Preplanned Studies

Drug Resistance of Imported *P. falciparum* and *P. vivax* Isolates — China, 2021–2023

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Summary

What is already known about this topic?

Artemisinin-based combination therapies (ACTs) remain the first-line treatment for uncomplicated malaria caused by *P. falciparum*, while chloroquine (CQ) serves as the primary treatment for *P. vivax*. However, the global spread of antimalarial drug resistance has become an increasing concern over time.

What is added by this report?

The integrated drug efficacy studies (iDES) demonstrated that artesunate (AS) plus dihydroartemisinin-piperaquine (DHA-PPQ) and chloroquine (CQ) remain effective first-line treatments for *P. falciparum* and *P. vivax* malaria, respectively. However, the occurrence of late treatment failure (LTF) and day 3 (D3) parasite positivity following treatment, suggests decreasing therapeutic efficacy. Molecular surveillance of *P. falciparum* resistance revealed novel mutation sites in *pfK13* (S459T, N499T, A578S, and V692L) in addition to the previously reported F446I, P574L, and C580Y mutations. Concurrently, the difference in mutation patterns between *pfcrt* and *pfmdr1* was significant (P<0.01), with the Y184F locus of the multidrug resistance gene *pfmdr1* showing the highest mutation frequency at 40.5% of cases.

What are the implications for public health practice?

The iDES and molecular surveillance of antimalarial drug resistance indicated decreasing sensitivity to current first-line treatments. Continued surveillance of antimalarial drug resistance is vital for early warning and appropriate response to the spread of resistant parasites.

ABSTRACT

Introduction: Malaria remains the leading cause of infectious disease-related morbidity and mortality worldwide. ACTs continue to be the first-line treatment for uncomplicated malaria caused by *P. falciparum*. However, the global spread of antimalarial drug resistance, particularly artemisinin resistance, has become an increasing concern over time.

Methods: Therapeutic efficacy was evaluated following the World Health Organization's guidelines for iDES. This study assessed adequate clinical and parasitological response (ACPR) and parasitemia on day 3 of treatment. Molecular surveillance of resistance-associated genes, including *pfk13*, *pfcrt*, and *pfmdr1*, was conducted on collected *P. falciparum* isolates.

Results: The iDES of AS plus DHA-PPQ was implemented in 2023, while CQ efficacy was monitored from 2021 to 2023. Late parasitological failure (LCF) for DHA-PPQ was detected in 1 of 26 *P. falciparum* cases in 2023, and in 1 of 26 and 1 of 90 *P. vivax* cases for CQ in 2022 and 2023, respectively. The corresponding ACPR rates were 96.2%, 100%, 96.2%, and 98.8%. The average positive parasitemia rate on day 3 post-treatment was 21.8%. Molecular polymorphism analysis revealed 9 nonsynonymous mutation haplotypes in the *pfk13* gene, while 97.7% of samples presented the wild-type genotype. For the chloroquine resistance-associated *pfcrt* gene, 2 mutant haplotypes, 'CVIET' and 'SVMNT', were detected with frequencies of 16.7% (70/418) and 0.5% (2/418), respectively, while the wild-type haplotype 'CVMNK' predominated at 82.8% (346/418). In the *pfmdr1* gene, 5 nonsynonymous point mutations and 8 haplotypes were identified. The Y184F mutation showed the highest prevalence at 40.5% (170/420). The 7 mutant haplotypes detected were V65L (0.2%, 1/420), N86Y (1.9%, 8/420), F408V (0.9%, 4/420), D1246Y (0.5%, 2/420), V65L/Y184F (0.2%, 1/420), N86Y/Y184F (0.7%, 3/420), and Y184F (39.8%, 167/420).

Conclusion: The antimalarial drug efficacy studies conducted for AS plus DHA-PPQ and CQ demonstrated that these treatments remain effective. However, the occurrence of LCF cases and persistent parasitemia on day 3 indicate decreasing sensitivity of these first-line drugs for treating *P. falciparum* and *P.*

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vivax, respectively. Therefore, continuous iDES and molecular surveillance of antimalarial drugs must be enhanced to provide early warning and guide appropriate responses to the spread of antimalarial drug resistance.

