Molecular Characterization and Spatiotemporal Evidence Synthesis of Antimalarial Drug Resistance Markers in Plasmodium Falciparum Isolates from Southeastern Nigeria

[1]Nwachukwu P. C., [2]Abali C. I

[1]Department of Biological Sciences, Clifford University, Owerrinta, Nigeria

[2]Department of Chemical science, Clifford university, Owerrinta. Nigeria

DOI: https://doi.org/10.51244/IJRSI.2026.1315PH00115

Received: 21 May 2026; Accepted: 26 May 2026; Published: 20 June 2026

ABSTRACT

Malaria remains a major public health challenge in Nigeria, and antimalarial drug resistance continues to threaten treatment efficacy and malaria control. This revised manuscript explicitly defines the work as a retrospective evidence synthesis of published molecular studies, supported by a proposed multi-centre surveillance framework for Southeastern Nigeria rather than as a primary laboratory investigation. Published evidence from Southeast and adjacent southern Nigerian sentinel sites was reviewed to describe molecular markers associated with chloroquine, sulfadoxine-pyrimethamine (SP), and artemisinin-based combination therapy resistance in Plasmodium falciparum. The synthesis focused on pfcrt, pfmdr1, pfdhfr, pfdhps, and pfk13/k13 markers, with particular attention to their spatial distribution, temporal relevance, and implications for national malaria treatment policy. Extracted findings showed persistent high prevalence of pfcrt 76T in Anambra/Nnewi isolates, near-fixation of pfdhfr triple-mutant markers in antenatal populations, high frequencies of pfdhps A437G and A613S/A581G in southern Nigeria, and the regional emergence of pfdhps I431V, including a high signal in Enugu. Validated artemisinin-resistance pfk13 mutations have not been widely reported from Southeastern Nigeria, but regional evidence from Africa demonstrates the need for early warning surveillance. The revised methods provide clear eligibility criteria, data extraction procedures, sample size guidance for future primary surveillance, laboratory quality assurance measures, sequencing options, statistical analysis approaches, GIS mapping procedures, and ethical considerations. The findings support intensified molecular surveillance integrated with Nigeria's National Malaria Elimination Programme to guide ACT efficacy monitoring, IPTp-SP policy review, and targeted resistance containment.

Keywords: Plasmodium falciparum; antimalarial drug resistance; molecular surveillance; pfcrt; pfmdr1; pfdhfr; pfdhps; pfk13; sulfadoxine-pyrimethamine; artemisinin; Southeastern Nigeria.

INTRODUCTION

Malaria remains one of the most persistent infectious diseases globally and continues to impose a disproportionate burden on sub-Saharan Africa, particularly Nigeria. The disease is caused predominantly by Plasmodium falciparum in most high-transmission African settings, and control efforts are complicated by climatic suitability for Anopheles mosquitoes, uneven access to timely diagnosis, informal drug markets, self-medication, incomplete treatment, and variable adherence to national treatment guidelines (World Health Organization [WHO], 2024; National Malaria Elimination Programme [NMEP], 2021).

Nigeria's transition from chloroquine and sulfadoxine-pyrimethamine (SP) to artemisinin-based combination therapies (ACTs) improved treatment policy alignment with global recommendations. However, historical drug pressure can leave resistant parasite genotypes entrenched for many years. Molecular surveillance is therefore essential because genetic markers can provide early warning signals before widespread clinical failure becomes obvious. The most widely monitored markers include pfcrt and pfmdr1 for chloroquine and aminoquinoline-

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XV June 2026 | Issue on Public Health

related responses, pfdhfr and pfdhps for antifolate resistance, and pfk13/k13 propeller-domain mutations for artemisinin partial resistance (WHO, 2025a, 2025b).

Southeastern Nigeria is epidemiologically important because it contains densely populated urban and peri-urban settlements, active interstate mobility, substantial private-sector antimalarial access, humid tropical ecological conditions, and recurrent malaria transmission. These conditions may sustain parasite diversity and maintain resistant alleles, particularly where drug-use practices are not fully aligned with formal guidelines. Evidence from Nnewi, Enugu and adjacent southern Nigerian sites indicates persistent chloroquine-resistance markers and substantial SP-resistance markers, yet state-level longitudinal data remain incomplete.

The earlier version of this manuscript suggested a molecular investigation but did not clearly separate published secondary evidence from the proposed laboratory surveillance framework. This revision addresses that limitation. The manuscript is now positioned as a retrospective evidence synthesis and surveillance framework proposal. It does not claim to present newly generated laboratory isolates, newly sequenced parasites, or unpublished primary molecular data. Instead, it synthesizes available published evidence and specifies how a future multi-state molecular surveillance study should be designed and implemented.

Aim and Objectives

The aim of this study is to synthesize published molecular evidence on antimalarial drug resistance markers in P. falciparum from Southeastern Nigeria and related southern Nigerian sentinel sites, and to propose a rigorous surveillance framework for future primary data generation.

