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Translating Mosquito Insecticide Resistance into Operational Decisions: A Critical Review of Bioassays, Mechanisms, Thresholds, and Control Failure

Fatima Zahra Amrani[1]*, Youssef Benali[1], Bruno Martins[2], Lucas Pereira[2]

[1]Department of Bio-Nano Sciences and Applied Technologies, Faculty of Sciences and Engineering, Mohammed V University, Rabat, Morocco.
[2]Department of Nano-Biomedical Engineering, Faculty of Engineering, University of Minho, Braga, Portugal.

ABSTRACT

Insecticide resistance is increasingly detected in mosquito populations targeted by public-health interventions, yet the operational meaning of a resistant classification remains difficult to determine. Standard bioassays provide essential evidence of survival under controlled exposure, but they do not reproduce the insecticide dose, contact duration, mosquito behaviour, product condition, intervention coverage, or transmission environment encountered during implementation. This critical methods review examines how resistance evidence should be translated into operational decisions by integrating four connected domains: what mosquito bioassays measure, the biological mechanisms underlying resistance, resistance intensity in relation to operational exposure, and the evidential pathway from laboratory phenotype to intervention failure. The synthesis distinguishes resistance frequency from resistance intensity, mechanism detection from field-level contribution, biological efficacy from operational effectiveness, and observed associations from causal evidence. The strongest defensible conclusion is that no single phenotype, molecular marker, intensity category, or product bioassay can independently establish control failure. Decision-relevant interpretation instead requires linked evidence describing the assay construct, vector population, physiological and behavioural mechanisms, product-specific performance, realized exposure, implementation conditions, and relevant entomological or epidemiological outcome. Important limitations include uneven surveillance, non-equivalent assay systems, incomplete calibration of intensity categories, uncertain contributions of co-occurring mechanisms, and weak linkage between laboratory measurements and programme outcomes. Resistance-informed vector control should therefore use staged evidence integration rather than universal thresholds, with uncertainty retained explicitly at each transition from detection to mechanism attribution, product evaluation, and operational action. The central implication is that resistance surveillance becomes operationally valuable only when measurements are interpreted within defined products, populations, exposure pathways, intervention objectives, and decision contexts.

Keywords: Mosquito insecticide resistance, Medical entomology, Vector-borne disease ecology, Resistance bioassays, Resistance intensity, Operational exposure.

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Corresponding author: Fatima Zahra Amrani
E-mail ✉ fatima.amrani@gmail.com
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INTRODUCTION

Mosquito insecticide resistance has become a geographically extensive but biologically heterogeneous challenge for vector control. Resistance phenotypes occur across major *Aedes* vectors and African malaria-vector populations, but their prevalence, intensity, mechanisms, and temporal trajectories vary among species and settings [1, 2]. This heterogeneity matters because a resistant classification does not represent a uniform biological state. The same mortality result may arise from different combinations of target-site alteration,

detoxification, reduced penetration, physiological condition, or assay exposure, while populations carrying apparently similar mechanisms may experience different operational insecticide doses.

The central interpretive problem is therefore not whether resistance exists, but what a particular measurement establishes. Diagnostic-dose mortality can indicate that a population no longer responds like a susceptible reference population under a specified protocol. It does not directly quantify the strength of resistance, identify the responsible mechanism, predict the efficacy of a particular formulation, or determine whether an intervention will fail to protect people. These are distinct constructs situated at different biological and operational scales. Treating them as interchangeable compresses uncertainty and encourages decisions that are more precise than the evidence permits.

Field evidence reinforces this non-equivalence. Long-lasting insecticidal nets may retain protective value in areas where phenotypic resistance is detected, while their effectiveness may also be weakened by resistance in combination with poor physical condition, inconsistent use, inadequate coverage, altered vector behaviour, ecological change, or declining insecticide availability. Field effectiveness therefore cannot be inferred from bioassay mortality alone because net use, physical integrity, coverage, mosquito behaviour, and transmission ecology jointly shape outcomes [3, 4]. Conversely, preserved epidemiological protection in one context does not show that resistance is operationally unimportant elsewhere or that increasing resistance intensity will remain inconsequential.

This review develops a decision-centred interpretation of mosquito insecticide-resistance evidence. It critically compares bioassay constructs, target-site, metabolic, behavioural, and cuticular mechanisms, resistance intensity, operational exposure, product performance, and control outcomes. Its central argument is that translation from laboratory phenotype to operational action requires an explicit chain of evidence. Bioassay resistance is not equivalent to operational control failure; resistance frequency is not equivalent to resistance intensity; a detected mechanism is not equivalent to its field-level contribution; and laboratory exposure is not equivalent to the dose, behaviour, coverage, and environment of operational use.

What mosquito resistance bioassays measure

A resistance bioassay measures mosquito survival or mortality after a defined experimental exposure. Its result is conditional on the insecticide, concentration, exposure duration, delivery surface, route of uptake, post-exposure observation period, mosquito species, age, physiological state, and handling conditions. Diagnostic-dose assays are useful for identifying reduced susceptibility relative to a standardized expectation, whereas escalating-dose assays probe the strength of survival beyond that diagnostic exposure. Escalating-dose intensity assays consequently add information that frequency-based classification cannot provide, but their categories remain laboratory phenotypes rather than direct epidemiological thresholds [5]. A population with many survivors at the diagnostic dose may contain resistance of different strengths, and identical resistance frequencies can conceal markedly different dose–response relationships.

