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RESEARCH

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Artesunate–amodiaquine and artemether–lumefantrine for the treatment of uncomplicated falciparum malaria in Liberia: in vivo efficacy and frequency of molecular markers

Victor S. Koko^{1*} , Marian Warsame², Benjamin Vonhm³, Moses K. Jeuronlon⁴, Didier Menard^{5,6,7}, Laurence Ma⁸, Fahn Taweh³, Lekilay Tehmeh⁹, Paye Nyansaiye¹, Oliver J. Pratt¹, Sei Parwon¹⁰, Patrick Kamara¹¹, Magnus Asinya¹², Aaron Kollie¹³ and Pascal Ringwald¹⁴

Abstract

Background: Artesunate–amodiaquine (ASAQ) and Artemether–lumefantrine (AL) are the recommended treatment for uncomplicated *Plasmodium falciparum* malaria in Liberia. Intermittent preventive treatment with sulfadoxine/pyrimethamine is also recommended for pregnant women. The therapeutic efficacy of Artesunate–amodiaquine and Artemether–lumefantrine, and the frequency of molecular markers associated with anti-malarial drug resistance were investigated.

Methods: The therapeutic efficacy of ASAQ and AL was evaluated using the standard World Health Organization protocol (WHO. Methods for Surveillance of Antimalarial Drug Efficacy. Geneva: World Health Organization; 2009. <https://www.who.int/malaria/publications/atoz/9789241597531/en/>). Eligible children were recruited and monitored clinically and parasitologically for 28 days. Polymorphisms in the *Pfkelch 13*, chloroquine resistance transporter (*Pfcr1*), multidrug resistance 1 (*Pfmdr-1*), dihydrofolate reductase (*Pfdhfr*), and dihydropteroate synthase (*Pfdhps*) genes and copy number variations in the plasmepsin-2 (*Pfpm2*) gene were assessed in pretreatment samples.

Results: Of the 359 children enrolled, 180 were treated with ASAQ (89 in Saclepea and 91 in Bensonville) and 179 with AL (90 in Sinje and 89 in Kakata). Of the recruited children, 332 (92.5%) reached study endpoints. PCR-corrected per-protocol analysis showed ACPR of 90.2% (95% CI: 78.6–96.7%) in Bensonville and 92.7% (95% CI: 83.4–96.5%) in Saclepea for ASAQ, while ACPR of 100% was observed in Kakata and Sinje for AL. In both treatment groups, only two patients had parasites on day 3. No artemisinin resistance associated *Pfkelch13* mutations or multiple copies of *Pfpm2* were found. Most samples tested had the *Pfcr1* 76 T mutation (80/91, 87.9%), while the *Pfmdr-1* 86Y (40/91, 44%) and 184F (47/91, 51.6%) mutations were less frequent. The *Pfdhfr* triple mutant (51I/59R/108 N) was the predominant allele (49.2%). For the *Pfdhps* gene, it was the 540E mutant (16.0%), and the 436A mutant (14.3%). The quintuple allele (51I/59R/108 N-437G/540E) was detected in only one isolate (1/357).

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Conclusion: This study reports a decline in the efficacy of ASAQ treatment, while AL remained highly effective, supporting the recent decision by NMCP to replace ASAQ with AL as first-line treatment for uncomplicated falciparum malaria. No association between the presence of the mutations in *Pfcr1* and *Pfmdr-1* and the risk of parasite recrudescence in patients treated with ASAQ was observed. Parasites with signatures known to be associated with artemisinin and piperazine resistance were not detected. The very low frequency of the quintuple *Pfdhfr/Pfdhps* mutant haplotype supports the continued use of SP for IPTp. Monitoring of efficacy and resistance markers of routinely used anti-malarials is necessary to inform malaria treatment policy.

Trial registration ACTRN12617001064392.

Keywords: Artesunate–amodiaquine, Artemether–lumefantrine, *Plasmodium falciparum*, Efficacy, Molecular markers of antimalarial drug resistance, Liberia

Background

Malaria is a major health problem, with an estimated 241 million cases and 627,000 deaths worldwide, increasing from 227 million and 558,000 cases and deaths, respectively, in 2020 [1]. Countries in the World Health Organization (WHO) African Region accounted for 95% of the global malaria burden. Effective malaria treatment with artemisinin-based combination therapy is a critical component of recommended malaria interventions [2]. These anti-malarials combine potent and fast-acting artemisinin derivatives with long half-life partner drug, and include Artemether–lumefantrine (AL), Artesunate–amodiaquine (ASAQ), artesunate–mefloquine (ASMQ), dihydroartemisinin–piperazine (DP), artesunate–sulfadoxine/pyrimethamine (ASSP) and artesunate–pyronaridine (ASPY) for the treatment of uncomplicated falciparum malaria. Recent studies have shown that ASAQ and AL, the most used artemisinin-based combinations, remain highly effective in Africa, achieving cure rates of >90%, the recommended threshold for treatment policy change [3]. However, unacceptably high rates of treatment failure with ASSP in Somalia [4] and India [5] and DP in several Southeast Asian countries [6–8] have led to the abandonment of both first-line treatments in these countries. These findings are a reminder that anti-malarial drug resistance remains a serious threat to effective case management. Therefore, it is critical for malaria endemic countries to regularly monitor the efficacy of recommended artemisinin-based combinations to inform national treatment policy. Therapeutic efficacy study (TES), prospective evaluations of clinical and parasitological responses to treatment of uncomplicated malaria is the gold standard for generating evidence for national treatment guidelines [3].

In addition to TES, molecular markers (*i.e.*, genetic changes in *Plasmodium falciparum* genome), associated with anti-malarial drug resistance are complementary tools. Partial artemisinin resistance, defined as delayed parasite clearance following artesunate monotherapy or ACT [3], has emerged and spread in Southeast Asia

[9]. Non-synonymous mutations in the propeller region of the *P. falciparum kelch13* (*Pfkelch13*) gene have been demonstrated to be a major determinant associated with artemisinin resistance [10, 11]. Since 2014, more than 260 non-synonymous *Pfkelch13* mutations have been detected worldwide, but only 21 have been validated (F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L, C580Y) or are suspected to be associated (P441L, G449A, C469F/Y, A481V, R515K, P527H, N537I/D, G538V, R622I, V568G, A675V) with partial artemisinin resistance [3]. The presence of *Pfkelch13* mutations known to be associated with artemisinin resistance in Africa has historically been rare and sporadic [3]. However, results of recent studies suggest the emergence and spread of indigenous *Pfkelch13* mutants (R561H in Rwanda and A675V and C469Y in Uganda) associated with delayed parasite clearance and in vitro artemisinin resistance [12–14].

High prevalence of multiple copies of the *plasmepsin 2* (*Pfpm2*) gene, a marker of piperazine resistance [15], has been observed to be associated with DP treatment failure in Southeast Asian countries, where artemisinin resistance is frequent [16–18]. Studies on African isolates showed varying frequencies of multiple copies of *Pfpm2* gene: <5% in Mozambique [19], 30.5% and 33.9% in Burkina Faso and Uganda, respectively [20], and up to 50% in Burundi [3]. However, in high transmission areas like Africa, the detection of minor clones with amplified *Pfpm2* in polyclonal infections (MOI > 1) are challenging. Single Nucleotide Polymorphisms (SNP) in the *Plasmodium chloroquine resistance transporter* (*Pfcr1*) and *Plasmodium multi drug resistance 1* (*Pfmdr-1*) have been suspected to be associated with resistance to ACT partner drugs, lumefantrine and amodiaquine [21, 22], but robust molecular markers have not yet been validated [23].

Sulfadoxine/pyrimethamine (SP) is the recommended drug for intermittent preventive treatment of pregnant women (IPTp) living in areas of moderate to high malaria transmission in Africa to prevent the deleterious effects

of malaria on maternal and fetal outcomes [24]. Accumulation of point mutations at several codons in the dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*) genes increases the risk of SP treatment failure [25]. A quintuple mutant (51I, 59R and 108 N in the *Pfdhfr* and 437G + 540E in the *Pfdhps* genes) is a significant predictor of SP treatment failure [26, 27]. Acquisition of an additional mutation in *Pfdhps* (A581G) on the genetic background of the quintuple mutant has been shown to confer a higher degree of resistance to SP [28] and is associated with reduced efficacy of IPT-SP in pregnant women [29, 30] and in infants [31] when its frequency is above 10%.

In Liberia, malaria transmission is perennial with an estimated 1,809,994 cases and 2,232 deaths in 2019 [1, 3]. Children and pregnant women are the most affected groups. Based on a recent health facility survey, it was estimated that 33.9% of all outpatient attendance, 47.6% of admissions and 22.6% of inpatient deaths were due to malaria [32]. The Malaria Indicator Survey 2016 showed that 45% of children aged 6–59 months tested positive for malaria by rapid diagnostic test [33]. ASAQ was recommended as first-line and AL as alternative first-line (for patients who cannot tolerate ASAQ) treatments for the management of uncomplicated *P. falciparum* malaria [34]. Currently, the National Malaria Control Programme (NMCP) has reduced procurement of ASAQ and replaced it with AL as the first-line drug of choice for treatment of uncomplicated malaria and the treatment guideline will be updated (NMCP, pers. commun.). The last TES conducted in 2008–2009 showed high PCR corrected cure rates of above 97% at day 28 for both combinations [35]. Since then, the NMCP has not been able to monitor the efficacy of the recommended artemisinin-based combination due to operational challenges, including the Ebola outbreak in 2014–2015. In addition to case management and vector control, IPTp with SP is a critical component of nationally recommended interventions to reduce the burden of malaria in pregnant women [34]. To provide up to date evidence for national malaria treatment policy, the clinical and parasitological efficacy of ASAQ and AL were assessed as well as the frequency of SNP (*Pfkelch13*, *Pfcr1*, *Pfmdr-1*, *Pfdhfr*, *Pfdhps*) and CNV (*Pfpm2*) associated or suspected to be associated with resistances to anti-malarial drugs.