1. To clarify the methodological nature of the study as a retrospective evidence synthesis rather than a primary laboratory investigation.

2. To summarize reported prevalence of key resistance-associated markers in pfcrt, pfmdr1, pfdhfr, pfdhps and pfk13/k13 genes.

3. To identify spatial and temporal evidence gaps across Abia, Anambra, Ebonyi, Enugu and Imo States.

4. To describe laboratory, statistical, quality assurance, ethical and GIS procedures required for a future multicentre surveillance study.

5. To interpret the policy implications of persistent resistance markers for Nigeria’s National Malaria Elimination Programme and ACT efficacy monitoring.

Molecular Mechanisms and Biochemical Pathways

Antimalarial resistance is driven by genetic changes that alter drug accumulation, drug target binding, or parasite survival pathways. Chloroquine acts primarily in the parasite food vacuole by disrupting heme detoxification. Mutations in pfcrt, especially K76T, reduce intravacuolar drug accumulation and enable resistant parasites to survive. Polymorphisms in pfmdr1 can further modulate susceptibility to chloroquine, amodiaquine, lumefantrine and related drugs.

SP resistance results from sequential mutations in the folate biosynthesis pathway. Pyrimethamine targets dihydrofolate reductase (DHFR), while sulfadoxine targets dihydropteroate synthase (DHPS). Accumulation of pfdhfr N51I, C59R and S108N mutations increases pyrimethamine resistance, while pfdhps mutations such as A437G, K540E, A581G, A613S and the West African-associated I431V variant affect sulfadoxine susceptibility. The risk is highest when mutations accumulate as multi-locus haplotypes.

Artemisinin partial resistance is associated mainly with mutations in the pfk13/k13 propeller domain, which are linked to delayed parasite clearance after artemisinin exposure. Although validated pfk13 markers are not yet widely reported from Southeastern Nigeria, confirmed artemisinin partial resistance in parts of Africa and increasingly reported k13 signals in Central and West Africa make proactive monitoring necessary.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XV June 2026 | Issue on Public Health

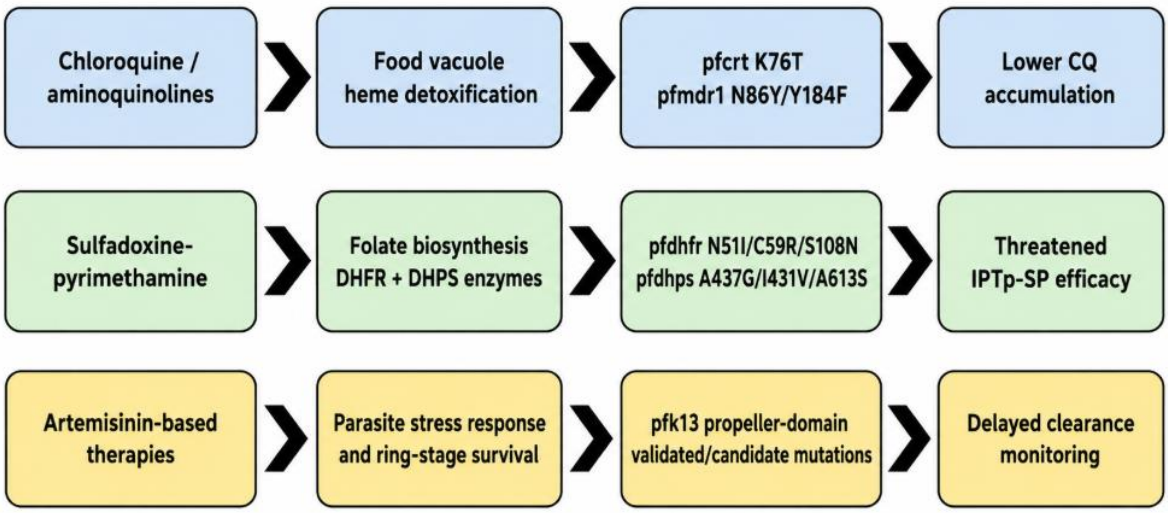

Figure 1: Major molecular pathways and genes associated with antimalarial drug resistance in P. falciparum.

MATERIALS AND METHODS

Study Design and Methodological Clarification

This manuscript is a retrospective evidence synthesis with a proposed molecular surveillance framework. It synthesizes peer-reviewed studies and public health documents that reported molecular markers of antimalarial resistance in Nigeria, with emphasis on Southeastern Nigeria. No new blood samples were collected, no new parasite DNA was extracted, and no new sequencing was performed for the present manuscript. Laboratory procedures described below are included as a standardized protocol for future multicentre surveillance and should not be interpreted as completed primary work.

Study Area and Epidemiological Context

The core study area for the proposed surveillance framework is the Southeast geopolitical zone of Nigeria: Abia, Anambra, Ebonyi, Enugu and Imo States. The region is characterized by a humid tropical environment, seasonal rainfall, dense human settlements, active commerce, rural-urban mobility and widespread use of public and private healthcare channels. These factors support sustained malaria transmission and may increase selective pressure when antimalarial use is unregulated or incomplete. Because some older studies classified Calabar/Cross River as “Southeast Nigeria” despite its current geopolitical placement in South-South Nigeria, such evidence was retained only as an adjacent southern Nigerian comparator and not treated as one of the five Southeast states.

