Global Scientific and Academic Research Journal of Multidisciplinary Studies
ISSN: 2583-4088 (Online)
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journals-gsarjms-home/

Changes in Knockdown Resistance (kdr) Allele Frequencies in Anopheles gambiae Populations Before and After Long-Lasting Insecticidal Net Distribution in Nasarawa and Plateau States, North-Central Nigeria

By

Barminas A. Kahansim[1,*], Hayward B Mafuai[1] and Dung D. Pam[1].
[1]Department of Zoology, University of Jos, Plateau State, Nigeria.

Article History

Received: 05/07/2026
Accepted: 16/07/2026
Published: 18/07/2026

Vol – 5 Issue –7

PP: - 19-27

Abstract

Background: *The effectiveness of long-lasting insecticidal nets (LLINs) is threatened by the emergence of pyrethroid resistance in malaria vectors. This study assessed changes in knockdown resistance (kdr) allele frequencies in Anopheles gambiae populations before and after LLIN distribution in selected communities of Nasarawa and Plateau States, Nigeria.*

Methods:*A total of 1,152 Anopheles gambiae sensu lato mosquitoes collected from Gbuwhen, Maiganga, Lankan, and Seri were analyzed. Mosquitoes obtained during pre-LLIN and post-LLIN periods were genotyped for the kdr mutation using polymerase chain reaction (PCR). Allelic frequencies calculated and compared between periods using Pearson's chi-square test.*

Results: *Overall kdr prevalence increased significantly from 143 positive mosquitoes during the pre-LLIN period to 247 during the post-LLIN period ($\chi^2 = 36.44$, $p < 0.001$). Marked increases were observed in Maiganga (28.5% to 79.0%) and Seri (44.9% to 100%), whereas Gbuwhen showed a slight decline (9.3% to 5.7%) and no kdr-positive mosquitoes were detected in Lankan after LLIN distribution.*

Conclusion: *LLIN deployment was associated with significant changes in kdr allele frequencies across study communities. Continuous molecular surveillance and integrated insecticide resistance management are required to sustain malaria vector control interventions in North-Central Nigeria.*

Keywords: *Anopheles gambiae, knockdown resistance, kdr mutation, pyrethroid resistance, LLINs, malaria vector control/surveillance, Molecular epidemiology and Alleleic frequency.*

INTRODUCTION

Malaria remains a major public health challenge in Nigeria, accounting for a substantial proportion (27%) of the global malaria burden with nearly 32% of all malaria deaths. Long-lasting insecticidal nets (LLINs) remain a cornerstone of global malaria control and relying solely on pyrethroid insecticides, widespread mosquito resistance in high-burden regions like Nigeria requires upgraded tools [1]. The increasing emergence of insecticide resistance in Anopheles mosquitoes threatens the effectiveness of these interventions. Resistance may arise through metabolic detoxification mechanisms or target-site mutations. One of the most important target-site mechanisms is knockdown resistance (kdr), caused by mutations in the voltage-gated sodium channel gene, particularly at codon 1014 [2,3]. Previous studies have documented the occurrence of kdr mutations and pyrethroid resistance in several regions of Nigeria. However, information on resistance patterns in Nasarawa and Plateau States remains limited [4].

Malaria prevention heavily relies on two primary strategies: long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). Because pyrethroid insecticides are highly durable and safe for humans, the World Health Organization endorsed them as the core chemical for these nets. However, this vital defense is now threatened as mosquitoes develop widespread resistance to insecticides. The crisis is worsened by a lack of chemical diversity; only four insecticide classes exist for indoor spraying, and just one is used for bed nets. If mosquitoes become fully resistant to these limited options, controlling malaria will become incredibly difficult [5]

Insects primarily develop insecticide resistance through two biological mechanisms: metabolic resistance, which degrades the chemical before it takes effect, and **target-site**

*Corresponding Author: **Barminas A. Kahansim**

resistance, which structurally alters the insecticide's intended binding site [6].

Effective malaria control requires comprehensive entomological surveillance. Control programs must identify local mosquito species, their abundance, and their behavior before intervening. Understanding vector susceptibility to insecticides is critical to prevent resistance and ensure the success of strategies like Indoor Residual Spraying (IRS) and Insecticide-Treated Nets (ITNs) [7].

Pre-intervention surveillance and resistance monitoring are critical cornerstones for any successful malaria control program. Implementing vector control without baseline data often leads to sub-optimal resource allocation and accelerates insecticide resistance, rendering interventions ineffective [8 - 11].

The documented resistance of *Anopheles gambiae* to critical vector control chemicals in Nigeria remains an escalating public health crisis. Historical studies established early warnings of pyrethroid and DDT resistance in the southwest. However, current data highlights a widespread, nationwide crisis threatening the stability of the National Malaria Elimination Programme (NMEP) [12,13].

Research in Nigeria highlights that *Anopheles coluzzii* adaptation to urban agricultural environments is driving metabolic resistance to traditional pyrethroid nets. To counter this, studies confirm that dual-active nets containing chlorfenapyr disrupt mitochondrial energy production, proving effective against pyrethroid-resistant, *kdr*-positive populations. This ongoing study is highly critical because it addresses the precise evolutionary bottleneck of malaria control in Nigeria. Tracking the genetic shifts in wild vector populations represents the transition from reactive vector control to proactive genomic surveillance, which is the only way to save lives and maintain the efficacy of public health interventions [14].