Methods

Study sites and study design

Study patients were recruited from four health facilities in four counties: (i) Bensonville Hospital, Bensonville, Montserrado County; (ii) Saclepea Comprehensive Health Center, Saclepea, Nimba County; (iii) Charles Henry Rennie Hospital, Kakata, Margibi County; and

(iv) Sinje Health Center, Garwula, Grand Cape Mount County (Fig. 1). The efficacy of ASAQ was assessed at the Bensonville and Saclepea sites while the efficacy of AL was evaluated at the Kakata and Sinje sites. The study was one-arm cohorts evaluating the efficacy of both artemisinin-based combinations in the treatment of uncomplicated falciparum infections. Children who met the inclusion and exclusion criteria of the study were enrolled, treated with ASAQ or AL on site and assessed clinically and parasitologically for 28 days according the 2009 WHO protocol [36].

Recruitment, treatment and follow-up procedure

Potential study children who visited the study health facilities between December 2017 and May 2018 were screened for the following criteria: age 6–59 months, axillary temperature ≥ 37.5 °C and/or history of fever in the past 24 h, and *P. falciparum* mono-infection with parasitaemia of 2000 to 200,000 asexual parasites/ μ l by microscopy. Other inclusion criteria included willingness to comply with the study visit schedule and informed consent from parents or guardians. Children with exclusion criteria, including the presence of general danger signs or signs of severe falciparum malaria, mixed or mono-infection with non-falciparum species, or severe malnutrition and febrile conditions due to diseases other than malaria, received appropriate care and treatment according to national guidelines.

Children recruited at the Bensonville and Saclepea sites received daily dose of ASAQ for 3 days according to the recommended weight bands: one tablet of 25 artesunate + 67.5 amodiaquine for 4.5 to < 9 kg body weight, one tablet of 50 artesunate + 135 amodiaquine for 9–< 18 kg body weight, and one tablet of 100 artesunate + 270 amodiaquine for 18 to < 36 kg body weight. A dose of artesunate 4 (range 2–10) mg/kg + amodiaquine 10 (range 7.5–15) mg/kg body weight once daily for 3 days was the target. Children from Kakata and Sinje were given twice daily dose of AL for 3 days according to recommended weight bands: one tablet for those weighing 5–14 kg and two tablets for 15–24 kg. AL was given with milk or fatty meal. All treatments were administered under direct observation by the study team and patients were observed for 30 min. If the first dose was vomited, the treatment was administered again. If vomiting recurred, the patient was given artesunate injection according to national guidelines and the patient was withdrawn from the study. Patient with treatment failure were treated with quinine 10 mg/kg BW three times a day for seven days. Prequalified ASAQ (manufactured by Sanofi with Batch numbers 5MA082 for 25 mg artesunate/67.5 mg amodiaquine and 6MA113 for 50 mg artesunate/135 mg

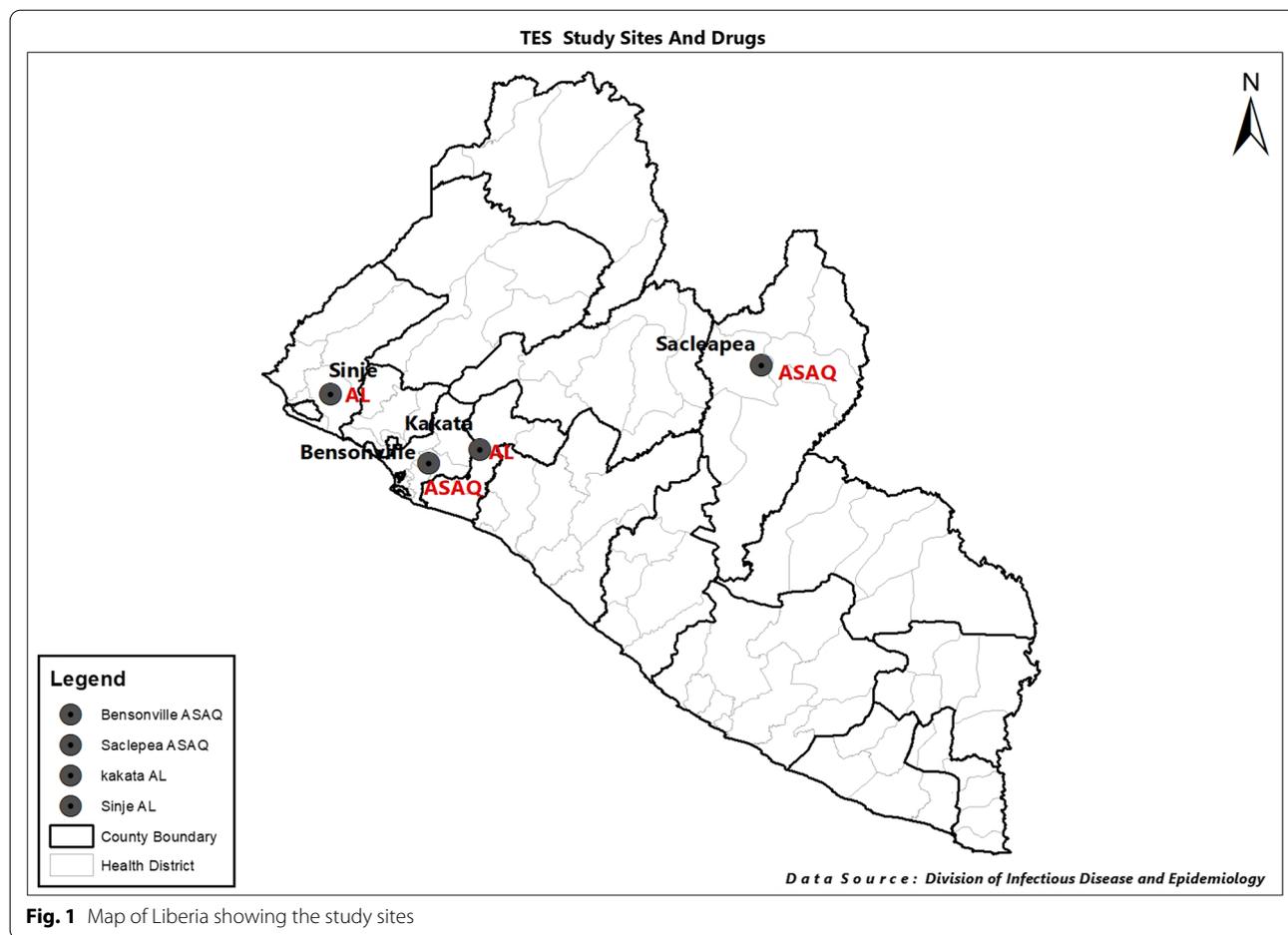


Fig. 1 Map of Liberia showing the study sites

amodiaquine, and AL (manufactured by Ipca Laboratories LTD with batch number DYI2036341) were obtained from WHO /HQ.

Study children were followed for up to 28 days at scheduled visits on days 1, 2, 3, 7, 14, 21, and 28, and at unscheduled visits when symptoms worsened or recurred. The allowable time window for the weekly follow-up was ± 1 day. A clinical assessment and parasitological examination were performed at each visit. Existing general Community Health Volunteers (gCHVs) in the study areas were engaged and trained to trace patients if they did not show up for the scheduled appointment.

Laboratory investigation

Thick and thin blood smears collected on the day of recruitment (day 0) and follow-up days were stained with Giemsa and asexual parasites were counted against White Blood Cells (WBC) in the thick blood smear using the WHO procedure [36]. Assuming WBC count of 6,000 WBCs/ μ L, parasite density (asexual parasites per μ L of blood) was calculated by dividing the number of asexual parasites counted by the number of WBCs and then

multiplying by 6000. Two microscopists independently read all blood slides. A third microscopist re-examined the blood slides with discordant results (species diagnosis, parasite density of $> 50\%$, or presence of parasites). The final parasite density was calculated by taking the average of the two closest counts. A blood smear was declared negative if no asexual parasites were observed after examination of 1,000 WBCs.

Filter paper blood samples were collected from each patient on day 0 and on the day of parasite recurrence (from day 7 onward), stored in individual plastic bags with desiccant and protected from light, moisture and extreme temperature until analysis. Each dried blood spot was cut out sterilely and placed in an Eppendorf tube. DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen) as previously described [12]. Paired DNA from patients with recurrent parasites (day-0 and day of recurrence) were genotyped using nested polymerase chain reaction (PCR) targeting the highly polymorphic genes *msp1*, *msp2* and *glurp* [37]. The fragment sizes were estimated by capillary electrophoresis (Fragment analyzer, Agilent) and the cut-off settings for PCR

artefacts and stutter peaks was defined for peaks <10% of the low and upper control bands. The bins used to define a match were ± 10 bp for *msp1/msp2* and ± 20 bp for *glurp*. The genotypes of parasites on day 0 and on the day of parasite recurrence were compared to distinguish recrudescence (same genotype) from new infection (different genotype). Both the current WHO-recommended algorithm [38] and the newly proposed two out of three (2/3) algorithm [39] were used. In the WHO algorithm, recurrent parasitaemia was classified as recrudescence if at least one allele at all 3 markers (3/3) was common to both paired samples. In the 2/3 algorithm, recurrent parasitaemia was classified as recrudescence, if at least one allele at 2 among 3 markers (2/3) was common to both paired samples.