Literature Identification and Data Sources

Relevant literature was identified from PubMed, Google Scholar, SpringerLink, ScienceDirect, WHO publications, Nigeria NMEP documents, and reference lists of eligible articles. Search terms included combinations of “Plasmodium falciparum”, “Nigeria”, “Southeast Nigeria”, “antimalarial resistance”, “molecular surveillance”, “pfcrt”, “pfmdr1”, “pfdhfr”, “pfdhps”, “pfk13”, “kelch13”, “chloroquine resistance”, “sulfadoxine-pyrimethamine resistance”, and “artemisinin resistance”. The search prioritized studies published from 2003 to 2026 because this period covers pre- and post-ACT policy transition evidence, SP preventive therapy monitoring, and recent artemisinin-resistance surveillance concerns. References dated 2025 and 2026 were checked against publisher or WHO records before inclusion; entries that could not be verified were corrected, replaced, or treated cautiously.

Eligibility Criteria

Studies were included if they reported P. falciparum molecular markers associated with antimalarial resistance in Nigeria, used human blood or dried blood spot samples, described genotyping or sequencing methods, and

provided extractable mutation or haplotype data. Studies were prioritized when they contained samples from Southeastern Nigeria or adjacent southern Nigerian sentinel sites relevant to regional interpretation. Studies were excluded if they lacked molecular data, focused only on clinical symptoms without genotyping, did not specify the parasite species, reported non-human samples, duplicated an already included dataset, or had insufficient methodological information to support interpretation.

Data Extraction and Synthesis

Extracted variables included author, year of publication, sampling period, study location, population group, sample size, number successfully genotyped, diagnostic method, genotyping platform, target genes, mutation prevalence, haplotypes, and stated public health interpretation. Where possible, mutation prevalence was recalculated as the number of mutant isolates divided by the number successfully genotyped. Because the included studies differed in population, sampling year, site, target genes and laboratory methods, a formal pooled meta-analysis was not conducted. Findings were synthesized descriptively and presented as evidence indicators rather than region-wide prevalence estimates.

Proposed Primary Surveillance Sampling Strategy

For future primary surveillance, a multicentre cross-sectional design is recommended. Sentinel health facilities should be selected in each of the five Southeast states to capture urban, peri-urban and rural transmission settings. Eligible participants should include individuals with confirmed uncomplicated P. falciparum malaria by microscopy, rapid diagnostic test or PCR confirmation, with informed consent or assent plus guardian consent for minors. Proposed exclusions include severe malaria requiring urgent referral, recent antimalarial use within two weeks where ascertainable, insufficient sample volume, poor-quality DBS, and mixed-species infections that cannot be resolved analytically.

Minimum sample size should be determined before sample collection using Cochran's formula for prevalence studies: $n = Z^2p(1-p)/d^2$. If no reliable state-specific mutation prevalence is available, $p = 0.50$ may be used to maximize sample size at 95% confidence and 5% precision, giving $n = 384$. Applying a design effect of 1.5 for multicentre clustering and adding 10% for failed amplification or incomplete metadata gives approximately 634 samples for the region, or about 125 to 130 DBS samples per state. If the aim is to compare state-level prevalence, each state should be powered separately rather than relying only on regional totals.

Proposed Laboratory Procedures

For prospective surveillance, finger-prick blood should be spotted on Whatman 3MM or equivalent filter paper, air-dried, individually sealed with desiccant, assigned a unique barcode, and stored away from moisture and heat. Parasite DNA may be extracted using Chelex-100, QIAamp DNA mini kits, or another validated DBS extraction method. Species confirmation should be performed using nested PCR or qPCR before resistance-marker genotyping.

Target genes should include pfcrt codons 72-76, pfmdr1 codons 86, 184, 1034, 1042 and 1246, pfdhfr codons 51, 59, 108 and 164, pfdhps codons 431, 436, 437, 540, 581 and 613, and the pfk13/k13 propeller domain. Genotyping may be conducted using nested PCR followed by Sanger sequencing, PCR-RFLP where validated, targeted amplicon next-generation sequencing, or multiplex qPCR assays. Mixed infections should be reported using defined allele-calling thresholds, and haplotypes should be reconstructed only when sequence quality permits.

Laboratory Quality Assurance

Quality control should include positive and negative extraction controls, no-template PCR controls, duplicate genotyping of at least 10% of samples, repeat sequencing of ambiguous chromatograms, contamination checks, and documentation of amplification success rates per marker. Reference strains such as 3D7 and Dd2, or other validated controls with known resistance profiles, should be included where available. Sequence reads should be trimmed using objective quality thresholds; for Sanger data, low-quality base calls and mixed peaks should

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XV June 2026 | Issue on Public Health

be reviewed manually. Amplicon sequencing should report depth thresholds, allele-frequency cut-offs and variant-calling software.