Controlling malaria requires a deep, scientifically grounded understanding of the intricate relationships between mosquitoes, environmental factors, and insecticides. While the task is complex, sustained research into mosquito behavior and chemical resistance enables the development of targeted, highly effective prevention programs. By prioritizing scientific data collection and strategic planning, researchers can significantly reduce transmission rates, protect vulnerable populations, and advance global health equity [15 – 18]. Research conducted in Ibadan, Nigeria, examined how the major malaria-carrying mosquitoes (*An. coluzzii* and *An. gambiae*) react to carbamate and organophosphate insecticides. The study provides critical baseline data to help health authorities make informed decisions about vector control. Understanding these specific chemical responses allows for more targeted strategies, which are essential for reducing mosquito populations and preventing malaria transmission in vulnerable communities [19].

Currently, there is a lack of published data regarding knockdown resistance (*kdr*) gene frequencies within the mosquito populations of Plateau and Nasarawa states. This shortage of data is highly critical because the Nigerian Federal Ministry of Health (FMOH) is scaling up the distribution of Long-Lasting Insecticidal Nets (LLINs). Furthermore, because these communities are predominantly agrarian and heavily rely on agricultural pesticides and herbicides to boost crop yields, extensive localized resistance testing is vital. Assessing statewide resistance levels is necessary to confirm if current public health interventions, such as LLINs, remain fully effective against local malaria vectors.

MATERIALS AND METHODS

Study Area

Fieldwork was conducted across four communities in North-Central Nigeria: Gbuwhen and Maiganga in Nasarawa State, alongside Lankan and Seri in Plateau State. These communities have historically maintained universal Long-Lasting Insecticidal Net (LLIN) coverage and are co-endemic for both malaria and lymphatic filariasis. Geographically, both states are situated within the Guinea Savannah eco-zone. Nasarawa State spans 26,875.59 square kilometers [7°45'–9°45' N, 7°00'–9°37' E] and shares borders with Kaduna, Plateau, Taraba, Benue, Kogi, and the Federal Capital Territory. In contrast, Plateau State represents a high-altitude topography [8°24' N, 8°32'–10°38' E], with elevations peaking at 1,829 meters above sea level. This elevated position drives a unique sub-tropical climate, characterized by cool average temperatures (13°C to 22°C) and robust annual precipitation varying from 131.75 cm to 146 cm.

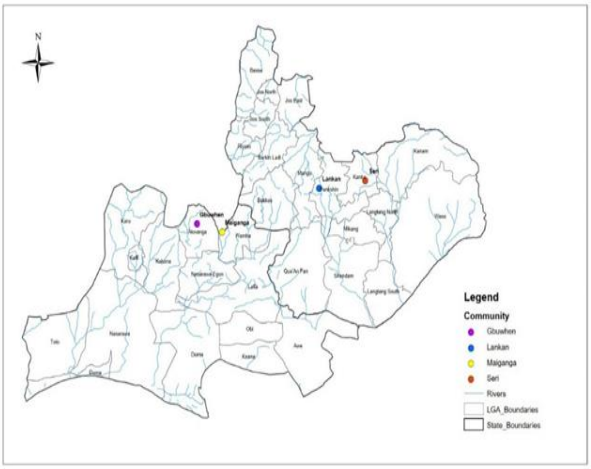

Fig. 1 Map of Nasarawa and Plateau States showing the study areas

Study design

This longitudinal study (2000–2023) analyzed 18,157 mosquitoes collected from fixed household compounds in Plateau (8,325) and Nasarawa (9,832) states retrospectively during the wet season, following methods from Richards et al. [20] and Coetzee [21]. Molecular analysis of 1,152 *Anopheles gambiae* complex specimens was conducted to identify species-level, with DNA samples screened for *kdr* mutations following procedures similar to Aneth et al. [22].

*Corresponding Author: **Barminas A. Kahansim**

Specimen Collection and Processing

Field sampling required essential materials, including a torchlight with spare batteries, petri dishes, a white cloth sheet (4m x 3m), hand sprayers, pyrethrum insecticide, forceps, cotton wool, labels, 1.5ml Eppendorf tubes, glassine bags, Ziplock bags, and silica gel desiccants. Knocked-down mosquitoes were collected from the white sheets using forceps and a torchlight, then transferred to petri dishes labeled with the household number and collection timestamp Mosquitoes were sampled via indoor pyrethrum spray catches (PSC) following standardized guidelines [23]. Prior to spraying, all doors, windows, and structural openings in randomly numbered target households were sealed. The specimens were morphologically examined under a stereomicroscope to group them by genus and species complex. Identified *Anopheles gambiae* complex vectors were stored individually in 1.5ml}\) Eppendorf tubes containing cotton wool and silica gel as a desiccant [24]. These tubes were secured in Ziplock bags and transported to the laboratory for molecular testing [25]. The final sample consisted of adult *An. gambiae* s.l. specimens pulled from historical routine collections across pre-LLIN (baseline) and post-LLIN (post-intervention campaign) eras. To maintain a balanced study design, exactly 144 specimens were processed per community for each temporal period, yielding a total sample size of 1,152 mosquitoes.

Molecular Analysis and Genomic DNA Extraction

Genomic deoxyribonucleic acid (DNA) was isolated from individual dried mosquito specimens using a NIMR-Biotech purification kit (Nigerian Institute of Medical Research, Lagos State). For each specimen, the head and thorax were mechanically homogenized with a sterile pestle inside a 2 mL Eppendorf tube. The extraction protocol was supplemented with Proteinase K and Qiagen Buffer AW1. Prior to extraction, Qiagen Wash Buffers AW1 and AW2 were reconstituted with absolute ethanol, and heating blocks were pre-heated to 56°C and 70°C. To maintain sample integrity and mitigate cross-contamination during all post-maceration steps, a dedicated tube-opening tool was utilized [25,26]. Specimens that exhibited zero PCR amplification were classified as non-amplified and systematically omitted from downstream allelic frequency calculations.