Day 0 DNA was also analysed for the presence of point mutations in the *Pfkelch13* gene associated with artemisinin resistance [11], the *Pfprt* and *Pfmdr-1* genes associated or suspected to be associated with 4-aminoquinolines and aminoalcohol resistance [23], and the *Pfdhfr* and *Pfdhps* genes linked to pyrimethamine and sulfadoxine resistance. Amplicons from targeted sequences were generated using nested PCR assays as previously described [40, 41] and sent to Eurofins (Germany) for sequencing. Mutations at codons 440–680 for *Pfkelch13*, at codons 72–76, 93, 97, 145, 218, 343, 350 and 353 for *Pfprt*, at codons 86, 184, 1034, 1042 and 1246 for *Pfmdr-1*, at codons 51, 59, 108, 164 for *Pfdhfr* and at codons 436, 437, 540, 581, 613 for *Pfdhps* were assessed with the CLC Main Workbench 20 software (Qiagen). Electropherograms with mixed alleles were considered as mutant for the purpose of mutation frequency estimation. Quality control was assessed by including blinded quality control samples (parasites with wild-type, C580Y and R539T alleles for *Pfkelch13* and 3D7, Dd2, 7G8 laboratory strains for *Pfdhfr*; *Pfdhps*, *Pfprt* and *Pfmdr-1*) in each 96-well sequencing plate. DNA from D0 samples were also analysed to estimate copy number variations in the *Pfpm2* gene, which is associated with piperazine resistance, using the method described previously [15]. *Pfpm2* gene copy number was estimated samples without assessing the number of clones (MOI) and a *Pfpm2* copy number > 1.5 was defined as an amplification of the gene. All D0 samples were screened for *Pfkelch13* and *Pfdhfr/Pfdhps* mutations, while a subsample of 30% from each site was analysed for *Pfprt* and *Pfmdr-1* mutations and *Pfpm2* copy number variations. The samples for genotyping and molecular markers were analysed at the Institut Pasteur, Paris, France.

Outcome measures

Treatment response was classified based on parasitological and clinical response as recommended by WHO [36]: early

treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR). The primary endpoint of the study was 28-day PCR-corrected ACPR. Secondary endpoints included day 3 positivity, 28-day PCR-uncorrected ACPR, and frequencies of SNP in *Pfkelch13*, *Pfdhfr*, *Pfdhps*, *Pfprt*, *Pfmdr-1*, and CNV in *Pfpm2* genes.

Ethical considerations

Ethical approval for the study protocol was obtained from the University of Liberia-Pacific Institute for Research & Evaluation Institutional Review Board (UL-PIRE IRB) and the WHO Research Ethics Review Committee (ERC.0002892). Parents/guardians were informed of the study procedure, its benefits and potential risks and gave written informed consent for their children to participate in the study prior to enrollment.

Sample size and data management

A minimum sample of 73 children per site was estimated based on a 5% treatment failure rate for ASAQ and AL and with a 95% confidence level and 5% precision. Twenty percent (n=15) was added to account for loss to follow-up and withdrawal during the 28-day follow-up period. The final target sample was 88 per site. Data were double-entered, validated against the case sheet in case of discrepancies, and analysed using the WHO excel software programme (<http://www.who.int/malaria/publications/atoz/9789241597531/en/>). Per-protocol and Kaplan–Meier analyses were used to analyse treatment outcomes according to the WHO protocol [36]. The per-protocol analysis was performed excluding patients who discontinued treatment, stopped treatment, withdrawn, or had new infections during follow-up. In the KM analysis, these cases were censored on the last day of follow-up, withdrawal, or re-infection. Recurrent cases with indeterminate PCR results were excluded from both the per-protocol and KM analyses. Descriptive statistics including percentages, mean, standard deviation, and range were presented. Patient characteristics at the time of enrollment and treatment outcomes were compared between sites within each study drug. Chi-square and Fisher exact tests were used to compare categorical data and t-tests were used to compare continuous variables. The presence of *Pfprt* K76T, *Pfmdr1* N86Y and Y184F mutations was compared between day 0 samples from cured and recrudescence patients using Fisher's exact test and an estimation of relative risk. A difference is considered significant if the p-value < 0.05.

Table 1 Characteristics of study children at enrolment

Characteristic	Artesunate–amodiaquine		Artemether–lumefantrine	
	Bensonville (n = 91)	Saclepea (n = 89)	Kakata (n = 89)	Sinje (n = 90)
Male, n (%)	52 (57.14)	43 (48.86)	45 (50.56)	41 (45.56)
Female, n (%)	39 (42.86)	46 (51.14)	44 (49.44)	49 (54.44)
Age (years): Mean (SD ^a)	2.6 (1.2)	2.5 (1.2)	2.6 (1.3)	2.1 (1.1)
Axillary temp Mean (SD ^a)	37.1 (0.9)	38 (0.7)	37.5 (1.0)	37.4 (0.9)
Parasite density (per μ L):				
Geometric mean	28,592	27,625	16,425 ^b	14714 ^c
CI 95%	11,781–18,375	22,303–34,217	12,625–21,369	14,712–14,714

^a SD: standard deviation

^b Parasitemia in Kakata was significantly lower than that of Bensonville ($t = 3.8$; $df = 177$; $p = 0.0002$) and Saclepea ($t = 3.9$; $df = 177$; $P = 0.0001$)

^c Parasite density in Sinje was significantly lower than that of Bensonville ($t = 3.0$; $df = 178$; $p = 0.003$) and Saclepea ($t = 3.0$; $df = 169$; $p = 0.003$)

Results

Baseline characteristics of enrolled patients

The study was conducted from December 2017 to May 2018. A total of 359 children, 180 (91 in Bensonville and 89 Saclepea) and 179 (89 in Kakata and 90 in Sinje) were recruited for the ASAQ and AL clinical efficacy studies, respectively. Baseline characteristics of the recruited children were comparable between the sites except for mean parasite density (Table 1). Geometric mean parasite density in Sinje was significantly lower than in Saclepea ($t = 3.9$; $df = 177$; $p = 0.0001$) and Bensonville ($t = 3.8$; $df = 177$; $p = 0.0002$). In addition, lower parasite density was observed in Kakata compared to Saclepea ($t = 3.0$; $df = 169$; $p = 0.003$) and Bensonville ($t = 3.0$; $df = 178$; $p = 0.003$).

Treatment outcomes

Of the 359 children enrolled, 332 (92.5%) reached the study endpoints. Of the remaining 27 cases, 24 (17 in Bensonville, 5 in Kakata and 2 in Sinje) were lost to

follow-up and three (2 in Bensonville and 1 in Saclepea) were withdrawn during follow-up due to missing treatment dose for day-1 (one case) or for day-2 (two cases). Before PCR correction, per-protocol analysis revealed an ACPR of 63.9% (51.7–74.9%) and 86.4% (77.4–92.8%) for ASAQ treatment in Bensonville and Saclepea, respectively, while ACPR of 94.0% (86.7–98.0%) and 100% (95.9–100%) were observed for AL in Kakata and Sinje, respectively (Table 2). Forty-three patients experienced parasite recurrences, most of which occurred in the ASAQ group in Bensonville (26/43, 60.5% recurrences) and Saclepea (12/43, 27.9% recurrences). Most of these parasite recurrences were new infections (31/43, 74.1%), of which the majority (20/31, 64.5%) occurred in Bensonville.

Using the PCR analysis method recommended by the WHO [38], the PCR-corrected per protocol results showed an ACPR of 90.2% (78.6–96.7%) in Bensonville and 92.7% (84.8–97.3%) in Saclepea for ASAQ (Table 3). PCR-corrected KM analysis revealed cumulative cure

Table 2 28-day PCR-unadjusted treatment efficacy of study children after treatment with Artesunate–amodiaquine or Artemether–lumefantrine

PCR-unadjusted outcome	Artesunate–amodiaquine				Artemether–lumefantrine			
	Bensonville (n = 91)		Saclepea (n = 89)		Kakata (n = 89)		Sinje (n = 90)	
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI
LCF	6 (8.3)	3.1–17.3	3 (3.4)	0.7–9.6	1 (1.2)	0.0–6.5	0 (0)	0.0–4.1
LPF	20 (27.8)	17.9–39.6	9 (10.2)	4.8–18.5	4 (4.8)	1.3–11.7	0 (0)	0.0–4.1
ACPR	46 (63.9)	51.7–74.9	76 (86.4)	77.4–92.8	79 (94.0)	86.7–98.0	88 (100)	95.9–100
Total per protocol	72		88		84		88	
Withdrawn/lost	19 (20.9)		1 (1.1)		5 (5.6)		2 (2.2)	
Kaplan Meier: cure rate	65.1	53.0–74.8	86.4	77.2–92.0	94.0	86.3–97.5	100	n/a

LCF late clinical failure, LPF late parasitological failure, ACPR adequate clinical and parasitological response