Assay design also determines the exposure construct being measured. Tests based on treated papers, coated bottles, direct topical application, or contact with insecticidal netting differ in how insecticide reaches the mosquito and in the uniformity of the delivered dose. Alternative contact formats can reveal effects of long-lasting insecticidal nets that may be underestimated or obscured by short standardized exposures [6]. Similarly, comparisons among the Centers for Disease Control and Prevention bottle assay, the World Health Organization tube assay, and topical application demonstrate differences in delivered exposure and mortality variability; their classifications should not be treated as interchangeable simply because all report mortality [7]. Agreement between methods may strengthen confidence in a phenotype, but disagreement may reflect assay geometry, dose distribution, absorption route, or experimental variability rather than a biological contradiction.

Interpretation is further weakened when mortality percentages are converted into coarse categories without retaining dose–response shape or uncertainty. Continuous probabilistic analysis can preserve information about the

estimated dose–mortality relationship and the uncertainty surrounding resistance intensity, whereas broad low, moderate, or high labels may imply boundaries that have not been calibrated to product performance or disease outcomes [8]. A bioassay result should therefore be reported as a method-specific observation, not as an autonomous decision rule. At minimum, interpretation requires the assay protocol, delivered exposure, mosquito characteristics, replication, uncertainty, and the operational question to which the measurement is being applied. The evidence dimensions and interpretive boundaries for mosquito resistance bioassays measure are summarized in **Table 1**.

Table 1. What Mosquito Resistance Bioassays Measure: Vector Systems, Biological Mechanisms, Exposure Pathways, Evidence Requirements, Uncertainty, and Interpretive Boundaries

Vector or transmission domain	Environmental or operational driver	Biological mechanism	Human-exposure pathway	Evidence required	Context dependency	Uncertainty	Interpretive boundary
Anopheles malaria vectors assessed with diagnostic-dose assays	Routine susceptibility surveillance using a standardized insecticide exposure	Composite survival phenotype integrating all mechanisms active under the test conditions	Indirect; the assay estimates potential loss of insecticidal killing rather than human protection	Protocol-specific mortality, controls, mosquito age and physiological state, insecticide identity, and test quality	Species, population, season, insecticide, and laboratory conditions	Relative contributions of biological resistance and procedural variation	Detection of reduced susceptibility does not establish resistance intensity or operational failure
Anopheles malaria vectors assessed with escalating-dose assays	Need to distinguish weak survival at a diagnostic dose from survival under stronger exposure	Increasing dose challenges the strength of the composite resistance phenotype	Indirect; stronger laboratory survival may indicate greater concern but does not define disease risk	Mortality across multiple concentrations, reproducible dose delivery, and uncertainty around the dose–response relation	Reference strain, population composition, physiological state, and insecticide class	Calibration between assay multiples and operationally encountered doses	Resistance frequency is not equivalent to resistance intensity
Anopheles vectors tested against insecticidal netting	Product evaluation and concern that standardized short contact may misrepresent net interaction	Combined effects of contact time, surface insecticide availability, uptake, irritation, and delayed mortality	Contact during host seeking through or upon treated net material	Product identity and condition, contact format, exposure duration, immediate and delayed outcomes, and susceptible comparison	Net formulation, textile condition, mosquito strain, and contact behaviour	Artificial exposure may not reproduce natural duration, repetition, or avoidance	Laboratory net bioefficacy is not equivalent to household or community effectiveness
Aedes aegypti assessed using bottle, tube, or topical assays	Selection of surveillance method and comparison among laboratories or programmes	Each method exposes mosquitoes through a different surface, route, and dose distribution	Indirect; results inform susceptibility to control insecticides but do not measure actual human–vector contact	Within-method replication, delivered concentration, baseline mortality, variance, and method-specific controls	Colony or field origin, insecticide, solvent, handling, and assay platform	Cross-method differences may reflect measurement design rather than biological change	Mortality values from different assay systems are not automatically interchangeable
Malaria vectors analysed with continuous intensity models	Need to avoid information loss from categorical intensity classifications	Estimated dose–response describes the probability of mortality across increasing exposure	Indirect; probabilistic estimates may improve comparison but require operational calibration	Adequate observations across doses, model checking, transparent assumptions, and uncertainty intervals	Data quality, dose spacing, sample size, population heterogeneity, and model assumptions	Dependence on priors, sparse responses, and external validation	A modelled intensity estimate is not a validated intervention-failure threshold
Aedes resistance surveillance across geographic settings	Local insecticide selection pressure, control history, and uneven surveillance coverage	Variable combinations of target-site, metabolic, and other resistance processes contribute to observed mortality	Potentially reduced efficacy of adulticides or treated materials used to limit human–vector contact	Repeated local phenotype data supported by mechanism and intervention-performance evidence	Species, geography, insecticide history, and surveillance density	Incomplete spatial coverage and heterogeneous testing practices	Widespread resistance does not imply uniform severity or identical control consequences

