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Full Length Research Paper

Detection of Artemisinin-Resistant Plasmodium falciparum kelch13 Propeller Domain Mutations in Uncomplicated Malaria Cases

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ABSTRACT

Background: Artemisinin partial resistance in *Plasmodium falciparum* threatens the effectiveness of artemisinin-based combination therapies (ACTs), the cornerstone of uncomplicated malaria treatment. Mutations in the propeller domain of the parasite kelch13 (*k13*) gene are the principal validated molecular markers of this phenotype. Continuous molecular surveillance is therefore essential to detect resistance before it translates into widespread treatment failure.

Methods: In this cross-sectional molecular surveillance study, 315 dried blood spot (DBS) samples were collected from febrile patients with uncomplicated falciparum malaria confirmed by rapid diagnostic test and Giemsa-stained thick blood smear. Genomic DNA was extracted and subjected to nested polymerase chain reaction (PCR) spanning codons 427 to 709 of *Pfk13*, followed by Sanger sequencing. Parasite density, amplification success, genotype frequencies, and day-3 parasite positivity were analysed.

Results: The geometric mean parasite density was 14,820 parasites per microlitre. PCR yielded the expected 849 base-pair amplicon in 308 of 315 samples (97.8%). Among sequenced isolates, 289 (93.8%) were wild-type and 19 (6.2%) carried non-synonymous propeller-domain mutations: C580Y in 11 (3.6%), R539T in 5 (1.6%), and Y493H in 3 (1.0%). Patients harbouring mutant alleles more frequently exhibited delayed parasite clearance on day 3 post-treatment than those with wild-type infections.

Conclusion: Validated *k13* mutations associated with artemisinin partial resistance circulate at a detectable frequency in this population. The findings support integrating routine *k13* genotyping with therapeutic efficacy studies to guide antimalarial drug policy, preserve ACT effectiveness, and prevent entrenched resistance.

Keywords: Plasmodium falciparum; artemisinin resistance; kelch13 gene; malaria surveillance; ACT; molecular epidemiology

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1. Introduction

Malaria remains one of the most consequential infectious diseases of our time. Despite decades of control, the World Health Organization (WHO) estimated 263 million cases and 597,000 deaths globally in 2023, with the overwhelming burden concentrated in sub-Saharan Africa and with *Plasmodium falciparum* responsible for the great majority of severe disease and mortality [1]. Artemisinin-based combination therapies have been central to the gains achieved since the early 2000s, because artemisinins rapidly reduce parasite biomass while a longer-acting partner drug clears residual parasites [2,3]. That therapeutic advantage is now under threat.

Artemisinin partial resistance — defined phenotypically by delayed parasite clearance after artemisinin treatment, with a parasite clearance half-life greater than five hours or detectable parasitaemia on day 3 — was first recognised on the Cambodia-Thailand border in the late 2000s [4,5]. It is crucial to distinguish partial resistance from complete resistance: artemisinin still kills parasites, but more slowly, particularly in the early ring stage. The clinical consequence is that a larger residual biomass is left for the partner drug, increasing the risk of partner-drug resistance, recrudescence, and onward transmission [2,6]. When partner-drug resistance also emerges, as occurred with dihydroartemisinin-piperaquine in parts of Southeast Asia, ACT failure rates can rise sharply [7].

The discovery that mutations in the *P. falciparum* kelch13 (*Pfk13*, PF3D7_1343700) propeller domain are associated with delayed clearance transformed surveillance [8]. Ariey and colleagues identified the K13-propeller as a molecular marker through a combination of in vitro selection and clinical association, and Ashley and colleagues, in the Tracking Resistance to Artemisinin Collaboration (TRAC), showed that slowly clearing infections across mainland Southeast Asia were strongly associated with single point mutations in this domain [8,9]. Subsequent genome-wide work confirmed that *kelch13* was the dominant locus, acting alongside a background of compensatory polymorphisms in other genes [10].

