference Manager**.** Titles and abstracts were initially assessed for relevance, followed by full-text screening of articles meeting the inclusion criteria. Data were systematically extracted using a standardized template that included the following: author (s), publication year, country of study, omics approach used, *Anopheles* species studied, insecticide class investigated, resistance mechanisms identified and key findings relevant to vector control. Any discrepancies between reviewers were resolved through discussion and consensus, ensuring accuracy and consistency in the extracted data. Descriptive statistics were performed using Microsoft Excel 2021. Bar charts were used to visualize the species distribution, while pie charts illustrated the geographic distribution of studies and omics techniques employed.

## Results

### Study selection

A total of 539 records were retrieved through the database search. After removing duplicates (n=32), 507 unique records were screened. Following title and abstract review, 343 articles were excluded for not meeting the inclusion criteria. Full texts of 164 articles were assessed for eligibility, and 22 studies published between 2016 and April 2025 met all inclusion criteria and were included in the systematic review. The selection process is illustrated in the PRISMA flow diagram (Fig. 1).

### Study characteristics

The included studies were conducted across multiple countries in sub-Saharan Africa, providing broad geographical representation. Table 2 summarizes the characteristics of the selected studies, including the *Anopheles* species studied, omics approaches applied, insecticide classes investigated, identified resistance mechanisms, and key findings. The included studies also examined various insecticide classes. Pyrethroids were the most studied, particularly deltamethrin and permethrin, due to their widespread use in LLINs and IRS. Several studies also evaluated resistance to carbamates, organophosphates and organochlorines.

Geographic analysis revealed that 13 of the 22 studies were multi-country collaborations in sub-Saharan Africa. Ethiopia and Burkina Faso each contributed two country-specific studies, while Ghana, Uganda, Côte d'Ivoire, Benin and Tanzania contributed one study each, as presented in Fig. 2. This pattern indicates a strong regional focus and emphasizes collaborative research efforts in areas with high malaria transmission and resistance prevalence.

The most frequently studied mosquito vector was *An. funestus* (n=9), followed by *An. gambiae* (n=7) and *An. coluzzii* (n=4). These species are primary malaria vectors in Africa due to their wide distribution and high vectorial capacity. Less frequently studied species included *An. stephensi* (n= 2), a recently emerging urban vector and *An. arabiensis* (n=1). Several studies also examined *An. gambiae* s.l. (n=3), which includes multiple sibling species such as *An. gambiae* and *An. coluzzii*, it is shown in Fig. 3.


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Genomics was the most common omics approach (n=9), followed by transcriptomics (n=7) and multi-omics, which combines both genomic and transcriptomic analyses (n=6), as presented in Fig. 4.

Genomics studies primarily utilize whole-genome sequencing (WGS), single-nucleotide polymorphism (SNP) detection and copy number variation (CNV) analysis to uncover target-site mutations and detoxification gene amplifications. Transcriptomics involved RNA sequencing (RNA-seq) and quantitative PCR (qPCR) to assess gene expression changes associated with metabolic, cuticular and behavioral resistance. Multi-omics approaches provided a more integrative view, identifying networks of co-regulated genes and linking genomic alterations to transcriptional responses.

Across these studies, the most commonly identified resistance mechanisms included overexpression of CYP450s, GSTs, and esterases, target-site mutations in the VGSC gene and increased expression of cuticular and behavioral genes.

**Table 1.** Eligibility criteria using the PICO framework

| Pico element | Description |
|---|---|
| **Population** | *Anopheles* species populations exhibiting insecticide resistance. |
| **Intervention** | Uses of omics tools (genomics, transcriptomics) to investigate resistance. |
| **Comparison** | Comparative analyses between insecticide-resistant and susceptible *Anopheles* populations |
| **Outcome** | Identification of resistance mechanisms, discovery of molecular targets for novel interventions and Improvement of vector control strategies based on omics studies. |