African malaria-vector surveillance across space and time	Changing intervention pressure, ecology, test distribution, and vector composition	Population-level changes in phenotypic susceptibility	Potential modification of protection provided by indoor insecticidal interventions	Standardized longitudinal surveillance with spatial, temporal, species, and intervention information	Sparse observations, non-random sampling, changing vector composition, and shifting test coverage	Model uncertainty where observations are limited	Modelled resistance probability is not observed product or programme failure

Target-site, metabolic, behavioural, and cuticular mechanisms

Target-site resistance modifies the mosquito protein upon which an insecticide acts, thereby reducing toxicodynamic sensitivity. Even within this apparently discrete category, interpretation is not straightforward. Population-genomic evidence in African *Anopheles gambiae* and *Anopheles coluzzii* shows that target-site resistance is structured by multiple variants and haplotypes whose phenotypic meanings depend on species, genetic background, and geography [9]. Allele frequency can therefore support mechanistic surveillance, but it is not a substitute for phenotypic testing, resistance-intensity measurement, or product evaluation. A frequent allele may be weakly predictive in one background and more consequential in another, particularly when metabolic or penetration mechanisms co-occur.

Metabolic resistance acts before sufficient insecticide reaches its target, commonly through increased detoxification or sequestration. Functional evidence demonstrates that particular cytochrome P450 alleles can increase pyrethroid survival and reduce mortality caused by pyrethroid-treated nets [10]. Such evidence provides a stronger causal chain than expression data alone because it connects a defined genetic variant to phenotype and product response. Nevertheless, the field-level contribution of a validated allele remains population- and product-dependent. Multiple detoxification enzymes may be co-expressed, substrate specificity may differ among active ingredients, and apparent metabolic resistance may interact with target-site or cuticular processes. Mechanism detection consequently indicates biological plausibility, not a universal or quantitatively fixed contribution to control loss.

Cuticular and behavioural processes further complicate mechanism attribution. Reduced penetration can delay insecticide uptake while enzymatic detoxification removes part of the absorbed dose, allowing the two pathways to act jointly rather than independently [11]. Measuring only enzyme activity may consequently overattribute resistance to metabolism, whereas measuring cuticular change without uptake kinetics may not establish its functional importance. Behaviour operates at a different point in the exposure pathway by altering whether, when, where, and for how long mosquitoes contact treated surfaces. Physiological resistance and shifts in biting or resting behaviour have been observed together after long-lasting insecticidal-net deployment, but temporal co-occurrence does not identify a single causal mechanism [12]. Environmental change, host availability, vector-species turnover, or human behaviour may produce similar patterns. Forced-contact bioassays cannot measure this behavioural component of operational exposure.

Resistance intensity and operational exposure

Resistance frequency describes the proportion of mosquitoes surviving a specified diagnostic exposure; resistance intensity describes how survival changes as exposure increases. The distinction is operationally important because two populations with similar diagnostic-dose mortality may differ in their susceptibility at higher concentrations and in their response to particular formulations. In Accra, high pyrethroid-resistance intensity occurred alongside low mortality in tests of pyrethroid-only long-lasting insecticidal nets, providing a coherent entomological warning signal [13]. However, this concurrence does not independently establish loss of population protection. The evidence remains specific to the tested vector population, products, exposure methods, and ecological setting, and it does not quantify intervention use, coverage, physical barrier effects, or disease outcomes.

Intensity is also dynamic rather than a fixed property of a species or region. Surveillance across Kinshasa and other provinces of the Democratic Republic of Congo showed

substantial variation among insecticides, locations, and survey periods, demonstrating that diagnostic-dose frequency cannot stand in for a stable or spatially uniform estimate of resistance strength [14]. Variation may represent different selection histories, vector composition, mechanism combinations, or sampling conditions. Mosquito physiological state further modifies observed intensity: age and blood feeding can change apparent resistance and subsequent longevity [15]. Comparisons made with non-equivalent age structures, feeding states, rearing histories, or environmental conditions may therefore misclassify biological differences as geographic or temporal changes in resistance.

Operational exposure is the insecticide dose actually encountered by a mosquito during intervention use. It depends on contact probability, duration, route, repetition, product condition, active-ingredient availability, household use, coverage, and mosquito behaviour. Laboratory intensity assays deliberately standardize these factors to improve comparison, but standardization does not create equivalence with field contact. Longitudinal evidence from Malawi linked increasing resistance intensity partly to greater expression of P450 alleles and declining entomological performance of tested nets, while leaving the magnitude of population-level epidemiological effect unresolved [16]. The defensible interpretation is therefore exposure–response based: resistance intensity identifies the strength of survival under specified laboratory challenge, whereas operational significance emerges only when that phenotype is connected to product-specific dose, natural contact, intervention condition, coverage, vector ecology, and an explicitly defined control objective.

From laboratory phenotype to control failure

Translation from a resistant laboratory phenotype to operational failure requires several evidential transitions. The phenotype must first affect the performance of a relevant product under a realistic contact pathway; the resulting reduction in killing, deterrence, or feeding inhibition must then persist at the coverage achieved by the programme; and that change must be sufficient to alter an explicitly defined entomological or epidemiological objective. In Kinshasa, reduced net-induced mortality co-occurred with high *Plasmodium* infection in resistant vectors, a concerning field pattern that does not alone establish a causal resistance-to-transmission pathway [17]. **Figure 1** integrates the sequential evidence transitions linking laboratory resistance measurements, interacting biological mechanisms, realized operational exposure, product performance, and the probability that a vector-control objective will not be achieved.

