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Ex vivo drug susceptibility and resistance mediating genetic polymorphisms of *Plasmodium falciparum* in Bobo-Dioulasso, Burkina Faso

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ABSTRACT Malaria remains a leading cause of morbidity and mortality in Burkina Faso, which utilizes artemether–lumefantrine as the principal therapy to treat uncomplicated malaria and seasonal malaria chemoprevention with monthly sulfadoxine–pyrimethamine plus amodiaquine in children during the transmission season. Monitoring the activities of available antimalarial drugs is a high priority. We assessed the *ex vivo* susceptibility of *Plasmodium falciparum* to 11 drugs in isolates from patients presenting with uncomplicated malaria in Bobo-Dioulasso in 2021 and 2022. IC_{50} values were derived using a standard 72 h growth inhibition assay. Parasite DNA was sequenced to characterize known drug resistance-mediating polymorphisms. Isolates were generally susceptible, with IC_{50} values in the low-nM range, to chloroquine (median IC_{50}10 nM, IQR 7.9–24), monodesethylamodiaquine (22, 14–46) piperaquine (6.1, 3.6–9.2), pyronaridine (3.0, 1.3–5.5), quinine (50, 30–75), mefloquine (7.1, 3.7–10), lumefantrine (7.1, 4.5–12), dihydroartemisinin (3.7, 2.2–5.5), and atovaquone (0.2, 0.1–0.3) and mostly resistant to cycloguanil (850, 543–1,290) and pyrimethamine (33,200, 18,400–54,200), although a small number of outliers were seen. Considering genetic markers of resistance to aminoquinolines, most samples had wild-type PfCRT K76T (87%) and PfMDR1 N86Y (95%) sequences. For markers of resistance to antifolates, established PfDHFR and PfDHPS mutations were highly prevalent, the PfDHPS A613S mutation was seen in 19% of samples, and key markers of high-level resistance (PfDHFR I164L; PfDHPS K540E) were absent or rare (A581G). Mutations in the PfK13 propeller domain known to mediate artemisinin partial resistance were not detected. Overall, our results suggest excellent susceptibilities to drugs now used to treat malaria and moderate, but stable, resistance to antifolates used to prevent malaria.

KEYWORDS *Plasmodium falciparum*, susceptibility, antimalarial drugs, *ex vivo*, Burkina Faso

Malaria, primarily due to *Plasmodium falciparum*, remains a leading cause of morbidity and mortality in Burkina Faso, with about 37% of clinic consultations and 15% of deaths reported as due to malaria in 2021 (1). Since 2005, artemisinin-based combination therapies (ACTs), initially artemether–lumefantrine (AL) and artesunate–amodiaquine (AS-AQ), and more recently including dihydroartemisinin–piperaquine (DP) and artesunate–pyronaridine, have been approved to treat uncomplicated malaria (2–4). Following World Health Organization (WHO) recommendations for the Sahel sub-region (5), seasonal malaria chemoprevention (SMC) with sulfadoxine–pyrimethamine–amodiaquine (SP-AQ) during the malaria transmission season has been widely utilized in children under 5 years of age (6). Most studies have

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reported excellent efficacy of ACTs in treating uncomplicated malaria in Burkina Faso (7–10), but one recent trial reported suboptimal efficacies of AL and DP (11), although these results may have been influenced by methodological factors (12). Since the implementation of SMC in 2014, studies have demonstrated excellent efficacy in the prevention of uncomplicated malaria, hospital admissions, and deaths in Burkina Faso (13–16).

The emergence and spread of *P. falciparum* resistant to artemisinin, ACT partner drugs, and components of SMC is of great concern, although the prevalence of highly resistant parasites has generally been lower in West Africa than in eastern and southern Africa (17). Mutations in PfK13 that have mediated artemisinin partial resistance, manifesting as delayed parasite clearance and abnormal ring survival assays in Southeast Asia (18) and, more recently, in East Africa (19–24), have not been reported in Burkina Faso (11, 25, 26). Resistance to amodiaquine, associated with the PfCRT K76T and PfMDR1 N86Y mutations, has been uncommon in Burkina Faso in recent years (27–29), and resistance to lumefantrine or piperaquine, the latter associated with novel PfCRT mutations and duplication of *plasmepsin* genes in Southeast Asia, has not been described (27). Regarding resistance to the SP component of SMC in Burkina Faso, some resistance-mediating mutations in the target proteins dihydrofolate reductase (PfDHFR) and dihydropteroate synthase (PfDHPS) have been common, but mutations mediating high-level resistance, notably PfDHFR I164L and PfDHPS K540E and A581G, have been detected rarely (30).

