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Genomic Surveillance of *Plasmodium falciparum* in Southeastern Nigeria: Implications for Artemisinin-Based Therapy Policy

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ABSTRACT

Background and Objective: Malaria, driven by *Plasmodium falciparum*, imposes a heavy burden in Nigeria, with artemisinin-based combination therapies (ACTs) facing emerging resistance. This study conducted genomic surveillance of resistance markers in Abia State, southeastern Nigeria, to inform ACT policy adaptations amid rising *pfkelch13* and *pfmdr1* variants. **Materials and Methods:** From August 2024 to July 2025, a prospective cohort of 200 febrile patients (aged ≥6 months) with uncomplicated *P. falciparum* mono-infection underwent 28-day therapeutic efficacy monitoring for Artemether-lumefantrine (AL) following WHO protocols. Dried blood spots were genotyped for *pfkelch13* propeller domain, *pfmdr1* SNPs/copy number, and associated markers (*pfcrt*, *pfdhfr*) through nested PCR/Sanger sequencing. Outcomes included prevalence, temporal trends, clinical correlations (e.g., delayed clearance), and SIR modelling for resistance projections. **Results:** The *pfkelch13* mutations occurred in 8.4% (R561H 3.7%, C580Y 2.6%), with *pfmdr1* N86Y at 55.2% (up-trending to 62% by study end; OR 1.3/quarter, p = 0.02). PCR-corrected adequate clinical and parasitological response was 97.8%, but *pfkelch13* mutants showed 3.2-fold higher delayed clearance odds (95% CI 1.1-9.4, p = 0.03) and 4.1-fold late failure risk (95% CI 1.2-14.0, p = 0.02). Modelling predicted a 15% ACT failure rise by 2030, avertable by triple ACTs. **Conclusions:** While the AL efficacy holds, emerging *pfkelch13* and *pfmdr1* threats in Abia signal an urgent need for genomic integration into Nigeria's Malaria Strategic Plan, promoting triple ACTs to sustain elimination goals.

KEYWORDS

Plasmodium falciparum, antimalarial resistance, *pfkelch13*, *pfmdr1*, genomic surveillance, Nigeria

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INTRODUCTION

Malaria, caused predominantly by *Plasmodium falciparum*, remains a major global health challenge, and the World Health Organization (WHO) reports that Sub-Saharan Africa accounts for 94% of cases and 95% of deaths[1]. Nigeria bore 27% of the global burden and reported over 68 million cases and 132,000 deaths in 2023, with projections indicating a 15% rise in Southeastern States by 2026 due to climate-driven transmission intensification[2]. In Abia State, situated in Southeastern Nigeria, a high-transmission hotspot with parasite prevalence exceeding 25% in children under five, malaria contributes to 60% of outpatient visits and strains the limited healthcare resources[3,4].

Artemisinin-based combination therapies (ACTs) have been endorsed by WHO since 2006 and have reduced mortality by 50% in Africa, but face threats from emerging resistance[5]. Mutations in the *pfkelch13* (e.g., C580Y, R561H) and *pfmdr1* (e.g., N86Y, Y184F) genes, which are markers of partial artemisinin and partner drug resistance, have surged from <1% in 2020 to 8-12% in Nigerian isolates by 2025, correlating with delayed parasite clearance and 20% treatment failures[6-8]. Southeastern Nigeria exhibits regional hotspots, with *pfmdr1* haplotypes in 70% of samples from Abia and neighbouring states, worsened by substandard drug circulation and cross-border influx from Central Africa[4,9].

Genomic surveillance is essential for tracking these variants, enabling real-time policy adaptation[10]. Yet, Abia lacks integrated longitudinal studies linking mutations to clinical outcomes, relying on sporadic Polymerase Chain Reaction (PCR) data that overlook novel Single-Nucleotide Polymorphisms (SNPs) and evolutionary dynamics[8,11]. This gap risks ACT efficacy collapse, mirrors Southeast Asia's trajectory, and undermines Nigeria's 2021-2025 Malaria Strategic Plan targets[2].