Table 3 28-day PCR corrected treatment outcomes: WHO algorithm

PCR adjusted Outcome	Artesunate–amodiaquine					Artemether–lumefantrine				
	Bensonville (n = 91)		Saclepea (n = 89)		Overall (n = 180)	Kakata (N = 89)		Sinje (N = 90)		Overall (n = 179)
	n (%)	CI 95%	n (%)	95% CI		% (CI 95%)	n (%)	CI 95%	n (%)	
LCF ^a	0	0.0–7.1	2 (2.4)	0.3–8.5	1.5 (0.2–5.3)	0	0.0–4.6	0 (0)	0.0–4.1	0 (0.0–2.2)
LPF ^b	5 (9.8)	3.3–21.4	4 (4.9)	1.3	7.5 (3.6–13.3)	0	0.0–4.6	0 (0)	0.0–4.1	0 (0.0–2.2)
ACPR ^c	46 (90.2)	78.6–96.7	76 (92.7)	84.8–97.3	91.0 (84.9–95.3)	79 (100)	95.4–100	88 (100)	95.9–100	100 (97.8–100)
Total per-protocol	51		82		134	79		88		167
Withdrawn/lost:	19 (20.9)		1 (1.1)		20 (11.1)	5 (5.6)		2 (2.2)		7 (3.9)
Re-infection	20 (23.1)		5 (5.6)		25 (13.9)	5 (5.6)		0		5
Unknown	1 (1.1)		0		1 (0.6)	0		0		0
KM ^d cure rate	92.1	81.9–96.6	93.0	85.1–96.8	92.0 (86.4–95.4)	100	NA	100	NA	100 (NA)

^a LCF late clinical failure, ^bLPF late parasitological failure, ^cACPR adequate clinical and parasitological response, ^dKM Kaplan Meier

rate of 92.1% (81.9–96.6%) and 93.0% (85.1–96.8%) in Bensonville and Saclepea, respectively. For AL, both the per-protocol and KM analysis showed 100% efficacy at both sites. Compared with the WHO algorithm, the 2/3 algorithm showed that all recurrences classified as recrudescence (n = 11) remained the same while 25.8% (8/31) of the new infections were reclassified as recrudescence (see Additional file 1: Genotyping Raw data). These changes primarily affected the Bensonville site, where 30% (6/20) of the new infections were re-assigned to recrudescence, reducing the efficacy rate from 90.2% to 80.7% (Table 4). On day2, 4.5% (4/89) of patients at Bensonville, 2.3% (2/88) at Saclepea, 17.0% (15/88) at Sinje and 4.6% (4/87) at Kakata were still slide positive. Except for two cases (0.6%, 2/352), children in both treatment groups were free of parasites on day3.

Markers for partial artemisinin resistance

Of the 359 pretreatment samples, 358 (99.7%) yielded interpretable results, most of which (353/358) carried the *Pfkelch13* wild-type allele, ranging from 95.6 to 100% depending on the site. The remaining five isolates carried synonymous mutants (C469C in one sample at Bensonville, G548G in two samples at Bensonville and Kakata, and Y493Y in one sample at Bensonville) and one non-synonymous mutant (V637I in one sample at Saclepea).

Markers for piperazine resistance

Estimation of *Pfpm2* copy number was performed on 112 randomly selected day-0 samples (25% of day-0 samples per site). Interpretable data were obtained for 100 samples (89.3%). Samples that were not interpretable were likely samples with insufficient DNA quantity (due to

Table 4 28-day PCR corrected treatment outcome: 2/3 algorithm

PCR adjusted Outcome	Artesunate–amodiaquine					Artemether–lumefantrine				
	Bensonville (n = 91)		Saclepea (n = 89)		Overall (n = 180)	Kakata (N = 89)		Sinje (N = 90)		Overall (N = 179)
	n (%)	CI 95%	n (%)	95% CI		% (CI 95%)	n (%)	95% CI	n (%)	
LCF ^a	1 (1.8)	0.0–9.4	2 (2.4)	0.3–8.4	2.1 (0.4–6.1)	0	0.0–4.5	0 (0)	0.0–4.1	0 (0.0–2.2)
LPF ^b	10 (17.5)	8.7–29.9	5 (6.0)	2.0–13.5	10.7 (6.1–17.1)	1 (1.3)	0.0–6.8	0 (0)	0.0–4.1	0.6 (0.0–3.3)
ACPR ^c	46 (80.7)	68.1–90.0	76 (91.6)	83.4–96.5	87.1 (80.4–92.2)	79 (98.8)	93.2–100	88 (100)	95.9–100	99.4 (96.7–100)
Total per-protocol	57		83		140	80		88		168
Withdrawn/lost:	19 (20.9)		1 (1.1)		20 (11.1)	5 (5.6)		2 (2.2)		7 (3.9)
Re-infection	13		5		18	4		0		4
PCR NI ^d	2		0		2	0		0		0
^e KM cure rate	84.1	73.0–90.9	91.8	83.6–96.0	88.4 (82.2–92.5)	98.8		100		99.4 (95.9–99.9)

^a LCF late clinical failure, ^bLPF late parasitological failure, ^cACPR adequate clinical and parasitological response, ^eKM Kaplan Meier

initial parasite density) or poor DNA quality. Parasites in all samples carried a single copy of the *Pfpm2* gene (CNV < 1.5).

Associations between mutations in the *Pfcr*t and *Pfmdr*-1 genes and ASAQ or AL clinical and parasitological outcomes

A total of 91 day 0 samples from patients treated with AL or ASAQ were selected and used to assess the frequency of mutations in the *Pfcr*t and *Pfmdr*-1 genes. These samples included all the day 0 isolates from patients classified as recurrent (n = 40, 5 in the AL group and 35 in the ASAQ group) and a random selection of day-0 samples from patients classified as cured (n = 43, 28 in the AL group and 15 in the ASAQ group) or lost to follow-up (n = 8, 3 in the AL group and 5 in the ASAQ group).

Molecular analysis revealed that the *Pfcr*t 76 T mutant was highly frequent (87.9%, 80/91), with little variation in frequencies between sites (80.0% to 95.2%) (Table 5). The 74I/75E/76 T/356 T (44.0%, 40/91), followed by 74I/75E/76 T (31.9%, 29/91) and the wild-type allele (12.1%, 11/91) were the most frequent *Pfcr*t alleles. For the *Pfmdr*-1 gene, the 184F mutation was

the most common mutation (51.6%, 47/91), followed by the 86Y mutation (44.0%, 40/91). Across the study sites, the proportions of the two mutants were not much different (Table 5), except in Sinje site, where the frequencies of 184F and 86Y were different (66.5% and 26.7%, respectively). Next, the association of *Pfcr*t K76T, *Pfmdr*-1 N86Y, and Y184F mutations with adjusted 28-day clinical outcomes were examined. As shown in Table 6, no association between the presence of the mutations in *Pfcr*t and *Pfmdr*-1 and the risk of parasite recrudescence in patients treated with ASAQ (defined by the WHO and the 2/3 algorithms) was observed. *Pfmdr*-1 N86 and 86Y were equally distributed in the AL group and none of the genotype was associated with treatment failure.

Markers for SP resistance

The *Pfdhfr* and *Pfdhps* genes were successfully amplified in 99.7% (358/359) and 99.4% (357/359), respectively. For the *Pfdhfr* gene, the triple mutant (51I/59R/108 N) was the predominant allele and accounted for 49.2%, with little variation between sites (Table 7). Other alleles detected included the 108 N allele (36.3%) and the wild-type allele (14.5%). For the *Pfdhps* gene, the

Table 5 *Pfcr*t and *Pfmdr*-1 mutation and alleles observed in *P. falciparum* obtained from blood samples collected prior antimalarial treatment

		Bensonville		Saclepea		Kakata		Sinje		Total	
		n	%	n	%	n	%	n	%	N	%
Mutation	<i>Pfcr</i> t										
	76 T	29	85.3	19	90.5	20	95.2	12	80.0	80	87.9
	<i>Pfmdr</i> -1										
	86Y	16	47.1	8	38.1	12	57.1	4	26.7	40	44.0
	184F	16	47.1	10	47.6	11	52.4	10	66.7	47	51.6
Alleles	<i>Pfcr</i> t										
	WT	5	14.7	2	9.5	1	4.8	3	20.0	11	12.1
	74I/75E/76 T	11	32.4	8	38.1	6	28.6	4	26.7	29	31.9
	74I/75E/76 T/362 V	0	0.0	0	0.0	0	0.0	1	6.7	1	1.1
	74I/75E/76 T/356 T	16	47.1	8	38.1	10	47.6	6	40.0	40	44.0
	74I/75E/76 T/217F/356 T	0	0.0	1	4.8	0	0.0	0	0.0	1	1.1
	74I/75E/76 T/141L	0	0.0	1	4.8	2	9.5	0	0.0	3	3.3
	74I/75E/76 T/141L/356 T	2	5.9	1	4.8	2	9.5	1	6.7	6	6.6
	Total	34	100.00	21	100.00	21	100.00	15	100.00	91	100.00
	<i>Pfmdr</i> -1										
	WT	9	26.5	5	23.8	5	23.8	2	13.3	21	23.1
	86Y	6	17.6	5	23.8	5	23.8	1	6.7	17	18.7
	184F	9	26.5	8	38.1	4	19.0	9	60.0	30	33.0
86Y/1246Y	3	8.8	1	4.8	0	0.0	2	13.3	6	6.6	
86Y/184F	6	17.6	2	9.5	7	33.3	1	6.7	16	17.6	
86Y/184F/1276Y	1	2.9	0	0.0	0	0.0	0	0.0	1	1.1	
Total	34	100.00	21	100.00	21	100.00	15	100.00	91	100.00	

Table 6 Associations between *Pfcr* K76T, *Pfmdr-1* N86Y and Y184F mutations with unadjusted (recurrences) and adjusted (recrudescences) 28-day clinical outcomes following ASAQ treatment