To characterize current resistance trends, we measured *ex vivo* susceptibilities to important antimalarials, characterized genetic polymorphisms relevant to drug resistance, and assessed associations between these parasitological and genetic measures of drug resistance in fresh clinical isolates collected in Bobo-Dioulasso in 2021 and 2022.

RESULTS

Study samples

We collected 118 *P. falciparum* isolates in 2021 and 100 in 2022 in Bobo-Dioulasso, located in the Houet province of the southwest region of the country and the second largest city in Burkina Faso. The median age of subjects was 13 years, and 59% of the subjects were male (Table 1). The mean parasitemia of samples was 3.1%.

Ex vivo drug susceptibility

Of the 218 isolates, 158 (72.4%) successfully grew in culture and yielded drug susceptibility data. We measured *ex vivo* susceptibilities to 11 antimalarial drugs (Fig. 1; Table 2). Analyses of Dd2 and 3D7 reference strains yielded IC_{50} values consistent with those reported previously (31) (Table 3). Except for the antifolates pyrimethamine and cycloguanil, median isolate IC_{50} values were in the low-nanomolar range and similar to those for the drug-susceptible control strain 3D7, indicating potent activity (Fig. 1; Tables 2 and 3). For chloroquine, eight of 157 tested isolates (5.1%) had IC_{50} values at or above 100 nM, a value generally considered the threshold for chloroquine resistance (32). Considering piperaquine, no isolate showed the biphasic dose–response curves associated with piperaquine resistance in southeast Asia (33). Results differed for the PfDHFR inhibitors pyrimethamine and cycloguanil, with IC_{50} values consistent with widespread antifolate resistance and known genotypes of parasites circulating in Africa.

TABLE 1 Baseline characteristics of patients and samples

Year	Number of samples	Sites		Gender N (%)		Age, median years (min–max)	Parasitemia (mean ± SD)
		Hamdalay	Sakaby	M	F		
2021	118	97	15	62 (59)	44 (42)	12 (3–60)	3.1 ± 3.2
2022	100	52	48	59 (60)	40 (40)	13 (5–37)	2.9 ± 4.1
Total	218	149	63	121 (59)	84 (41)	13 (3–60)	3.1 ± 3.7