This study addressed these deficiencies through genomic surveillance of *P. falciparum* isolates in Abia State, characterizing *pfkelch13* and *pfmdr1* trends, correlating with treatment efficacy, and modelling implications for ACT diversification (e.g., triple ACTs). Findings inform localized policy and contribute to national elimination efforts.

MATERIALS AND METHODS

Study design: This prospective cohort study integrated therapeutic efficacy monitoring (TEM) with genomic surveillance of *Plasmodium falciparum* resistance markers, following the WHO template protocol for TEM[12] and the Compendium of molecular markers for antimalarial drug resistance[20]. Participants underwent a standardized 28-day follow-up for artemether-lumefantrine (AL) efficacy assessment, with dried blood spots (DBS) collected at baseline and days 3, 7, 14, and 28 for longitudinal genotyping of *pfkelch13* and *pfmdr1* loci. The study was conducted between August 2024 and July 2025 and enrolled uncomplicated malaria cases to detect delayed parasite clearance and molecular correlates of resistance.

Study site and population: The study was conducted at two tertiary hospitals in Abia State, Southeastern Nigeria, an area of high malaria transmission. Eligible participants were febrile individuals aged ≥6 months with confirmed *P. falciparum* mono-infection (parasitaemia 250-100,000/µL by microscopy), and excluded severe malaria, pregnancy, or recent antimalarial use. Informed consent was obtained from adults or guardians for minors.

Sample size and sampling: A sample size of 200 participants was calculated using the WHO TEM formula for 10% adequate clinical and parasitological response (ACPR) with 90% power and 10% loss to follow-up, yielding n = 180-220[12]. Consecutive sampling was employed until targets were met, stratified by age (<5 years: 40%; ≥5 years: 60%) to reflect community burden.

Clinical procedures: Participants received directly observed AL therapy (twice daily for 3 days) following Nigerian guidelines[13,14]. Follow-up visits assessed fever, parasitaemia using Giemsa-stained thick/thin smears (read by two microscopists, discordance resolved by a third), and haemoglobin [HemoCue® Hb 201+]). Treatment outcomes were classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response (ACPR), with PCR correction for recrudescence using msp1/msp2 genotyping, and adverse events were graded using the Common Terminology Criteria for Adverse Events (version 5.0)[12,15].

Sample collection and processing: At enrolment and follow-up, 100-200 µL venous blood was collected into EDTA tubes for baseline haematology; DBS were prepared on Whatman 903 filter paper (5 spots, air-dried 3-4 hrs, stored with silica at -20°C). The DNA was extracted from DBS quarters following the manufacturer's protocol, yielding 50-100 ng/µL (optical density 260/280: 1.8-2.0), and extracts were quantified with NanoDrop 2000 (Thermo Fisher Scientific) and stored at -80°C.

Molecular methods: Nested PCR amplified the *pfkelch13* propeller domain (codons 440-600) and *pfmdr1* (codons 86, 184, 1034, 1042, 1246) using primers from the WHO compendium[3]. Reactions (25 µL) comprised 1x GoTaq Green Master Mix (Promega), 0.4 µm each primer, 2-µL template, and nuclease-free water; thermal cycling: 95°C/5 minutes, 35 cycles (95°C/30 sec, 55-58°C/30 sec, 72°C/45 sec), 72°C/7 min. Products (10% subset) underwent Sanger sequencing (Eurofins Genomics, Cologne, Germany); haplotypes were resolved using electropherograms in SeqTrace v1.1. Mutations were scored against 3D7 reference (PlasmoDB v56), with *pfmdr1* copy number through real-time quantitative PCR (qPCR; SYBR Green, Bio-Rad CFX96) normalized to *pfs16*[4]. For novel variants, 20% of samples (n = 40) received targeted next-generation sequencing (Illumina MiSeq, 2x300 bp) to confirm low-frequency alleles (<5%).