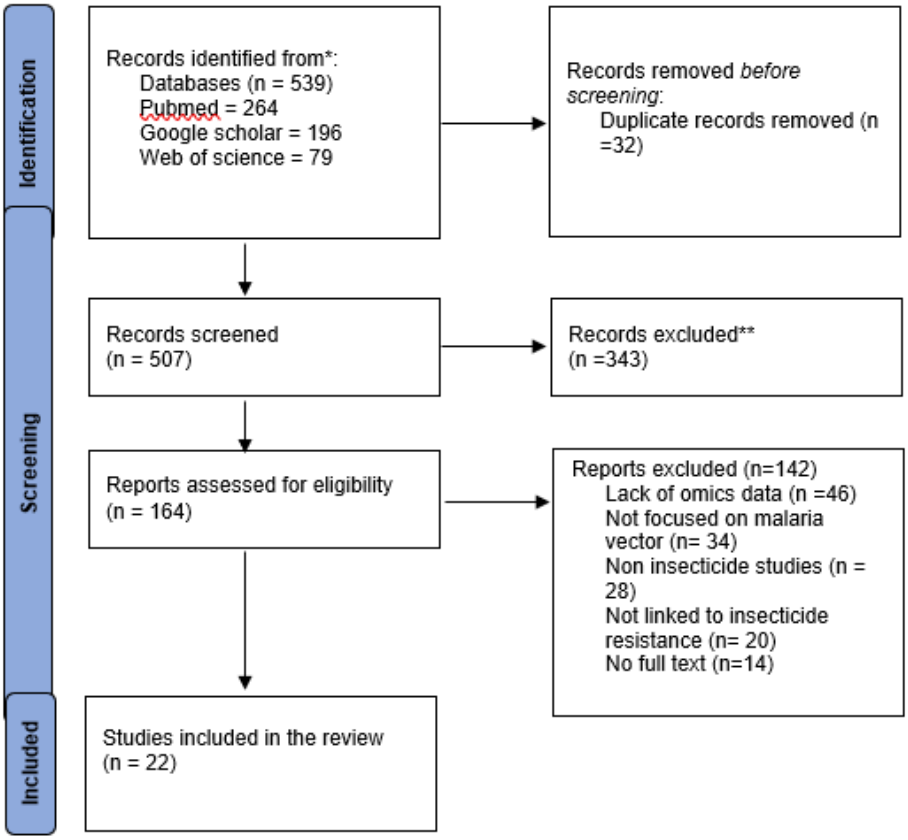



**Fig. 1.** Preferred Reporting Items for Systematic reviews and Meta-Analyses flow diagram showing the study selection process of included articles


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**Table 2.** Summary of included studies investigating insecticide resistance mechanisms in *Anopheles* spp. using Omics approaches