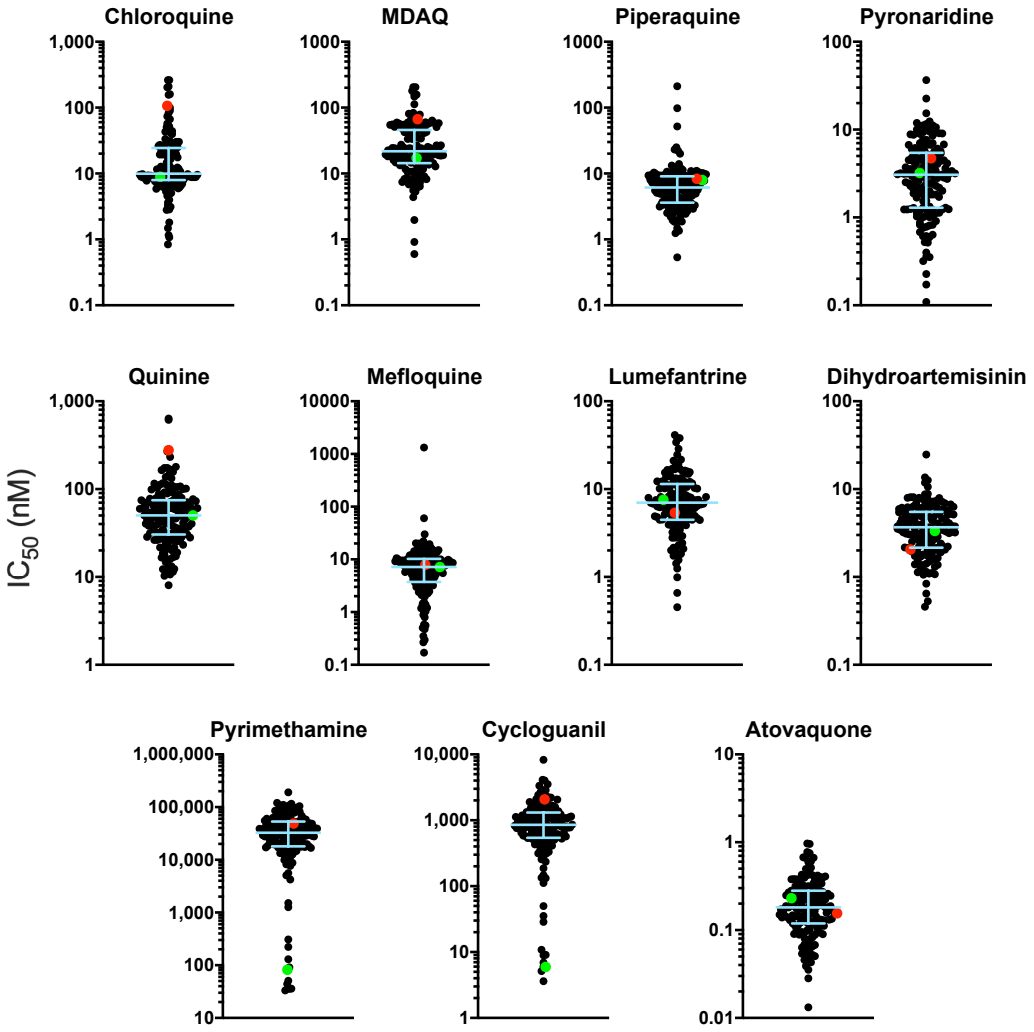

FIG 1 *Ex vivo* drug susceptibilities. Each black point represents the result for a single isolate. Points for the 3D7 (green) and Dd2 (red) control strains represent means. Bars and whiskers represent median IC_{50} values and IQRs respectively.

We tested for pairwise correlations between *ex vivo* drug susceptibilities (Fig. 2). Correlations were seen between results for aminoquinolines; the strongest correlation was between pyronaridine, a compound structurally related to the quinolines, and monodesethylamodiaquine (MDAQ), the principle active metabolite of amodiaquine (Spearman's rank-order coefficient 0.52; $P < 0.001$). Modest correlations were observed between results for lumefantrine and mefloquine (0.42), quinine and its analog mefloquine (0.38), and the antifolates cycloguanil and pyrimethamine (0.41). All other pairwise associations were relatively weak.

Resistance-mediating genetic polymorphisms

We sequenced genes associated with altered drug susceptibility from 200 isolates (Fig. 3); the number of successfully sequenced samples for each allele is shown in Table S1. Among genetic markers of resistance to aminoquinolines, most samples had wild-type PfCRT K76T and PfMDR1 N86Y sequences. Other known PfCRT/PfMDR1 resistance mutations were not seen. The PfMDR1 Y184F mutation was common, as seen previously, but this mutation appears to be associated with parasite fitness, not drug resistance (34). For markers of resistance to antifolates, previously common PfDHFR (N51I, C59R, and

TABLE 2 *Ex vivo* drug susceptibilities (nM) of *P. falciparum* isolates

Drug	N	Median IC_{50} (nM)	IQR	Min–Max
Chloroquine	157	10	7.9–24	0.8–263
MDAQ	156	22	14–46	0.6–206
Piperaquine	157	6.1	3.6–9.2	0.5–212
Pyronaridine	156	3.0	1.3–5.5	0.1–37
Quinine	160	50	30–75	8.1–638
Mefloquine	154	7.1	3.7–10	0.2–60
Lumefantrine	146	7.1	4.5–12	0.5–41
Dihydroartemisinin	158	3.7	2.2–5.5	0.5–25
Pyrimethamine	158	33,200	18,400–54,200	33–190,000
Cycloguanil	157	850	543–1,290	3.6–8,270
Atovaquone	158	0.2	0.1–0.3	0.01–1.0

S108N), and PfDHPS (S436A and A437G) mutations were highly prevalent (Fig. 3; Tables 4 and S1). The PfDHPS I431V and A613S mutations, which have been seen previously in West Africa (29), were found at low prevalence, and key markers of high-level resistance (PfDHFR I164L; PfDHPS K540E) were absent or rare (PfDHPS A581G) (Fig. 3; Tables 5 and S1). Considering polymorphisms in PfK13, the principal marker of artemisinin partial resistance, only three propeller domain mutations were identified, each in one isolate (C532S, V534L, and E606D). None of these PfK13 polymorphisms are validated or candidate markers of artemisinin partial resistance (35), and all have been reported as rare mutations elsewhere in West and Central Africa (17, 36).