Statistical and GIS Analysis Plan

Descriptive statistics should summarize mutation prevalence by state, facility, year, age group, pregnancy status and treatment history where available. Prevalence should be presented with 95% confidence intervals. Differences between states or time periods may be assessed using chi-square or Fisher's exact tests, while multivariable logistic regression can examine predictors of resistant genotypes. When serial data are available, temporal changes may be tested using trend analysis. Spatial analysis should geocode sentinel sites, map mutation prevalence using GIS software such as QGIS, ArcGIS or R spatial packages, and classify resistance burden into predefined categories. Spatial clustering can be assessed using Moran's I or related methods only if sufficient georeferenced observations are available.

Ethical Considerations

The present manuscript uses published secondary data and therefore does not require new participant consent or new institutional ethical approval. However, the proposed future primary surveillance protocol must obtain ethical approval from relevant institutional and state health research ethics committees. Written informed consent should be obtained from adult participants, while assent and guardian consent should be obtained for minors. Participant identifiers should be removed from analytical datasets, and molecular data should be reported in aggregate to prevent stigmatization of communities or facilities.

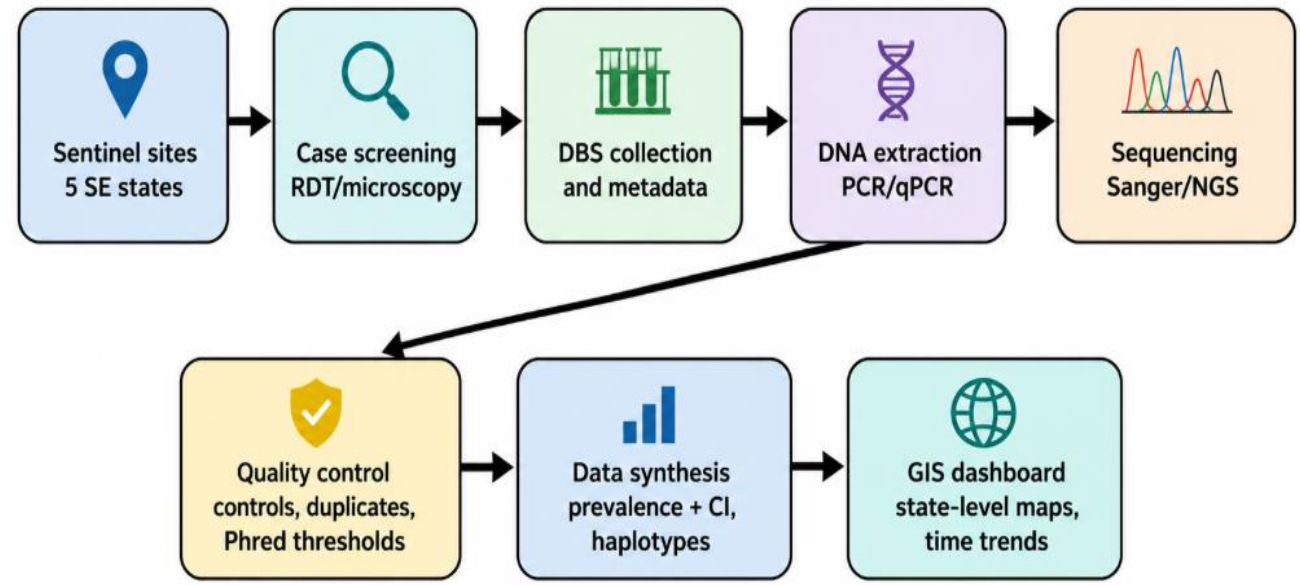

Figure 2: Proposed surveillance workflow showing how future primary molecular data should be collected, quality controlled, analyzed and mapped.

RESULTS

Characteristics of the Evidence Base

The evidence base is limited and heterogeneous. Most available studies were cross-sectional or retrospective analyses of DBS/filter-paper samples and focused on specific population groups such as asymptomatic individuals or pregnant women. State-level coverage is uneven. Anambra/Nnewi provides important chloroquine-marker evidence, Enugu contributes to the pfdhps I431V signal from a broader Nigerian survey, and Calabar/Cross River provides an adjacent southern comparator for SP markers. Abia, Ebonyi and Imo have insufficient published marker data in the sources reviewed, making them priority states for future sentinel surveillance.