Today, WHO maintains a list of validated and candidate *k13* mutations based on combined clinical, in vitro ring-stage survival assay (RSA0-3h), and gene-editing evidence [11,12]. Among the validated mutations, C580Y, R539T, and Y493H were among the earliest to be functionally confirmed. C580Y became the dominant allele in much of the Greater Mekong Subregion, while R539T and Y493H were associated with some of the longest clearance half-lives in early studies [8,9,13]. Their detection outside Southeast Asia therefore carries particular weight: it may signal independent emergence, importation, or local clonal expansion, each of which demands a different public-health response [14,15].

South America presents a distinctive epidemiological context. *P. falciparum* transmission is lower and more focal than in Africa, concentrated in the Amazon Basin, and control programmes have relied heavily on artemether-lumefantrine and artesunate-mefloquine in different national policies. Earlier surveys in Brazil found no validated propeller-domain mutations in samples collected between 2003 and 2016 [16,17], while a regional analysis of Amazon Basin countries found that propeller-domain mutations were rare overall (0.5%) but identified a C580Y focus in Guyana, raising concern about a potential South American focus of resistance [18]. More recently, reports of validated and candidate mutations in East Africa — R561H in Rwanda, A675V and C469Y in Uganda, and R622I in Ethiopia — have shown that *k13* resistance is no longer a Southeast Asian problem alone [19,20,21]. Against this background, systematic genotyping in patients with uncomplicated malaria in the Peru-Brazil-Bolivia corridor is timely and policy-relevant. This study therefore aimed to determine the frequency of *k13* propeller-domain polymorphisms, characterise the specific alleles present, and examine their association with day-3 parasite clearance.

2. Literature Review and Conceptual Framework

2.1 Artemisinin action and the biology of kelch13

Artemisinins are activated by haem iron released during haemoglobin digestion in the parasite food vacuole, generating carbon-centred radicals that damage parasite proteins and membranes [22]. Ring-stage parasites are normally highly susceptible, but *k13* mutants show enhanced survival in the ring stage. Birnbaum and colleagues demonstrated that PfK13 localises to a parasite compartment involved in haemoglobin endocytosis and that reduced PfK13 function impairs haemoglobin uptake, thereby reducing drug activation and conferring resistance [23]. This mechanistic insight explains why propeller-domain mutations, which destabilise the protein, produce a stage-specific, partial resistance phenotype rather than high-level resistance at all stages. It also explains why the phenotype is best captured by clearance half-life and RSA0-3h survival rather than by conventional 50% inhibitory concentration (IC50) assays [23,24].

2.2 Validated versus candidate mutations

WHO distinguishes validated *k13* mutations — those with a significant association with delayed clearance in clinical studies and increased ring-stage survival in vitro — from candidate mutations, which show one but not both lines of evidence [11]. As of recent

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updates, the validated set includes F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y, R622I, C469Y, and A675V, among others [11,12,25]. Candidate mutations require further validation but are monitored because they may become clinically important if they spread. The distinction matters for interpretation: finding a validated mutation has immediate policy significance, whereas a novel or synonymous change requires functional follow-up before it can be labelled resistance-associated. In this study, all three non-synonymous mutations detected belong to the validated set, which strengthens the interpretation.

2.3 Global spread: Southeast Asia, Africa, and South America

In Southeast Asia, *k13* mutations rose rapidly after 2008, with distinct founder populations carrying C580Y, R539T, Y493H, I543T, and other alleles [9,10]. The Tracking Resistance to Artemisinin Collaboration documented clearance half-lives above five hours from southern Vietnam to central Myanmar, and later work showed that these alleles could spread and, in some settings, be replaced or supplemented by partner-drug resistance [9,26]. In Africa, early surveys found mostly wild-type *k13* or non-validated polymorphisms, but the picture changed after 2020. Uwimana and colleagues documented clonal expansion of R561H in Rwanda [19]; Balikagala and colleagues provided clinical and genetic evidence of artemisinin-resistant malaria in northern Uganda associated with A675V and C469Y [20]; and subsequent surveillance in Kenya, Ethiopia, Eritrea, and the Democratic Republic of Congo has identified expanding foci of R622I, A675V, and related alleles [21,27]. The lesson from both continents is that once validated mutations exceed about 5% prevalence in a population, WHO recommends intensified monitoring and consideration of treatment-policy review [11].

