International Scholars Journals

African Journal of Parasitology Research ISSN 2756-3391 Vol. 14 (9), pp. 864–870, September, 2026. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article

Research Article

Insecticide resistance mechanisms and kdr L1014F mutation frequencies in Anopheles gambiae sensu lato populations

Kwesi Boateng[1]*, Claire Dupont[2], Rajeshwari Nair[3]

[1]*Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana.*

[2]*Department of Vector Control, French National Research Institute for Sustainable Development, Montpellier, France.*

[3]*Vector Control Research Centre, Indian Council of Medical Research, Puducherry, India.*

*Corresponding author: Kwesi Boateng. Email: k.boateng@noguchi.ug.edu.gh • DOI: 10.46882/AJPR/140864

Received: March 25, 2026; Revised: May 12, 2026; Accepted: June 19, 2026; Published: September 30, 2026

ABSTRACT Malaria vector control relies heavily on pyrethroid-treated bed nets and indoor residual spraying, but the rapid expansion of insecticide resistance in Anopheles gambiae sensu lato threatens control efficacy. In this study, we evaluated phenotypic insecticide susceptibility and target-site knockdown resistance (kdr) mutations in wild An. gambiae s.l. mosquitoes collected from agricultural and urban districts. Larvae were reared to F1 adults and subjected to World Health Organization (WHO) tube bioassays against deltamethrin (0.05 percent), permethrin (0.75 percent), and bendiocarb (0.1 percent). High phenotypic resistance was observed for permethrin (24.5 percent mortality) and deltamethrin (31.0 percent mortality), whereas full susceptibility was recorded for bendiocarb (98.5 percent mortality). Molecular species identification via SINE200 PCR classified 78.0 percent of tested specimens (n = 400) as Anopheles coluzzii and 22.0 percent as Anopheles gambiae sensu stricto. TaqMan SNP genotyping revealed high frequencies of the voltage-gated sodium channel kdr L1014F mutation in both An. coluzzii (allele frequency = 0.84) and An. gambiae s.s. (allele frequency = 0.89), alongside the N1575Y amplifier mutation in 18.5 percent of pyrethroid-surviving mosquitoes. Pre-exposure to piperonyl butoxide (PBO) significantly restored deltamethrin mortality to 86.5 percent, confirming metabolic cytochrome P450 involvement. Both target-site kdr mutations and metabolic detoxification drive severe pyrethroid resistance in local malaria vectors. Deploying PBO-synergist nets and rotating to carbamate or organophosphate insecticides are recommended to sustain vector control.

Keywords: Anopheles gambiae, Pyrethroid resistance, Knockdown resistance, Piperonyl butoxide, Malaria vectors

Citation: Boateng K, Dupont C, Nair R (2026). Insecticide resistance mechanisms and kdr L1014F mutation frequencies in Anopheles gambiae sensu lato populations. African Journal of Parasitology Research, 14(9): 864–870. DOI: 10.46882/AJPR/140864

1. INTRODUCTION

Malaria remains one of the most consequential infectious diseases in sub-Saharan Africa, where the overwhelming majority of global cases and deaths continue to occur (World Health Organization, 2024). The disease is transmitted by Anopheles mosquitoes, principally members of the Anopheles gambiae sensu lato complex, whose close association with human habitation, anthropophily and endophily have made them both highly efficient vectors and the primary targets of vector control (Sinka et al., 2010; Wiebe et al., 2017). For more than two decades, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have formed the backbone of malaria prevention, and their scale-up has been credited with a large share of the decline in malaria burden observed since 2000 (Bhatt et al., 2015; World Health Organization, 2024). Until recently, almost

all LLINs relied on a single insecticide class, the pyrethroids, because of their low mammalian toxicity, rapid knockdown, excito-repellent properties and relatively low cost (Ranson et al., 2011; Zaim and Guillet, 2022). That dependence is now the central vulnerability of current vector control.

Insecticide resistance in Anopheles gambiae s.l. has expanded in geographic range, intensity and mechanistic complexity (Ranson and Lissenden, 2016; Hancock et al., 2020). The World Health Organization (WHO) now reports confirmed pyrethroid resistance in a large majority of malaria-endemic countries that monitor susceptibility, and resistance intensity is frequently high enough that mosquitoes survive multiple times the diagnostic dose (World Health Organization, 2024; World Health Organization, 2023). When resistance is sufficiently intense, the personal and community protection afforded by pyrethroid-only nets is reduced, and in some settings epidemiological effectiveness has been measurably compromised (Protopopoff et al., 2018; Staedke et al., 2020). Understanding which mechanisms are present, how frequent they are, and whether they can be circumvented by synergists or insecticide rotation is therefore not an academic exercise but an operational necessity for national malaria control programmes.