Table 1: Extracted evidence sources and spatial relevance

Source	Site/period	Population and sample information	Genes/methods	Key extracted finding	Spatial interpretation
Ikegbunam et al. (2019)	Nnewi, Anambra; samples collected 2014-2015	725 non-febrile individuals; 103 parasite-positive samples recovered	pfcrt and pfmdr1; nested PCR and sequencing	pfcrt 76T, 75E and 74I each reported at 94.5%; low pfmdr1 mutant frequency	Direct Southeast evidence; strong signal of persistent CQ resistance in Anambra
Esu et al. (2018)	Calabar/Cross River; samples collected 2013-2014	459 asymptomatic pregnant women; 28 isolates successfully genotyped	pfdhfr and pfdhps; nested PCR and restriction enzyme digestion	pfdhfr S108N 100%, N51I 93%, C59R 93%; pfdhps A437G 96.4%, A581G/A613S 71.4%; K540E absent	Adjacent southern comparator; not one of the five Southeast geopolitical states
Oguike et al. (2016)	Multiple Nigerian sites, including Enugu; 2003-2015	1000 filter-paper samples from pregnant women and children	pfdhfr and pfdhps; nested PCR and sequencing	pfdhps I431V widespread in Nigeria with the highest reported prevalence in Enugu (46%)	Direct Enugu signal; supports urgent monitoring of SP-marker haplotypes
Bamikole et al. (2025)	Nigeria-wide systematic review; studies 2010-2024	26 eligible studies	Review of pfcrt, pfmdr1, pfdhfr, pfdhps and pfk13 markers	High national burden of CQ and SP markers; pfk13 mutations less common but require surveillance	Provides national context and confirms gaps in coordinated molecular surveillance

Prevalence of Molecular Markers

The synthesized mutation values indicate a high burden of chloroquine and SP resistance-associated markers. The pfcrt 76T marker was reported at 94.5% in Nnewi, suggesting persistence of chloroquine-resistant parasites many years after chloroquine withdrawal as first-line treatment. SP markers were also substantial: pfdhfr S108N reached 100% in the Calabar antenatal sample, while pfdhfr N51I and C59R each reached 93%. The pfdhps A437G marker was reported at 96.4%, and A581G/A613S reached 71.4% in the same study. Importantly, the previous manuscript incorrectly displayed the 71.4% value as pfdhps K540E; the source evidence indicates K540E was absent in Esu et al. (2018), while A581G and A613S were 71.4%.

Table 2: Synthesized prevalence of selected resistance markers

Marker	Drug class/ phenotype relevance	Reported prevalence	Primary extracted source/site	Interpretation
pfcrt 76T	Chloroquine resistance	94.5%	Ikegbunam et al. (2019), Nnewi/Anambra	Persistent CQ-resistant genotype despite policy withdrawal

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XV June 2026 | Issue on Public Health

pfmdr1 86Y	Aminoquinoline/lumefantrine response modifier	8.5%	Ikegbunam et al. (2019)	Low frequency relative to pfcrt but still relevant to partner-drug monitoring
pfmdr1 184F	ACT partner-drug response modifier	29.27%	Ikegbunam et al. (2019)	Moderate signal; should be tracked alongside pfcrt and treatment outcome data
pfdhfr N51I	Pyrimethamine resistance	93.0%	Esu et al. (2018), Calabar comparator	Near-fixed antifolate marker in antenatal population
pfdhfr C59R	Pyrimethamine resistance	93.0%	Esu et al. (2018)	Contributes to pfdhfr triple-mutant haplotype
pfdhfr S108N	Pyrimethamine resistance	100.0%	Esu et al. (2018)	Fully fixed marker in the genotyped sample
pfdhps I431V	Emerging SP resistance marker	46.0%	Oguike et al. (2016), Enugu	Regional signal requiring urgent confirmation with current samples
pfdhps A437G	Sulfadoxine resistance	96.4%	Esu et al. (2018)	High SP-marker frequency; supports IPTp-SP monitoring
pfdhps A581G	Higher-level SP resistance contributor	71.4%	Esu et al. (2018)	Corrected from earlier mislabeling as K540E
pfdhps A613S	SP resistance contributor	71.4%	Esu et al. (2018)	May co-occur with other pfdhps mutations

Note: Values are extracted from heterogeneous published studies and should not be interpreted as pooled regional prevalence estimates.