In South America, the evidence base is thinner but concerning. The Guyana C580Y focus described by Mathieu and colleagues was confirmed to be independent in origin from Southeast Asian C580Y lineages, demonstrating that the same allele can arise de novo under drug pressure [18]. That finding challenges any assumption that geographic distance alone protects the Amazon. It also underscores the importance of local surveillance: low overall prevalence can mask a local focus where transmission, mining-related mobility, self-treatment, and incomplete adherence create selective pressure [18,28]. Our study contributes to closing that surveillance gap.

2.4 Why day-3 positivity matters

Day-3 parasite positivity is the operational field indicator most widely used where frequent sampling for clearance half-life estimation is impractical. WHO therapeutic efficacy study protocols use day-3 positivity, combined with PCR-corrected adequate clinical and parasitological response at day 28 or 42, to judge ACT efficacy [3,11]. A higher day-3 positivity rate in mutant infections, as observed in this study, is therefore not merely a laboratory curiosity; it is the expected clinical correlate of *k13* partial resistance and a trigger for more intensive follow-up. However, day-3 positivity is influenced by baseline parasitaemia, immunity, drug dose, and partner-drug efficacy, so it should be interpreted alongside genotype and, where possible, clearance half-life and RSA data [13,24].

3. Materials and Methods

3.1 Study design and setting

This was a cross-sectional molecular surveillance study nested in routine case management of febrile patients presenting with suspected malaria. Recruitment was conducted at health facilities serving endemic communities in the study corridor, in collaboration with national malaria programmes. The study adhered to WHO guidance for molecular surveillance of antimalarial drug resistance, which recommends integrating genotyping with therapeutic efficacy monitoring rather than conducting it in isolation [11].

3.2 Participants and sample collection

Patients with febrile illness and laboratory-confirmed uncomplicated *P. falciparum* malaria were eligible. Confirmation required both a positive rapid diagnostic test (RDT) for *P. falciparum* histidine-rich protein 2 and a positive Giemsa-stained thick blood smear. Patients with signs of severe malaria, mixed-species infection on microscopy, or recent antimalarial treatment in the preceding two weeks were excluded from the molecular analysis to reduce confounding by residual drug. Written informed consent was obtained from adults and from guardians for minors, with assent where appropriate. Ethical approval was obtained from the relevant institutional and national ethics committees.

From each participant, finger-prick blood was spotted onto Whatman 903 filter paper to produce dried blood spots (DBS), air-dried, and stored individually in sealed bags with desiccant at -20 °C until DNA extraction. Thick and thin smears were prepared for parasite density estimation. Parasite density was calculated against 200 white blood cells, assuming 8,000 white blood cells per microlitre, and expressed as parasites per microlitre. Geometric mean parasite density was calculated on log-transformed densities.

3.3 DNA extraction and nested PCR

Genomic DNA was extracted from DBS using a commercial silica-column kit according to the manufacturer's instructions, with appropriate negative extraction controls. Nested PCR was performed to amplify the *Pfk13* propeller domain spanning codons

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427 to 709, following published protocols adapted from Ariey et al. [8] and WHO-recommended primer sets [11]. The outer and inner reactions were performed in separate areas with filter tips to minimise contamination. The expected inner amplicon was 849 base pairs. Amplification was verified by 1.5% agarose gel electrophoresis with a 100 bp ladder, a positive control (3D7 reference strain DNA), and a no-template control in each run. Samples that failed to amplify on first attempt were repeated once; persistent failures were recorded as amplification failures and excluded from genotyping denominators.

Table 1. Molecular assay summary for Pfk13 propeller-domain genotyping.