| Authors (year) | Omics Approaches | Countries | Vector species | Insecticide studies | Resistance mechanism identified/ mutation | Key findings | Citation |
|---|---|---|---|---|---|---|---|
| **Riveron et al., (2016)** | Transcriptomic | Ghana | *Anopheles funestus* s.s., | Pyrethroid, Carbamates, DDT, Organophosphate | Metabolic resistance via overexpression of P450s and GSTe2; also, target site resistance via LL19F-GSTe2 and A296S-RDL mutations. | The study reported multiple resistance in *An. funestus* showing high resistance to pyrethroids, DDT, and carbamates with different molecular mechanisms. | (29) |
| **Barnes et al. (2017)** | Genomics (whole genome sequencing) | Multi-country (Africa-wide) | *An. funestus* | Pyrethroids | Metabolic resistance via overexpression and selective sweep of CYP6P9a (P450) | Identified a strong selective sweep on chromosome 2R around the cyp6p9a gene in southern African populations post LLIN implementation. | (30) |
| **Ingham et al. (2018)** | Transcriptomic | Multi-country (Africa-wide) | *An. gambiae* s.l., *An. coluzzii*, *An. arabiensis* | Pyrethroid | Metabolic resistance via overexpression of GSTs, cytochrome P450s, and carboxylesterases<br>Novel non-enzymatic resistance | Novel mechanisms, including overexpression of α–crystallins and hexamerins, which are known to be associated with binding and storage roles. | (31) |
| **Isaacs et al. (2018)** | Transcriptomic | Uganda | *An. gambiae* s.l, | Carbamates (Bendiocarb) | Novel sequestration mechanism via overexpression of D7 salivary proteins and overexpression of cuticle protein. | Identification of significant overexpression of salivary gland proteins (D7r2, D7r4) in resistant mosquitoes and in silico modelling showed potential for D7 proteins to bind insecticides. | (32) |
| **Lucas et al. (2019)** | Genomic (Whole genome sequencing) | Multi-country (African countries) | *Anopheles gambiae* | Pyrethroid, DDT, Organophosphates, and carbamates | Metabolic resistance via Copy number variants (CNV) in detoxification genes. | Highlight CNVs as a critical but unrecognized mechanism of metabolic resistance. | (33) |
| **Yared et al (2020)** | Genomics | Ethiopia | *An. stephensi* | DDT, pyrethroid, Organophosphate, and carbamates. | Absence of the kdr mutation suggests Metabolic resistance as the primary resistance mechanism. | The study provides information about *An. stephensi* resistance to several insecticides and also identify the absence of a*ce-1* mutation in the genotyped *An. stephensi*. | (34) |
| **Clarkson et al. (2021)** | Genomics | Multi-country (African countries) | *An. gambiae* and *An. coluzzii* | Pyrethroids | Target site resistance resulting from mutations in the VGSC gene. | Identification of 20 novel alleles (non-synonymous substitution),<br>A novel mutation I1527T was found in tight linkage with V402L substitutions was also identified in *An. coluzzii* from 4 countries. | (35) |
| **Hearn et al. (2021)** | Multi Omics (Genomics and Transcriptomics) | Multi-country (East Africa) | *An. funestus* | Pyrethroids | Metabolic resistance: Overexpression of cytochrome P450 | The study identifies that the haplotype of cytochrome P450, *CYP9K1*, drives pyrethroid resistance in *An. funestus.* | (36) |
| **Ingham et al. (2021)** | Multi Omics (Genomics and Transcriptomics) | Burkina Faso | *An. coluzzii* | Deltamethrin, permethrin, DDT | Metabolic resistance and Elevated respiration/metabolism, oxidative stress, cuticular protein overexpression, SNP clusters, 2Rb inversion, and microbiome shifts. | Resistance is linked to increased expression of oxidative phosphorylation genes, elevated respiration rate, 2Rb/2Rc inversions, and altered microbiome. | (37) |



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**Table 2.** Continued ...

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Kouamo et al. (2021)** | Transcriptomics | Multi-country (African countries) | *An. funestus* | DDT, Permethrin and Deltamethrin | Metabolic resistance via overexpression of glutathione-S-transferase (GST) | The GST genes are differentially overexpressed in resistant populations across Africa. | (38) |
| **Njoroge et al. (2022)** | Genomics | Multi-country (African countries) | *An. gambiae* | Pyrethroids | Metabolic resistance (mutation within cytochrome P450 genes) | Identified a triple mutant haplotype associated with high-level pyrethroid resistance. Confirmed overexpression and metabolic activity of CYP6P4 and CYP6AA1. | (39) |
| **Mugenzi et al. (2023)** | Transcriptomics | Multi-country (African countries) | *An. funestus* | Carbamate Pyrethroid | Metabolic resistance (overexpression of cytochrome P450s, GSTs, transcription factors, ATP-binding cassette transporters) | The CYP6P9a and CYP6P9b are overexpressed in Malawi, and the CYP6P4a and CYP6P4b genes are overexpressed in resistant *An. funestus* in Ghana | (40) |
| **Zoh et al. (2023)** | Transcriptomics | Côte d'Ivoire | *An. gambiae* | Pyrethroid (Deltamethrin and Transfluthrin) | Target site insensitivity (kdr mutation) Metabolic resistance (differential transcription of detoxification enzymes) | There are over-transcribed genes in both insecticides, particularly the transfluthrin, which has multiple detoxification enzymes, and over-transcribed cuticle protein | (41) |
| **Lucas et al. (2023)** | Genome sequencing (GWAS) | Multi-country (West Africa) | *An. gambiae, An. coluzzii* | Deltamethrin, Pirimiphos-methyl (PM) | Target site mutations, metabolic resistance via CNVs. | Resistance is variable and highly multiallelic between populations. | (42) |
| **Kientega et al. (2024)** | Genomics | Burkina Faso | *An. gambiae,* | Pyrethroid and organophosphates | Target site resistance (over-expressed Pyrethroid resistance target site allele in *An. gambiae)* Metabolic resistance to organophosphates was also identified, and an SNP in *An. gambiae.* | Identification of novel voltage-gated sodium channel (VGSC) target site alleles at increasing frequencies along known alleles in *An. gambiae.* | (43) |
| **Samake et al. (2024)** | Genomics | Ethiopia | *An. stephensi* | Pyrethroids, Organophosphates, carbamates, Temephos | Metabolic resistance | High resistance to pyrethroids and carbamates, but susceptible to organophosphates (primiphos-methyl) | (44) |
| **Odero et al. (2024)** | Genomics | Tanzania | *An. funestus* | DDT, Deltamethrin | Target site mutation in DDT (knockdown resistance) | The study discovers eight novel VGSC mutation l976F (knockdown resistance), which showed association with resistance to DDT, not deltamethrin insecticide. | (45) |
| **Al-Yazeedi et al. (2024)** | Multi Omics (Genomics and Transcriptomics) | Multi-country (sub–Saharan Africa) | *An. funestus* | Pyrethroid (Permethrin) | Novel mechanism involving overexpression and nonsynonymous mutation in the UDP-glycosyltransferase (UGTs) genes. | The study reveals the role of UGTs in pyrethroid resistance and also reveals that they may contribute to cross-resistance. | (46) |
| **Saizonou et al. (2024)** | Transcriptomics | Benin | *An. gambiae* | Pyrethroids, Organophosphate | Metabolic resistance (differential expression of detoxification enzymes), overexpression of cuticular and Salivary gland proteins when comparing resistant and susceptible mosquitoes. | The study suggests that overexpression of salivary and cuticle proteins contributes to cross-resistance in multiple classes of insecticides. | (47) |