Outcome	Algorithm decision	Parameters	Mutation					
			<i>Pfcr</i>		<i>Pfmdr-1</i>			
			K76	76 T	N86	86Y	Y184	184F
Unadjusted outcome		Cured	1	18	4	15	6	13
		Recurrence	6	30	10	26	23	13
		Mean survival (SE), days	23.0 (2.5)	25.2 (0.6)	24.5 (1.4)	25.1 (0.7)	24.8 (0.9)	25.0 (0.9)
		Logrank test, <i>p</i> value	0.18		0.58		0.10	
		Hazard ratio (95% CI)	2.4 (0.7–8.5)		1.3 (0.5–3.2)		1.9 (0.9–4.3)	
Adjusted outcome	WHO/MMV	Cured	6	37	13	30	21	22
		Recrudescence	1	11	1	11	8	4
		Mean survival (SE), days	28.0 (0)	27.2 (0.4)	28.0 (0)	27.1 (0.5)	27.4 (0.4)	27.2 (0.7)
		Logrank test, <i>p</i> value	0.76		0.16		0.31	
		Hazard ratio (95% CI)	0.7 (0.1–5.3)		0.4 (0.08–1.5)		1.8 (0.5–6.4)	
	2/3	Cured	5	31	11	25	19	17
		Recrudescence	1	17	2	16	9	9
		Mean survival (SE), days	28.0 (0)	26.5 (0.5)	26.9 (1.4)	26.5 (0.5)	26.9 (0.6)	26.3 (0.7)
		Logrank test, <i>p</i> value	0.44		0.15		0.79	
		Hazard ratio (95% CI)	0.6 (0.1–2.7)		0.4 (0.1–1.3)		0.8 (0.3–2.4)	

wild-type allele was the most common (47.9%), followed by the 540E allele (16.0%), the 436A allele (14.3%), and the 436A/437G double mutant allele (9.0%). No significant differences were observed between sites. The 581G mutation, associated with loss of protective efficacy of IPTi and IPTp, was rare and observed in seven samples (7/357, 2%). The most frequent *Pfdhfr/Pfdhps* haplotypes were 511/59R/108 N-wild type (25.2%), followed by 108 N-wild type (14.8%) and wild type-wild-type (7.8%), as described in Table 8. The frequency of the quintuple mutant haplotype (511/59R/108 N-437G/540E) was found only in one (0.3%) isolate from Bensonville. No sextuple mutant haplotype (quintuple + 581G) was detected in the samples tested.

Discussion

Using the WHO recommended PCR analysis [38], the current study conducted eight years after the last therapeutic efficacy study showed cure rates of 90.2% (95% CI: 78.6–96.7%) in Bensonville and 91.6% (83.4–96.5%) in Saclepea for ASAQ, suggesting a possible decline in the efficacy of this ACT compared to the status in 2009 [35]. Bensonville is the capital of Montserrat with a high population movement. The contact information provided for follow-up sometimes not reachable, or the proxy contact telephone number provided for follow-up was sometimes not close to the patient/client. These factors may have contributed to the high rate (18.7%) of lost to follow-up in this study site. The study also revealed PCR

corrected AL cure rate of 100% observed in the study sites demonstrate that AL remained highly efficacious. In contrast to the results of the current study, ASAQ has maintained its high efficacy in neighbouring [42–44] and other West African countries [45–50] with cure rates of 98% and above. Similar high cure rates (96% and above) with the AL treatment have also been reported in the sub-region [42–51]. Both artemisinin-based combinations also remain highly effective in other African countries [52–61].

In the current study, parasite genotyping to distinguish between reinfection and recrudescence (true failures) was analysed using both the WHO [38] and the proposed 2/3 [39] algorithms. Compared to the WHO algorithm, the proposed 2/3 algorithm classified reinfection as recrudescence (25.8%), resulting in an increase in treatment failure rates. These changes primarily affected Bensonville, where 30% of new infections as per WHO algorithm were reclassified as recrudescence resulting in a drop of the efficacy rate to 80.7%. Based on both analysis (WHO or 2/3), the results seem to show a loss of efficacy of ASAQ in Bensonville lies between equal or below the 90% threshold at which a change in treatment policy should be initiated [3]. The results of this study support the recent decision of NMCP to replace ASAQ with AL as first-line treatment of uncomplicated falciparum malaria (NMCP, pers. commun.). The high rate of new infections in Bensonville shown by both algorithms could indicate an ongoing high malaria transmission. In such

Table 7 *dhfr* and *dhps* alleles observed in *P. falciparum* obtained from blood samples collected prior antimalarial treatment

	Bensonville (n = 91)		Saclepea (n = 89)		Kakata (n = 89)		Sinje Town (n = 90)		Total (n = 359)	
	n	%	n	%	n	%	n	%	N	%
<i>dhfr</i>										
WT	14	15.6%	5	5.6%	9	10.1%	24	26.7%	52	14.5%
108 N	43	47.8%	25	28.1%	32	36.0%	30	33.3%	130	36.3%
511/59R/108 N	33	36.7%	59	66.3%	48	53.9%	36	40.0%	176	49.2%
Total*	90	100.0%	89	100.0%	89	100.0%	90	100.0%	358	100.0%
<i>dhps</i>										
WT	26	29.2%	43	48.3%	52	58.4%	50	55.6%	171	47.9%
436A	7	7.9%	13	14.6%	12	13.5%	19	21.1%	51	14.3%
437G	15	16.9%	0	0	1	1.1%	1	1.1%	17	4.8%
K540E	15	16.9%	15	16.9%	15	16.9%	12	13.3%	57	16.0%
A613S	2	2.2%	3	3.4%	0	0	4	4.4%	9	2.5%
436A/437G	17	19.1%	8	9.0%	3	3.4%	4	4.4%	32	9.0%
436A/613S	0	0	4	4.5%	4	4.5%	0	0	8	2.2%
S436A/K540E	1	1.1%	0	0	0	0	0	0	1	0.3%
437G/581G	1	1.1%	0	0	1	1.1%	0	0	2	0.6%
437G/540E	1	1.1%	0	0	0	0	0	0	1	0.3%
436A/437G/540E	1	1.1%	0	0	0	0	0	0	1	0.3%
436A/437G/613S	2	2.2%	0	0	0	0	0	0	2	0.6%
436A/581G/613S	0	0	0	0	1	1.1%	0	0	1	0.3%
436A/437G/581G/613S	1	1.1%	3	3.4%	0	0	0	0	4	1.1%
Total**	89	100.0%	89	100.0%	89	100.0%	90	100.0%	357	100.0%

*One sample from Bensonville gave not interpretable data for *dhfr* sequence. ** two samples from Bensonville gave not interpretable data for *dhps* sequence

a setting, high multiplicity of infection (MOI) and high rates of new infection pose a challenge in distinguishing between recrudescence and reinfection and analysis copy numbers of markers of resistance. A similar significant difference in failure rates between the WHO and 2/3 algorithms in a high transmission area was recently reported from Equatorial Guinea [55].

In a recent WHO analysis, overall, the proportion of recurrent parasitemia classified as recrudescence was higher with the 2/3 algorithm than with the WHO method ($p < 0.001$). However, this did not always translate into a significant difference in Kaplan–Meier estimates of treatment outcome. Differences in the Kaplan–Meier estimates of treatment outcome were more evident in areas of moderate to high transmission than in areas of low to moderate transmission in particular for artemether–lumefantrine. Though there is no gold

standard, an expert committee recommended that WHO methodology [38] should be maintained as the primary analysis methodology for reporting and policy change. Bayesian and 2/3 algorithms may be applied for evaluation and comparison, but not for primary reporting.

In the current study, parasites were cleared by day 3 in all but two patients, which, together with the absence of the *Pfkelch13* mutation known to be associated with artemisinin resistance, indicates absence of artemisinin resistance in Liberia. Until recently, *Pfkelch13* mutations known to be associated with artemisinin resistance were rare or absent in Africa [19, 51, 55, 62–65]. However, this landscape has recently changed. For instance, indigenous *Pfkelch13* R561H mutant was detected in 7.3% of samples collected from the Masaka site in Rwanda between 2013 and 2015, but without delayed parasite clearance [12]. A subsequent study in 2018 showed a higher prevalence of

Table 8 *dhfr/dhps* haplotypes observed in *P. falciparum* obtained from blood samples collected prior antimalarial treatment