Associations between *ex vivo* susceptibilities and genetic polymorphisms

We tested for associations between *ex vivo* drug susceptibilities and key genetic polymorphisms associated with drug resistance (Fig. 4). The PfCRT K76T mutation was significantly associated with decreased susceptibility to the aminoquinolines chloroquine and MDAQ and increased susceptibility to piperaquine, mefloquine, and lumefantrine (Fig. 4A). An isolate containing the K76T mutation had the highest IC_{50} value for MDAQ (206 nM) and also had a high IC_{50} for chloroquine (156 nM). Despite relatively few mutant genotypes for comparison, the PfMDR1 N86Y mutation was significantly associated with increased susceptibility to lumefantrine and mefloquine (Fig. 4B). Isolates containing the common PfMDR1 Y184F mutation had significantly increased susceptibility to quinine (Fig. 4B). Amplification of genes associated with decreased susceptibility to mefloquine (*pfmdr1*) and piperaquine (*plasmepsin II–III*) in southeast Asia was not detected in 175 out of 176 isolates successfully analyzed; one isolate had a depth-of-coverage suggestive of two *plasmepsin II* copies. Responses to the antifolates

TABLE 3 Mean IC_{50} values (nM) for *P. falciparum* laboratory control strains

	3D7		Dd2	
Drug	N	IC_{50} ±SD (nM)	N	IC_{50} ±SD (nM)
Chloroquine	4	8.9 ± 1.4	4	107 ± 44
MDAQ	5	17 ± 8.1	5	67 ± 42
Piperaquine	5	8.0 ± 7.5	5	8.3 ± 6.0
Pyronaridine	5	3.2 ± 2.8	5	4.7 ± 2.0
Quinine	5	50 ± 30	5	279 ± 108
Mefloquine	4	7.2 ± 4.6	5	7.9 ± 4.8
Lumefantrine	3	7.6 ± 3.6	5	5.4 ± 5.5
Dihydroartemisinin	5	3.3 ± 2.0	5	2.1 ± 1.3
Pyrimethamine	5	82 ± 78	5	48,800 ± 19,000
Cycloguanil	4	5.9 ± 2.3	5	2,090 ± 670
Atovaquone	5	0.2 ± 0.2	5	0.2 ± 0.1

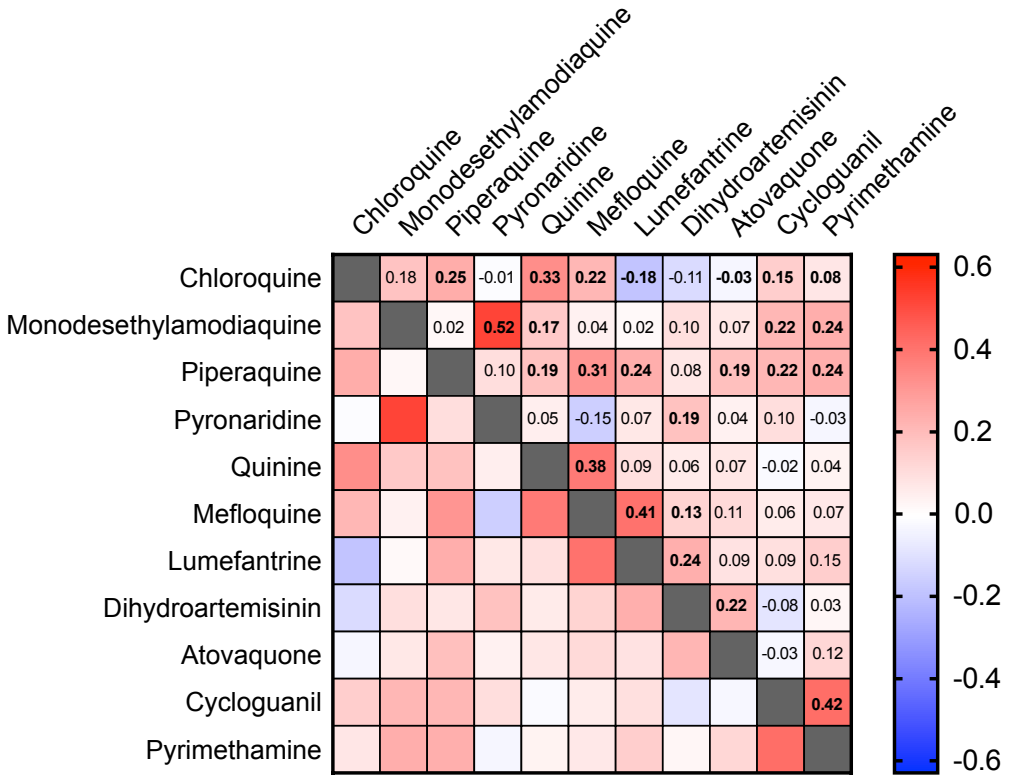

FIG 2 Correlations of *ex vivo* susceptibilities between drugs. Magnitude and direction of Spearman associations between IC_{50} values are indicated by the colors and values, respectively. Numbers in bold indicate statistically significant associations ($P < 0.05$).

pyrimethamine and cycloguanil were strongly associated with PFDHR haplotypes, with the nine fully wild-type isolates highly sensitive to both drugs (Fig. 4C). Consistent with results of earlier research from Uganda (37), PfDHFR double-mutant C59R/S108N isolates were more susceptible to both drugs than N51I/C59R/S108N triple-mutant isolates (Table 4).