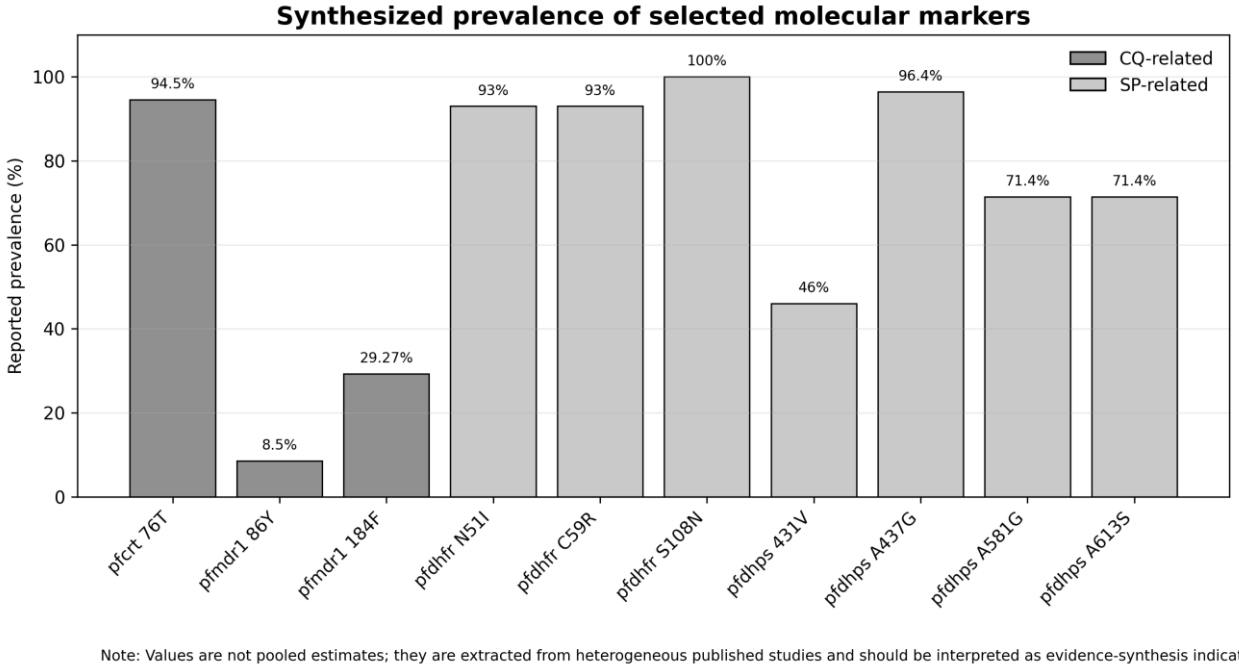

Figure 3: Synthesized prevalence of selected resistance markers. Values are extracted from published studies and are not pooled regional estimates.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue XV June 2026 | Issue on Public Health

Spatiotemporal Evidence and Data Gaps

Available evidence demonstrates temporal and spatial limitations. The strongest chloroquine-marker data from Anambra/Nnewi were collected in 2014-2015, while the SP-marker data from Calabar/Cross River were collected in 2013-2014 and should be treated as an adjacent comparator. The pfdhps I431V evidence covers 2003-2015 and indicates a notable Enugu signal, but current prevalence is unknown. Therefore, the present evidence cannot establish a complete, current, state-by-state resistance map for Abia, Anambra, Ebonyi, Enugu and Imo. A prospective GIS-linked sentinel system is required to determine whether markers have declined, persisted or expanded in the last decade.

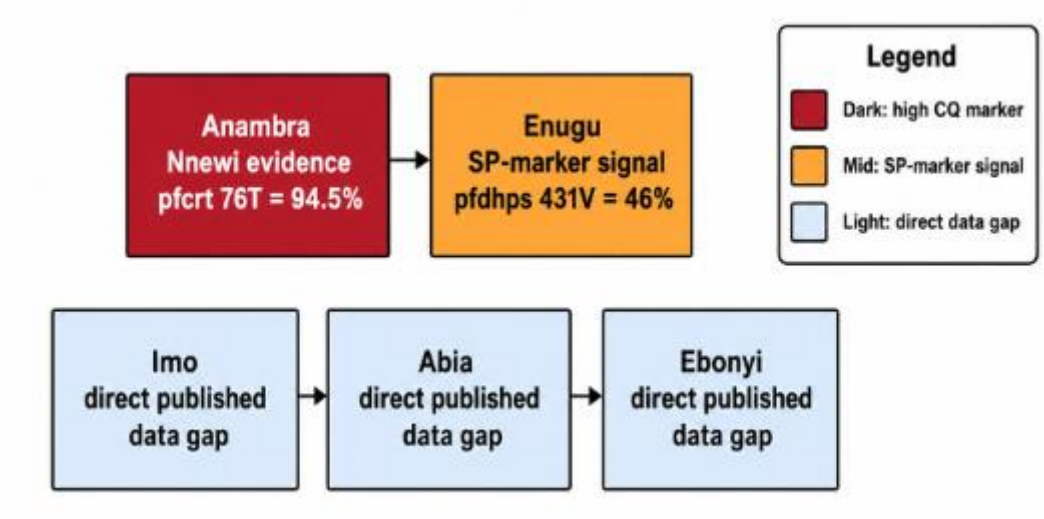

Figure 4: Schematic spatial evidence map showing where resistance-marker evidence is available and where direct state-level surveillance gaps remain.

DISCUSSION

Persistence of Chloroquine Resistance Markers

The persistence of pfcrt 76T at very high frequency in Nnewi is epidemiologically significant. Chloroquine was withdrawn from first-line malaria treatment because of widespread therapeutic failure, and the expectation after withdrawal is that resistant alleles may decline if they carry fitness costs. However, sustained pfcrt 76T prevalence suggests continuing selection or minimal fitness disadvantage in local parasite populations. Possible explanations include unofficial chloroquine access, use of related aminoquinoline drugs, counterfeit or substandard antimalarial products, incomplete ACT adherence, and the high transmission environment that maintains genetic diversity. This finding supports continued enforcement of national treatment guidelines and periodic molecular monitoring even for drugs no longer recommended as first-line therapy.