Haplotype			Bensonville (n = 91)		Saclepea (n = 89)		Kakata (n = 89)		Sinje Town (n = 90)		Total (n = 359)	
Combined	<i>dhfr</i>	<i>dhps</i>	n	%	n	%	n	%	n	%	N	%
Wild type	WT	WT	5	5.6%	1	1.1%	7	7.9%	15	16.7%	28	7.8%
Single mutant	WT	436A	0	0	1	1.1%	0	0	4	4.4%	5	1.4%
	WT	437G	2	2.2%	0	0	0	0	0	0	2	0.6%
	WT	613S	1	1.1%	0	0	0	0	1	1.1%	2	0.6%
	WT	K40E	3	3.4%	1	1.1%	1	1.1%	4	4.4%	9	2.5%
	108 N	WT	9	10.1%	8	9.0%	21	23.6%	15	16.7%	53	14.8%
Double mutant	WT	436A/437G	3	3.4%	2	2.2%	0	0	0	0	5	1.4%
	108 N	436A	5	5.6%	5	5.6%	4	4.5%	7	7.8%	21	5.9%
	108 N	437G	8	9.0%	0	0	0	0	1	1.1%	9	2.5%
	108 N	540E	10	11.2%	4	4.5%	5	5.6%	4	4.4%	23	6.4%
	108 N	613S	1	1.1%	2	2.2%	0	0	0	0	3	0.8%
Triple mutant	WT	S436A/A581G/A613S	0	0	0	0	1	1.1%	0	0	1	0.3%
	51I/59R/108 N	WT	12	13.5%	34	38.2%	24	27.0%	20	22.2%	90	25.2%
	108 N	436A/437G	8	9.0%	2	2.2%	0	0	3	3.3%	13	3.6%
	108 N	436A/613S	0	0	1	1.1%	1	1.1%	0	0	2	0.6%
Quadruple mutant	108 N	437G/581G	1	1.1%	0	0	1	1.1%	0	0	2	0.6%
	51I/59R/108 N	436A	2	2.2%	7	7.9%	8	9.0%	8	8.9%	25	7.0%
	51I/59R/108 N	437G	5	5.6%	0	0	1	1.1%	0	0	6	1.7%
	51I/59R/108 N	540E	2	2.2%	10	11.2%	9	10.1%	4	4.4%	25	7.0%
Quintuple mutant	51I/59R/108 N	613S	0	0	1	1.1%	0	0	3	3.3%	4	1.1%
	51I/59R/108 N	436A/437G	6	6.7%	4	4.5%	3	3.4%	1	1.1%	14	3.9%
	51I/59R/108 N	436A/540E	1	1.1%	0	0	0	0	0	0	1	0.3%
	51I/59R/108 N	436A/613S	0	0	3	3.4%	3	3.4%	0	0	6	1.7%
Sextuple mutant	51I/59R/108 N	437G/540E	1	1.1%	0	0	0	0	0	0	1	0.3%
	108 N	437G/581G/613S	0	0	0	0	3	3.4%	0	0	3	0.8%
Septuple mutant	51I/59R/108 N	436A/437G/613S	2	2.2%	0	0	0	0	0	0	2	0.6%
Septuple mutant	51I/59R/108 N	436A/437G/581G/613S	1	1.1%	0	0	0	0	0	0	1	0.3%
Total *			89	100.0%	89	100.0%	89	100.0%	90	100.0%	357	100.0%

*Two samples from Bensonville gave not interpretable data for *dhfr/dhps* sequences

the R561H mutant in Masaka (16%) and Rukara (15%), which was associated with delayed parasite clearance as measured by parasitaemia on day3 [13]. In addition, an association between *Pfkelch13* A675V or C469Y, candidate mutations, and prolonged parasite clearance half-life following artemisinin monotherapy was reported from Uganda [14]. These findings are concerning and highlight the need for frequent monitoring of ACT efficacy, including clearance of parasitaemia, and *Pfkelch13* mutations in Africa, as recommended by the WHO [3].

Dihydroartemisinin–piperaquine has recently been adopted as a second-line treatment for uncomplicated malaria and as a drug for Mass Drug Administration (MDA) in Africa. No parasites with multiple copies of the *Pfpm2* gene were detected in the current study. CNV data, however, from polyclonal infections are not completely reliable. Indeed, it remains a challenge to assess copy number variations in multi-genome infections, as minor variants with amplified plasmepsin 2–3 maybe be missed. A recent study found a high frequency of multiple copies of the *Pfpm2* gene in African samples, varying from 11 to 34% [20]. Given the experience in Southeast Asia, where piperaquine resistance has emerged and spread rapidly, resulting in high treatment failure rates after DP treatment, *Pfpm2* gene amplification should be closely monitored in Africa.

A medium to high frequency of parasites carrying polymorphisms in the *Pfprt* and *Pfmdr-1* genes was observed, which could be explained by the predominant use of ASAQ in the country. However, no association between the presence of the mutations in *Pfprt* and *Pfmdr-1* and the risk of parasite recrudescence in patients treated with ASAQ was observed. Overall, this clearly points out that robust molecular markers associated with amodiaquine and lumefantrine are still lacking.

Intermittent preventive treatment of malaria in pregnancy with SP (IPTp- SP) is one of the recommended core interventions in areas of moderate to high malaria transmission in Africa, including Liberia [24, 34]. As the effectiveness of this strategy is threatened by SP resistance, monitoring of mutations in the *Pfdhfr* and *Pfdhps* genes is an important tool to determine the status of SP resistance and guide IPTp policy. Although quintuple *Pfdhfr/Pfphs* mutations (N511/C59R/S108N-A437G/K540E) have been associated with clinical SP treatment failure [26, 27], evidence suggests that IPTp-SP remains effective in areas with high prevalence of quintuple mutation [24]. However, reduced effectiveness of IPT-SP has been reported in infants and pregnant women in areas where parasites with sextuple mutation (quintuple + 581G) are present [66, 67]. In the current study, the *Pfdhfr* triple mutation

(N511/C59R/S108N) was the most common *Pfdhfr* allele detected, accounting for 49.2%. The very low frequency (1/357, 0.3%) of the quintuple mutant haplotype and the absence of the sextuple mutation (quintuple-581G) support the continued use of SP for IPTp in Liberia. Due to the high proportion of polyclonal infections detected by *msp1/msp2/gdurp* genotyping, we cannot infer *Pfdhfr/Pfdhps* haplotypes with absolute certainty because the combination of SNPs could be deduced from different clones. The prevalence of *Pfdhfr* and *Pfdhps* mutations varies across the continent from absent to a low prevalence of quintuple mutations in West Africa [68–71] and a very high prevalence (>70%) in East Africa [72–76]. As expected, mutations in *Pfdhfr* and *Pfdhps* genes will continue to evolve to saturation under SP drug pressure in moderate to high transmission settings in Africa, where IPT with SP is recommended [77]. Therefore, it is important to continuously monitor the markers for SP resistance.

Conclusion

The findings of this study report a decline in the efficacy of ASAQ, while AL remains highly effective, supporting the recent decision by NMCP to replace ASAQ with AL as first-line treatment for uncomplicated falciparum malaria. There are no parasites carrying signatures known to be associated with artemisinin and piperaquine resistance. No association between the presence of the mutations in *Pfprt* and *Pfmdr-1* and the risk of parasite recrudescence in patients treated with ASAQ was observed. The very low frequency of the *Pfdhfr/Pfdhps* five-mutant haplotype supports the continued use of SP as an IPTp. The therapeutic efficacy of recommended artemisinin-based combination, molecular markers of resistance to artemisinin, partner drugs and SP should be closely monitored for early detection of resistant parasites and development of evidence-based malaria treatment and chemoprevention strategies.

Abbreviations

ACPR: Adequate clinical and parasitological response; AL: Artemether–lumefantrine; ASAQ: Artesunate–amodiaquine; ASMQ: Artesunate–mefloquine; ASPY: Artesunate–pyronaridine; ASSP: Artesunate–sulfadoxine/pyrimethamine; DP: Dihydroartemisinin–piperaquine; ETF: Early treatment failure; gCHVs: General Community Health Volunteers; *glurp*: Glutamate-rich protein; IPTp: Intermittent preventive treatment of pregnant women; LCF: Late clinical failure; LPF: Late parasitological failure; MDA: Mass Drug Administration; *Pfprt*: *Plasmodium falciparum* chloroquine resistance transporter; *Pfmdr-1*: *Plasmodium falciparum* multi drug resistance 1; *Pfkelch13*: *Plasmodium falciparum* Kelch13; *Pfpm2*: Plasmepsin-2; *Pfdhfr*: *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase; *Pfdhps*: *Plasmodium falciparum* dihydropteroate synthetase; HQ: Headquarters; MOH: Ministry of Health; *msp1*: Merozoite surface proteins 1; *msp2*: Merozoite surface proteins 2; NMCP: National Malaria Control Programme; PCR: Polymerase chain reaction; WBC: White Blood Cell; WHO: World Health Organization.

Supplementary Information

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Additional file 1. Liberia genotyping raw data.

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Disclaimer

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Authors' contributions

VSK, BV, MKJ and PN conceived and designed the study. BV, VSK, MKJ, FT, LT, PN and OJP implemented the study, supervised data collection, and ensured quality control of the study. SP, PK, MA, AK managed, coordinated and supervised their respective study sites. FT and LT supervised and monitored laboratory and clinical staff for the purpose of quality control. MW and PR provided technical support and ensured data validation and analysis. LM and DM performed parasite genotyping to distinguish between recrudescence and reinfection, as well as analysis of molecular markers for artemisinin and partner drugs, and SP resistance. MW led the writing of the manuscript with contributions from PR, VSK, BV and DM. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset used in this study is available and can be shared upon reasonable request to NMCP through the corresponding author.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by the University of Liberia-Pacific Institute for Research & Evaluation the Institution Review Board (UL-PIRE IRB_ FWA00004982) and the WHO Research Ethics Review Committee (ERC.0002892). Written informed consent was obtained from parent/guardian before enrolling their children in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

- WHO. World Malaria Report 2020. Geneva, World Health Organization; 2020. <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020>.
- WHO. Guidelines for malaria. Geneva, World Health Organization; 2021. <https://www.who.int/publications/i/item/guidelines-for-malaria>
- WHO. 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>.
- Warsame M, Hassan AH, Hassan AM, Arale AM, Jibril AM, Mohamud SA, et al. Efficacy of artesunate + sulphadoxine/pyrimethamine and artemether + lumefantrine and dhfr and dhps mutations in Somalia: evidence for updating the malaria treatment policy. *Trop Med Int Health*. 2017;22:415–22.
- Mishra N, Kaitholia K, Srivastava B, Shah NK, Narayan JP, Dev V, et al. Declining efficacy of artesunate plus sulphadoxine-pyrimethamine in northeastern India. *Malar J*. 2014;13:284.
- Phyo AP, Ashley EA, Anderson TJC, Bozdech Z, Carrara I, Sriprawat K, et al. Declining efficacy of artemisinin combination therapy against *P. falciparum* malaria on the Thai-Myanmar Border (2003–2013): the role of parasite genetic factors. *Clin Infect Dis*. 2016;63:784–91.
- Imwong M, Suwannasin K, Kunasol C, Sutawong K, Mayxay M, Rekol H, et al. The spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis*. 2017;17:491–7.
- van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, et al. Determinants of dihydroartemisinin-piperazine treatment failure in *Plasmodium falciparum* malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *Lancet Infect Dis*. 2019;19:952–61.
- Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 2009;361:455–67.
- 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;505:50–5.
- Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med*. 2014;371:411–23.
- Uwimana A, Legrand E, Stokes BH, Ndikumana JM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat Med*. 2020;26:1602–8.
- Uwimana A, Umulisa N, Venkatesan M, Svigel SS, Zhou Z, Munyaneza T, et al. Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicentre, therapeutic efficacy study. *Lancet Infect Dis*. 2021;1:1473.
- Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, et al. Evidence of artemisinin-resistant malaria in Africa. *N Engl J Med*. 2021;385:1163–71.
- Witkowski B, Duru V, Khim N, Ross LS, Saintpierre B, Beghain J, et al. A surrogate marker of piperazine-resistant *Plasmodium falciparum* malaria: a phenotype-genotype association study. *Lancet Infect Dis*. 2017;17:174–83.