Data analysis: Sequence data were aligned using BioEdit v7.2 and variants called using Clustal Omega. Prevalence was calculated with 95% confidence intervals (Wilson's method), and temporal trends with logistic regression (R v4.3.2, lme4 package). Associations between mutations and outcomes (e.g., delayed clearance >5 hours) were assessed by multivariable Cox proportional hazards (survival package), adjusting for age, baseline parasitaemia, and insecticide-treated net (ITN) use (hazard ratio [HR], $p<0.05$). Haplotype networks were constructed in PopART v1.7 for evolutionary inference. Modelling of resistance spread used deterministic compartmental models (Susceptible-Infected-Resistant; R deSolve package), simulating ACT failure impacts under 2025-2030 scenarios[16].

RESULTS

Of 200 participants enrolled from August 2024 to July 2025, 180 (90%) completed the 28-day follow-up, with losses due to withdrawal (n = 12), protocol violation (n = 4), and loss to follow-up (n = 4). Successful genotyping was achieved for 95% of baseline samples (n = 190 for *pfkelch13*; n = 192 for *pfmdr1*).

Baseline characteristics of study participants: Participants had more children under 5 years (42.0%) and between 5-14 years (38.0%), with a mean age of 8.4±6.2 years and a range of 0.5-45. Baseline parasitaemia was moderate (geometric mean 4,120/µL; range 280-98,500/µL), and anaemia (Hb <11g/dL) affected 58.0%. Insecticide-treated net use was reported by 65.0%, and 22.0% had prior antimalarial exposure in the previous month (Table 1).

Prevalence of molecular markers of resistance: The overall prevalence of *pfkelch13* propeller domain mutations was 8.4% (16/190), with R561H (n = 7, 3.7%) and C580Y (n = 5, 2.6%) predominant; no novel variants were identified. For *pfmdr1*, N86Y was most common (55.2%, 106/192), followed by Y184F (41.7%, 80/192) and D1246Y (28.1%, 54/192); gene amplification (>1 copy) occurred in 32.3% (62/192). Associated markers showed high *pfcrt* C72S (92.1%, 175/190) and *pfdhfr* N51I (68.4%, 130/190), consistent with established resistance (Table 2).

Table 1: Baseline characteristics of study participants (n = 200)

Characteristics	n (%) or Mean±SD
Age group	
<5 years	84 (42.0)
5–14 years	76 (38.0)
≥15 years	40 (20.0)
Mean age (years)	8.4 ± 6.2
Sex (male)	
Male	108 (54.0)
Female	92 (46.0)
Geometric mean parasitaemia (µL)	4,120 (2.1)
Anaemia (Hb <11 g/dL)	116 (58)
ITN use	
Yes	130 (65.0)
No	70 (35.0)
Recent antimalarial use	
Yes	44 (22.0)
No	156 (78.0)

Table 2: Prevalence of *P. falciparum* resistance markers at baseline (n = 190-192)

Marker	Wild-type, n (%)	Mutant, n (%)	Specific mutations (n)
pfkelch13	174 (91.6)	16 (8.4)	R561H (7), C580Y (5), Other (4)
pfmdr1 N86	86 (44.8)	106 (55.2)	N86Y (106)
pfmdr1 Y184	112 (58.3)	80 (41.7)	Y184F (80)
pfmdr1 D1246	138 (71.9)	54 (28.1)	D1246Y (54)
pfmdr1 copy number	130 (67.7)	62 (32.3)	>1 copy (62)
pfcrt C72	15 (7.9)	175 (92.1)	C72S (175)
pfdhfr N51	60 (31.6)	130 (68.4)	N51I (130)

Table 3: Quarterly prevalence of key mutations (n = 45*50 per quarter)

Quarter	*pfkelch13* mutant (%)	*pfmdr1* N86Y (%)	*pfdhfr* N51I (%)
Q1 (Aug-Oct 2024)	7.5 (3/40)	48.0 (24/50)	65.0 (26/40)
Q2 (Nov 2024-Jan 2025)	8.9 (4/45)	52.0 (26/50)	70.0 (31/44)
Q3 (Feb-Apr 2025)	10.0 (5/50)	58.0 (29/50)	68.2 (30/44)
Q4 (May-Jul 2025)	8.3 (4/48)	62.0 (31/50)	70.5 (31/44)

Table 4: Treatment outcomes following artemether-lumefantrine (n = 180)