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**Table 2.** Continued ...

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ingham et al. (2024)** | Multi Omics (Genomics and Transcriptomics) | Multi-country (sub–Saharan Africa) | *An. gambiae* s.l and *An. funestus* | Pyrethroids | Metabolic resistance, cuticular resistance/ behavioral genes. | Highlights many known and novel resistance genes from detoxification, cuticle, and behavioral clusters, and also emphasizes the role of transcription factors in resistance regulation. | (48) |
| **Nagi and Ingham (2025)** | Multi Omics (Genomics and Transcriptomics) | Multi-country (African countries) | *An. gambiae* s.l and *An. funestus* | Pyrethroids, Organophosphates | Metabolic resistance (Overexpression of P45Os, GSTs, COEs), Cuticular resistance, Oxidative stress response, and transcriptional regulation. | Provide a correlation between genetic diversity and overexpressed genes that are associated with selective sweep loci, and also created AnoExpress for data exploration. | (49) |
| **Gadji et al. (2025)** | Multi Omics (Genomics and Transcriptomics) | Multi-country (African countries) | *An funestus* | Pyrethroid | - Metabolic resistance: overexpression of detoxification genes, P450s, and esterase.<br>Overexpression of cuticular proteins.<br>- Overexpression of novel genes (V-ATPase, Tubulin alpha-1, transposase)<br>Overexpression of epigenetic regulators (histone H3/4, glycine N-methyltransferase in resistant mosquitoes.<br>Novel Genomic variations at the cytochrome P450 reductase (CPR) gene | Novel genes like V-ATPase, tubulin α-1, transposase, and epigenetic regulators are highly overexpressed in resistant mosquitoes<br><br>Variation in the cytochrome P450 reductase (CPR) gene, particularly the N701 mutation, when co-expressed with CYP6P9a-P450, enhanced pyrethroid resistance. | (50) |