PCR Amplification of the *kdr* Gene

Polymerase chain reaction (PCR) amplification to detect knockdown resistance (*kdr*) mutations was executed in accordance with the cycling profile established by Martinez-Torres et al. [25]. The thermal cycling conditions consisted of an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, primer annealing at 48°C for 2 minutes, and extension at 72°C for 2 minutes. The protocol concluded with a final extension step at 72°C for 10 minutes.

Gel Electrophoresis and Amplicon Detection

PCR amplicons were resolved using a 1.5% (w/v) agarose gel prepared by dissolving 1.5 g of agarose in 100 mL of 1X TAE buffer. The mixture was heated in a microwave for approximately 2 minutes until completely clear, ensuring the solution did not boil over. After cooling the gel for 5 minutes, 11 µL of ethidium bromide was added for amplicon visualization. The molten gel was then cast into a tray containing an electrophoresis comb and allowed to solidify. Next, 10 µL of each sample, DNA ladder, and control were mixed with Ficoll loading dye and loaded into the wells. Electrophoresis was executed at 100 V and 150 mA for 45 minutes [27,28]. Band visualization, image capture, and gel documentation were performed using a ChemiGenius® Gel Documentation System (Syngene®).

Statistical Analysis

All computational and statistical operations were executed using R version 4.3.1 [29]. Population counts of *Anopheles gambiae* complex specimens were compiled across four distinct communities during two temporal intervals: the pre-intervention phase (2000–2003) and the post-intervention phase (2017–2023). Within each community, specimen counts were aggregated by calculating the cumulative sum across all operational years per respective period, establishing the total vector density per community and era.

A contingency table cross-tabulating community by study period was constructed utilizing the xtabs function in R. To determine if the structural distribution of mosquito counts across the pre- and post-intervention periods varied significantly among the four communities, a Pearson's Chi-squared (X^2) test of independence was performed. Statistical significance was evaluated at an alpha level of $\alpha = 0.05$. Additionally, the absolute and relative magnitudes of population shifts from the baseline to the post-intervention era were quantified to visualize community-specific trajectory differences.

Descriptive Statistics

To measure changes in overall mosquito abundance between the pre- and post-net distribution intervals, total counts were compared descriptively within each

target site. The percentage change in total vector counts from the baseline to the post-net era was calculated using the following formula:

$$Precentage\ Change = \frac{PoNC - PrNC}{PrNC} \times 100$$

Where $PrNC$ = pre-net count and $PoNC$ = Post-net count

For objective two, the sporozoite rates of *Anopheles* mosquitoes between the pre-net and post-net distribution periods across sampled communities were compared. Sporozoite rates were calculated for each community and year using the formula:

$$\text{Sporozoite Rate} = \frac{Number\ of\ positive\ mosquitoes}{Total\ number\ of\ mosquitoes\ tested}$$

Summary statistics were generated by aggregating these counts by community, year, and period (pre-net vs post-net).

To evaluate the impact of long-lasting insecticidal net (LLIN) distributions on pyrethroid resistance, the knockdown resistance (kdr) allele frequency was assessed in *Anopheles gambiae* populations across four communities (Gbuwhen, Lankan, Maiganga, and Seri) before and after net introduction. Mosquitoes were tested for the presence of the kdr mutation using standard molecular assays.

For each community and sampling period (pre-net vs. post-net), the Allelic frequency was calculated for each community and period using the formula:

$$\text{Allelic Frequency} = \frac{2 \times (\text{Homozygote}) + (\text{Heterozygote})}{2N}$$

where N is the total number of successfully genotyped specimens.

RESULTS

Knockdown Resistance (*kdr*) Allele Frequencies Across Communities

The allelic frequency of the *kdr* gene demonstrated marked variations across the four study communities and between the two sampling epochs (Table 1). In Gbuwhen, a minor reduction in resistance was observed, with frequencies declining from 9.3% during the pre-net era to 5.7% post-intervention. Similarly, Lankan exhibited a complete regression of the resistance marker, dropping from a baseline of 27.5% to 0% in the post-net distribution period. Conversely, Maiganga and Seri experienced profound selective sweeps following net distribution; *kdr* allelic frequencies escalated dramatically from 28.5% to 79.0% in Maiganga, and from 44.9% to complete fixation at 100.0% in Seri.

Pooling data across all four communities revealed that the total number of knockdown resistance (*kdr*)-positive mosquitoes increased from 143 individuals in the pre-net baseline era to 247 specimens in the post-net distribution phase. A Pearson's Chi-squared (X^2) test of independence demonstrated a highly significant shift in *kdr* prevalence between the two experimental periods X^2) = 36.44, df = 1, $p < 0.001$). This statistical outcome indicates a profound, nationwide increase in the proportion of *kdr*-positive *Anopheles gambiae* vectors following the scale-up of Long-Lasting Insecticidal Nets (LLINs) (Figure 3).

Visualization of *kdr* Dynamics

Bar plots were utilized to visually contrast community-specific shifts in *kdr* allelic frequencies alongside absolute *kdr*-positive specimen counts between the pre- and post-intervention periods (Figure 4). The graphical outputs reveal that Maiganga and Seri experienced the most pronounced escalations in both relative allele frequency and absolute vector numbers following net distribution. Conversely, Lankan demonstrated a stark, complete regression of resistance, displaying zero *kdr*-positive individuals during the post-intervention era (Figure 4).