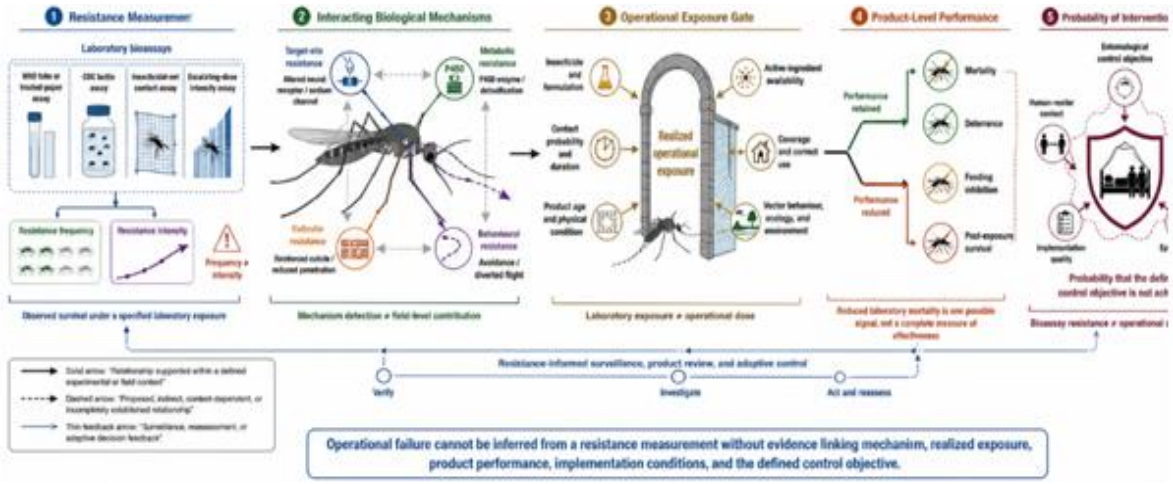

Figure 1. Resistance measurement, biological mechanisms, operational exposure, and the probability of intervention failure

Product-specific evidence can strengthen the translation chain without completing it. In southeast Côte d'Ivoire, poor conventional-net bioefficacy was associated with intense pyrethroid resistance and overexpression of several P450 genes, whereas next-generation

nets retained greater activity [18]. In Mozambique, escalating P450-associated resistance reduced mortality even to a piperonyl-butoxide–pyrethroid net, demonstrating that a product intended to mitigate one resistance pathway can be overtaken by sufficiently strong or complex resistance [19]. These findings connect phenotype, mechanism, and product response, but they remain entomological evidence. They do not independently establish inadequate household use, reduced community coverage, increased human exposure, or higher disease incidence.

Epidemiological evidence can also diverge from entomological warning signals. Health-facility data from Benin did not reveal a simple association between standard phenotypic-resistance frequency and clinical malaria incidence, underscoring the limits of a single-marker failure rule [20]. Such a result should not be interpreted as evidence that resistance is operationally harmless; routine-data quality, ecological linkage, intervention coverage, immunity, healthcare access, climate, and vector composition can obscure an effect. Control failure should instead be defined as failure to achieve a specified objective under documented implementation conditions, with evidence identifying where the chain from product contact to population protection weakened. The evidence dimensions and interpretive boundaries for from laboratory phenotype to control failure are summarized in **Table 2**.

Table 2. From Laboratory Phenotype to Control Failure: Vector Systems, Biological Mechanisms, Exposure Pathways, Evidence Requirements, Uncertainty, and Interpretive Boundaries

Vector or transmission domain	Environmental or operational driver	Biological mechanism	Human-exposure pathway	Evidence required	Context dependency	Uncertainty	Interpretive boundary
Resistant *Anopheles* populations with reduced net mortality	Use of insecticidal nets in settings with multiple resistant vector species	Combined target-site, metabolic, penetration, or behavioural resistance may reduce insecticidal killing	Surviving infected mosquitoes may retain opportunities for human contact	Resistance phenotype, product bioefficacy, infection measurements, contact data, and transmission outcomes	Vector species, infection prevalence, product condition, net use, and local transmission	Direction and magnitude of the resistance–infection relationship	Co-occurrence of poor net mortality and mosquito infection is not proof that resistance caused transmission failure
Anopheles coluzzii exposed to conventional and next-generation nets	Product choice under intense pyrethroid resistance	Overexpression of CYP6P4, CYP6P3, and CYP6Z1 contributes to reduced pyrethroid susceptibility	Lower insecticidal mortality may increase the probability of continued host seeking	Molecular expression, intensity assays, comparative net bioefficacy, and field-contact evidence	Active ingredient, synergist, resistance intensity, vector population, and net condition	Relative contribution of each enzyme and co-occurring mechanisms	Association between P450 expression and reduced bioefficacy does not quantify population-health impact
Anopheles funestus exposed to PBO–pyrethroid nets	Escalation of metabolic resistance after deployment of pyrethroid-based tools	Strong or complex P450-mediated detoxification can erode synergist-supported efficacy	Surviving mosquitoes may continue contact attempts, but natural exposure is not reproduced by forced assays	Longitudinal phenotype, validated mechanisms, product-specific bioassays, durability, use, and epidemiological outcomes	Resistance strength, enzyme profile, PBO availability, product age, and contact duration	Whether efficacy loss persists under household and community conditions	Loss of laboratory bioefficacy is not identical to operational programme failure
Malaria transmission assessed through routine clinical data	Net coverage, healthcare use, climate, immunity, vector ecology, and resistance surveillance	Resistance may affect transmission through reduced mosquito mortality, but multiple pathways intervene	Human infection depends on mosquito survival, biting, intervention use, and host factors	Linked entomological, implementation, clinical, temporal, and spatial data	Surveillance quality, ecological matching, diagnostic practice, and baseline transmission	Confounding and measurement error may conceal or exaggerate associations	Absence of a simple resistance–incidence association is not evidence of no resistance effect