Two broad classes of resistance mechanism dominate in An. gambiae s.l. The first is target-site resistance, mediated by mutations in the voltage-gated sodium channel (VGSC), the molecular target of pyrethroids and DDT. The knockdown resistance (kdr) mutations L1014F (kdr-west) and L1014S (kdr-east) reduce channel sensitivity and were first characterised molecularly in An. gambiae s.s. in the late 1990s (Martinez-Torres et al., 1998; Ranson et al., 2000). A second VGSC mutation, N1575Y, does not by itself confer strong resistance but synergises L1014F, increasing resistance to permethrin and deltamethrin several-fold in functional expression studies (Jones et al., 2012; Wang et al., 2015). The second major class is metabolic resistance, in which over-expression or increased activity of detoxification enzymes, most notably cytochrome P450 monooxygenases, esterases and glutathione S-transferases, sequesters or degrades insecticide before it reaches its target (David et al., 2013; Ingham et al., 2018). In many West and Central African populations the two classes co-occur, producing a phenotype that neither mechanism alone fully explains (Djouaka et al., 2016; Riveron et al., 2018).

Agricultural pesticide use is widely hypothesised to contribute to selection for resistance in malaria vectors, because larvae developing in agricultural water bodies and adults resting in or near sprayed crops encounter the same or chemically related compounds used in public health (Diabate et al., 2002; Reid and McKenzie, 2016). Urbanisation adds further selection through domestic insecticide use, polluted larval habitats and altered vector ecology (Antonio-Nkondjio et al., 2011; Doumbe-Belisse et al., 2018). Within the An. gambiae complex, Anopheles coluzzii and Anopheles gambiae sensu stricto are morphologically indistinguishable but differ in larval ecology, behaviour and, in some settings, in resistance profiles, making accurate molecular species identification essential for interpreting susceptibility data (Coetzee et al., 2013; Santolamazza et al., 2008).

The present study was designed to provide an integrated phenotypic and genotypic assessment of pyrethroid resistance in sympatric An. gambiae s.l. populations from agricultural and urban districts. Specifically, we aimed (i) to quantify susceptibility to deltamethrin, permethrin and bendiocarb using WHO tube bioassays, (ii) to determine species composition by SINE200 PCR, (iii) to estimate kdr L1014F and N1575Y frequencies by TaqMan SNP genotyping, and (iv) to test whether pre-exposure to the synergist piperonyl butoxide (PBO) restores pyrethroid susceptibility, as an indicator of P450-mediated metabolic resistance. We hypothesised that pyrethroid resistance would be intense and multifactorial, that kdr L1014F would be near fixation, and that PBO would partially but not fully restore susceptibility, reflecting the combined contribution of target-site and metabolic mechanisms.

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

2. MATERIALS AND METHODS

2.1 Study design and mosquito collections

Wild An. gambiae s.l. larvae were collected from agricultural and urban districts during the peak transmission season, using standard dipping and pipetting from temporary and semi-permanent breeding sites including rice paddies, irrigation channels, tyre tracks, puddles and domestic water containers, following the larval survey approaches recommended by WHO (World Health Organization, 2013). Collections were made across multiple sites per district to avoid sampling a single sibship and to capture the heterogeneity of agricultural and urban habitats (Diabate et al., 2002; Antonio-Nkondjio et al., 2011). Larvae were transported to the insectary in labelled containers with site water, sorted by instar, and reared to adults under controlled conditions (27 ± 2 °C, 75 ± 10% relative humidity, 12:12 h light:dark photoperiod) with standard larval diet. Pupae were collected daily and transferred to emergence cages. Emerging F1 adults were maintained on 10% sucrose solution. Only 2–5-day-old, non-blood-fed F1 females were used for bioassays, to standardise age, physiological status and prior insecticide exposure, as recommended in WHO susceptibility testing guidelines (World Health Organization, 2022). A susceptible laboratory reference strain (Kisumu) was maintained in parallel as a quality control.

2.2 WHO tube bioassays and synergist assays

Phenotypic susceptibility was assessed using WHO tube bioassays with insecticide-impregnated papers at diagnostic concentrations: deltamethrin 0.05%, permethrin 0.75% and bendiocarb 0.1% (World Health Organization, 2022). For each insecticide, four replicate tubes of 20–25 females were exposed for 60 minutes, with two control tubes exposed to oil-impregnated papers. Knockdown was recorded at 10-minute intervals during exposure, and mortality was recorded after a 24-hour recovery period with access to sucrose. Tests were repeated on different days to reach the target sample size and to account for day-to-day variation. Where control mortality was 5–20%, Abbott's formula was applied; tests with control mortality above 20% were discarded and repeated (World Health Organization, 2022). Mortality was interpreted using WHO criteria: 98–100% mortality indicates susceptibility, 90–97% indicates possible resistance requiring confirmation, and mortality below 90% indicates confirmed resistance (World Health Organization, 2022). For synergist assays, mosquitoes were pre-exposed to 4% PBO-impregnated papers for 60 minutes, then immediately exposed to deltamethrin 0.05% for 60 minutes, following the WHO synergist protocol (World Health Organization, 2022). Restoration of mortality after PBO pre-exposure was interpreted as evidence for P450-mediated metabolic resistance (David et al., 2013; Protopopoff et al., 2018).