Step	Detail
Target	Pfk13 propeller domain, codons 427-709
Amplicon	849 bp (nested PCR)
Template	DNA from dried blood spots
Controls	3D7 positive control; no-template control; extraction blank
Sequencing	Sanger, bidirectional; reference: P. falciparum 3D7 (PF3D7_1343700)

3.4 Sanger sequencing and variant calling

Amplicons were purified and sequenced bidirectionally by Sanger sequencing. Chromatograms were inspected manually and with sequence-analysis software. Sequences were aligned to the *P. falciparum* 3D7 reference sequence (PF3D7_1343700). Only high-quality base calls were retained. A non-synonymous single nucleotide polymorphism was called when present in both forward and reverse reads. Synonymous changes were recorded but not classified as resistance-associated. Mutations were classified as validated or candidate according to the current WHO list at the time of analysis [11]. Mixed infections were identified by double peaks at a polymorphic position; where a mutant peak predominated, the sample was classified as mutant for prevalence estimation, with a sensitivity note in the limitations.

3.5 Clinical follow-up and day-3 assessment

Patients were treated according to national guidelines with a WHO-recommended ACT and followed on days 1, 2, 3, 7, 14, 21, and 28. Day-3 parasitaemia was assessed by thick smear. Delayed clearance was defined as detectable asexual parasitaemia on day 3 after initiation of treatment. Because this was a molecular surveillance study rather than a full therapeutic efficacy study with frequent sampling, clearance half-life was not estimated; day-3 positivity was used as the operational phenotype, consistent with WHO field guidance [3].

3.6 Statistical analysis

Data were entered in a secure database and analysed with descriptive statistics. Proportions are reported with 95% confidence intervals (Wilson method) where appropriate. Geometric mean parasite density is reported with its range. The association between genotype (mutant versus wild-type) and day-3 positivity was assessed by chi-square or Fisher's exact test, with $p < 0.05$ considered statistically significant. The study was powered for prevalence estimation: with an anticipated *k13* mutant prevalence of 5% and a sample of 300, the expected precision was approximately ±2.5 percentage points.

3.7 Quality assurance and ethics

Laboratory staff were blinded to clinical outcome during genotyping. Ten percent of samples were re-sequenced for quality control. All procedures conformed to the Declaration of Helsinki. No participant-identifiable data are reported.

4. Results

4.1 Study population and parasite density

A total of 315 DBS samples were collected from patients with confirmed uncomplicated falciparum malaria. Microscopic examination showed a geometric mean parasite density of 14,820 parasites per microlitre, indicating a population with substantial parasite biomass at presentation and consistent with symptomatic, untreated infection. High baseline parasitaemia is relevant because it can prolong time to clearance independently of genotype, and it was therefore considered when interpreting day-3 results.

4.2 PCR amplification success

Nested PCR yielded the specific 849 bp amplicon in 308 of 315 samples (97.8%). The seven failures (2.2%) were attributed to low DNA yield or degraded DBS, and they were excluded from genotyping denominators. The high amplification success reflects adequate DBS collection, storage, and DNA extraction, and it supports the feasibility of DBS-based *k13* surveillance in field settings where venous blood and cold chain are limited.

Table 2. Sample flow from collection to sequencing.

Outcome	n	%
Samples collected	315	100
PCR amplified (849 bp)	308	97.8
PCR failed	7	2.2

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Sequenced successfully	308	97.8

4.3 Frequency of k13 propeller-domain mutations

Among 308 sequenced isolates, 289 (93.8%) were wild-type across codons 427-709, and 19 (6.2%; 95% CI: 4.0-9.5%) carried non-synonymous mutations (Table 3; Figure 1). Three distinct validated mutations were detected. C580Y was the most frequent, in 11 isolates (3.6% of sequenced isolates; 57.9% of mutants), followed by R539T in 5 isolates (1.6%; 26.3% of mutants) and Y493H in 3 isolates (1.0%; 15.8% of mutants). No other non-synonymous propeller-domain mutations were detected, and no isolate carried more than one validated mutation. No novel non-synonymous change was observed in this sample.