Table 1. Genotypic distribution and allelic frequencies of knockdown resistance (*kdr*) in *Anopheles gambiae* s.l. populations across four communities in North-Central Nigeria during the pre- and post-intervention periods.

Community	Period	Total Tested	Kdr Positive	Kdr Negative	Non-amplified	Allelic Frequency (%)
Gbuwhen	Pre-Net	141	12	117	15	9.3
Gbuwhen	Post-Net	144	8	132	4	5.7
Lankan	Pre-Net	144	39	103	2	27.5
Lankan	Post-Net	144	0	137	7	0
Maiganga	Pre-Net	144	39	98	7	28.5
Maiganga	Post-Net	144	109	29	6	79

*Corresponding Author: **Barminas A. Kahansim**

Community	Period	Total Tested	Kdr Positive	Kdr Negative	Non-amplified	Allelic Frequency (%)
Seri	Pre-Net	144	53	65	26	44.9
Seri	Post-Net	144	130	0	14	100

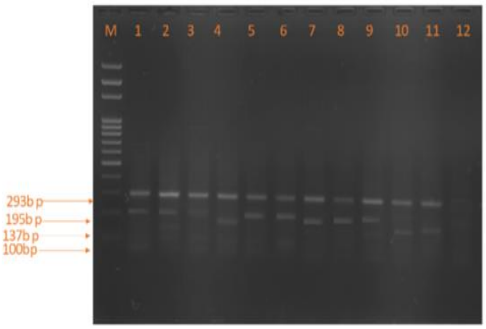

Fig.2 Lane M = molecular DNA lader; Lane 1,2,3,5,6,8,9 = homozygous resistant (RR); Lane 4,10,11 = homozygous susceptible (SS); Lane 12 = Negative control

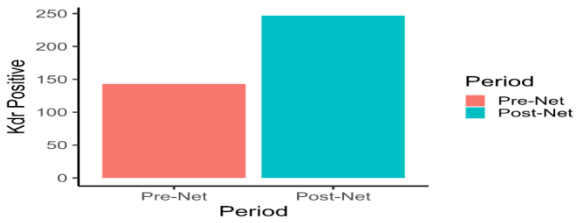

Figure 3. Total kdr-positive and kdr-negative counts in Pre- and Post-Net periods across all communities

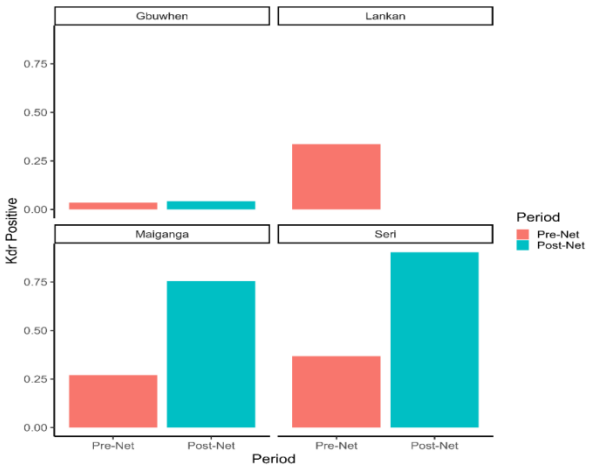

Figure 4. Comparative analysis of knockdown resistance (*kdr*) allelic frequencies across four endemic communities in Plateau and Nasarawa States, Nigeria, during the pre- and post-intervention periods.

DISCUSSION

Our research investigated the emergence and spread of insecticide resistance within local *Anopheles* populations, focusing specifically on the *kdr* gene mutation. This genetic marker directly allows malaria vectors to survive exposure to the pyrethroid insecticides coated on Long-Lasting Insecticidal Nets (LLINs). Given the widespread reliance on LLINs for national malaria suppression, evaluating these community-level changes in *kdr* frequency provides essential data on the ongoing durability of standard vector control tools.

Prior to the mass distribution of Long-Lasting Insecticidal Nets (LLINs), baseline *kdr* allelic mutations were already circulating within the local vector populations, though prevalence exhibited distinct spatial heterogeneity across the study sites. While Gbuwhen displayed a relatively low baseline resistance frequency of 9.3%, substantially higher initial frequencies were observed in Maiganga (28.5%) and Seri (44.9%). This pre-existing genetic resistance strongly indicates that selection pressure was operating well before widespread public health LLIN deployments. This early emergence of target-site insensitivity was likely driven by historical exposure to localized public health vector control initiatives or the extensive application of agrochemicals within these predominantly agrarian communities [4,29].

Following the deployment of Long-Lasting Insecticidal Nets (LLINs), divergent post-intervention shifts in knockdown resistance (*kdr*) frequencies were observed across the study sites. In Maiganga, and most notably in Seri, the prevalence of the resistance allele escalated precipitously. In Seri, the *kdr* mutation achieved complete phenotypic fixation, reaching 100.0% among all successfully genotyped vectors. This sharp evolutionary sweep was driven by intense, continuous insecticidal selection pressure; individuals carrying the *kdr* mutation possessed a distinct survival advantage, allowing them to withstand lethal chemical exposure, reproduce successfully, and disproportionately transmit the resistance allele to downstream generations. Consequently, homozygous resistant phenotypes rapidly replaced susceptible variants as the dominant population stratum. These trends strongly align with established findings reported across northern Nigeria and broader West African corridors, where the extensive concurrent utilization of pyrethroid chemistries in both commercial agriculture and public health vector control initiatives has accelerated the spread of resistance alleles [17,30,31].