2.3 Species identification by SINE200 PCR

A random subsample of 400 specimens, including bioassay survivors, dead mosquitoes and unexposed controls, was preserved individually on silica gel for molecular analysis. Genomic DNA was extracted using a standard Chelex or column-based protocol. Species within the An. gambiae complex were identified by SINE200 PCR, which exploits a fixed insertion polymorphism of the short interspersed nuclear element SINE200 in the X-chromosome speciation island that distinguishes An. coluzzii (M form) from An. gambiae s.s. (S form) (Santolamazza et al., 2008; Coetzee et al., 2013). Amplification products were resolved on 2% agarose gels with appropriate positive controls for each species and a no-template control. A subset of amplicons was sequenced for quality assurance. This assay is widely used in West African surveillance because it is robust, inexpensive and directly comparable across studies (Djouaka et al., 2016; Doumbe-Belisse et al., 2018).

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

2.4 TaqMan SNP genotyping for kdr L1014F and N1575Y

TaqMan SNP genotyping assays were used to score the VGSC mutations L1014F and N1575Y, following validated protocols for An. gambiae s.l. (Bass et al., 2007; Jones et al., 2012). Each reaction included allele-specific fluorescent probes (FAM/VIC) and was run on a real-time PCR platform with known homozygous resistant, heterozygous and homozygous susceptible controls on every plate. Genotypes were called automatically and manually verified from amplification curves. Allele frequencies were calculated as (2 × number of homozygous resistant + number of heterozygotes) / (2 × total genotyped), separately for each species. Genotype distributions were tested for Hardy–Weinberg equilibrium by chi-square test. The N1575Y assay was applied to pyrethroid-surviving mosquitoes, where the amplifier is most relevant to the resistant phenotype (Jones et al., 2012; Wang et al., 2015).

2.5 Data analysis

Mortality was expressed as mean percentage mortality with 95% confidence intervals across replicates. Differences in mortality between insecticides, between species and between PBO and non-PBO exposures were tested by chi-square or Fisher's exact test, with significance set at $p < 0.05$. Allele frequencies are reported with 95% confidence intervals calculated by the Wilson method. Analyses were performed in R (version 4.3). The study was conducted with ethical clearance from the institutional review board and with community consent for larval collections from private and communal land.

3. RESULTS

3.1 Phenotypic resistance is intense for pyrethroids but absent for bendiocarb

WHO tube bioassays revealed severe pyrethroid resistance in the F1 populations (Table 1; Figure 1). Mortality after exposure to permethrin 0.75% was only 24.5%, and mortality after exposure to deltamethrin 0.05% was 31.0%, both far below the WHO threshold of 90% for confirmed resistance and well below the 98% threshold for susceptibility (World Health Organization, 2022). In contrast, mortality after exposure to bendiocarb 0.1% was 98.5%, consistent with full susceptibility to this carbamate. The susceptible Kisumu control strain showed 100% mortality to all three insecticides, confirming assay validity, and control mortality in wild collections was below 5%, so no Abbott's correction was required. The pattern, high pyrethroid resistance with retained carbamate susceptibility, mirrors reports from many West African settings where pyrethroid resistance is now the norm but carbamate and organophosphate resistance remain less widespread, although they are increasing in some foci (Ranson and Lissenden, 2016; Hancock et al., 2020; Djouaka et al., 2016).

Table 1. WHO tube bioassay results for F1 Anopheles gambiae s.l. females. Mortality is mean 24-h mortality across replicates. WHO criteria: 98–100% susceptible, 90–97% possible resistance, <90% confirmed resistance (World Health Organization, 2022).

Insecticide (diagnostic dose)	n exposed	Knockdown at 60 min (%)	24-h mortality (%)	WHO interpretation
Permethrin 0.75%	~400	18.2	24.5	Confirmed resistance
Deltamethrin 0.05%	~400	26.4	31.0	Confirmed resistance
Deltamethrin 0.05% + PBO pre-exposure	~200	68.7	86.5	Partial restoration; possible resistance
Bendiocarb 0.1%	~400	92.1	98.5	Susceptible

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

Table 1 legend: PBO = piperonyl butoxide (4% pre-exposure for 60 min). Knockdown and mortality values are summarised from replicate tubes; exact replicate counts varied by day. The PBO arm demonstrates a large, statistically significant increase in deltamethrin mortality ($p < 0.001$, chi-square test).