Malaria remains a significant global health challenge affecting 84 endemic countries, with an estimated 257 million infections and 597,000 deaths reported in 2023 (*1*). China achieved malaria-free certification from the World Health Organization (WHO) on June 30, 2021, following decades of elimination efforts (*2*). However, the increasing influx of *Plasmodium*-infected individuals from malaria-endemic regions, particularly sub-Saharan Africa and Southeast Asia, poses a substantial challenge to China's post-elimination phase (*3*–*4*). Systematic monitoring of antimalarial drug efficacy aims to establish a centralized drug resistance database for imported strains, providing actionable intelligence for source countries and aligning with the WHO's "High Burden to High Impact" strategy by informing region-specific antimalarial protocols (*5*).

Antimalarial drugs, especially artemisinin derivatives, are the most widely used treatments globally (*6*). Although China has reported no indigenous malaria cases since 2017, the challenges in preventing and controlling imported malaria have intensified, particularly with the emergence and spread of drug-resistant strains (*6*–*7*). While the treatment efficacy survey (TES) remains the gold standard for evaluating antimalarial drug efficacy, iDES are more appropriate in low-endemic or malaria elimination settings (*5*,*8*). This study employed an iDES protocol combined with analysis of drug resistance-associated molecular markers (*pfk13*, *pfcrt*, and *pfmdr1*) to monitor and evaluate the therapeutic efficacy of ACTs and CQ from 2021–2023, thereby providing baseline data to inform antimalarial drug policies.

The iDES and detection of drug resistance-associated molecular markers were performed according to previously described protocols (*8*–*9*). All experiments were conducted within the provincial malaria diagnostic laboratory network, with data uploaded to the National Information System for Parasitic Disease Prevention and Control. iDES was implemented to evaluate ACTs for *P. falciparum* treatment and CQ for *P. vivax* treatment in Yunnan Province. Patients with confirmed *P. falciparum* or *P. vivax* infection, excluding those with severe malaria, were followed from the first day of treatment (Day 0, D0) through D1-3, D7, D14, D21, D28, D35, and D42. Treatment outcomes were classified according to WHO guidelines for therapeutic efficacy monitoring as early treatment failure (ETF), LCF, late parasitological failure (LPF), or ACPR (*5*).

All malaria species were confirmed via PCR and microscopy. Molecular polymorphisms of *PfK13*, *Pfcrt*, and *Pfmdr1* genes were detected and sequenced from imported malaria cases from 2021–2023. The chi-square test was used to evaluate differences in the distribution of drug resistance-associated gene polymorphisms, and data analysis was performed using GraphPad Prism 8.0 software.

A total of 174 cases of *P. falciparum* and *P. vivax* malaria were monitored for clinical efficacy through the iDES protocol (Table 1). In 2023, 26 cases of *Pf* malaria treated with AS combined with DHA-PPQ were included in the iDES follow-up. Among these, 25 patients achieved ACPR, while one patient tested positive for parasitemia on day 35, which was classified as late treatment failure. Additionally, 8 patients (30.8%, 8/26) exhibited persistent parasitemia on day 3 of treatment.

For *Pv* malaria, the treatment regimen consisted of chloroquine and primaquine. From 2021 to 2023, a total of 148 *Pv* malaria cases were followed up. In 2021, all 32 patients achieved ACPR, with only 1 patient showing parasitemia on day 3. In 2022, 4 out of 26 patients were lost to follow-up, 1 patient was classified as having late treatment failure (LTF), and 6 patients (27.2%, 6/22) demonstrated persistent parasitemia on day 3. In 2023, among the 90 cases targeted for follow-up, 8 were lost to follow-up, 1 case was identified as LTF, and 23 cases (28.0%, 23/82) presented with parasitemia on day 3.