Programme-level determination of control failure	Product degradation, incomplete use, poor coverage, behavioural avoidance, or biological resistance	Several failures can produce similar reductions in intervention effect	Reduced personal protection or community-level suppression can increase exposure	Defined objective, comparator, implementation fidelity, product quality, vector outcomes, and human outcomes	Intervention type, target vector, transmission setting, resources, and evaluation period	Attribution among biological and implementation causes	A resistant bioassay classification alone cannot establish control failure

Decision thresholds and evidence interpretation

A decision threshold is a rule linking evidence to an action, not a universal biological constant. The consequences of acting too early or too late, the availability of alternative products, the reversibility of the decision, and the uncertainty of the evidence should determine how much support is required. Decision rules should therefore distinguish technical resistance detected under standardized exposure from practical resistance that materially changes control performance in a defined programme context [21]. A diagnostic-dose result may appropriately trigger confirmation or intensified surveillance without automatically triggering product withdrawal.

Threshold interpretation also depends on the intervention being evaluated. A mortality value obtained from an insecticidal net can reflect net sampling, washing, storage, manufacturing variation, active-ingredient migration to the surface, textile condition, and mosquito susceptibility. These factors may change during transport and field use, so product and vector measurements must be interpreted jointly [22]. Separate thresholds are consequently needed for assay validity, resistance alert, product investigation, operational action, and post-decision review. Collapsing these functions into one mortality cutoff hides the evidential transition between detection and action.

Molecular and model-based indicators can extend surveillance but do not remove the need for calibration. Target-site allele-frequency maps can support anticipatory surveillance, although their operational meaning remains conditional on vector species, genetic background, phenotype, product, and local exposure [23]. Data-informed deployment models can combine resistance, ecology, intervention efficacy, cost, and resource constraints, but their rankings remain dependent on structural assumptions and setting-specific inputs [24]. Decision thresholds should therefore be tiered: an alert threshold initiates verification, an investigation threshold triggers product and exposure assessment, and an action threshold requires evidence that the expected benefit of changing strategy exceeds its costs and risks.

Critical methodological synthesis

The most consistent finding across the evidence base is non-equivalence between resistance detection and operational failure. Bioassays, molecular markers, product tests, field entomology, and epidemiological outcomes measure different parts of the causal chain. Across synthesis and cluster-randomized evidence, piperonyl-butoxide–pyrethroid nets can improve entomological or epidemiological outcomes in high-resistance settings, but the magnitude and persistence of benefit vary by product, setting, coverage, and time since deployment [25–27]. These findings demonstrate that mechanism-aware products can restore part of the effect lost to resistance; they do not establish that one product class is universally superior or evolutionarily durable.

Comparative intervention evidence further shows that next-generation nets cannot be treated as a homogeneous category. A four-arm trial in Tanzania found that some dual-active-ingredient nets improved malaria outcomes and cost-effectiveness relative to pyrethroid-only nets [28]. The inference is product- and setting-specific because active ingredients differ in mode of action, resistance vulnerability, wash durability, manufacturing characteristics, price, and dependence on sustained household use. A positive result for one combination cannot be transferred automatically to another combination or to a population with a different mechanism profile.

Time since deployment is a central but often undermeasured modifier. Third-year follow-up of a cluster-randomized trial showed that the advantage of piperonyl-butoxide nets weakened as net use and piperonyl-butoxide content declined [29]. This finding illustrates why laboratory susceptibility cannot be isolated from

operational exposure. Textile deterioration, declining surface availability of active ingredients, loss of synergist, net attrition, inconsistent use, and replacement practices may alter effectiveness even when the underlying mosquito phenotype is unchanged.

The strongest supported inference is therefore conditional rather than threshold-based: resistance becomes operationally important when a defined phenotype and mechanism reduce the performance of a relevant product under realized exposure sufficiently to compromise a specified programme objective. The unresolved question is which combinations of assay phenotype, intensity, mechanism, product condition, contact behaviour, coverage, and ecology best predict that transition. Comparative models can help organize these variables, but their recommendations remain dependent on local inputs and assumptions [24]. The convergent findings, context-dependent results, methodological limitations, and remaining uncertainties are synthesized in **Table 3**.