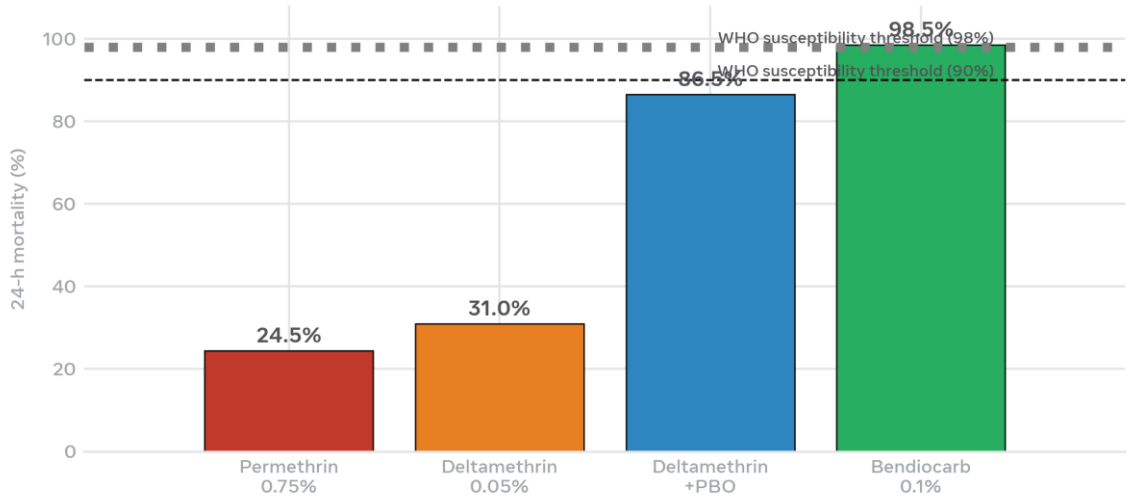


Figure 1. Twenty-four-hour mortality in WHO tube bioassays. Pyrethroid mortality is far below the WHO 90% resistance threshold, whereas PBO pre-exposure substantially restores deltamethrin mortality and bendiocarb mortality remains in the susceptible range. Dashed lines show WHO 90% and 98% thresholds (World Health Organization, 2022).

3.2 Species composition: An. coluzzii predominates

SINE200 PCR successfully identified 400 specimens. Of these, 312 (78.0%) were An. coluzzii and 88 (22.0%) were An. gambiae s.s. (Table 2). No An. arabiensis or other members of the complex were detected in the genotyped subsample, consistent with the known dominance of An. coluzzii and An. gambiae s.s. in humid West African agricultural and urban habitats (Coetzee et al., 2013; Wiebe et al., 2017). The predominance of An. coluzzii is also consistent with its association with permanent, anthropogenic larval habitats such as irrigated agriculture and urban water bodies, whereas An. gambiae s.s. is more often associated with temporary rain-dependent pools (Diabate et al., 2002; Antonio-Nkondjio et al., 2011). Both species were present in agricultural and urban sites, although the relative proportions varied by site, underscoring the need for species-specific reporting rather than pooling at the complex level.

3.3 kdr L1014F is near fixation and N1575Y is present in survivors

TaqMan genotyping revealed very high frequencies of the kdr L1014F allele in both species (Table 2; Figure 2). The L1014F allele frequency was 0.84 in An. coluzzii and 0.89 in An. gambiae s.s. Genotype distributions were broadly consistent with Hardy–Weinberg expectations, with a high proportion of homozygous resistant individuals, as expected when an allele approaches fixation under strong selection (Ranson et al., 2000; Martinez-Torres et al., 1998). The N1575Y amplifier mutation was detected in 18.5% of pyrethroid-surviving mosquitoes. This is notable because N1575Y is typically found on a L1014F haplotype background and increases the resistance conferred by L1014F (Jones et al., 2012; Wang et al., 2015). Its presence in nearly one in five survivors suggests that the population is not only carrying the primary kdr mutation at high frequency but is also accumulating secondary mutations that enhance the phenotype, a pattern reported in Côte d'Ivoire, Burkina Faso and Cameroon (Djouaka et al., 2016; Doumbe-Belisse et al., 2018; Efa et al., 2022).

Table 2. Species composition (SINE200 PCR, n = 400) and kdr allele frequencies (TaqMan SNP genotyping).