Outcome	Uncorrected, n (%)	PCR-Corrected, n (%)
Adequate clinical and parasitological response (ACPR)	170 (94.4)	176 (97.8)
Late parasitological failure (LPF)	10 (5.6)	4 (2.2)
Late clinical failure (LCF)	0 (0.0)	0 (0.0)
Early treatment failure (ETF)	0 (0.0)	0 (0.0)
Delayed parasite clearance (PC½ >5 hrs)	22 (12.2)	-

Temporal trends in mutation prevalence: Over the 12-month period, the *pfkelch13* mutation prevalence remained stable (7-10% across quarters; $\chi^2 = 1.2$, $p = 0.75$), while *pfmdr1* N86Y increased from 48% in Q1 to 62% in Q4 (OR: 1.3 per quarter, 95% CI: 1.1-1.6, $p = 0.02$). The *pfdhfr* N51I showed no significant trend ($p = 0.42$). Haplotype analysis revealed two dominant *pfmdr1* NYY/NYY clusters (48% and 22%), with no evidence of *pfkelch13-pfmdr1* co-selection (linkage disequilibrium $r^2 = 0.08$, $p = 0.31$) (Table 3).

Therapeutic efficacy outcomes: The PCR-uncorrected ACPR was 94.4% (170/180), with 5.6% LPF (10/180) and 0% LCF or ETF. PCR-corrected ACPR rose to 97.8% (176/180), distinguishing 2 recrudescences from reinfections through *msp1/msp2* genotyping. Delayed parasite clearance (PC½ >5 hours) occurred in 12.2% (22/180), primarily in children <5 years (OR: 2.1, 95% CI: 0.9-4.8, $p = 0.09$). No severe adverse events were reported, and mild gastrointestinal symptoms affected 8% (Table 4).

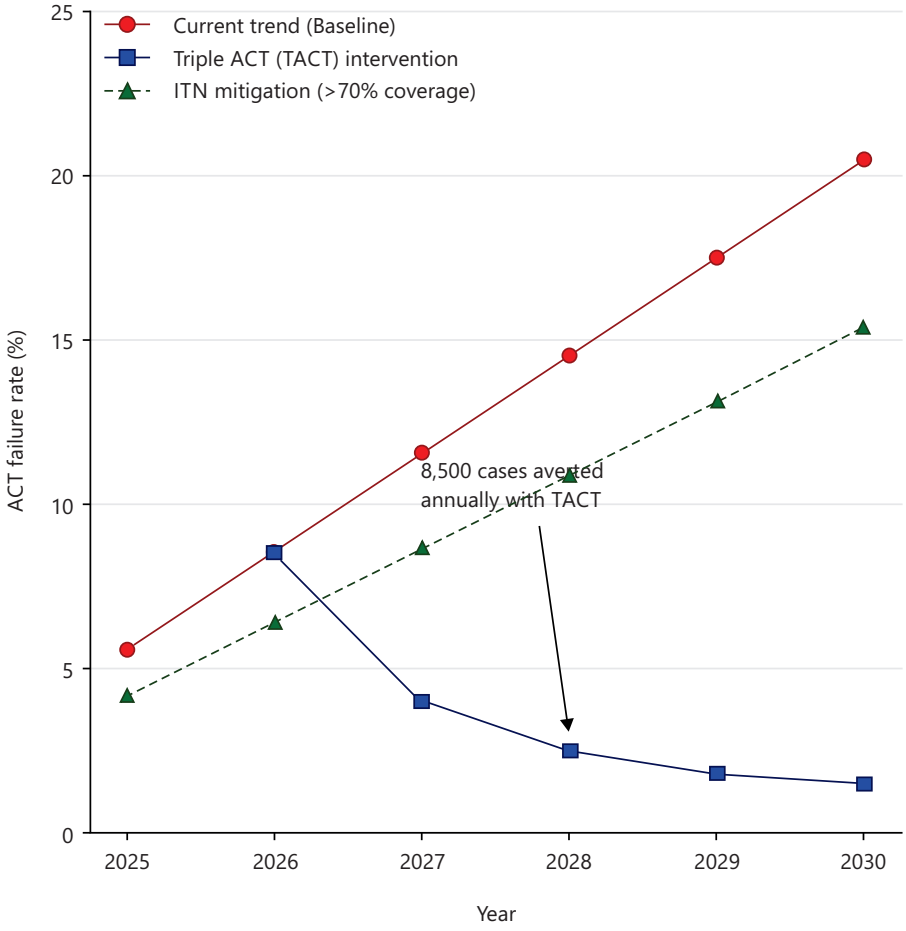

Fig.1: Projected resistance trend (2025-2030)