Table 3. Critical Methodological Synthesis: Convergent Findings, Context Dependence, Methodological Limitations, Evidence Confidence, and Residual Uncertainty

Evidence domain	Convergent finding	Contradictory or context-dependent finding	Study-design basis	Main methodological limitation	Strength of inference	Residual uncertainty	Implication
Resistance bioassays	Standardized assays detect survival under defined exposure	Assay platforms deliver non-equivalent doses and variability	Comparative laboratory and intensity-bioassay studies	Limited external validity for natural contact	Strong for measurement differences; limited for operational prediction	Which assay characteristics best predict product performance	Preserve method, dose–response, mosquito-state, and uncertainty data
Target-site resistance	Variants and haplotypes can contribute to reduced toxicodynamic sensitivity	Phenotypic meaning varies with species, background, geography, and co-mechanisms	Population genomics and spatiotemporal modelling	Incomplete genotype–phenotype–product linkage	Strong genetic inference; moderate operational inference	Product-specific predictive value of allele trends	Validate markers against intensity and product response
Metabolic resistance	Functionally validated P450 pathways can reduce pyrethroid and net efficacy	Enzyme combinations and resistance strength differ among populations and products	Functional molecular, expression, and field-bioefficacy studies	Expression alone does not quantify causal contribution	Strong for selected alleles and settings	Generalizability and capacity to overcome synergists	Pair molecular diagnostics with product-specific monitoring
Behavioural and cuticular resistance	Avoidance and reduced penetration can lower the internal dose received	Relative effects depend on contact opportunity, duration, ecology, and mosquito state	Longitudinal field observation and physiological experiments	Difficult causal attribution under natural exposure	Moderate mechanistic inference	Persistence and transmission relevance	Measure contact behaviour and uptake kinetics separately from forced mortality
Resistance intensity	Escalating-dose assays add information beyond diagnostic-dose frequency	Measured intensity changes with site, insecticide, age, feeding state, and assay design	Dose–response, surveillance, and physiological studies	No universal epidemiological calibration	Strong measurement inference	Operationally relevant exposure range	Report continuous response and sample-state modifiers
Operational exposure	Coverage, use, product chemistry, durability, and behaviour determine realized dose	High laboratory resistance may coexist with retained protection, while product advantage may decay	Cohorts, product-method studies, and trial follow-up	Individual contact histories are rarely measured directly	Strong evidence of multifactoriality	Contribution of each exposure component	Monitor product condition, use, contact, and phenotype jointly
Control failure	Resistance can reduce product mortality and weaken intervention effect	Epidemiological outcomes do not show a universal monotonic relationship with assay mortality	Field entomology, observational cohorts, routine data, and trials	Confounding and incomplete linkage across scales	Strong for context dependence	Predictive combination of evidence layers	Define the objective and comparator before declaring failure
Decision thresholds	Action requires consideration of consequences,	Marker cutoffs and model rankings are setting- and	Critical methods analysis and	Limited prospective	Moderate decision-level inference	False-positive and false-	Separate alert, investigation, action, and

	alternatives, feasibility, and uncertainty	assumption-dependent	deployment modelling	validation of action thresholds		negative performance	review thresholds
Resistance-informed products	Mechanism-aware and dual-active tools can improve outcomes in resistant settings	Benefit varies with formulation, mechanism, durability, coverage, cost, and time	Evidence synthesis and cluster-randomized trials	Transferability between products and ecological settings	Strong for selected product–setting combinations	Evolutionary durability and long-term comparative value	Use adaptive product portfolios with continuing surveillance

Recommendations for resistance-informed vector control

Resistance-informed control should be local, mechanism-aware, and integrated rather than reduced to changing insecticides after a single resistant classification [30]. Programmes should begin by defining the operational objective and the product or strategy under consideration. Standardized phenotype and intensity testing can then function as an alert, followed by confirmatory assays, mechanism characterization, product-specific bioefficacy testing, assessment of product condition, and measurement of coverage and vector-contact patterns. A strategy change should be linked to pre-specified review criteria so that ineffective or impractical decisions can be revised.

Genetic surveillance can improve timeliness by detecting the emergence and spread of resistance variants before conventional phenotype data become geographically dense. Its decision value is greatest when markers are connected to measured phenotype, resistance intensity, active-ingredient vulnerability, and product performance rather than reported as isolated molecular detections [31]. A real-time diagnostic for a rapidly spreading triple-mutant haplotype associated with high-level metabolic resistance illustrates the potential for anticipatory monitoring, but intervention decisions still require evidence that the haplotype is prevalent and functionally important in the local vector population and relevant to the products being used [32].

Operational choice should be framed as a transparent trade-off among expected effectiveness, resistance mechanism, product durability, achievable coverage, cost, implementation feasibility, supply continuity, and evolutionary risk [33]. Immediate protection may justify deploying a more effective product even when its long-term resistance-management value is uncertain, whereas rotation, mixtures, mosaics, or integrated non-chemical measures may be preferred when comparable protection can be preserved. Programmes should document the rationale, uncertainty, expected failure modes, monitoring indicators, and conditions for changing course. This is an adaptive decision process, not a deployment-ready universal framework.

CONCLUSION

Mosquito insecticide resistance becomes operationally meaningful only through its interaction with a specific product, realized mosquito exposure, implementation quality, vector ecology, and a defined control objective. Bioassay resistance is therefore not equivalent to operational control failure; resistance frequency is not equivalent to resistance intensity; a detected mechanism is not equivalent to its field-level contribution; and laboratory exposure is not equivalent to the dose, behaviour, coverage, and environment of operational use. The strongest defensible approach is staged evidence integration, beginning with standardized detection and progressing through intensity, mechanism, product performance, operational exposure, and outcome assessment. The highest-priority research need is prospective linkage of these evidence layers within the same populations and intervention settings. Until such calibration is available, resistance-informed decisions should remain product-specific, locally revisable, explicit about uncertainty, and proportionate to the consequences of both action and delay.