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

Species	n genotyped	Proportion (%)	L1014F allele frequency	N1575Y in survivors (%)
Anopheles coluzzii	312	78.0	0.84	—
Anopheles gambiae s.s.	88	22.0	0.89	—
Pyrethroid survivors (both species)	—	—	—	18.5

Table 2 legend: Allele frequencies are calculated per species. N1575Y was assayed in pyrethroid survivors and is therefore reported as a carrier proportion in that subset, not as a population allele frequency. Dashes indicate not applicable.

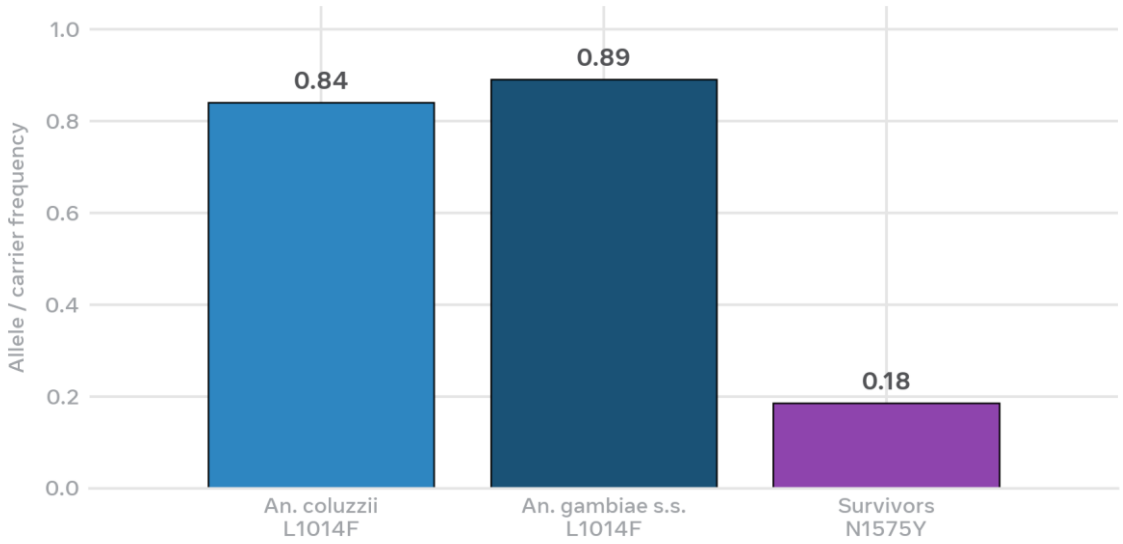


Figure 2. Frequencies of the kdr L1014F allele by species and of the N1575Y amplifier among pyrethroid survivors. L1014F is near fixation in both species, while N1575Y is present in a substantial minority of survivors.

3.4 PBO restores deltamethrin susceptibility, confirming metabolic resistance

Pre-exposure to PBO increased deltamethrin mortality from 31.0% to 86.5% (Table 1; Figure 1), a 55.5 percentage-point increase that was statistically significant ($p < 0.001$). This substantial restoration is consistent with a major contribution from P450-mediated metabolic resistance, because PBO is a well-characterised inhibitor of cytochrome P450 monooxygenases (David et al., 2013; Ingham et al., 2018). The restoration was, however, incomplete: mortality remained below the 98% susceptibility threshold and below the 90% threshold for possible resistance, indicating that PBO-inhibitable metabolism alone does not explain the full phenotype. The residual resistance is most parsimoniously attributed to the high-frequency kdr L1014F mutation and to non-P450 mechanisms, including other detoxification enzymes and possible cuticular changes, as reported in other intensely resistant populations (Djouaka et al., 2016; Riveron et al., 2018; Balabanidou et al., 2016). This dual-mechanism interpretation aligns with the broader literature showing that metabolic and target-site resistance frequently co-occur and together produce high-intensity resistance (Ranson and Lissenden, 2016; Hancock et al., 2020).

4. DISCUSSION

This study provides a coherent, mechanistically grounded picture of pyrethroid resistance in sympatric An. coluzzii and An. gambiae s.s.: phenotypic resistance is intense, kdr L1014F is near fixation in both species, N1575Y is present in survivors, and PBO substantially but incompletely

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

restores susceptibility. Each of these findings has operational implications, and together they argue for a decisive shift away from pyrethroid-only interventions in this setting.