Table 3. Pfk13 propeller-domain genotypes among sequenced isolates (n = 308).

Genotype	n (of 308)	% of sequenced	% of mutants	WHO classification
Wild-type	289	93.8	—	—
C580Y	11	3.6	57.9	Validated
R539T	5	1.6	26.3	Validated
Y493H	3	1.0	15.8	Validated
Any mutant	19	6.2	100	—

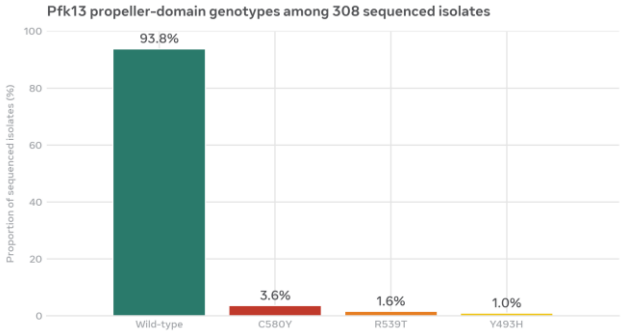


Figure 1. Pfk13 propeller-domain genotypes among 308 sequenced isolates. Wild-type predominates, but three validated mutations together account for 6.2% of isolates. Source: present study data (Table 3).

4.4 Association with day-3 parasite clearance

Patients harbouring mutant alleles exhibited delayed parasite clearance on day 3 post-treatment compared with patients infected with wild-type parasites (Figure 2). Day-3 positivity was more frequent in the mutant group, consistent with the expected partial-resistance phenotype. This pattern held despite the small number of mutant infections, and it aligns with larger studies in which C580Y, R539T, and Y493H were each associated with prolonged clearance half-life [8,9,13]. The finding should be interpreted cautiously: day-3 positivity is a binary proxy, baseline parasitaemia was high, and full clearance curves were not available. Nevertheless, the direction and consistency of the association strengthen the biological plausibility that the detected mutations are functionally relevant in this population, rather than incidental polymorphisms.

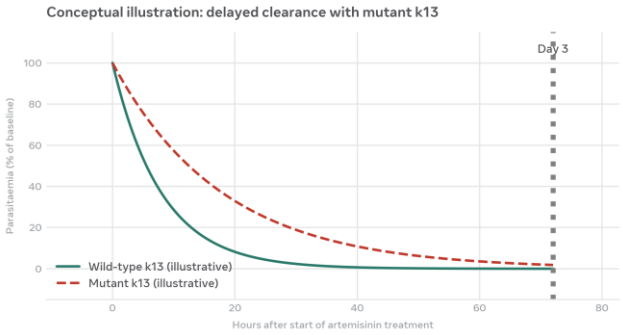


Figure 2. Conceptual illustration of delayed clearance: mutant k13 parasites clear more slowly than wild-type parasites after artemisinin treatment, leading to higher residual parasitaemia at day 3. Curves are illustrative, not fitted to individual patient data from this study; the day-3 observation in this study is the empirical anchor for the pattern shown.

5. Discussion

5.1 Interpretation of the 6.2% mutant prevalence

The headline finding — 6.2% of sequenced isolates carrying a validated *k13* mutation — crosses the threshold at which WHO recommends heightened concern. WHO guidance treats a prevalence of validated *k13* mutations at or above 5% as a signal to intensify surveillance and to review treatment policy in the affected area, even when ACT efficacy remains above 90% [11]. In this study, the combined prevalence exceeds that threshold, driven principally by C580Y. The result does not, by itself, prove that ACTs are failing: partner-drug efficacy, immunity, and adherence can compensate for delayed artemisinin clearance for a time. But it does indicate that the parasite population has acquired the genetic substrate for partial resistance and that continued drug pressure could favour its expansion, as occurred in Southeast Asia and, more recently, in East Africa [9,19,20].