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REFERENCES

1. Moyes CL, Vontas J, Martins AJ, Ng LC, Koou SY, Dusfour I, et al. Contemporary status of insecticide resistance in the major Aedes vectors of arboviruses infecting humans. PLoS Negl Trop Dis. 2017;11(7). doi:10.1371/journal.pntd.0005625
2. Hancock PA, Hendriks CJM, Tangena JAA, Gibson H, Hemingway J, Coleman M, et al. Mapping trends in insecticide resistance phenotypes in African malaria vectors. PLoS Biol. 2020;18(6). doi:10.1371/journal.pbio.3000633
3. Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, Mbogo C, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: A WHO-coordinated, prospective, international, observational cohort study. Lancet Infect Dis. 2018;18(6):640-9. doi:10.1016/S1473-3099(18)30172-5
4. Lindsay SW, Thomas MB, Kleinschmidt I. Threats to the effectiveness of insecticide-treated bednets for malaria control: Thinking beyond insecticide resistance. Lancet Glob Health. 2021;9(9). doi:10.1016/S2214-109X(21)00216-3
5. Venter N, Oliver SV, Muleba M, Davies C, Hunt RH, Koekemoer LL, et al. Benchmarking insecticide resistance intensity bioassays for Anopheles malaria vector species against resistance phenotypes of known epidemiological significance. Parasit Vectors. 2017;10(1):198. doi:10.1186/s13071-017-2134-4
6. Grossman MK, Oliver SV, Brooke BD, Thomas MB. Use of alternative bioassays to explore the impact of pyrethroid resistance on long-lasting insecticidal net efficacy. Parasit Vectors. 2020;13(1):179. doi:10.1186/s13071-020-04055-9
7. Althoff RA, Huijben S. A comparison of the mortality variability generated by the CDC bottle, WHO tube and topical application bioassays in Aedes aegypti. Parasit Vectors. 2022;15(1):476. doi:10.1186/s13071-022-05583-2
8. Kont MD, Lambert B, Sanou A, Williams J, Ranson H, Foster GM, et al. Characterising the intensity of insecticide resistance: A novel Bayesian framework to improve the interpretation of resistance intensity bioassays. Curr Res Parasitol Vector Borne Dis. 2023;4:100125. doi:10.1016/j.crpvbd.2023.100125
9. Clarkson CS, Miles A, Harding NJ, O'Reilly AO, Weetman D, Kwiatkowski D, et al. The genetic architecture of target-site resistance to pyrethroid insecticides in the African malaria vectors Anopheles gambiae and Anopheles coluzzii. Mol Ecol. 2021;30(21):5303-17. doi:10.1111/mec.15845
10. Weedall GD, Mugenzi LMJ, Menze BD, Tchouakui M, Ibrahim SS, Amvongo-Adjia N, et al. A cytochrome P450 allele confers pyrethroid resistance on a major African malaria vector, reducing insecticide-treated bednet efficacy. Sci Transl Med. 2019;11(484). doi:10.1126/scitranslmed.aat7386
11. Yahouédo GA, Chandre F, Rossignol M, Ginibre C, Balabanidou V, Mendez NGA, et al. Contributions of cuticle permeability and enzyme detoxification to pyrethroid resistance in the major malaria vector Anopheles gambiae. Sci Rep. 2017;7(1):11091. doi:10.1038/s41598-017-11357-z
12. Sanou A, Nelli L, Guelbéogo WM, Cissé F, Tapsoba M, Ouédraogo P, et al. Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors. Sci Rep. 2021;11(1):17556. doi:10.1038/s41598-021-96759-w
13. Pwalia R, Joannides J, Iddrisu A, Addae C, Acquah-Baidoo D, Obuobi D, et al. High insecticide resistance intensity of Anopheles gambiae (s.l.) and low efficacy of pyrethroid long-lasting insecticidal nets in Accra, Ghana. Parasit Vectors. 2019;12(1):299. doi:10.1186/s13071-019-3556-y
14. Wat'senga F, Agossa F, Manzambi EZ, Illombe G, Mapangulu T, Muyembe T, et al. Intensity of pyrethroid resistance in Anopheles gambiae before and after a mass distribution of insecticide-treated nets in Kinshasa and in 11 provinces of the Democratic Republic of Congo. Malar J. 2020;19(1):169. doi:10.1186/s12936-020-03240-6
15. Oliver SV, Lyons CL, Brooke BD. The effect of blood feeding on insecticide resistance

intensity and adult longevity in the major malaria vector Anopheles funestus (Diptera: Culicidae). Sci Rep. 2022;12(1):3877. doi:10.1038/s41598-022-07798-w