The profound escalation of *kdr* resistance observed in Seri is strongly linked to localized commercial farming practices, specifically the intensive and unregulated application of chemical insecticides and herbicides on agricultural crops.

*Corresponding Author: **Barminas A. Kahansim**

This continuous deposition of agrochemicals into the environment creates an aggressive, non-target selective sieve within vector larval habitats. Consequently, highly susceptible wild-type mosquitoes suffer absolute mortality, while individuals harboring pre-adaptive resistance alleles survive, proliferate, and rapidly saturate the ecological niche. This phenomenon of cross-resistance driven by overlapping agricultural and public health chemical selection pressures has long been established [32]. Crucially, this mechanism continues to be validated by recent systematic reviews and meta-analyses across Nigeria, which document entrenched, widespread pyrethroid resistance in *Anopheles gambiae* s.l. populations bred within heavily treated rice agroecosystems and polluted farming basins [33,34,12]

Similarly, Maiganga exhibited a profound post-intervention escalation in *kdr* frequency following the deployment of Long-Lasting Insecticidal Nets (LLINs). This aggressive trajectory mirrors historical entomological trends documented in Cameroon, Uganda, and Kenya, where prolonged operational reliance on pyrethroid-impregnated netting strongly correlated with escalating vector resistance [35,36]. Collectively, these findings reinforce the established paradigm that chronic, widespread public health exposure to pyrethroids drives the selection and expansion of resistant mosquito populations. Crucially, contemporary pan-African genomic surveillance confirms that this pyrethroid-driven selection pressure continues to undermine standard vector tools, prompting an urgent continent-wide transition toward next-generation dual-active ingredient nets [37,38].

Interestingly, vector populations in Gbuwhen and Lankan demonstrated an inverse trend, characterized by a post-LLIN decrease in resistance. Post-intervention surveillance showed a minor decline in *kdr* allelic frequency in Gbuwhen and the complete eradication of resistant genotypes in Lankan. These results contrast with traditional Nigerian and West African studies that typically report a spike in resistance following mass net distribution [39,40]. This downward shift could be driven by a lower localized usage of agricultural pesticides, eliminating the selection pressure required to maintain a high *kdr* frequency against its inherent biological fitness cost. Alternatively, this anomaly may stem from the inward migration of susceptible vectors or distinct environmental factors that restricted the breeding success of resistant populations

Alternatively, the complete absence of *kdr* mutations in the Lankan vector population post-intervention does not definitively indicate absolute insecticide susceptibility; rather, it may signal a shift toward alternative, non-target-site resistance mechanisms. Mosquitoes frequently circumvent pyrethroid-induced mortality by upregulating the production of specialized detoxification enzymes [36]. These metabolic pathways—predominantly driven by cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and carboxylesterases—allow the insect to rapidly degrade or sequester insecticidal compounds before they can interact with the voltage-gated sodium channels. This hypothesis is strongly supported by recent Nigerian vector surveillance, which confirms that metabolic mechanisms are increasingly driving pyrethroid resistance in populations where traditional target-site *kdr* mutations remain low or absent [33,34]

In the present study, approximately 8.0% of the extracted genomic DNA samples failed to yield detectable PCR products. This non-amplification rate is expected when processing field-collected entomological specimens preserved via air-drying, as molecular assays remain highly sensitive to nucleic acid fragmentation, template concentration, storage duration, and the co-extraction of endogenous tissue inhibitors. Compared to fresh material, desiccation can accelerate DNA degradation over multi-year archives. Consequently, absolute amplification efficiency is rarely achieved in molecular epidemiology field surveys, with published literature demonstrating baseline success rates between 85% and 98% depending on specimen integrity and the specific genetic locus targeted.

Prior vector research utilizing degraded or field-preserved biological materials reports comparable amplification success rates of 88% to 95%, translating to non-amplification rates of 5% to 12% [41 - 43]. Thus, our recorded 92.0% amplification success rate falls strictly within acceptable epidemiological thresholds for preserved insect cohorts. To maintain analytical rigor and prevent the artificial skewing of *kdr* allelic frequencies, non-amplified samples were systematically excluded from downstream statistical calculations. This quality-control measure ensures that the reported resistance frequencies are accurate, methodologically sound, and aligned with standard molecular entomology consensus guidelines [41 - 43].

Overall, this study demonstrates that insecticide resistance develops heterogeneously, driven by localized ecological conditions, agricultural practices, and vector species composition. The findings highlight significant threats to the long-term efficacy of pyrethroid-only LLINs, particularly in high-resistance areas like Seri and Maiganga [44,45].

Consequently, this study recommends the establishment of continuous, high-resolution entomological surveillance to track evolving vector resistance profiles dynamically. Furthermore, there is an urgent need to transition from traditional pyrethroid-only interventions toward integrated insecticide resistance management (IRM) frameworks. Implementing these multi-sectoral strategies—including the deployment of next-generation dual-active ingredient or PBO-synergized nets alongside targeted indoor residual spraying (IRS)—is imperative to safeguard the long-term efficacy of national malaria control initiatives across heterogeneous transmission settings [5,46 - 49].

Conclusion

The mass deployment of Long-Lasting Insecticidal Nets (LLINs) across Nasarawa and Plateau States has exerted a profound selective pressure on the primary vectors of malaria and lymphatic filariasis. This public health intervention significantly altered target-site *kdr* allele frequencies within the mosquito populations. Although cumulative datasets

indicate a nationwide escalation in insecticide resistance post-intervention, the distinct trajectories recorded across individual study sites demonstrate that vector adaptation is highly heterogeneous. These divergent outcomes underscore the complex evolutionary dynamics of mosquito populations in response to chemical control measures. Consequently, these findings emphasize the critical need for continuous entomological monitoring, the institutionalization of integrated vector management, and the implementation of localized, site-specific strategies to sustain malaria control efficacy.