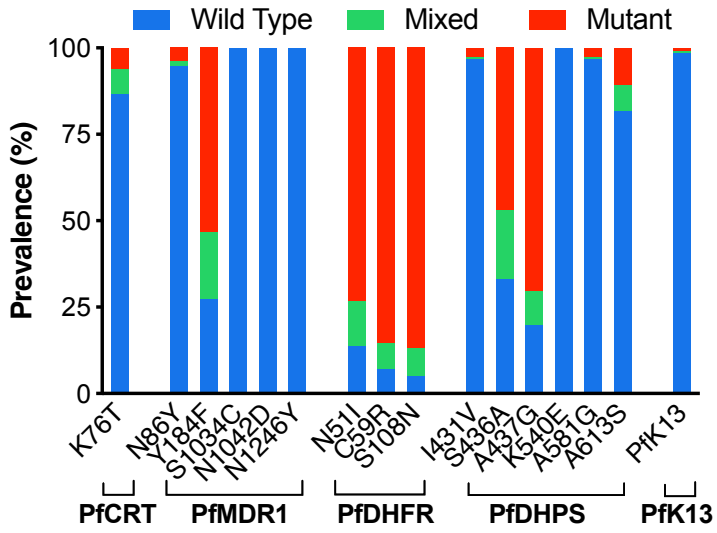

FIG 3 Prevalence of key drug resistance-associated polymorphisms. PfK13 data are for the propeller domain of the protein.

TABLE 4 PfDHFR haplotypes in Burkina Faso isolates and antifolate susceptibilities

PfDHFR haplotype[a]	N (%)	Median IC_{50} nM (N)[c]	
		Cycloguanil	Pyrimethamine
N51-C59-S108[b]	9 (5)	7.9 (6)	51 (7)
51I-C59-108N	3 (2)	316 (3)	20,910 (3)
N51-59R-108N	16 (9)	558 (15)	21,700 (15)
51I-59R-108N	148 (84)	935 (112)	36,840 (112)

[a]Isolates with mixed and mutant haplotypes were combined.
[b]Wild-type haplotype.
[c]IC_{50} data were not available for all isolates that were genotyped.

DISCUSSION

Prompt therapy of malaria with ACTs and regular administration of SP plus amodiaquine as SMC are key components of malaria control in Burkina Faso. Considering artemisinin, genomic studies have identified multiple PfK13 polymorphisms that are validated or candidate resistance markers in eastern Africa, with foci in Rwanda (19, 38), Uganda (20–22), and Ethiopia/Eritrea (23, 24). These markers have been associated with clinical and laboratory evidence of artemisinin partial resistance, but not with loss of ACT treatment efficacy (19, 20, 22, 23, 38). Considering antifolates, multiple mutations associated with a moderate level of resistance to the components of SP have been common across Africa; one key mutation (PfDHPS K540E) is common in eastern, but not West Africa; and additional mutations associated with high level resistance (PfDHFR 1I64L; PfDHPS A581G) have shown increasing prevalence in some regions in East Africa. Our new results from Burkina Faso are consistent with these findings, with potent *ex vivo* activity of all tested drugs, except the PfDHFR inhibitors pyrimethamine and cycloguanil and prevalence of resistance markers consistent with other recent findings from West Africa (27, 29, 39, 40). These results offer confidence that for now, current regimens will continue to offer good treatment and preventive efficacy in Burkina Faso.

For malaria treatment, a number of recent studies have shown sub-optimal efficacies for ACTs, in particular artemether–lumefantrine. Importantly, studies in Burkina Faso (11), Angola (41), DRC (42), and Uganda (43) showed PCR-corrected AL treatment efficacies < 90% at some study sites. Most relevant to this study, treatment efficacies in children were 74% and 76% for artemether–lumefantrine and 89% and 84% for dihydroartemisinin–piperaquine in Nanoro and Gourcy in Burkina Faso, respectively (in the north of country, a different region than in the current study) in 2017–18 (11). Importantly, all of these reports of inadequate treatment efficacies were in regions without noteworthy prevalence of artemisinin resistance mediating PfK13 mutations. The low treatment efficacies may have been due to inadequate compliance with treatment regimens, especially for twice-daily dosing of artemether–lumefantrine; methodological issues leading to spurious results (12, 44); analytical details affecting outcome assignments (45); or resistance determinants independent of PfK13 mutations. Importantly, there has been inconsistency in molecular methods to correct treatment outcomes, potentially explaining some reports of relatively low efficacies (46). Overall, despite encouraging