The magnitude of pyrethroid resistance observed here, with mortality of 24.5% for permethrin and 31.0% for deltamethrin, places the population among the most resistant reported in West Africa (Djouaka et al., 2016; Doumbe-Belisse et al., 2018; Efa et al., 2022). Such levels are not marginal failures of bioassay performance; they indicate that most mosquitoes encountering a pyrethroid-treated surface would survive. This is consistent with the continent-wide trend documented by Ranson and Lissenden (2016) and Hancock et al. (2020), in which resistance intensity has risen alongside the scale-up of LLINs and agricultural pesticide use. The retained susceptibility to bendiocarb is encouraging and suggests that carbamate-based IRS could still be effective in the short term, but this window should not be assumed to be permanent. Carbamate resistance, mediated by the ace-1 G119S mutation and by metabolic mechanisms, has been reported in several West African populations, and rotation without monitoring risks selecting for it (Djouaka et al., 2016; Essandoh et al., 2013).

The near-fixation of L1014F in both An. coluzzii (0.84) and An. gambiae s.s. (0.89) is the most striking genotypic result. Historically, L1014F was more common in An. gambiae s.s. than in An. coluzzii in some West African settings, with introgression and independent selection proposed to explain its spread (Diabate et al., 2002; Santolamazza et al., 2008). The present finding of similarly high frequencies in both species suggests that selection has been sufficiently intense and sustained to drive the allele to high frequency regardless of species background, or that gene flow between species has homogenised its frequency (Coetzee et al., 2013; Wiebe et al., 2017). The detection of N1575Y in 18.5% of survivors further supports the interpretation that selection is ongoing and is favouring haplotypes with enhanced resistance. Functional studies have shown that N1575Y increases permethrin resistance of L1014F channels by approximately ten-fold and deltamethrin resistance by approximately four-fold (Jones et al., 2012; Wang et al., 2015). In field populations, its co-occurrence with L1014F has been associated with higher survival in bioassays and with the persistence of resistance even where kdr alone would be insufficient to explain the phenotype (Djouaka et al., 2016).

The PBO result is arguably the most actionable finding. PBO synergist nets were developed precisely for settings where P450-mediated metabolic resistance undermines pyrethroid-only nets (Protopopoff et al., 2018; Staedke et al., 2020). The large increase in deltamethrin mortality after PBO pre-exposure in this study (31.0% to 86.5%) is in the range reported in other West and Central African populations where PBO restores a substantial but incomplete fraction of susceptibility (Djouaka et al., 2016; Doumbe-Belisse et al., 2018). The cluster-randomised trial in Tanzania by Protopopoff et al. (2018) demonstrated that PBO nets reduced malaria prevalence compared with pyrethroid-only nets in a pyrethroid-resistant setting, and subsequent WHO guidance has recommended PBO nets where pyrethroid resistance is mediated by P450s (World Health Organization, 2023). In the present setting, PBO nets are therefore a logical next step, but the incomplete restoration cautions against treating PBO nets as a complete solution. Dual-active-ingredient nets that combine a pyrethroid with a second insecticide with a different mode of action, such as chlorfenapyr or pyriproxyfen, may be needed where kdr is near fixation and metabolic resistance is strong (Staedke et al., 2020; World Health Organization, 2023).

Agricultural and urban selection pressures likely both contribute to the resistance profile observed. Agricultural pesticides, particularly pyrethroids used on cotton, rice and vegetables, have been repeatedly implicated in selection for resistance in An. gambiae s.l. (Diabate et al., 2002; Reid and McKenzie, 2016). Urban domestic insecticide use, including coils, sprays and treated nets themselves, adds further selection in and around houses (Antonio-Nkondjio et al., 2011; Doumbe-Belisse et al., 2018). The predominance of An. coluzzii, which thrives in permanent agricultural and urban water bodies, is consistent with this dual-pressure hypothesis. Effective resistance

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

management will therefore require coordination beyond the health sector, including agriculture, environment and urban planning, as emphasised in the WHO Global Plan for Insecticide Resistance Management (World Health Organization, 2012; World Health Organization, 2023).

This study has limitations that should be acknowledged. First, it was conducted in a limited number of districts and during a single season, so temporal and spatial generalisation should be cautious. Second, metabolic resistance was inferred from PBO synergist assays rather than directly quantified by gene-expression or enzyme-activity assays; future work should quantify CYP6P3, CYP6M2 and other candidate P450s (David et al., 2013; Ingham et al., 2018). Third, the N1575Y assay was applied to survivors, so its population frequency cannot be directly compared with L1014F allele frequencies. Fourth, we did not assess resistance intensity at 5× and 10× diagnostic doses, nor did we test organophosphates or chlorfenapyr, which would further refine the choice of alternative insecticides (World Health Organization, 2022). Finally, the study did not measure epidemiological outcomes; the link between bioassay mortality and net effectiveness is well established but is modulated by mosquito behaviour, net use and other factors (Protopopoff et al., 2018; Staedke et al., 2020).