Acknowledgements

The authors express their profound gratitude to the [insert Funding Body/Institution if applicable, e.g., National Malaria Elimination Programme (NMEP) or University/Institute] for providing the necessary resources and administrative support to execute this longitudinal study. We extend our sincere appreciation to the field entomologists, laboratory technicians, and data analysts at the Nigeria Institute of Medical Research (NIMR), Lagos, for their invaluable technical expertise during the morphological and molecular screening of the mosquito specimens. Most importantly, we are deeply indebted to the community leaders, household heads, and residents of Gbuwhen, Maiganga, Lankan, and Seri for their exceptional cooperation, hospitality, and active participation during the multi-year field sampling exercises.

Conflict of Interest

The authors declare that this research was conducted in the absence of any commercial, financial, or personal relationships that could be construed as a potential conflict of interest.

REFERENCES

1. WHO (2023) *World malaria report 2023.* World Health Organization
2. Abbasi, E., & Daliri, S. (2025) Knockdown resistance (kdr) and organochlorine insecticide resistance in malaria vectors: a systematic review. *Malaria journal*, *24*(1), 415. https://doi.org/10.1186/s12936-025-05659-1
3. Gueye, A., Ngom, E. H. M., Ndoye, B. B., Dione, M. L., Diouf, B., Ndiaye, E. H., Sy, F. A., Guèye, M., Niang, M., Diallo, D., Diallo, M., & Dia, I. (2024). Insecticide Resistance and Target-Site Mutations *kdr*, *N1575Y*, and *Ace-1* in *Anopheles gambiae s.l.* Populations in a Low-Malaria-Transmission Zone in the Sudanian Region of Senegal. *Genes*, *15*(10), 1331. https://doi.org/10.3390/genes15101331
4. Awolola, T. S., Adeogun, A., Olakiigbe, A. K., Oyeniyi, T., Olukosi, Y. A., Okoh, H., Arowolo, T., Akila, J., Oduola, A., & Amajoh, C. N. (2018). Pyrethroids resistance intensity and resistance mechanisms in Anopheles gambiae from malaria vector surveillance sites in Nigeria. *PloS one*, *13*(12), e0205230. https://doi.org/10.1371/journal.pone.0205230
5. WHO (2022) *Global plan for insecticide resistance management in malaria vectors.* World Health Organization
6. Al Naggar, Y., Fahmy, N. M., Alkhaibari, A. M., Al-Akeel, R. K., Alharbi, H. M., Mohamed, A., Eleftherianos, I., El-Seedi, H. R., Giesy, J. P., & Alharbi, H. A. (2025). Mechanisms and Genetic Drivers of Resistance of Insect Pests to Insecticides and Approaches to Its Control. *Toxics*, *13*(8), 681. https://doi.org/10.3390/toxics13080681
7. Dobaño, C., Rogerson, S.J., Taylor, T.E. *et al.* Genotypic and antigenic diversity of *Plasmodium falciparum* merozoite proteins in Malawian children with severe and uncomplicated malaria. *Malar J* **24**, 402 (2025). https://doi.org/10.1186/s12936-025-05662-6
8. Okiring, J., Gonahasa, S., Maiteki-Sebuguzi, C. *et al.* LLIN Evaluation in Uganda Project (LLINEUP): modelling the impact of COVID-19-related disruptions on delivery of long-lasting insecticidal nets on malaria indicators in Uganda. *Malar J* **23**, 180 (2024). https://doi.org/10.1186/s12936-024-05008-8
9. Tabue, R. N., Gbalegba, C. G. N., Mwalimu, C. D., Tungu, P. K., Fodjo, B. K., Elanga-Ndille, E., Menze, B., Mosha, J., Wolie, R. Z., Clarke, S. E., Lines, J., & Abeku, T. A. (2025). Managing insecticide resistance in malaria vectors in Africa: case studies from Cameroon, Côte d'Ivoire and Tanzania. *Malaria journal*, *24*(1), 375. https://doi.org/10.1186/s12936-025-05606-0
10. Ugwu, C., Ugwu, N., Ogbu, O., Chukwu, O., Chika-Igwenyi, N., Afolabi, O., Igwe, D., Ekwe, E., Ezekwesili, N., Uneke, C., & Isiguzo, G. (2024). Malaria Control Programme in Nigeria: uptake of prevention strategies - a systematic review. *African health sciences*, *24*(2), 181–193. https://doi.org/10.4314/ahs.v24i2.21
11. Hemingway, J., & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual review of entomology*, *45*, 371–391. https://doi.org/10.1146/annurev.ento.45.1.371
12. Adeogun, A. O., Babalola, A. S., Babatunde, T. P., Oyelude, F. J., Fawole, J., Adediran, A., Olagundoye, O., Busari, L., Adesoye, O. A., Adeleke, M. A., & Idowu, O. A. (2025) Insecticide Resistance in Mosquito Vectors in Nigeria: A Geospatial Review of Published Evidence. *FUDMA Journal of Sciences*, *9*(10), 19-27. https://doi.org/10.33003/fjs-2025-0910-3938
13. High Resistance to Deltamethrin and DDT in Major Malaria Vector Anopheles gambiae s.l. from South-Western Nigeria is Driven by Metabolic Resistance Mechanisms. (2025). *Sahel Journal of Life Sciences FUDMA*, *3*(2), 410-419. https://doi.org/10.33003/sajols-2025-0302-46