TABLE 5 Prevalence of PfDHPS haplotypes in Burkina Faso isolates

PfDHPS haplotype (*N* = 148)	
Amino acids I431V-S436A-A437G-K540E-A581G-A613S	**N (%)**
ISAKAA (wild type)	3 (2)
ISGKAA	57 (39)
IYGKAS	1 (0.7)
IAGKAA	49 (33)
IAGKAS	16 (11)
IAAKAA	15 (10)
IAAKAS	2 (1.4)
VAGKGS	5 (3.4)

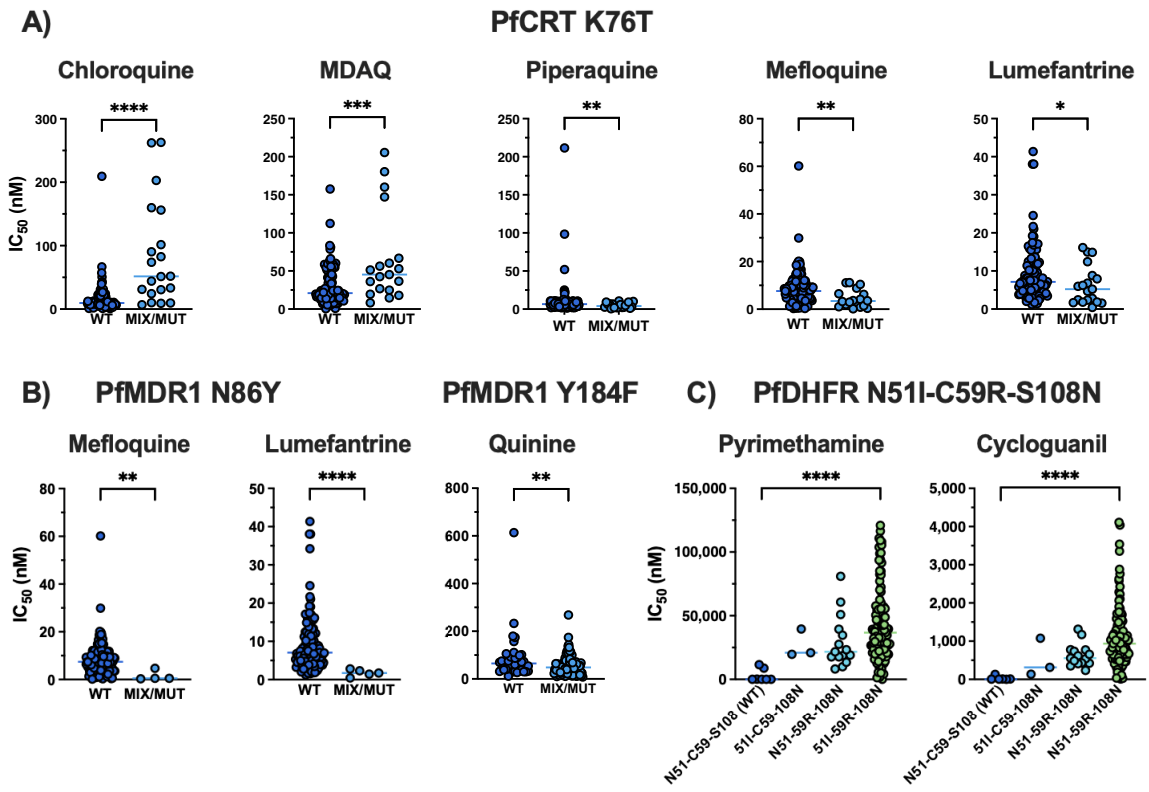

FIG 4 Associations between genetic polymorphisms in PfCRT, PfMDR1, and PfDHFR and *ex vivo* susceptibilities to the indicated drugs. Each point represents the result from a single isolate. WT = wild type; MIX/MUT = mixed or mutant genotype. Differences between IC_{50} values in (A) and (B) were assessed using the Mann–Whitney U test. Differences between IC_{50} values in (C) were assessed using the Kruskal–Wallis test with Dunn's post-hoc correction (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

ex vivo and genomic results from our study, reports from Africa of both unacceptably low treatment efficacies for ACTs in regions without PfK13 mutations and of increasing prevalence of resistance mediating PfK13 mutations are of great concern.