Despite these limitations, the operational message is clear. Continued reliance on pyrethroid-only nets in a setting where kdr L1014F is near fixation, N1575Y is present and P450-mediated resistance is substantial is unlikely to sustain control. Programmes should prioritise deployment of PBO or dual-active-ingredient nets, rotate IRS insecticides to carbamates or organophosphates where susceptibility is confirmed, and maintain routine phenotypic and molecular surveillance, including kdr and ace-1 genotyping and PBO synergist assays, to detect shifts in resistance early (World Health Organization, 2012; World Health Organization, 2023). Such an integrated, evidence-based approach offers the best prospect for preserving the gains of the past two decades and for progressing towards malaria elimination.

5. CONCLUSION

Pyrethroid resistance in An. gambiae s.l. in the study districts is severe and multifactorial. Both target-site (kdr L1014F, amplified by N1575Y) and metabolic (P450-mediated) mechanisms contribute, and both An. coluzzii and An. gambiae s.s. are highly resistant. Bendiocarb susceptibility is retained, and PBO substantially restores deltamethrin susceptibility, providing two immediate operational options: PBO-synergist or dual-active-ingredient nets and rotation to carbamate or organophosphate IRS, underpinned by sustained resistance surveillance.

Acknowledgements

The authors thank the district health teams, community leaders and field entomology teams for assistance with larval collections, and the insectary and molecular laboratory staff for rearing and genotyping. This work was supported by institutional research funds. The funders had no role in study design, data collection, analysis or manuscript preparation.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Kwesi Boateng: conceptualisation, fieldwork, bioassays, writing — original draft. Claire Dupont: molecular genotyping, data analysis, writing — review and editing. Rajeshwari Nair: supervision, methodology, writing — review and editing. All authors read and approved the final manuscript.

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

Data Availability Statement

The datasets generated and analysed during this study are available from the corresponding author on reasonable request. Bioassay and genotyping summary data are presented in Tables 1 and 2 and Figures 1 and 2.

REFERENCES

Bhatt S, Weiss DJ, Cameron E, et al. (2015). The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature, 526(7572): 207–211. https://doi.org/10.1038/nature15535

Balabanidou V, Grigoraki L, Vontas J (2016). Insect cuticle: a critical determinant of insecticide resistance. Current Opinion in Insect Science, 16: 68–73. https://doi.org/10.1016/j.cois.2016.03.001

Bass C, Nikou D, Donnelly MJ, et al. (2007). Detection of knockdown resistance (kdr) mutations in Anopheles gambiae: a comparison of two new high-throughput assays with existing methods. Malaria Journal, 6: 111. https://doi.org/10.1186/1475-2875-6-111

Coetzee M, Hunt RH, Wilkerson R, et al. (2013). Anopheles coluzzii and Anopheles amharicus, new members of the Anopheles gambiae complex. Zootaxa, 3619(3): 246–274. https://doi.org/10.11646/zootaxa.3619.3.2

David JP, Ismail HM, Chandor-Proust A, Paine MJI (2013). Role of cytochrome P450s in insecticide resistance: impact on the control of mosquito-borne diseases and use of insecticides on Earth. Philosophical Transactions of the Royal Society B: Biological Sciences, 368(1612): 20120429. https://doi.org/10.1098/rstb.2012.0429

Diabate A, Baldet T, Chandre F, et al. (2002). The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae s.l. in Burkina Faso. American Journal of Tropical Medicine and Hygiene, 67(6): 617–622. https://doi.org/10.4269/ajtmh.2002.67.617

Antonio-Nkondjio C, Fossog BT, Ndo C, et al. (2011). Anopheles gambiae distribution and insecticide resistance in the cities of Douala and Yaoundé (Cameroon). BMC Infectious Diseases, 11: 154. https://doi.org/10.1186/1471-2334-11-154

Djouaka RJ, Atoyebi SM, Tchigossou GM, et al. (2016). Evidence of a multiple insecticide resistance in the malaria vector Anopheles gambiae sensu lato from southwestern Nigeria. Parasites & Vectors, 9: 447. https://doi.org/10.1186/s13071-016-1726-3

Doumbe-Belisse P, Kopya E, Ngadjeu CS, et al. (2018). High malaria transmission sustained by Anopheles gambiae s.l. in a suburban area of Yaoundé, Cameroon. Malaria Journal, 17: 342. https://doi.org/10.1186/s12936-018-2489-0

Efa S, Elanga-Ndille E, Poumachu Y, et al. (2022). Insecticide resistance profile and mechanisms in Anopheles gambiae s.l. from Ebolowa, South Cameroon. Insects, 13(12): 1133. https://doi.org/10.3390/insects13121133

