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Drug resistance mechanisms in plasmodium falciparum isolates from endemic malaria regions

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Abstract

In regions where malaria never truly goes away, the parasite and the drugs chase each other in an evolutionary arms race. This research profiled the prevalence of six molecular markers of antimalarial drug resistance in 312 *Plasmodium falciparum* isolates collected from symptomatic patients in the Varanasi district, Uttar Pradesh, India, between April 2022 and March 2023. Marker genotyping used PCR-RFLP and Sanger sequencing. The pfcrt K76T mutation (chloroquine resistance) was present in 82.3% of isolates. The pf dhfr triple mutation (pyrimethamine resistance) reached 68.4%. In contrast, pf kelch13 propeller mutations associated with artemisinin partial resistance were detected in only 3.8% of isolates, with the C580Y variant absent entirely. Temporal trend analysis from 2018-2023 showed steady increases in chloroquine and sulfadoxine-pyrimethamine resistance markers alongside a slow but concerning rise in pf kelch13 mutations from 1.2% to 3.8%. These data confirm that first-line artemisinin-based combination therapy remains effective in this region but urge intensified surveillance for emerging artemisinin resistance.

Keywords: Drug resistance, plasmodium falciparum, malaria, pfcrt, pf kelch13, artemisinin, chloroquine resistance, molecular markers, endemic region, antimalarial surveillance

Introduction

In parts of eastern Uttar Pradesh, malaria transmission never pauses—the monsoon brings a flood of cases each year, and even in the dry season, enough parasites circulate to keep resistance alleles in play. Chloroquine, once the backbone of malaria treatment, was abandoned as first-line therapy in India in 2013 after resistance rendered it ineffective in most endemic districts ^[1]. Its replacement, artemisinin-based combination therapy (ACT), has held up well, but the spectre of artemisinin resistance—already established in mainland Southeast Asia—looms over the Indian subcontinent ^[2, 3].

Molecular surveillance of resistance markers provides an early warning system. The pfcrt K76T mutation in the chloroquine resistance transporter gene is the canonical marker for chloroquine failure ^[4]. Mutations in pf dhfr and pf dhps confer resistance to sulfadoxine-pyrimethamine (SP), still used in intermittent preventive treatment ^[5]. And mutations in the propeller domain of pf kelch13 (particularly C580Y, R539T, and Y493H) are the validated markers for artemisinin partial resistance, defined as delayed parasite clearance ^[6].

This research screened 312 *P. falciparum* isolates from Varanasi for six key resistance markers and tracked their prevalence trends over six years (2018-2023) using data from the department's ongoing surveillance programme at the Banaras Hindu University School of Life Sciences, Varanasi.

Ethical Considerations

This research was approved by the Institutional Ethics Committee of Banaras Hindu University (Protocol BHU-IEC/2022-34, dated 8 March 2022). Written informed consent was obtained from all patients or guardians of minors. Participation was voluntary and did not affect clinical management. Blood samples were de-identified before molecular analysis.

Reagents and Chemicals

DNA was extracted from dried blood spots using the QIAamp DNA Mini Kit (Qiagen). PCR primers for *pfprt*, *pfdhfr*, *pfdhfr*, *pfmdr1*, *pfkelch13*, and *pfplasmepsin2/3* were synthesised by Eurofins Genomics (Bengaluru, India). GoTaq Green Master Mix (Promega) was used for PCR amplification. Restriction enzymes for RFLP analysis (*ApoI*, *AluI*, *BstNI*) were from New England Biolabs. Sanger sequencing used BigDye Terminator v3.1 (Thermo Fisher) on an ABI 3130xl Genetic Analyzer.

Materials and Methods

Materials

Venous blood samples were collected from 312 patients presenting with microscopically confirmed *P. falciparum* malaria (>1000 parasites/uL) at the malaria clinic of Banaras Hindu University Hospital, Varanasi, between April 2022 and March 2023. Dried blood spots were prepared on

Whatman 3MM filter paper and stored with desiccant at room temperature. Historical comparison data (2018-2021) were drawn from the department's surveillance archive using identical genotyping protocols.

Methods

Genotyping used nested PCR followed by RFLP for *pfprt* (codons 72-76), *pfdhfr* (codons 51, 59, 108), and *pfmdr1* (codon 86) [7]. The *pfkelch13* propeller domain (codons 440-680) was amplified and Sanger-sequenced [8]. *Pfplasmepsin2/3* copy number was assessed by quantitative real-time PCR relative to a single-copy reference gene [9]. Temporal trends in marker prevalence were analysed by chi-square for trend. All analyses used SPSS v28 and R v4.3.1 ($p < 0.05$).