16. Menze BD, Tchouakui M, Mugenzi LMJ, Tchapga W, Tchoupo M, Wondji MJ, et al. Marked aggravation of pyrethroid resistance in major malaria vectors in Malawi between 2014 and 2021 is partly linked with increased expression of P450 alleles. BMC Infect Dis. 2022;22(1):660. doi:10.1186/s12879-022-07596-9
17. Riveron JM, Watsenga F, Irving H, Irish SR, Wondji CS. High Plasmodium infection rate and reduced bed net efficacy in multiple insecticide-resistant malaria vectors in Kinshasa, Democratic Republic of Congo. J Infect Dis. 2018;217(2):320-8. doi:10.1093/infdis/jix570
18. Meiwald A, Clark E, Kristan M, Edi C, Jeffries CL, Pelloquin B, et al. Association of reduced long-lasting insecticidal net efficacy and pyrethroid insecticide resistance with overexpression of CYP6P4, CYP6P3, and CYP6Z1 in populations of Anopheles coluzzii from southeast Côte d'Ivoire. J Infect Dis. 2022;225(8):1424-34. doi:10.1093/infdis/jiaa699
19. Riveron JM, Huijben S, Tchapga W, Tchouakui M, Wondji MJ, Tchoupo M, et al. Escalation of pyrethroid resistance in the malaria vector Anopheles funestus induces a loss of efficacy of piperonyl butoxide-based insecticide-treated nets in Mozambique. J Infect Dis. 2019;220(3):467-75. doi:10.1093/infdis/jiz139
20. Tokponnon FT, Sissinto Y, Ogouyemi-Hounto A, Adéothy AA, Adechoubou A, Houansou T, et al. Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: Evidence from health facility data from Benin. Malar J. 2019;18(1):37. doi:10.1186/s12936-019-2656-7
21. Namias A, Jobe NB, Paaijmans KP, Huijben S. The need for practical insecticide-resistance guidelines to effectively inform mosquito-borne disease control programs. Elife. 2021;10. doi:10.7554/eLife.65655
22. Skovmand O, Dang DM, Tran TQ, Bossellman R, Moore SJ. From the factory to the field: Considerations of product characteristics for insecticide-treated net bioefficacy testing. Malar J. 2021;20(1):363. doi:10.1186/s12936-021-03897-7
23. Hancock PA, Lynd A, Wiebe A, Devine M, Essandoh J, Wat'senga F, et al. Modelling spatiotemporal trends in the frequency of genetic mutations conferring insecticide target-site resistance in African mosquito malaria vector species. BMC Biol. 2022;20(1):46. doi:10.1186/s12915-022-01242-1
24. Sherrard-Smith E, Winskill P, Hamlet A, Ngufor C, N'Guessan R, Guelbeogo MW, et al. Optimising the deployment of vector control tools against malaria: A data-informed modelling study. Lancet Planet Health. 2022;6(2). doi:10.1016/S2542-5196(21)00296-5
25. Gleave K, Lissenden N, Chaplin M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. Cochrane Database Syst Rev. 2021;5(5). doi:10.1002/14651858.CD012776.pub3
26. Protopopoff N, Mosha JF, Lukole E, Charlwood JD, Wright A, Mwalimu CD, et al. 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. Lancet. 2018;391(10130):1577-88. doi:10.1016/S0140-6736(18)30427-6
27. Staedke SG, Gonahasa S, Dorsey G, Kamya MR, Maiteki-Sebuguzi C, Lynd A, et al. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda: A pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. Lancet. 2020;395(10232):1292-303. doi:10.1016/S0140-6736(20)30214-2
28. Mosha JF, Kulkarni MA, Lukole E, Matowo NS, Pitt C, Messenger LA, et al. Effectiveness and cost-effectiveness against malaria of three types of dual-active-ingredient long-lasting insecticidal nets compared with pyrethroid-only LLINs in Tanzania: A four-arm, cluster-randomised trial. Lancet. 2022;399(10331):1227-41. doi:10.1016/S0140-6736(21)02499-5

29. Protopopoff N, Mosha JF, Messenger LA, Lukole E, Charlwood JD, Wright A, et al. Effectiveness of piperonyl butoxide and pyrethroid-treated long-lasting insecticidal nets versus pyrethroid-only LLINs with and without indoor residual spray against malaria infection: Third-year results of a cluster, randomised controlled, two-by-two factorial design trial in Tanzania. Malar J. 2023;22(1):294. doi:10.1186/s12936-023-04727-8
30. Dusfour I, Vontas J, David JP, Weetman D, Fonseca DM, Corbel V, et al. Management of insecticide resistance in the major Aedes vectors of arboviruses: Advances and challenges. PLoS Negl Trop Dis. 2019;13(10). doi:10.1371/journal.pntd.0007615
31. Hancock PA, Ochomo E, Messenger LA. Genetic surveillance of insecticide resistance in African Anopheles populations to inform malaria vector control. Trends Parasitol. 2024;40(7):604-18. doi:10.1016/j.pt.2024.04.016
32. Njoroge H, van't Hof A, Oruni A, Pipini D, Nagi SC, Lynd A, et al. Identification of a rapidly-spreading triple mutant for high-level metabolic insecticide resistance in Anopheles gambiae provides a real-time molecular diagnostic for antimalarial intervention deployment. Mol Ecol. 2022;31(16):4307-18. doi:10.1111/mec.16591
33. Oxborough RM, Figueroa Chilito KC, Tokponnon F, Messenger LA. Malaria vector control in sub-Saharan Africa: Complex trade-offs to combat the growing threat of insecticide resistance. Lancet Planet Health. 2024;8(10). doi:10.1016/S2542-5196(24)00172-4