Regarding malaria chemoprevention, multiple mutations associated with moderate resistance to both components of SP have circulated in West Africa, including Burkina Faso, for many years (30, 47, 48). Despite the prevalence of these mutations, SMC has demonstrated excellent preventive efficacy in multiple countries, including Burkina Faso (16, 49–52). The strong efficacy of SMC with SP-AQ may have been facilitated by increasing susceptibility of parasites to AQ, with loss of resistance genotypes in Burkina Faso despite inclusion of artesunate–AQ in national treatment guidelines until recently. Remarkably, despite the heavy selective pressure of widespread coverage of SMC, we did not see selection of the PfDHFR I164L and PfDHPS K540E and A581G mutations (47, 48), all of which mediate a higher level of resistance to SP. These results are consistent with those from a broad survey of antifolate mutations in West Africa conducted in 2015–18 (30).

This study had some limitations. *Ex vivo* drug susceptibility assays are inherently imprecise, and IC_{50} results may be affected by varied growth in culture and the inability to repeat assays to improve precision. Additionally, to improve reliability, we limited our study to isolates with high parasitemias (≥ 1%); these results might not reflect those for low-density infections. Finally, in high malaria transmission settings, *P. falciparum* infections are typically polyclonal, and our *ex vivo* susceptibility values often represent an average of results for clones competing in culture.

In summary, our results suggest a stable situation regarding antimalarial drug resistance in Burkina Faso. However, reasons for concern include growing prevalence

of mutations mediating artemisinin partial resistance and increasing antifolate resistance in other parts of Africa, reports of decreased ACT treatment efficacy in Burkina Faso and elsewhere, and the likelihood that continued use of ACTs to treat malaria and SP-AQ for SMC may eventually lead to decreased susceptibility of circulating malaria parasites to important antimalarials. Thus, continued surveillance for *ex vivo* and genomic markers of decreased *P. falciparum* susceptibility to current antimalarial regimens is a high priority.

MATERIALS AND METHODS

Sample collection

P. falciparum isolates were collected from patients aged 6 months or older presenting at the health centers of Hamdalaye and Sakaby, both in Bobo-Dioulasso, during the malaria transmission seasons in 2021 and 2022. Patients presenting with fever (axillary temperature ≥37.5°C) or history of fever in the last 24 h were screened for *P. falciparum* malaria using a rapid diagnostic test (RDT; SD Bioline); those with positive RDT results had a Giemsa-stained thin blood smear evaluated to confirm the diagnosis before enrollment. The study protocol and all related documents were approved by the Comité d'Ethique Institutionnel pour la Recherche en Santé of the IRSS.

Subjects with *P. falciparum* mono-infection based on a thin blood smear with ≥1% parasitemia and providing informed consent were enrolled, and 3–5 mL of venous blood was collected in EDTA vacutainer tubes. Informed consent was provided by parents or guardians for children under 10 years of age; assents were also provided for children 10–16 years of age. Patients reporting use of antimalarial drugs in the previous 30 days were excluded to avoid the possibility of collecting parasites under drug selection. Parasitemia was determined from thin smears by counting 1,000 or more erythrocytes. Four blood spots were placed on Whatman FTA cards (Cytivia) for subsequent molecular analysis. Samples were transported within 18 h of collection to the laboratory of the Institut de Recherche en Sciences de la Santé (IRSS), Direction Régionale de l'Ouest in Bobo-Dioulasso.

Sample processing for *ex vivo* assays

Samples were usually analyzed the same day as collection; samples collected late in the day were stored at 4°C overnight and assayed the following day. We previously demonstrated that overnight refrigeration of freshly collected isolates had a minimal impact on IC_{50} values (53). Blood was centrifuged at 2,000 rpm for 10 min at room temperature, plasma and buffy coat were removed, and the erythrocyte pellet was washed three times with RPMI 1640 media (Thermo Fisher Scientific). The pellet was resuspended in complete medium consisting of RPMI 1640 with 25 mM HEPES, 24 mM NaHCO3, 0.1 mM hypoxanthine, 10 µg/mL gentamicin, and 0.5% AlbuMAX II (Thermo Fisher Scientific) to produce a hematocrit of 50%.