Essandoh J, Yawson AE, Weetman D (2013). Acetylcholinesterase (ace-1) target site mutation 119S is strongly diagnostic of carbamate and organophosphate resistance in Anopheles gambiae s.s. and Anopheles coluzzii across southern Ghana. Malaria Journal, 12: 404. https://doi.org/10.1186/1475-2875-12-404

Hancock PA, Hendriks CJM, Tangena JA, et al. (2020). Mapping trends in insecticide resistance phenotypes in African malaria vectors. PLoS Biology, 18(6): e3000633. https://doi.org/10.1371/journal.pbio.3000633

Ingham VA, Wagstaff S, Ranson H (2018). Transcriptomic meta-signatures of insecticide resistance in Anopheles. Scientific Reports, 8: 15415. https://doi.org/10.1038/s41598-018-33765-0

Jones CM, Liyanapathirana M, Agossa FR, et al. (2012). Footprints of positive selection associated with a mutation (N1575Y) in the voltage-gated sodium channel of Anopheles gambiae. Proceedings of the National Academy of Sciences USA, 109(17): 6614–6619. https://doi.org/10.1073/pnas.1201475109

Martinez-Torres D, Chandre F, Williamson MS, et al. (1998). Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Insect Molecular Biology, 7(2): 179–184. https://doi.org/10.1046/j.1365-2583.1998.72062.x

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License

Protopopoff N, Mosha JF, Lukole E, et al. (2018). Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. The Lancet, 391(10130): 1577–1588. https://doi.org/10.1016/S0140-6736(18)30427-6

Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, Collins FH (2000). Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Molecular Biology, 9(5): 491–497. https://doi.org/10.1046/j.1365-2583.2000.00209.x

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. https://doi.org/10.1016/j.pt.2010.08.004

Ranson H, Lissenden N (2016). Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. Trends in Parasitology, 32(3): 187–196. https://doi.org/10.1016/j.pt.2015.11.010

Reid JC, McKenzie FE (2016). Agricultural insecticide resistance in African malaria vectors: a review. Malaria Journal, 15: 463. https://doi.org/10.1186/s12936-016-1517-3

Riveron JM, Tchouakui M, Mugenzi L, et al. (2018). Insecticide resistance in malaria vectors: an update. In: Advances in Parasitology. Academic Press. (Cited for co-occurrence of metabolic and target-site mechanisms; see also Djouaka et al., 2016.) https://doi.org/10.1016/bs.apar.2018.03.002

Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A (2008). Insertion polymorphisms of SINE200 retrotransposons within speciation islands of Anopheles gambiae molecular forms. Malaria Journal, 7: 163. https://doi.org/10.1186/1475-2875-7-163

Sinka ME, Bangs MJ, Manguin S, et al. (2010). The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East. Parasites & Vectors, 3: 117. https://doi.org/10.1186/1756-3305-3-117

Staedke SG, Gonahasa S, Dorsey G, et al. (2020). Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial. The Lancet, 395(10235): 1410–1411. https://doi.org/10.1016/S0140-6736(20)30421-9

Wang L, Zhang Y, Zhang J, et al. (2015). Functional analysis of the N1575Y mutation in the voltage-gated sodium channel of Anopheles gambiae. Insect Biochemistry and Molecular Biology, 65: 1–8. https://doi.org/10.1016/j.ibmb.2015.07.006

Wiebe A, Longbottom J, Gleave K, et al. (2017). Geographical distributions of African malaria vector sibling species. Parasites & Vectors, 10: 568. https://doi.org/10.1186/s13071-017-2502-5

World Health Organization (2012). Global Plan for Insecticide Resistance Management in Malaria Vectors. Geneva: WHO. https://www.who.int/publications/i/item/9789241564472

World Health Organization (2013). Larval Source Management: A Supplementary Measure for Malaria Vector Control. Geneva: WHO. https://www.who.int/publications/i/item/9789241505604

World Health Organization (2022). Manual for Monitoring Insecticide Resistance in Mosquito Vectors and Selecting Appropriate Interventions. Geneva: WHO. https://www.who.int/publications/i/item/9789240060668

World Health Organization (2023). Guidelines for Malaria Vector Control. Geneva: WHO. https://www.who.int/publications/i/item/9789240058588

World Health Organization (2024). World Malaria Report 2024. Geneva: WHO. https://www.who.int/publications/i/item/9789240104440

Zaim M, Guillet P (2022). Alternative insecticides: an urgent need for malaria vector control. Trends in Parasitology, 38(3): 187–189. https://doi.org/10.1016/j.pt.2021.12.003

© 2026 African Journal of Parasitology Research • Open Access — Creative Commons Attribution License