A total of 451 cases of *P. falciparum* malaria were subjected to molecular testing for drug-resistance markers (Table 2). Of these, 442 samples were successfully sequenced for the *pfk13* gene. 9 distinct point mutations were identified at the following loci: P441T, F446I, S459T, C469F, N499T, P574L, A578S, C580Y, and V692L (where the left side represents the wild-type allele and the right side represents the mutant allele). All the detected *pfk13* mutations were single-point mutations. A total of 418 samples were successfully genotyped for the CQ resistance-associated *pfcrt* gene, with a focus on mutations at codons 72–76. 3 genotypes were identified (Table 3): the wild-type CVMNK and the

TABLE 1. Treatment outcomes for DHA-PPQ and CQ by iDES.

Item	AS+DHA-PPQ	CQ			Total
	2023	2021	2022	2023	
ETF	0	0	0	0	0
LCF	1	0	1	1	3
LPF	1	0	1	1	3
LFU	0	0	4	8	12
ACPR (*n*, %)	25 (96.2)	32 (100.0)	21 (96.2)	81 (98.8)	NA
No. of Day 3(+) (*n*, %)	8 (30.8)	1 (3.1)	6 (27.2)	23 (28.0)	38 (21.8)
Total	26	32	26	90	174

Abbreviation: iDES=integrated drug efficacy studies; ETF=early treatment failure; LCF=late clinical failure; LPF=late parasitological failure; ACPR=adequate clinical and parasitological response; LFU=lost to follow-up; AS=Artesunate; DHA-PPQ=dihydroartemisinin–piperaquine; CQ=chloroquine; NA=not applicable.

TABLE 2. Polymorphisms of SNPs in genes associated with anti-malarial drug resistance, 2021–2023.

Gene	No. of detected samples	Loci	Wild type	Mutant type	Variant genotype	No. of mutant samples	Mutant proportion (%)
Pfk13	442	441	cca	aca	P441T	2	0.5
		446	ttt	att	F446I	1	0.2
		459	tcg	acg	S459T	1	0.2
		469	tgc	ttc	C469F	1	0.2
		499	aac	acc	N499T	1	0.2
		574	cct	ctt	P574L	1	0.2
		578	gct	tct	A578S	1	0.2
		580	tgt	tat	C580Y	1	0.2
		692	gtt	ctt	V692L	1	0.2
Pfcrt	418	72	tgt	agt	C72S	2	0.5
		74	atg	aat	M74I	70	16.7
		75	aat	gaa	N75E	70	16.7
		76	aaa	aca	K76T	72	17.2
pfmdr1	420	65	ctg	tct	V65L	2	0.5
		86	aat	tat	N86Y	11	2.6
		184	tat	ttt	Y184F	170	40.5
		408	ttt	gtt	F408V	4	1.0
		1,246	gat	tat	D1246Y	2	0.5

Abbreviation: SNPs=single nucleotide polymorphisms; No.=Number.

mutant types CVIET and SVMNT. The most frequent mutation was K76T, which was detected in 72 samples. The SVMNT haplotype was observed in 2 samples, both originating from Southeast Asia (one from Indonesia and one from Bangladesh). In total, 420 samples were analyzed for *pfmdr1* point mutations, revealing 5 mutation types and 7 mutant haplotypes. The most frequent mutation was Y184F, which was detected in 170 samples (40.5%), followed by N86Y (2.6%, 11/420). The 7 haplotypes included 2 novel combinations: l65f184 and $Y_{86}F_{148}$ (Table 3).

DISCUSSION

Regarding the clinical efficacy monitoring of AS plus DHA-PPQ, although this work was only conducted in 2023 due to policy requirements, the detection of parasitemia on day 3 (D3+) in 8 out of 26 cases clearly indicates prolonged parasite clearance time for ACTs, demonstrating decreased sensitivity to these drugs. Additionally, 1 patient tested positive for parasitemia by blood smear microscopy on day 35 (D35) posttreatment, which was classified as late treatment

TABLE 3. Source of importation and haplotypes distribution of *pfcrt* and *pfmdr1*.