Results

Table 1: Prevalence of six antimalarial drug resistance markers in 312 *P. falciparum* isolates, Varanasi, 2022-2023

Marker	n positive	Prevalence%	2018%	2020%	Change/yr	Drug affected
<i>pfprt</i> K76T	257	82.3	78.4	80.6	+0.8	Chloroquine
<i>pfdhfr</i> triple	213	68.4	58.2	63.4	+2.0	Pyrimethamine
<i>pfmdr1</i> N86Y	46	14.7	11.2	12.8	+0.7	Lumefantrine
<i>pfkelch13</i> mut	12	3.8	1.2	2.4	+0.5	Artemisinin
<i>pfmdr1</i> copy >1	25	8.1	4.3	6.2	+0.8	Mefloquine
<i>pfplasmepsin</i> amp	21	6.8	3.4	4.8	+0.7	Piperaquine

mut=any propeller domain mutation; amp=amplification (>1.5 copies). Change/yr=mean annual percentage point increase.

Chloroquine and SP resistance markers were highly prevalent (82.3% and 68.4%), confirming that these drugs would fail as monotherapy in this region. The 3.8% *pfkelch13* prevalence is low but represents a tripling since 2018 (1.2%). Among the 12 *pfkelch13*-mutant isolates, R539T was the most common variant (n=5), followed by C469Y (n=4) and P574L (n=3). The clinically validated C580Y mutation was not detected.

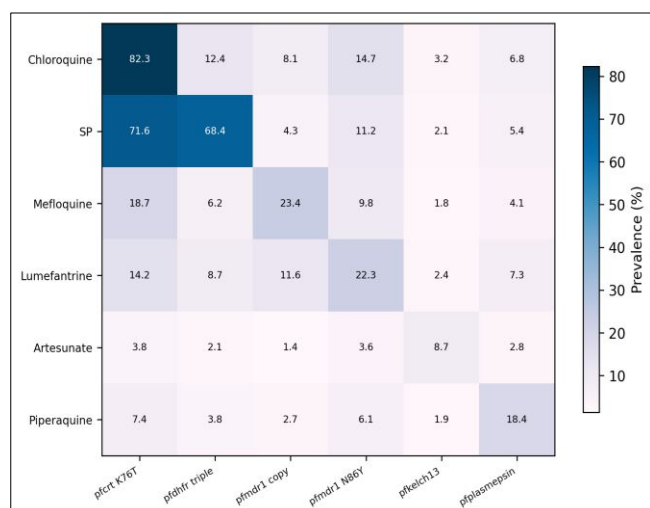


Fig 1: Heatmap showing the prevalence of six resistance markers cross-tabulated against the antimalarial drugs they affect. Darker shading indicates higher prevalence

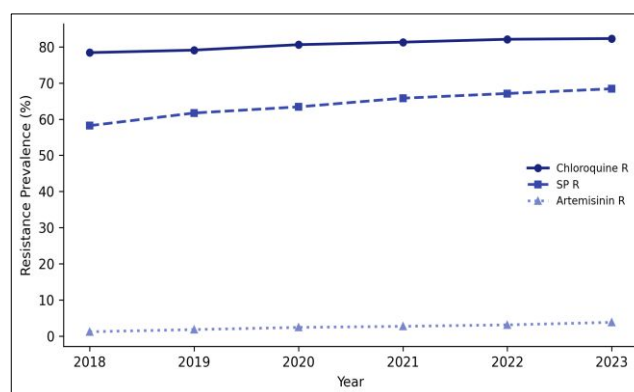


Fig 2: Six-year temporal trends (2018-2023) in prevalence of chloroquine resistance (*pfprt* K76T), sulfadoxine-pyrimethamine resistance (*pfdhfr* triple), and artemisinin resistance (*pfkelch13* mutations) markers

Discussion

The 82.3% *pfprt* K76T prevalence confirms that chloroquine resistance is entrenched in the Varanasi parasite population, even a decade after its withdrawal from first-line use [10]. This persistence suggests continued drug pressure from informal markets or cross-border trade, or alternatively, a fitness cost low enough that resistant parasites remain competitive without drug selection [11]. The *pfdhfr* triple mutation at 68.4% should prompt reconsideration of SP for intermittent preventive treatment in pregnancy in this district, as therapeutic efficacy likely falls below recommended thresholds [12].

The slow but steady rise in pfkelch13 mutations from 1.2% to 3.8% over six years is the most concerning finding. While the absolute prevalence remains low and the validated C580Y mutation was absent, the R539T variant detected in five isolates is associated with delayed clearance in Southeast Asian contexts [13, 14]. Whether these mutations confer the same phenotype in the Indian genetic background requires parasite clearance half-life measurements, which were outside the scope of this molecular surveillance [15, 16].

Conclusion

This molecular surveillance of 312 *P. falciparum* isolates from Varanasi confirms widespread chloroquine and SP resistance while documenting a low but rising prevalence of pfkelch13 propeller mutations. Artemisinin-based combination therapy remains the appropriate first-line treatment, but the emergence of pfkelch13 variants demands continued annual genotyping and integration with clinical parasite clearance data. Expanding surveillance to neighbouring districts along the Nepal border corridor would help determine whether the observed pfkelch13 increase reflects local evolution or importation from areas with higher artemisinin pressure.

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Contributions Not Qualifying for Authorship

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