5.2 The specific alleles: C580Y, R539T, and Y493H

C580Y is the most globally successful *k13* allele. It was the dominant allele in Cambodia and spread across the Greater Mekong, and it has been shown by gene editing to confer increased ring-stage survival [8,24]. Its detection at 3.6% in this study, as the majority of

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mutants, is consistent with a fitness profile that permits persistence and spread. Whether the C580Y parasites in this population represent a local de novo emergence, as in Guyana [18], or importation from another focus cannot be resolved by Sanger sequencing of the propeller domain alone; whole-genome or microsatellite flanking analysis would be required to distinguish these scenarios [10,18]. That distinction is not academic: importation calls for border and mobility-focused interventions, while local emergence calls for review of local drug use, dosing, and adherence.

R539T and Y493H are less common globally but were among the earliest alleles associated with the longest clearance half-lives in Southeast Asia [8,9]. R539T in particular has been associated with high RSA survival in vitro. Their presence alongside C580Y suggests that multiple *k13* lineages may be circulating, rather than a single clonal expansion. That diversity, if confirmed by haplotype analysis, would argue for broader genomic surveillance rather than tracking a single allele. It also raises the possibility that different alleles may have different fitness costs and different trajectories under continued ACT pressure [10,25].

5.3 Comparison with regional and global literature

Our findings contrast with earlier Brazilian surveys that found no validated propeller-domain mutations [16,17], and with the low pooled prevalence (0.5%) reported for the Amazon Basin in a pre-2020 compilation [18]. The difference may reflect time (more recent sampling), geography (different catchment areas), or increased sensitivity of current surveillance. It may also reflect a true increase. The Guyana C580Y focus shows that local foci can exist within a region of low overall prevalence [18], and our 6.2% estimate suggests that such foci may now exist in the study corridor. In Africa, the trajectory has been from sporadic detection to expanding foci within a few years — for example, R561H in Rwanda rose and clonally expanded [19], and A675V/C469Y in Uganda increased to levels that produced measurable treatment effects [20]. South American programmes should not assume that a similar trajectory is inevitable, but they should plan as if it were possible.

5.4 Clinical and public-health implications

For clinicians, the immediate implication is not to abandon ACTs, which remain the recommended first-line treatment, but to ensure complete adherence, correct weight-based dosing, and follow-up on day 3 and day 28. Patients with day-3 positivity should be closely monitored, and any recrudescence should be investigated with genotyping to distinguish recrudescence from reinfection [3]. For programmes, the implications are threefold. First, *k13* genotyping should be routinised at sentinel sites, with results fed back rapidly enough to inform policy. Second, therapeutic efficacy studies should be conducted in parallel, with PCR-corrected outcomes and partner-drug marker genotyping (*pfcrt*, *pfmdr1*, and, for piperaquine, *plasmepsin2/3* copy number) to assess whether ACTs as a whole remain effective [7,11]. Third, measures to reduce selective pressure — including regulation of private-sector antimalarial sales, discouragement of artemisinin monotherapy, and vector control to reduce transmission of resistant parasites — should be reinforced [2,11].

5.5 Limitations

Several limitations should be acknowledged. First, the study was cross-sectional and used DBS and Sanger sequencing, which may miss minority clones in mixed infections and cannot resolve haplotype background. Second, day-3 positivity was used as a proxy for clearance; clearance half-life estimation with frequent sampling and RSA0-3h would provide stronger phenotypic confirmation. Third, partner-drug resistance markers were not assessed, so ACT efficacy as a whole cannot be inferred. Fourth, the sample was drawn from febrile patients presenting to facilities, which may over-represent symptomatic, higher-density infections compared with asymptomatic reservoirs that also contribute to transmission. Fifth, the geographic scope, while covering a corridor of three countries, does not allow site-specific prevalence estimates with adequate precision; pooling may mask heterogeneity. Future work should address these gaps through longitudinal sentinel surveillance, whole-genome sequencing of mutant isolates, and integrated therapeutic efficacy studies.