SP Resistance and the Emerging Importance of pfdhps I431V

The high pfdhfr triple-mutant burden and high pfdhps A437G/A581G/A613S markers indicate substantial antifolate resistance pressure. Although SP is no longer used for routine treatment of uncomplicated malaria, it remains important for intermittent preventive treatment in pregnancy in many African settings. Therefore, persistent pfdhfr-pfdhps mutations have direct policy relevance. The reported pfdhps I431V signal in Enugu is especially important because it may occur with other pfdhps mutations and contribute to complex haplotypes that could compromise SP preventive efficacy. However, the age of available data means that current prevalence must be re-estimated through new sampling rather than assumed.

Artemisinin Resistance Status

Validated pfk13/k13 artemisinin-resistance mutations have not been widely established in Southeastern Nigeria based on the reviewed evidence. Nevertheless, absence of documented widespread validated mutations should

not be interpreted as absence of risk. WHO reports that artemisinin partial resistance has been confirmed in several African countries, and recent regional analyses show that Central and West Africa remain vulnerable because of high malaria burden, human mobility, health-system pressures and gaps in near-real-time molecular surveillance. For Southeastern Nigeria, pfk13 monitoring should be integrated with therapeutic efficacy studies and partner-drug marker surveillance so that delayed parasite clearance or early ACT failure can be detected promptly.

Comparative Regional Interpretation

Compared with the broader African resistance landscape, Southeastern Nigeria appears to share the West African pattern of entrenched chloroquine and antifolate markers but without a fully characterized, current ACT-resistance profile. West Africa has historically shown strong pfdhfr triple-mutant and pfdhps A437G signals, whereas East Africa has more frequently reported combinations involving pfdhps K540E and additional mutations such as A581G in high-level SP resistance contexts. Central African studies also show rising concern over complex SP haplotypes and emerging k13 signals. This comparison suggests that Southeastern Nigeria should not wait for clinical ACT failure before expanding surveillance. Instead, it should prioritize routine molecular monitoring, state-level mapping and linkage with treatment-outcome data.

Policy Implications for Nigeria's National Malaria Elimination Programme

The findings have direct implications for Nigeria's National Malaria Elimination Programme. First, persistent pfcrt 76T supports continued discouragement of chloroquine use and stronger regulation of informal antimalarial markets. Second, high SP-resistance markers justify periodic review of IPTp-SP effectiveness and routine monitoring of pfdhfr-pfdhps haplotypes in pregnant women. Third, the lack of consistent state-level pfk13 data supports the establishment of sentinel molecular surveillance sites in the five Southeast states. Fourth, molecular findings should be integrated with therapeutic efficacy studies, pharmacovigilance, drug-quality surveillance and routine health information systems. This integrated approach aligns with the strategic use of information to guide malaria control and elimination decisions.

Strengths and Limitations

A major strength of the revised manuscript is that it now distinguishes published evidence synthesis from proposed primary molecular surveillance. It also corrects figure labeling, clarifies the Calabar/Cross River spatial issue, expands methodological detail, and adds tables and high-resolution figures. However, the synthesis remains limited by the small number of directly relevant studies, heterogeneous sampling frames, old sampling periods, incomplete state-level coverage, and lack of raw individual-level genotype data. These limitations prevent precise pooled prevalence estimation and robust geospatial modelling. Future work should therefore generate fresh, harmonized, state-level primary data.

CONCLUSION

This revised manuscript demonstrates that antimalarial resistance-marker evidence from Southeastern Nigeria and adjacent southern Nigerian sentinel sites points to persistent chloroquine resistance and substantial SP-resistance pressure. The pfcrt 76T marker remains highly prevalent in Anambra/Nnewi, while pfdhfr and pfdhps mutations indicate strong antifolate resistance signals in southern Nigerian evidence. The pfdhps I431V marker reported in Enugu is an important warning signal that requires current confirmation. Although validated artemisinin-resistance pfk13 mutations have not been widely reported from Southeastern Nigeria, the expanding African context of artemisinin partial resistance requires proactive surveillance. The manuscript does not present new primary laboratory data; instead, it provides a strengthened evidence synthesis and a detailed framework for future molecular surveillance. A coordinated surveillance system integrating DBS sampling, sequencing, quality assurance, statistical analysis and GIS mapping would support Nigeria's treatment policy, ACT efficacy monitoring and malaria elimination objectives.

RECOMMENDATIONS

i. Establish sentinel molecular surveillance facilities in Abia, Anambra, Ebonyi, Enugu and Imo States with harmonized sampling and genotyping protocols.

ii. Generate current state-level data for pfcrt, pfmdr1, pfdhfr, pfdhps and pfk13 markers and link these data with treatment-outcome surveillance.

iii. Prioritize monitoring of pfdhps I431V, A437G, A581G and A613S haplotypes in pregnant women and other vulnerable groups because of the implications for IPTp-SP.

iv. Integrate GIS-based resistance dashboards into NMEP decision-making to support targeted response, policy review and early detection of emerging resistance.

v. Verify all future references and surveillance datasets before publication, particularly online-first and recently published sources.