16. Amato R, Lim P, Miotto O, Amaratunga C, Dek D, Pearson RD, et al. Genetic markers associated with dihydroartemisinin–piperaquine failure in *Plasmodium falciparum* malaria in Cambodia: a genotype-phenotype association study. *Lancet Infect Dis*. 2017;17:164–73.
17. Thanh NV, Thuy-Nhien N, Tuyen NT, Tong NT, Nha-Ca NT, Dong LT, et al. Rapid decline in the susceptibility of *Plasmodium falciparum* to dihydroartemisinin–piperaquine in the south of Vietnam. *Malar J*. 2017;16:27.
18. Phuc BQ, Rasmussen C, Duong TT, Dong LT, Loi MA, Ménard D, et al. Treatment failure of dihydroartemisinin/piperaquine for *Plasmodium falciparum* malaria. *Vietnam Emerg Infect Dis*. 2017;23:715–7.
19. Gupta H, Galatas B, Chidimatembue A, Huijben S, Cisteró P, Matambisso G, et al. Effect of mass dihydroartemisinin–piperaquine administration in southern Mozambique on the carriage of molecular markers of antimalarial resistance. *PLoS ONE*. 2020;15:e0240174.
20. Leroy D, Macintyre F, Adoke Y, Ouoba S, Barry A, Mombo-Ngoma G, et al. African isolates show a high proportion of multiple copies of the *Plasmodium falciparum* plasmepsin-2 gene, a piperaquine resistance marker. *Malar J*. 2019;18:126.
21. Menard D, Dondorp A. Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harb Perspect Med*. 2017;7:a025619.
22. Arya A, Kojom Foko LP, Chaudhry S, Sharma A, Singh V. Artemisinin-based combination therapy (ACT) and drug resistance molecular markers: a systematic review of clinical studies from two malaria endemic regions - India and sub-Saharan Africa. *Int J Parasitol Drugs Drug Resist*. 2021;15:43–56.
23. Rasmussen C, Alonso P, Ringwald P. Current and emerging strategies to combat antimalarial resistance. *Expert Rev Anti Infect Ther*. 2021;1:1–20.
24. WHO. Evidence Review Group: Intermittent Preventive Treatment of Malaria in Pregnancy (IPTp) with Sulfadoxine- Pyrimethamine (SP). Geneva: World Health Organization, 2012. http://www.who.int/malaria/mpac/sep2012/iptp_sp_erg_meeting_report_july2012.pdf.
25. Plowe CV. The evolution of drug-resistant malaria. *Trans R Soc Trop Med Hyg*. 2009;103(Suppl 1):S11–4.
26. Kublin JG, Dzinjalimala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, et al. Molecular markers for failure of sulfadoxinepyrimethamine and chlorproguanil-dapsone treatment of *Plasmodium falciparum* malaria. *J Infect Dis*. 2002;185:380–8.
27. Happi CT, Gbotosho GO, Folarin OA, Akinboye DO, Yusuf BO, Ebong OO, et al. Polymorphisms in *Plasmodium falciparum* dhfr and dhps genes and age related in vivo sulfadoxine-pyrimethamine resistance in malaria-infected patients from Nigeria. *Acta Trop*. 2005;95:183–93.
28. Gesase S, Gosling RD, Hashim R, Ord R, Naidoo I, Madebe R, et al. High resistance of *Plasmodium falciparum* to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at Codon 581. *PLoS One*. 2009;4:e4569.
29. Harrington W, Mutabingwa T, Muehlenbachs A, Sorensen B, Bolla M, Fried M, et al. Competitive facilitation of drug-resistant *Plasmodium falciparum* malaria parasites in pregnant women who receive preventive treatment. *Proc Natl Acad Sci USA*. 2009;106:9027–32.
30. Chico RM, Cano J, Ariti C, Collier TJ, Chandramohan D, Roper C, et al. Influence of malaria transmission intensity and the 581G mutation on the efficacy of intermittent preventive treatment in pregnancy: systematic review and meta-analysis. *Trop Med Int Health*. 2015;20:1621–33.
31. Gosling RD, Gesase S, Moshia JF, Carneiro I, Hashim R, Lemnge M, et al. Protective efficacy and safety of three antimalarial regimens for intermittent preventive treatment for malaria in infants: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2009;374:1521–32.
32. Ministry of Health. Liberia Health Facility Survey report 2018. National Malaria Control Programme. Monrovia, 2018.
33. Ministry of Health. Liberia Malaria Indicator Survey. National Malaria Control Programme. Monrovia, 2016. <https://dhsprogram.com/pubs/pdf/MIS27/MIS27.pdf>
34. Ministry of Health. Technical Guidelines for Malaria Case management and malaria in pregnancy. National Malaria Control Programme. Monrovia. 2020.
35. Schramm B, Valeh P, Baudin E, Mazinda CS, Smith R, Pinoges L, et al. Efficacy of Artesunate–amodiaquine and Artemether–lumefantrine fixed-dose combinations for the treatment of uncomplicated *Plasmodium falciparum* malaria among children aged six to 59 months in Nimba County, Liberia: an open-label randomized non-inferiority trial. *Malar J*. 2013;12:251.
36. WHO. Methods for Surveillance of Antimalarial Drug Efficacy. Geneva: World Health Organization; 2009. <https://www.who.int/malaria/publications/atoz/9789241597531/en/>
37. Cattamanchi A, Kyabayinze D, Hubbard A, Rosenthal PJ, Dorsey G. Distinguishing recrudescence from reinfection in a longitudinal antimalarial drug efficacy study: comparison of results based on genotyping of msp-1, msp-2, and glurp. *Am J Trop Med Hyg*. 2003;68:133–9.
38. WHO. Methods and techniques for clinical trials on antimalarial drug efficacy: genotyping to identify parasite populations: informal consultation organized by the Medicines for Malaria Venture and cosponsored by the WHO, 29–31 May 2007, Amsterdam, The Netherlands. Geneva: World Health Organization; 2008. <https://apps.who.int/iris/handle/10665/43824>.
39. Jones S, Kay K, Hodel EM, Chy S, Mbituyumuremyi A, Uwimana A, et al. Improving methods for analyzing antimalarial drug efficacy trials: molecular correction based on length-polymorphic markers msp-1, msp-2, and glurp. *Antimicrob Agents Chemother*. 2019;63:e00590-e619.
40. Andriantsoanirina V, Lascombes V, Ratsimbao A, Bouchier C, Hoffman J, Tichit M, et al. Rapid detection of point mutations in *Plasmodium falciparum* genes associated with antimalarial drugs resistance by using High-Resolution Melting analysis. *J Microbiol Methods*. 2009;78:165–70.
41. Stokes BH, Dhingra SK, Rubiano K, Mok S, Straimer J, Gnädig NF, et al. *Plasmodium falciparum* K13 mutations in Africa and Asia impact artemisinin resistance and parasite fitness. *Elife*. 2021;10:e66277.
42. Smith SJ, Kamara ARY, Sahr F, Samai M, Swaray AS, Menard D, et al. Efficacy of artemisinin-based combination therapies and prevalence of molecular markers associated with artemisinin, piperaquine and sulfadoxine-pyrimethamine resistance in Sierra Leone. *Acta Trop*. 2018;185:363–70.
43. Beavogui AH, Camara A, Delamou A, Diallo MS, Doumbouya A, Kourouma K, et al. Efficacy and safety of Artesunate–amodiaquine and Artemether–lumefantrine and prevalence of molecular markers associated with resistance, Guinea: an open-label two-arm randomised controlled trial. *Malar J*. 2020;19:223.
44. Konaté A, Barro-Kiki PCM, Angora KE, Bédia-Tanoh AV, Djohan V, Kassi KF, et al. Efficacy and tolerability of Artesunate–amodiaquine versus Artemether–lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria at two sentinel sites across Côte d'Ivoire. *Ann Parasitol*. 2018;64:49–57.
45. Ebenebe JC, Ntadom G, Ambe J, Wammanda R, Jiya N, Finomo F, et al. Efficacy of artemisinin-based combination treatments of uncomplicated falciparum malaria in under-five-year-old Nigerian children ten years following adoption as first-line antimalarials. *Am J Trop Med Hyg*. 2018;99:649–64.
46. Abuaku B, Duah-Quashie NO, Quaye L, Matrevi SA, Quashie N, Gyasi A, et al. Therapeutic efficacy of Artesunate–amodiaquine and Artemether–lumefantrine combinations for uncomplicated malaria in 10 sentinel sites across Ghana: 2015–2017. *Malar J*. 2019;18:206.
47. Grandesso F, Guindo O, Woi Messe L, Makarimi R, Traore A, Dama S, et al. Efficacy of artesunate–amodiaquine, dihydroartemisinin–piperaquine and artemether–lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Maradi Niger. *Malar J*. 2018;17:52.
48. Lingani M, Bonkian LN, Yerbanga I, Kazienga A, Valéa I, Sorgho H, et al. In vivo/ex vivo efficacy of Artemether–lumefantrine and Artesunate–amodiaquine as first-line treatment for uncomplicated falciparum malaria in children: an open label randomized controlled trial in Burkina Faso. *Malar J*. 2020;19:8.
49. Diallo MA, Yade MS, Ndiaye YD, Diallo I, Diongue K, Sy SA, et al. Efficacy and safety of artemisinin-based combination therapy and the implications of Pfk13 and Pfcoronin molecular markers in treatment failure in Senegal. *Sci Rep*. 2020;10:8907.
50. Dama S, Niangaly H, Djimde M, Sagara I, Guindo CO, Zeguime A, et al. A randomized trial of dihydroartemisinin–piperaquine versus Artemether–lumefantrine for treatment of uncomplicated *Plasmodium falciparum* malaria in Mali. *Malar J*. 2018;17:347.
51. Souleymane I, Clément KH, Denis MM, Berenger A, Baba C, Offianan TA, et al. Therapeutic efficacy of Artesunate–amodiaquine and polymorphism of *Plasmodium falciparum* k13-propeller gene in Pala (Tchad). *Int J Open Access Trials*. 2017;1:1–6.
52. de Wit M, Funk AL, Moussally K, Nkuba DA, Siddiqui R, Bil K, et al. In vivo efficacy of Artesunate–amodiaquine and Artemether–lumefantrine for the treatment of uncomplicated falciparum malaria: an