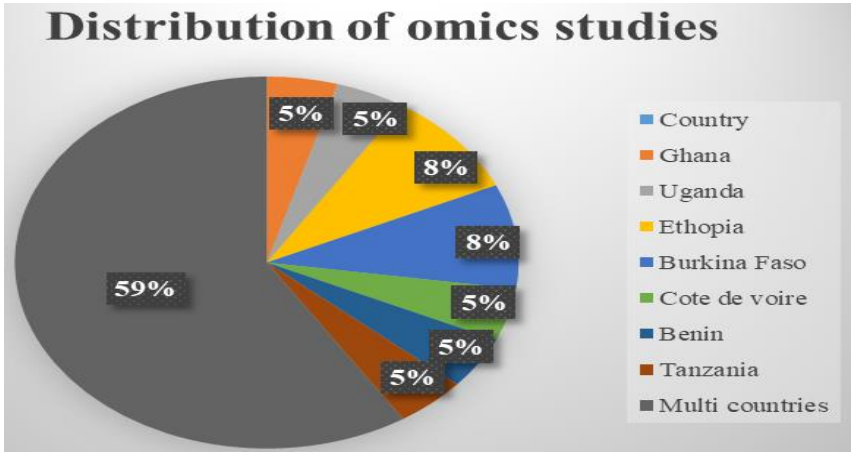



**Fig. 2.** Distribution of Omics-based insecticide resistance studies by country in Sub-Saharan Africa


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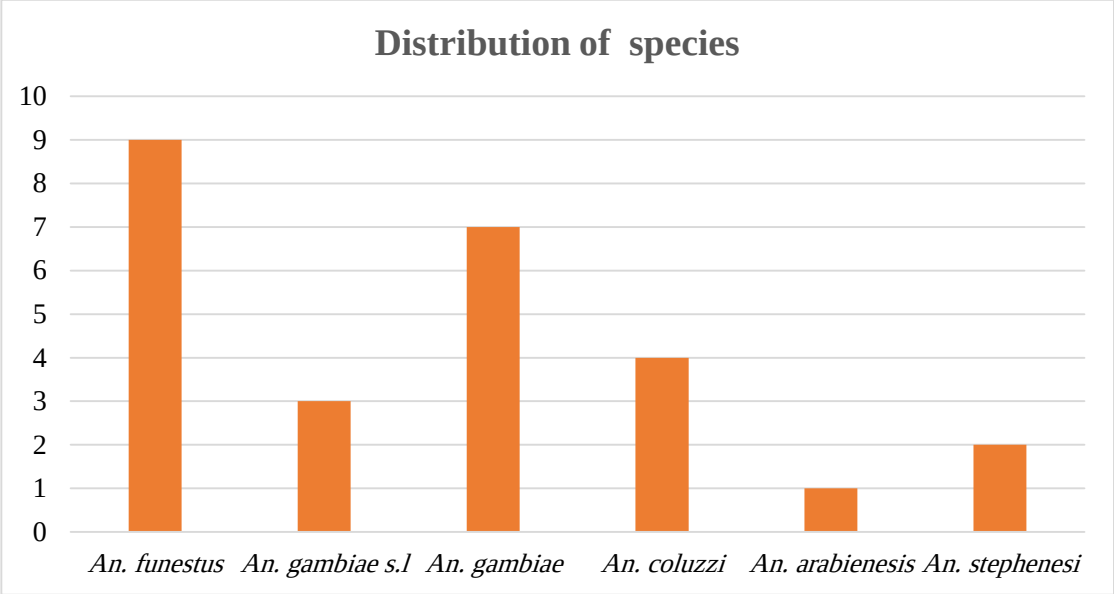