*Corresponding Author: **Barminas A. Kahansim**

14. Assenga AA, Ahoua Alou LP, Camara S, Koffi AA, N'Guessan R, Kamande DS, Ngonyani S, Kibondo UA, Odufuwa OG, Ntabaliba WS, Lekundayo RG, Abilah F, Madumla EP, Muganga JB, Moore J and Moore SJ (2025) PRONet Duo insecticide-treated net incorporated with chlorfenapyr and bifenthrin is superior to Interceptor® G2 nets against pyrethroid-resistant Anopheles gambiae sensu lato: a randomized experimental hut trial in Côte d'Ivoire and Tanzania using non-inferiority design. Front. Malar. 3:1540884. doi: 10.3389/fmala.2025.1540884
15. Okorie, P. N., McKenzie, F. E., Ademowo, O. G., Bockarie, M., & Kelly-Hope, L. (2011). Nigeria *Anopheles* vector database: An overview of 100 years' research. *PLoS ONE*, *6*(12), e28347
16. Nalinya, S., Musoke, D., & Deane, K. (2022). Malaria prevention interventions beyond long-lasting insecticidal nets and indoor residual spraying in low- and middle-income countries: a scoping review. *Malaria journal*, *21*(1), 31. https://doi.org/10.1186/s12936-022-04052-6
17. Ibrahim, E. A., Wamalwa, M., Odindi, J., & Tonnang, H. E. Z. (2024). Insights and challenges of insecticide resistance modelling in malaria vectors: a review. *Parasites & vectors*, *17*(1), 174. https://doi.org/10.1186/s13071-024-06237-1
18. Charavanamuttu, Y., Agyeman Wamba, A., Taylor-Robinson, A. W., & Lampejo, T. (2026). The Evolving Landscape of Malaria Prevention Strategies: A Review of Recent Developments. *Pathogens (Basel, Switzerland)*, *15*(2), 137. https://doi.org/10.3390/pathogens15020137
19. Karunamoorthi K, Sabesan S. Insecticide Resistance in Insect Vectors of Disease with Special Reference to Mosquitoes: A Potential Threat to Global Public Health. Health Scope. 2013;2(1):4-18. doi: https://doi.org/10.17795/jhealthscope-9840
20. Okorie, P. N., Ademowo, G. O., Irving, H., Kelly-Hope, L. A., & Wondji, C. S. (2015). Insecticide susceptibility of *Anopheles coluzzii* and *Anopheles gambiae* in Ibadan, south-west Nigeria. *Medical and Veterinary Entomology*, *29*(1), 44–50
21. Richards, F. O., Pam, D. D., Kal, A., Gerlong, G. Y., Onyeka, J., Sambo, Y., et al. (2005). Significant decrease in the prevalence of *Wuchereria bancrofti* infection in anopheline mosquitoes following the addition of albendazole to annual ivermectin-based mass treatments in Nigeria. *Annals of Tropical Medicine & Parasitology*, *99*(2), 155–164
22. Coetzee, M. (2020) Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malaria Journal*, *19*, 1-20
23. Aneth, M. M., Isabelle, D., Jonathan, R. M., & Eliningaya, J. K. (2012). Knockdown resistance, kdr alleles, and the annual entomological inoculation rate of wild mosquito populations from Moshi, northern Tanzania. *Journal of Global Infectious Diseases*, *4*(2), 114–119
24. FAO. (2012). *Training manual on malaria entomology for entomology and vector control technicians (basic level).* Food and Agriculture Organization
25. Ahmed, U. A., & Ahmed, M. M. (2011). Morphological identification of malaria vectors within *Anopheles* species in parts of Kano State, Nigeria. *Bayero Journal of Pure and Applied Sciences*, *4*(2), 160–163
26. Busari, L. O., Iwalewa, Z. O., Adeogun, A. O., Surakat, O. A., Rufai, A. M., Fasasi, K. A., & Adeleke, M. A. (2024). Molecular detection of the infectivity status of *Anopheles gambiae sensu lato* in Osun State, Nigeria. *Dutse Journal of Pure and Applied Sciences*, *10*(4a), 1–8
27. Martinez-Torres, D., Chandre, F., Williamson, M. S., Darriet, F., Bergé, J. B., Devonshire, A. L., Guillet, P., Pasteur, N., & Pauron, D. (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae s.s. Insect Molecular Biology*, *7*(2), 179–184
28. Snounou, G., Viriyakosol, S., Zhu, X. P., Jarra, W; Pinheiro, L., Do Rosario, V. E., Thaithong, S., & Brown, K.N. (1993). High sensitivity of detection of human malaria parasites using nested polymerase chain reaction. *Molecular and Biochemical Parasitology* 61, 315-320
29. Wilkins, E. E., Howell, P. I., & Benedict, M. Q. (2006). IMP PCR primers detect single-nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in Anopheles arabiensis. *Malaria Journal*, *5*, 1-7
30. R Core Team. (2023). R: A language and environment for statistical computing (Version 4.3.1) [Computer software]. R Foundation for Statistical Computing
31. Oduola, A. O., Otubanjo, O. A., Olojede, J. B., Oyewole, I. O., & Awolola, T. S. (2012). Malaria transmission risk indices of three *Anopheles* species in selected rural communities in Oyo State, southwestern Nigeria. *International Journal of Tropical Medicine*, *7*(1), 42–48
32. Ibrahim, S. S., Manu, Y. A., Tukur, Z., Irving, H., & Wondji, C. S. (2014). High frequency of kdr L1014F is associated with pyrethroid resistance in *Anopheles coluzzii* in northern Nigeria. *BMC Infectious Diseases*, *14*, 441
33. Yadouleton, A. W., Asidi, A., Djouaka, R. F., Braïma, J., Agossou, C. D., & Akogbéto, M. C. (2010). Development of vegetable farming: A

cause of the emergence of insecticide resistance in populations of *Anopheles gambiae* in urban areas of Benin. *Malaria Journal, 8*, 103