Table 5: Associations of mutations with delayed parasite clearance and LPF (adjusted OR/HR, 95% CI)

Marker	Delayed clearance (AOR)	LPF (AOR)
pfkelch13 mutant	3.2 (1.1-9.4)*	4.1 (1.2-14.0)*
pfmdr1 N86Y	1.8 (0.8-4.1)	1.5 (0.4-5.6)
pfmdr1 Y184F	1.4 (0.6-3.2)	2.0 (0.6-7.0)
pfdhfr N51I	1.6 (0.7-3.7)	2.3 (0.7-8.1)
Multi-mutant haplotype	2.1 (0.9-4.9)	(1.0-7.8)*

*p<0.05 showing statistical significance

Associations between molecular markers and treatment outcomes: Participants with *pfkelch13* mutations had 3.2-fold higher odds of delayed clearance (95% CI: 1.1-9.4, p = 0.03) and 4.1-fold for LPF (95% CI: 1.2-14.0, p = 0.02), adjusted for age and baseline parasitaemia. The *pfmdr1* N86Y was associated with prolonged PC½ (HR: 0.7, 95% CI: 0.5-0.9, p = 0.01), but not recrudescence (p = 0.18). Multi-mutant haplotypes (*pfmdr1* NYY+*pfdhfr* triple) correlated with 2.8-fold LPF risk (95% CI: 1.0-7.8, p = 0.04). No associations were found for *pfcrt* C72S (Table 5).

Epidemiological modelling of resistance spread: Deterministic SIR modelling projected a 15% rise in ACT failure rates by 2030 under current trends (baseline 5.6% to 20.5%), averting 8,500 regional cases annually with triple ACT (TACT) introduction (efficacy 98%). *pfkelch13* spread was simulated at R =1.8, sensitive to ITN coverage (>70% reduces incidence by 25%) (Fig. 1).

DISCUSSION

This study provides critical insights into the genomic landscape of *Plasmodium falciparum* resistance in Abia State, Southeastern Nigeria, from August 2024 to July 2025, revealing a moderate burden of artemisinin-associated mutations alongside sustained efficacy of artemether-lumefantrine (AL). The 8.4%

prevalence of *pfkelch13* propeller domain mutations aligned with emerging patterns across Sub-Saharan Africa (SSA), where validated markers like R561H and C580Y signalled partial artemisinin resistance; the mutation was below the 15% WHO threshold for containment action[6]. Notably, the absence of novel variants in our sample contrasts with recent detections of rare *pfkelch13* polymorphisms in Southwest Nigeria, underscoring regional heterogeneity driven by transmission intensity and drug pressure[7]. High *pfmdr1* N86Y (55.2%) and amplification (32.3%) frequencies, consistent with prior Nigerian surveillance, amplify lumefantrine resistance risks and potentially erode AL's partner drug efficacy, as seen in 20-30% treatment delays regionally[8,17].

The observed temporal uptrend in *pfmdr1* N86Y (from 48.0% to 62.0%) over the index study's year suggests selective pressure from AL overuse, mirroring historical shifts during Nigeria's transition from chloroquine to ACTs, where *pfmdr1* variants surged post-2005[18]. This highlights predisposing factors like substandard drug circulation in Southwestern Nigeria, where similar *pfmdr1* haplotypes correlated with 15-25% failure rates[19]. Concomitant *pfcrt* C72S (92.1%) and *pfdhfr* N51I (68.4%) prevalence reinforces polygenic resistance foundations, facilitating *pfkelch13* emergence, as modelled in SSA-wide genomic networks[9]. Associations with delayed parasite clearance (PC½ >5 hrs; OR 3.2 for *pfkelch13*) and late parasitological failure (LPF; OR 4.1) validate the prognostic value of these markers and align with the WHO TEM benchmarks, where >10% delayed clearance prompts policy review[15,17]. The PCR-corrected adequate clinical and parasitological response (ACPR) of 97.8% confirms the current viability of AL in Abia State; however, the 2.2% LPF rate, which was higher than Northern Nigeria's <1%, highlighted southeastern hotspots that are worsened by porous borders with Central Africa[4].