- open-randomized, non-inferiority clinical trial in South Kivu Democratic Republic of Congo. *Malar J.* 2016;15:455.
53. Davlantes E, Dimbu PR, Ferreira CM, Florinda Joao M, Poda D, Félix J, et al. Efficacy and safety of Artemether–lumefantrine, Artesunate–amodiaquine, and dihydroartemisinin–piperazine for the treatment of uncomplicated *Plasmodium falciparum* malaria in three provinces in Angola, 2017. *Malar J.* 2018;17:144.
 54. Adegbite BR, Edoa JR, Honkpehedji YJ, Zinsou FJ, Dejon-Agobe JC, Mbong-Ngwese M, et al. Monitoring of efficacy, tolerability and safety of Artemether–lumefantrine and Artesunate–amodiaquine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Lambaréné, Gabon: an open-label clinical trial. *Malar J.* 2019;18:424.
 55. Riloha Rivas M, Warsame M, Mbā Andeme R, Nsue Esidang S, Ncogo PR, Phiri WP, et al. Therapeutic efficacy of Artesunate–amodiaquine and Artemether–lumefantrine and polymorphism in *Plasmodium falciparum* kelch13-propeller gene in Equatorial Guinea. *Malar J.* 2021;20:275.
 56. Roth JM, Sawa P, Makio N, Omweri G, Osoti V, Okach S, et al. Pyronaridine-artesunate and Artemether–lumefantrine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Kenyan children: a randomized controlled non-inferiority trial. *Malar J.* 2018;17:199.
 57. Mandara CI, Francis F, Chiduo MG, Ngasala B, Mandike R, Mkude S, et al. High cure rates and tolerability of Artesunate–amodiaquine and dihydroartemisinin–piperazine for the treatment of uncomplicated falciparum malaria in Kibaha and Kigoma Tanzania. *Malar J.* 2019;18:99.
 58. Ishengoma DS, Mandara CI, Francis F, Talundzic E, Lucchi NW, Ngasala B, et al. Efficacy and safety of Artemether–lumefantrine for the treatment of uncomplicated malaria and prevalence of Pfkclh13 and Pfm-dr-1 polymorphisms after a decade of using artemisinin-based combination therapy in mainland Tanzania. *Malar J.* 2019;18:88.
 59. Yeka A, Kigozi R, Conrad MD, Lugemwa M, Okui P, Katureebe C, et al. Artesunate/amodiaquine versus artemether/lumefantrine for the treatment of uncomplicated malaria in Uganda: a randomized trial. *J Infect Dis.* 2016;213:1134–42.
 60. Yeka A, Wallender E, Mulebeke R, Kibuuka A, Kigozi R, Bosco A, et al. Comparative efficacy of Artemether–lumefantrine and dihydroartemisinin–piperazine for the treatment of uncomplicated malaria in Ugandan children. *J Infect Dis.* 2019;219:1112–20.
 61. Uwimana A, Penkunas MJ, Nisingizwe MP, Warsame M, Umulisa N, Uyizwe D, et al. Efficacy of Artemether–lumefantrine versus dihydroartemisinin–piperazine for the treatment of uncomplicated malaria among children in Rwanda: an open-label, randomized controlled trial. *Trans R Soc Trop Med Hyg.* 2019;113:312–9.
 62. Mvumbi DM, Bobanga TL, Kayembe JN, Mvumbi GL, Situakibanza HN, Benoit-Vical F, et al. Molecular surveillance of *Plasmodium falciparum* resistance to artemisinin-based combination therapies in the Democratic Republic of Congo. *PLoS One.* 2017;12:e0179142.
 63. Hemming-Schroeder E, Umukoro E, Lo E, Fung B, Tomás-Domingo P, Zhou G, et al. Impacts of antimalarial drugs on *Plasmodium falciparum* drug resistance markers, Western Kenya, 2003–2015. *Am J Trop Med Hyg.* 2018;98:692–9.
 64. Raman J, Kagoro FM, Mabuza A, Malatje G, Reid A, Freaun J, et al. Absence of kelch13 artemisinin resistance markers but strong selection for lumefantrine-tolerance molecular markers following 18 years of artemisinin-based combination therapy use in Mpumalanga Province, South Africa (2001–2018). *Malar J.* 2019;18:280.
 65. Ahouidi A, Oliveira R, Lobo L, Diedhiou C, Mboup S, Nogueira F. Prevalence of Pfkclh13 and Pfm-dr-1 polymorphisms in Bounkiling. *Southern Senegal PLoS One.* 2021;16:e0249357.
 66. Braun V, Rempis E, Schnack A, Decker S, Rubaihayo J, Tumwesigye NM, et al. Lack of effect of intermittent preventive treatment for malaria in pregnancy and intense drug resistance in western Uganda. *Malar J.* 2015;14:372.
 67. Gutman J, Kalilani L, Taylor S, Zhou Z, Wiegand RE, Thwai KL, et al. The A581G mutation in the gene encoding *Plasmodium falciparum* dihydropteroate synthetase reduces the effectiveness of sulfadoxine-pyrimethamine preventive therapy in Malawian pregnant women. *J Infect Dis.* 2015;211:1997–2005.
 68. Naidoo I, Roper C. Mapping “partially resistant”, “fully resistant”, and “super resistant” malaria. *Trends Parasitol.* 2013;29:505–15.
 69. Dicko A, Sagara I, Djimdé AA, Touré SO, Traore M, Dama S, et al. Molecular markers of resistance to sulfadoxine-pyrimethamine one year after implementation of intermittent preventive treatment of malaria in infants in Mali. *Malar J.* 2010;9:9.
 70. Afutu LL, Boampong JN, Quashie NB. High prevalence of molecular markers of *Plasmodium falciparum* resistance to sulphadoxine-pyrimethamine in parts of Ghana: a threat to IPTp-SP? *J Trop Pediatr.* 2021;67:120.
 71. Fagbemi KA, Adebunsi SA, Nderu D, Adedokun SA, Pallerla SR, Amoo AOJ, et al. Analysis of sulphadoxine-pyrimethamine resistance-associated mutations in *Plasmodium falciparum* isolates obtained from asymptomatic pregnant women in Ogun State Southwest Nigeria. *Infect Genet Evol.* 2020;85:104503.
 72. Pacheco MA, Schneider KA, Cheng Q, Munde EO, Ndege C, Onyango C, et al. Changes in the frequencies of *Plasmodium falciparum* dhps and dhfr drug-resistant mutations in children from Western Kenya from 2005 to 2018: the rise of Dhps S436H. *Malar J.* 2020;19:378.
 73. Gikunju SW, Agola EL, Ondondo RO, Kinyua J, Kimani F, LaBeaud AD, et al. Prevalence of pfdhfr and dhps mutations in *Plasmodium falciparum* associated with drug resistance among pregnant women receiving IPTp-SP at Msambweni County Referral Hospital, Kwale County. *Kenya Malar J.* 2020;19:190.
 74. Bwire GM, Mikomangwa WP, Kilonzi M. Occurrence of septuple and elevated Pfdhfr-Dhps quintuple mutations in a general population threatens the use of sulfadoxine-pyrimethamine for malaria prevention during pregnancy in eastern-coast of Tanzania. *BMC Infect Dis.* 2020;20:530.
 75. Tumwebaze P, Tukwasibwe S, Taylor A, Conrad M, Ruhamyankaka E, Asua V, et al. Changing antimalarial drug resistance patterns identified by surveillance at three sites in Uganda. *J Infect Dis.* 2017;215:631–5.
 76. Asua V, Conrad MD, Aydemir O, Duvalsaunt M, Legac J, Duarte E, et al. Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. *J Infect Dis.* 2021;223:985–94.
 77. Amimo F, Lambert B, Magit A, Sacarlal J, Hashizume M, Shibuya K. *Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine in Africa: a systematic analysis of national trends. *BMJ Glob Health.* 2020;5:e003217.

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