**Fig. 3.** Number of Omics studies by *Anopheles* species

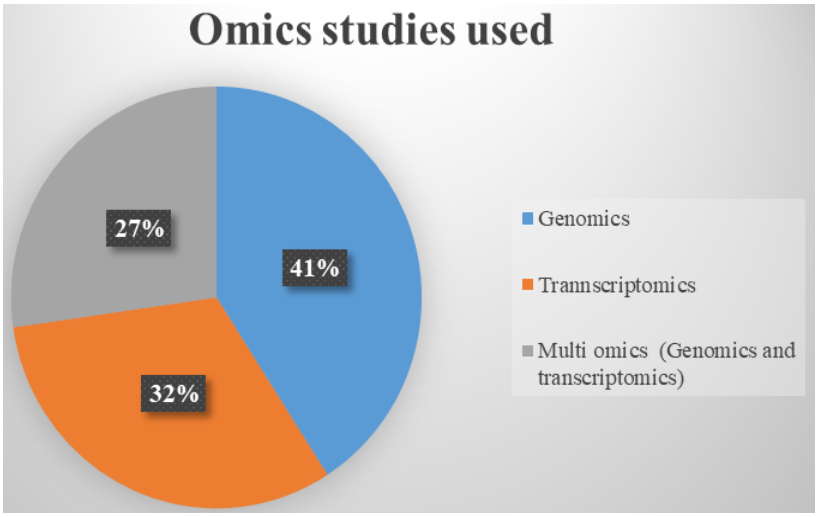



**Fig. 4.** Omics approaches used to investigate insecticide resistance in *Anopheles* species

## Discussion

The widespread emergence of insecticide resistance is a major challenge for malaria vector control, particularly in sub-Saharan Africa, where vector-borne transmission remains intense. Understanding the resistance mechanism is crucial for developing novel, sustainable control strategies. While traditional phenotypic assays remain valuable for resistance surveillance, they offer limited insight into the genetic and biochemical mechanisms (51). This review highlights how omics technologies, genomics, transcriptomics and multi-omics have advanced our understanding of resistance mechanisms in *Anopheles* mosquitoes and revealed both well-established and novel contributors to resistance, as shown in Table 2.


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Genomics studies were the most widely applied, as presented in Fig. 4, often utilizing whole-genome sequencing (WGS), single-nucleotide polymorphism (SNP) analysis and copy number variation (CNV) mapping to detect selective sweeps and mutations associated with metabolic resistance. For instance, Barnes et al. (30) reported a selective sweep around the CYP6P9a gene in *An. funestus* from Africa, indicating intense selection pressure from insecticide exposure. Njoroge et al. (39) confirmed widespread overexpression of CYP450 genes in *An. gambiae* across several African countries, while Lucas et al. (33) conducted genome sequencing across multiple countries and identified copy number variants (CNVs) in detoxification gene clusters as a previously under-recognized but important mechanism of metabolic resistance in *An. gambiae* and *An. coluzzii*. In a follow-up study, Lucas et al. (42) further demonstrated that CNVs contribute to high levels of metabolic resistance in both *An. gambiae* and *An. coluzzii*, underscoring their broad relevance across species and regions. These findings demonstrate that metabolic resistance is both widespread and driven by complex genetic changes such as gene amplifications and selective sweeps, particularly in CYP450 gene families, and highlight the importance of genomic surveillance in identifying emerging resistance threats.

Target-site resistance was also frequently uncovered through genomic studies and it has revealed multiple novel kdr-associated alleles. Clarkson et al. (35) identified 20 novel mutations in the VGSC gene, including I1527T and V402L, which are strongly associated with knockdown resistance (kdr) in *An. coluzzii* across Africa. Similarly, Kientega et al. (43) reported the emergence of novel VGSC alleles in *An. gambiae* populations in Burkina Faso, further confirming the role of target-site mutations in pyrethroid resistance. In a related study, Odero et al. (45) identified eight novel VGSC mutations in *An. funestus* populations from Tanzania. These findings indicate that VGSC mutations are not only widespread but also evolving independently across different vector species and regions in response to insecticide pressure.

Transcriptomics approaches provided insights into gene expression changes associated with detoxification, cuticular modification, salivary gland expression and oxidative stress responses. Riveron et al. (29) demonstrated the overexpression of CYP450s and GSTe2 in *An. funestus* from Ghana, in conjunction with target-site mutations, highlights the coexistence of multiple resistance mechanisms. These studies also suggest that insecticide resistance can be mediated by various mechanisms simultaneously, rather than through a single pathway. Similarly, Kouamo et al. (38) and Mugenzi et al. (40) further supported the role of GST genes and P450s in *An. funestus* populations across multiple African countries. Additionally, Zoh et al. (41) identified over-transcription of detoxification and cuticular protein genes in *An. gambiae* exposed to deltamethrin and transfluthrin in Côte d'Ivoire, illustrating the combined role of metabolic and cuticular resistance. Multi-omics approaches provided an integrative perspective by correlating genomic variation with transcriptomic profiles, uncovering resistance networks that are not easily discernible using single-omics methods, enabling the identification of both known and novel mechanisms. Hearn et al. (52) linked a CYP9K1 haplotype to resistance in *An. funestus*, supported by elevated gene expression. Ingham et al. (37, 48) demonstrated co-expression of metabolic, cuticular and behavioral genes in *An. coluzzii* and *An. gambiae* s.l., emphasizing the multilayered nature of resistance and the role of transcriptional regulation.