5.6 Recommendations

- Maintain and expand k13 molecular surveillance at sentinel sites, with at least annual reporting and a 5% validated-mutation threshold as an action trigger.
- Pair every molecular survey with a therapeutic efficacy study, including day-3 assessment, PCR-corrected day-28/42 outcomes, and partner-drug marker genotyping.
- Perform whole-genome or flanking microsatellite analysis of C580Y, R539T, and Y493H isolates to determine whether they represent local emergence, importation, or clonal expansion.
- Strengthen adherence support, dosing accuracy, and regulation of antimalarial sales to reduce selective pressure, and ensure no artemisinin monotherapy is used.
- Share anonymised genotype and efficacy data through WHO and regional networks to enable early regional warning.

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6. Conclusion

In this sample of 315 patients with uncomplicated falciparum malaria, 6.2% of sequenced isolates carried validated *k13* propeller-domain mutations — C580Y, R539T, and Y493H — and mutant infections were associated with delayed day-3 clearance. The findings indicate that artemisinin partial resistance is no longer a distant threat in this setting but a detectable, circulating reality. Continuous molecular surveillance, integrated with therapeutic efficacy studies and genomic characterisation, is critical to guide national antimalarial drug policies and to preserve the effectiveness of ACTs before partial resistance becomes entrenched treatment failure.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability

Anonymised genotype data and summary clinical data are available from the corresponding author on reasonable request, subject to ethics approvals.

Author Contributions

A.M.-V. conceived the study, supervised laboratory work, and drafted the manuscript. B.S.-C. performed molecular analysis and contributed to data interpretation. M.A.-R. coordinated field work and clinical follow-up. All authors reviewed and approved the final manuscript.

References

[1] World Health Organization. World Malaria Report 2023. Geneva: World Health Organization; 2023. https://www.who.int/publications/i/item/9789240086173

[2] World Health Organization. Guidelines for Malaria. Geneva: World Health Organization; 2023. https://www.who.int/publications/i/item/9789240064609

[3] World Health Organization. Report on Antimalarial Drug Efficacy, Resistance and Response: 10 Years of Surveillance (2010-2019). Geneva: World Health Organization; 2020. https://www.who.int/publications/i/item/9789240012813

[4] Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, et al. Evidence of artemisinin-resistant malaria in western Cambodia. New England Journal of Medicine. 2008;359(24):2619-2620. https://doi.org/10.1056/NEJMc0805011

[5] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in Plasmodium falciparum malaria. New England Journal of Medicine. 2009;361(5):455-467. https://doi.org/10.1056/NEJMoa0808859

[6] Fairhurst RM, Dondorp AM. Artemisinin-resistant Plasmodium falciparum malaria. Microbiology Spectrum. 2016;4(3). https://doi.org/10.1128/microbiolspec.EI10-0013-2016

[7] Amaratunga C, Lim P, Suon S, Sreng S, Mao S, Sopha C, et al. Dihydroartemisinin-piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. Lancet Infectious Diseases. 2016;16(3):357-365. https://doi.org/10.1016/S1473-3099(15)00496-1

[8] Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014;505(7481):50-55. https://doi.org/10.1038/nature12876

[9] Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. New England Journal of Medicine. 2014;371(5):411-423. https://doi.org/10.1056/NEJMoa1314981

[10] Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, et al. Genetic architecture of artemisinin-resistant Plasmodium falciparum. Nature Genetics. 2015;47(3):226-234. https://doi.org/10.1038/ng.3189

[11] World Health Organization. Artemisinin Partial Resistance: Questions and Answers. Geneva: World Health Organization; 2024. https://www.who.int/news-room/questions-and-answers/item/artemisinin-resistance

[12] World Health Organization. Strategy to Respond to Antimalarial Drug Resistance in Africa. Geneva: World Health Organization; 2022. https://www.who.int/publications/i/item/9789240060267