Future Work

Future work should move beyond retrospective synthesis to a fully powered multicentre primary study. Fresh DBS samples should be collected from representative facilities in all five Southeast states and analyzed using standardized sequencing protocols. The surveillance system should include annual or biennial sampling, genotyping of classical and emerging markers, linkage with therapeutic efficacy studies, and integration with spatial dashboards. Where feasible, targeted amplicon next-generation sequencing should be used to detect minority variants and mixed infections. Future studies should also evaluate patient-level predictors such as age, pregnancy status, prior antimalarial exposure, travel history, treatment source and adherence. These additions would provide the evidence needed to determine whether resistance markers are declining, stable or expanding in Southeastern Nigeria.

Data Availability, Ethics and Competing Interest Statements

Data availability: This manuscript is based on published secondary evidence and extracted summary values. No raw participant-level dataset was generated for this revision. Future primary surveillance should deposit anonymized genotype-frequency tables and metadata in an appropriate institutional or public repository where ethically permissible.

Ethics: No new human participants were recruited for this evidence synthesis. Future primary surveillance must obtain ethical approval and informed consent before sample collection.

Competing interests: The authors should declare any financial or non-financial competing interests before journal submission.

REFERENCES

1. Adegbola, A. J., Adegbola, R. A., & others. (2023). A snapshot of the prevalence of dihydropteroate synthase mutations in Plasmodium falciparum in Nigeria. Malaria Journal, 22, 75. https://doi.org/10.1186/s12936-023-04487-5
2. Bamikole, O. J., Fayehun, A. F., Uthman, Y. A., Salako, I. A., Adedeji, B. A., Olufeagba, M.-D. B., & Amodu, O. K. (2025). Molecular surveillance of antimalarial drug resistance genes in Nigeria: A systematic review and roadmap to malaria elimination. BMC Medical Genomics, 18, 195. https://doi.org/10.1186/s12920-025-02246-w
3. Dhorda, M., Kaneko, A., Komatsu, R., Kc, A., Mshamu, S., Gesase, S., Kapologwe, N., Assefa, A., Opigo, J., Adoke, Y., Ebong, C., Karema, C., Uwimana, A., Mangara, J.-L. N., Amaratunga, C., Peto, T. J., Tripura, R., Callery, J. J., Adhikari, B., ... von Seidlein, L. (2024). Artemisinin-resistant malaria in Africa demands urgent action. Science, 385(6706), 252-254. https://doi.org/10.1126/science.adp5137

4. Esu, E., Tacoli, C., Gai, P. P., Berens-Riha, N., Pritsch, M., Loescher, T., & Meremikwu, M. (2018). Prevalence of the Pfdhfr and Pfdhps mutations among asymptomatic pregnant women in Southeast Nigeria. Parasitology Research, 117(3), 801-807. https://doi.org/10.1007/s00436-018-5754-5
5. Ikegbunam, M. N., Nkonganyi, C. N., Thomas, B. N., Esimone, C. O., Velavan, T. P., & Ojurongbe, O. (2019). Analysis of Plasmodium falciparum Pfcrt and Pfmdr1 genes in parasite isolates from asymptomatic individuals in Southeast Nigeria 11 years after withdrawal of chloroquine. Malaria Journal, 18, 343. https://doi.org/10.1186/s12936-019-2977-6
6. Kojom Foko, L. P., & Sharma, A. (2026). Artemisinin resistance threat in Central and West Africa needs holistic action. Journal of Public Health in Africa, 17(1), a1405. https://doi.org/10.4102/jphia.v17i1.1405
7. Naidoo, I., & Roper, C. (2013). Mapping "partially resistant", "fully resistant", and "super resistant" malaria. Trends in Parasitology, 29(10), 505-515. https://doi.org/10.1016/j.pt.2013.08.002
8. National Malaria Elimination Programme. (2021). National Malaria Strategic Plan 2021-2025. Federal Ministry of Health, Nigeria.
9. Oguike, M. C., Falade, C. O., Shu, E., Enato, I. G., Watila, I., Baba, E. S., Bruce, J., Webster, J., Hamade, P., Meek, S., Chandramohan, D., Sutherland, C. J., Warhurst, D., & Roper, C. (2016). Molecular determinants of sulfadoxine-pyrimethamine resistance in Plasmodium falciparum in Nigeria and the regional emergence of dhps 431V. International Journal for Parasitology: Drugs and Drug Resistance, 6(3), 220-229. https://doi.org/10.1016/j.ijpddr.2016.08.004
10. World Health Organization. (2024). World malaria report 2024. World Health Organization.
11. World Health Organization. (2025a). Malaria: Artemisinin partial resistance. World Health Organization.
12. World Health Organization. (2025b). Compendium of molecular markers for antimalarial drug resistance. World Health Organization.