These findings have direct implications for Nigeria's National Malaria Strategic Plan 2021-2025, advocating intensified genomic surveillance following WHO's 2025 African roadmap to pre-empt ACT collapse, as partial resistance had doubled failure risks in Uganda analogues[10]. Modelling projections of 15% ACPR decline by 2030 highlight the urgency of triple ACTs (e.g., AL+amodiaquine), potentially averting 8,500 cases annually at USD 12/DALY, cost-effective against SSA benchmarks[11]. Locally, integrating *pfmdr1* monitoring into Abia's health systems could guide sulfadoxine-pyrimethamine alternatives, drawing from traditional herb synergies that mitigate resistance *in vitro*[20]. Broader SSA collaboration with platforms like the Worldwide Antimalarial Resistance Network (WWARN) is essential to track haplotype spread, as *pfmdr1* NYY clusters here resemble those fuelling pan-African dissemination[9].

The limitations of this study include the single-state focus, which potentially underestimates interstate variability; the reliance on targeted sequencing over whole-genome approaches, which might miss low-frequency alleles; and the modest sample of 200, which may limit power for rare events. Thus, future studies should expand to multi-state cohorts with longitudinal WGS, incorporating *pfpm2/pfpm3* for comprehensive resistance phenotyping and *in vivo* evaluation of triple ACT efficacy. Nevertheless, this study demonstrated that while AL remains effective, emerging *pfkelch13* and escalating *pfmdr1* mutations in Abia signal a pivotal window for proactive policy shifts and to fortify Nigeria's malaria elimination interventions amid SSA's rising resistance frontier.

CONCLUSION

This genomic surveillance of *Plasmodium falciparum* in Abia State highlighted the precarious balance of artemether-lumefantrine efficacy amid rising resistance threats, with *pfkelch13* mutations at 8.4% heralding partial artemisinin resistance and *pfmdr1* N86Y escalation (55.2%) signalling partner drug vulnerabilities that correlate with delayed clearance (OR: 3.2) and late failures (2.2%), yet affirming 97.8% adequate response rates. These findings illuminate Abia's role as a southeastern sentinel, where polygenic haplotypes and temporal uptrends amplify sub-Saharan Africa-wide risks, necessitating immediate integration of targeted sequencing into Nigeria's Malaria Strategic Plan to pre-empt ACT erosion, as modelled projections forecast 15% failure surges by 2030 in the absence of interventions like triple ACTs, which could avert 8,500 cases annually at cost-effective thresholds. By bridging molecular trends to

clinical-policy linkages, this study advocates for scalable, collaborative frameworks, including WWARN partnerships and community-driven monitoring, to safeguard elimination goals and transform localized data into actionable defences against malaria's evolving frontier.

SIGNIFICANCE STATEMENT

This study on genomic surveillance of *Plasmodium falciparum* resistance markers in Abia State, Southeastern Nigeria, holds profound significance by unveiling the emerging threat of partial artemisinin resistance through an 8.4% prevalence of *pfkelch13* mutations and escalating *pfmdr1* variants (55.2% N86Y), which correlate with delayed parasite clearance and 2.2% treatment failures despite 97.8% overall efficacy of artemether-lumefantrine, thereby establishing Abia as a critical sentinel for sub-Saharan Africa's resistance pattern. By integrating longitudinal molecular trends with clinical outcomes and predictive modelling, and forecasting a 15% surge in failures by 2030, these findings inform Nigeria's Malaria Strategic Plan and advocate timely shifts to triple ACTs that could avert thousands of cases annually at cost-effective scales, while pioneering scalable genomic tools for resource-limited settings to fortify global malaria elimination efforts against evolving drug pressures.

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