34. Chandre, F., Darriet, F., Manga, L., Akogbéto, M., Faye, O., Mouchet, J., & Guillet, P. (1999). Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bulletin of the World Health Organization, 77*(3), 230–234
35. Ukonze, C.B., Ezihe, C.K., Egbuche, C.M., Hasan, H.A. (2025) The insecticide resistance status of Anopheles mosquitoes in the rice agroecosystems of Anambra State, Nigeria. Tropical Biomedicine 43(1): 83-89 (2026) https://doi.org/10.47665/tb.43.1.011
36. Mohammed AK, Dangambo MA, Muhammad A, Alhassan AJ (2026) Systematic review and meta-analysis of insecticide resistance status and mechanisms in the arbovirus vector Aedes aegypti from Nigeria. PLoS Negl Trop Dis 20(6): e0014421. https://doi.org/10.1371/journal.pntd.0014421
37. Nwane, P., Etang, J., Chouaibou, M., Toto, J. C., Koffi, A., Mimpfoundi, R., & Simard, F. (2011). Trends in DDT and pyrethroid resistance in *Anopheles gambiae* populations from Cameroon. *BMC Infectious Diseases, 11*, 319
38. Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: What are the implications for malaria control? *Trends in Parasitology, 27*(2), 91–98
39. WHO (2025) Guidelines for malaria, 13 August 2025. Geneva: World Health Organization; 2025. https://doi.org/10.2471/ B09514. Licence: CC BY-NC-SA 3.0 IGO
40. WHO (2021) *Global technical strategy for malaria 2016–2030, 2021 update*. Geneva: World Health Organization. https://iris.who.int/handle/10665/342995
41. Awolola, T. S., Oduola, A. O., Oyewole, I. O., Obansa, J. B., Amajoh, C. N., Koekemoer, L. L., & Coetzee, M. (2007). Dynamics of knockdown pyrethroid insecticide resistance alleles in a field population of *Anopheles gambiae s.s.* in southwestern Nigeria. *Journal of Vector Borne Diseases, 44*(3), 181–188
42. Owusu-Asenso, C.M., Sraku, I.K., Eyeson, N.A.S. *et al*. Environmental contaminants drive insecticide resistance in *Anopheles* mosquitoes in Ghana. *Sci Rep* **15**, 32201 (2025). https://doi.org/10.1038/s41598-025-14239-x
43. Hougbe, S. Z., Ahouandjinou, M. J., Affolabi, Z., Koumodji, K. D., Fassinou, A. J., Koukpo, C. Z., Towakinou, L., Agbo-Ola, A., Chitou, F. T., Sagbohan, H., Kpanou, C., Padonou, G. G., Akogbeto, M., Baba-Mousa, L., & Osse, R. A. (2025). Susceptibility of chlorfenapyr on wild populations of *Anopheles gambiae s.l.* resistant to pyrethroids along the south-north transect of Benin. *The Journal of Basic and Applied Zoology, 86*, 76
44. Mwagira-Maina, S., Runo, S., Wachira, L., Kitur, S., Nyasende, S., Kemei, B., Ochomo, E., Matoke-Muhia, D., Mbogo, C., & Kamau, L. (2021). Genetic markers associated with insecticide resistance and resting behavior in *Anopheles gambiae* mosquitoes in selected sites in Kenya. *Malaria Journal, 20*, 461
45. Awolola, T. S., Brooke, B. D., Koekemoer, L. L., & Coetzee, M. (2005). Absence of the kdr mutation in the molecular "M" form suggests different pyrethroid resistance mechanisms in the malaria vector mosquito *Anopheles gambiae s.s. Tropical Medicine & International Health, 10*(5), 417–422
46. WHO (2026) *Operational manual on larval source management: control of Anopheles and Aedes mosquito vectors*. Geneva: World Health Organization. Retrieved from WHO IRIS Repository
47. Lukole, E. A., Cook, J., Mosha, J. F., Matowo, N. S., Kulkarni, M. A., Mallya, E., Aziz, T., Martin, J., Rowland, M., Kleinschmidt, I., Manjurano, A., Mosha, F. W., & Protopopoff, N. (2025). Community benefits of mass distribution of three types of dual-active-ingredient long-lasting insecticidal nets against malaria prevalence in Tanzania: evidence from a 3-year cluster-randomized controlled trial. *BMC public health*, *25*(1), 346. https://doi.org/10.1186/s12889-025-21586-x
48. Lukole, E.A., Gobin, S., Cook, J. *et al.* (2026) Acceptability and preferences for dual-active ingredient long-lasting insecticidal nets in rural Tanzania: a mixed-methods study. *Malar J* **25**, 44. https://doi.org/10.1186/s12936-025-05723-w
49. Monroe, A., Moore, S., Olapeju, B., Merritt, A. P., & Okumu, F. (2021). Unlocking the human factor to increase effectiveness and sustainability of malaria vector control. *Malaria journal*, *20*(1), 404. https://doi.org/10.1186/s12936-021-03943-4