Ex vivo growth inhibition assays

Drug susceptibilities were measured using a 72 h microplate growth inhibition assay with SYBR green detection, as previously described (30). Study compounds (chloroquine, monodesethylamodiaquine, piperaquine, pyronaridine, quinine, mefloquine, lumefantrine, dihydroartemisinin, pyrimethamine, cycloguanil, and atovaquone), supplied by Medicines for Malaria Venture, were dissolved in dimethyl sulfoxide (distilled water for chloroquine) as 10-mM stocks (50 mM for pyrimethamine) and stored at −20°C. Drugs were serially diluted 3-fold in complete medium in 96-well microplates (50 µL per well), including drug-free and parasite-free control wells, with concentration ranges optimized to capture full dose–response curves (chloroquine and quinine, 10,000–0.5 nM; mefloquine and piperaquine, 5,000–0.25 nM; monodesethylamodiaquine, lumefantrine, and dihydroartemisinin 1,000–0.05 nM; pyronaridine, 500–0.025 nM; pyrimethamine, 500,000–25 nM; cycloguanil 50,000–2.5 nM; atovaquone 100–0.005 nM). Cultures were

adjusted to 0.2% parasitemia and 2% hematocrit using uninfected O^+ erythrocytes, obtained from a local blood bank to a total volume of 200 µL per well. Plates were maintained at 5% CO_2, 5% O_2, and 90% N_2 for 72 h at 37°C in a humidified modular incubator (Billups Rothenberg). After 72 h, plates were stored at −20°C until reading. After thawing the plates, wells were resuspended, and 100 µL culture per well was transferred to black 96-well plates containing 100 µL per well of SYBR Green lysis buffer (20 mM Tris, 5 mM EDTA, 0.008 % saponin, 0.08 % Triton X-100, and 0.2 µL/ml SYBR Green I [Invitrogen]), and mixed. Plates were incubated for 1 h in the dark at room temperature, and fluorescence was measured with a FLUOstar Omega plate reader (BMG LabTech; 485 nm excitation and 530 nm emission). Laboratory control *P. falciparum* Dd2 (MRA-156) and 3D7 (MRA-102) strains (BEI Resources) were assayed regularly during the study period. IC_{50} values were derived by plotting the fluorescence intensity against log drug concentration and fit to a non-linear curve using a four-parameter Hill equation in Prism (GraphPad Software, version 10.1).

Genomic analyses

DNA was extracted from filter paper blood spots using Chelex 100 (Bio-Rad) as previously described (31). All samples were characterized by molecular inversion probe (MIP) capture and next-generation sequencing (22). The probes spanned the *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps*, and *pfK13* genes; the copy number of the *plasmepsin II/III* and *pfmdr1* genes was assessed from isolates based on the depth of coverage. MIP capture, library preparation, and sequencing details were as previously described (54).

Statistical analysis

To quantify associations between *ex vivo* drug susceptibilities, we calculated correlations between median IC_{50} values using Spearman's rank-order correlation coefficient to account for non-parametric distributions of IC_{50} values in GraphPad Prism 10.1. Associations between transporter or enzyme polymorphisms and *ex vivo* susceptibilities were determined by comparing IC_{50} values using a Mann–Whitney U test or Kruskal–Wallis test with Dunn's post-hoc test in Prism. Statistical tests were two-tailed, and results were considered significant at $P < 0.05$.

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A.F.S., J.B.O., R.A.C., and P.J.R. designed the study. A.F.S., K.Z., F.A., and R.S.Y. monitored field and laboratory activities. A.F.S., K.Z., F.A., R.S.Y., B.T., N.E.C., J.T.K., and M.S. performed field work and laboratory analysis of samples. R.A.C. verified and analyzed data and performed statistical analysis of the results. M.D.C., A.F.S., J.L., S.G., and J.A.B. performed and analyzed genotyping data. A.F.S. wrote the first draft of the manuscript. J.B.O., P.J.R., and R.A.C. revised the manuscript. All authors read and approved the final version of the manuscript.

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A. Fabrice Somé, Conceptualization, Project administration, Supervision, Writing – original draft | Melissa D. Conrad, Data curation, Formal analysis, Methodology, Software | Zachari Kabré, Investigation | R. Serge Yerbanga, Conceptualization, Investigation, Methodology, Project administration, Supervision | Thomas Bazié, Data curation, Investigation | Catherine Neya, Investigation | Jenny Legac, Data curation, Investigation | Shreeya Garg, Data curation, Investigation | Jeffrey A. Bailey, Methodology, Resources, Software | Jean-Bosco Ouédraogo, Project administration, Supervision | Philip J. Rosenthal, Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review and editing | Roland A. Cooper, Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review and editing.

DATA AVAILABILITY

Raw sequencing reads for target genes are available in the NCBI Sequence Read Archive under BioProject accession number PRJNA1067191. MIP primers used in this study are described in previous publications (19, 20, 30). MIPWrangler and MIPTools software are available on GitHub. Additional data are available from the authors upon request.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Table S1 (AAC01534-23-S0001.docx). Prevalence of mutations in key drug resistance genes.

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