This review also identified unconventional resistance mechanisms. Transcriptomic analysis by Ingham et al. (54) reported overexpression of hexamerins and α-crystallins, proteins typically involved in storage and stress responses, now implicated in insecticide sequestration. Isaacs et al. (31) highlighted the role


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of salivary gland proteins, specifically the D7 family, in *An. gambiae*. Supporting this, Saizonou et al. (47) in Benin demonstrated concurrent overexpression of salivary gland and cuticular proteins in *An. gambiae*. Behavioral adaptations such as the upregulation of sensory appendage proteins in mosquito legs may enhance avoidance of insecticide-treated surfaces, as shown by Ingham et al. (41) and Zoh et al. (48).

Multi-omics approaches further expanded our understanding of novel metabolic pathways. For example, Al-Yazeedi et al. (46) identified the overexpression of UDP-glycosyltransferases (UGTs), enzymes involved in Phase II detoxification, as contributors to cross-resistance in *An. funestus* populations across multiple sub-Saharan countries. Furthermore, Gadji et al. (50) identified upregulation of V-ATPase (a proton pump implicated in cuticle biosynthesis), Tubulin alpha-1 and key epigenetic regulators such as histone H3/H4 and glycine N-methyltransferase, expanding the catalog of novel resistance-associated genes.

One of the most notable findings was the emergence of CYP450 reductase (CPR) as a synergistic cofactor enhancing P450 enzyme activity. In Ghana, CPR mutations (e.g., N701) enhanced detoxification capacity when co-expressed with CYP6P9a in *An. funestus* (39, 55).

Collectively, these findings illustrate that insecticide resistance is driven not by a single mechanism, but by multilayered genetic, metabolic, structural and behavioral adaptations involving genetic, metabolic, structural and behavioral components. Also, the growing complexity of insecticide resistance highlights the importance of integrative omics approaches in revealing unconventional yet operationally relevant resistance markers for future surveillance and intervention.

Omics-based research has direct implications for designing and implementing malaria control strategies. Genomic surveillance can inform early detection of resistance mutations, enabling preemptive adaptation of insecticide strategies. Tools such as AnoExpress, developed for real-time resistance monitoring, further demonstrate the operational value of omics research (49, 59). By identifying specific resistance genes and pathways, omics data support the strategic rotation and combination of insecticides, reducing the likelihood of resistance development and preserving treatment efficacy (60, 61). Molecular markers derived from these studies also enable real-time tracking of resistance mutations, facilitating proactive, region-specific vector control (62). Finally, the identification of novel resistance-associated genes such as CPR, salivary gland proteins and oxidative phosphorylation regulators offers new targets for next-generation insecticides and synergists. Regional variations in resistance mechanisms, as reflected in Figs. 2 and 3, underscore the need for tailored interventions that align with the local vector ecology and resistance profile (51).

Beyond *Anopheles*, transcriptomic analyses in *Culex quinquefasciatus* have revealed both target-site mutations and concurrent upregulation of detoxification enzymes, including elevated CYP450s, esterases and cuticle proteins (36, 53). Similar molecular patterns have also been observed in *Drosophila melanogaster* (54) and *Triatoma infestans* (57, 58). The recurrence of these mechanisms across diverse insect taxa suggests that certain genes and pathways act as universal resistance enhancers. These cross-species parallels highlight the broader significance of omics approaches in revealing conserved resistance processes that could inform integrated pest and vector management strategies.

Although numerous biochemical and molecular studies on insecticide resistance have been conducted across Africa (26, 27), they were not included in this review because our eligibility criteria were limited to studies employing omics methodologies such as genomics, transcriptomics and multi-omics. Nevertheless, these earlier studies remain valuable for under-


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standing resistance mechanisms and complement the omics-based insights summarized here.

## Conclusion

This review underscores the critical role of omics technologies in elucidating insecticide resistance mechanisms in malaria vectors. Omics approaches, particularly genomics and transcriptomics, have been instrumental in identifying known and novel resistance mechanisms, revealing complex molecular networks that underpin resistance phenotypes. These insights extend beyond traditional mechanisms, offering new opportunities for designing, optimizing and monitoring vector control strategies. Future research should expand multi-omics studies to understudied regions and lesser-known vector species, ensuring a more equitable and comprehensive understanding of resistance across diverse ecological settings. Ultimately, incorporating omics into routine resistance surveillance and vector management programs will be essential to sustaining the gains made in malaria control. As insecticide resistance continues to evolve, leveraging omics-based insights will be crucial to guiding evidence-based interventions, preserving the efficacy of current tools and achieving long-term malaria elimination goals.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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