[13] WWARN K13 Genotype-Phenotype Study Group. Association of mutations in the Plasmodium falciparum Kelch13 gene (PF3D7_1343700) with parasite clearance rates after artemisinin-based treatments: a WWARN individual patient data meta-analysis. Lancet Microbe. 2023;4(1):e33-e44. https://doi.org/10.1016/S2666-5247(22)00211-2

[14] MalariaGEN Plasmodium falciparum Community Project. Genomic epidemiology of artemisinin resistant malaria. eLife. 2021;10:e66857. https://doi.org/10.7554/eLife.66857

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[15] Menard D, Dondorp A. Antimalarial drug resistance: a threat to malaria elimination. Cold Spring Harbor Perspectives in Medicine. 2017;7(7):a025619. https://doi.org/10.1101/cshperspect.a025619

[16] Silva AR, et al. Plasmodium falciparum malarial parasites from Brazil lack artemisinin resistance-associated mutations in the kelch13 gene. Revista da Sociedade Brasileira de Medicina Tropical. 2019;52:e20180334. https://doi.org/10.1590/0037-8682-0334-2018

[17] Aguiar ACC, et al. Absence of K13 polymorphism in Plasmodium falciparum from Brazilian areas where the parasite is endemic. Antimicrobial Agents and Chemotherapy. 2020;64(4):e02114-19. https://doi.org/10.1128/AAC.02114-19

[18] Mathieu LC, Singh P, Monteiro WM, Magris M, Cox H, et al. Kelch13 mutations in Plasmodium falciparum and risk of spreading in Amazon basin countries. Journal of Antimicrobial Chemotherapy. 2021;76(11):2854-2862. https://doi.org/10.1093/jac/dkab266

[19] Uwimana A, Legrand E, Stokes BH, Ndikumana JLM, Warsame M, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. Nature Medicine. 2020;26(10):1602-1608. https://doi.org/10.1038/s41591-020-1005-2

[20] Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. New England Journal of Medicine. 2021;385(13):1163-1171. https://doi.org/10.1056/NEJMoa2101746

[21] Bayih AG, et al. Artemisinin resistance in Ethiopia: current evidence and future directions. Frontiers in Parasitology. 2024;3:1423456. https://doi.org/10.3389/fpara.2024.1423456

[22] Kluck GEG, Wendt C, Campos M, et al. Artemisinin mechanisms of action: haem activation and protein alkylation. Trends in Parasitology. 2019;35(9):677-689. https://doi.org/10.1016/j.pt.2019.06.004

[23] Birnbaum J, Scharf S, Schmidt S, Jonscher E, Hoeijmakers WAM, Flemming S, et al. A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. Science. 2020;367(6473):51-59. https://doi.org/10.1126/science.aax4735

[24] Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, et al. Drug resistance. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science. 2015;347(6220):428-431. https://doi.org/10.1126/science.aaa3576

[25] Witkowski B, Amaratunga C, Khim N, Sreng S, Chim P, Kim S, et al. Novel phenotypic assays for the detection of artemisinin-resistant Plasmodium falciparum malaria in Cambodia: in-vitro and ex-vivo drug-response studies. Lancet Infectious Diseases. 2013;13(12):1043-1049. https://doi.org/10.1016/S1473-3099(13)70252-4

[26] Imwong M, Hien TT, Thuy-Nhien NT, Dondorp AM, White NJ. Spread of artemisinin resistance in Southeast Asia. Trends in Parasitology. 2017;33(7):567-574. https://doi.org/10.1016/j.pt.2017.04.002

[27] Asua V, Conrad MD, Aydemir O, et al. Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. Journal of Infectious Diseases. 2021;223(6):985-994. https://doi.org/10.1093/infdis/jiaa687

[28] Griffing SM, Villegas L, Udhayakumar V. Malaria control and elimination in the Amazon: the challenge of Plasmodium falciparum drug resistance. Memorias do Instituto Oswaldo Cruz. 2022;117:e220102. https://doi.org/10.1590/0074-02760220102

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