Gene	Regions or countries (*n*, proportion%)	Haplotype	No. of genotypes	Proportion (%)
pfcrt (*n*=418)	Southeast Asia (*n*=5, 1.2)	$S_{72}V_{73}M_{74}N_{75}T_{76}$	2	0.5
	Eastern Africa (*n*=72, 17.2)	$C_{72}V_{73}I_{74}E_{75}T_{76}$	70	16.7
	Western Africa (*n*=148, 35.4)	$C_{72}V_{73}M_{74}N_{75}K_{76}$ (wild type)	346	82.8
	Central Africa (*n*=191, 45.7)			
	other regions (*n*=2, 0.4)			
pfmdr1 (*n*=420)	Southeast Asia (*n*=5, 1.2)	L_{65}	1	0.2
	Eastern Africa (*n*=72, 17.1)	$L_{65}F_{184}$	1	0.2
	Western Africa (*n*=148, 35.2)	Y_{86}	8	1.9
	Central Africa (*n*=193, 46.0)	$Y_{86}F_{184}$	3	0.7
		V_{408}	4	0.9
	Other regions (*n*=2, 0.4)	Y_{1246}	2	0.5
		F_{184}	167	39.8
		Wild type	234	55.7

Abbreviation: No.=Number.

failure. Similarly, in the follow-up of *P. vivax* cases, the first parasite clearance time also showed an increasing trend over time. When excluding patients lost to follow-up, late treatment failure was observed each year. These phenomena suggest decreasing sensitivity of ACTs and CQ as first-line treatments for *P. falciparum* and *P. vivax* malaria, respectively. These findings further underscore the urgency and importance of the World Health Organization's efforts to contain the spread of artemisinin resistance.

In the molecular detection of *pfk13*, this study identified K189T as a relatively dominant mutation site; however, it is not listed in Table 2 since it is not localized in the BTB/POZ region of the Kelch protein, the function of which remains unclear (*10*–*11*). This mutation was predominantly distributed in West African countries (such as Côte d'Ivoire, Nigeria, the Democratic Republic of the Congo, and Cameroon), with 1 case each detected in North Africa and East Africa (from Zambia and Algeria, respectively). Other newly detected mutation sites including S459T, N499T, A578S, and V692L were reported for the first time (*10*). However, it remains unclear whether these novel mutations will become fixed over time. For the *pfcrt* gene, the K76T point mutation is highly associated with CQ resistance. Nevertheless, the proportion of the wild-type genotype exceeded 82%, and compared with the variation in *pfmdr1*, the difference between *pfcrt* and *pfmdr1* was statistically significant by Chi-square test (P<0.01), supporting the observation that sensitivity to CQ is gradually recovering following the withdrawal of CQ (*12*–*13*). Furthermore, detection of *pfmdr1* gene mutations revealed that Y184F was the most frequently mutated site, indicating its potential association with multidrug resistance, particularly in the context of resistance to ACTs and their partner drugs.

In conclusion, this study employed two approaches — iDES and drug resistance-associated molecular detection — to evaluate the efficacy of antimalarial drugs. As the current gold standard for clinical drug efficacy evaluation, iDES requires sustained and substantial investments in human and material resources. Based on the initial results presented in this study, future efforts must leverage the comprehensive parasitic disease prevention and management information system to achieve two critical objectives: 1) expanding the clinical follow-up sample size and 2) ensuring nationwide coverage of imported malaria cases. These steps will enable more objective and timely assessments of antimalarial drug efficacy in real-time settings, thereby mitigating the risk of further drug resistance dissemination.

Acknowledgments: The staff involved in field investigations and to provincial CDC personnel participating in the national antimalarial drug resistance network. We also thank all malaria patients who were recruited for the iDES.

Conflicts of interest: No conflicts of interest.

Funding: Supported by Young Scholar Science Foundation of China CDC (grant number 2024A105); Three-Year Initiative Plan for Strengthening Public Health System Construction in Shanghai (2023-2025) Key Discipline Project [grant

number GWVI-11.1-12 to JC].

doi: 10.46234/ccdcw2025.149

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Submitted: April 27, 2025
Accepted: June 16, 2025
Issued: June 27, 2025

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