Prevalence of *Plasmodium falciparum* haplotypes associated with resistance to sulfadoxine–pyrimethamine and amodiaquine before and after upscaling of seasonal malaria chemoprevention in seven African countries: a genomic surveillance study

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Summary

Background Seasonal malaria chemoprevention is used in 13 countries in the Sahel region of Africa to prevent malaria in children younger than 5 years. Resistance of *Plasmodium falciparum* to seasonal malaria chemoprevention drugs across the region is a potential threat to this intervention.

Methods Between December, 2015, and March, 2016, and between December, 2017, and March, 2018, immediately following the 2015 and 2017 malaria transmission seasons, community surveys were done among children younger than 5 years and individuals aged 10–30 years in districts implementing seasonal malaria chemoprevention with sulfadoxine–pyrimethamine and amodiaquine in Burkina Faso, Chad, Guinea, Mali, Nigeria, Niger and The Gambia. Dried blood samples were collected and tested for *P falciparum* DNA by PCR. Resistance-associated haplotypes of the *P falciparum* genes *crt*, *mdr1*, *dhfr*, and *dhps* were identified by quantitative PCR and sequencing of isolates from the collected samples, and survey-weighted prevalence and prevalence ratio between the first and second surveys were estimated for each variant.

Findings 5130 (17·5%) of 29274 samples from 2016 and 2176 (7·6%) of 28546 samples from 2018 were positive for *P falciparum* on quantitative PCR. Among children younger than 5 years, parasite carriage decreased from 2844 of 14345 samples (19·8% [95% CI 19·2–20·5]) in 2016 to 801 of 14019 samples (5·7% [5·3–6·1]) in 2018 (prevalence ratio 0·27 [95% CI 0·24–0·31], p<0·0001). Genotyping found no consistent evidence of increasing prevalence of amodiaquine resistance-associated variants of *crt* and *mdr1* between 2016 and 2018. The *dhfr* haplotype IRN (consisting of 51Ile-59Arg-108Asn) was common at both survey timepoints, but the *dhps* haplotype ISGEAA (431Ile-436Ser-437Gly-540Glu-581Ala-613Ala), crucial for resistance to sulfadoxine–pyrimethamine, was always rare. Parasites carrying amodiaquine resistance-associated variants of both *crt* and *mdr1* together with *dhfr* IRN and *dhps* ISGEAA occurred in 0·05% of isolates. The emerging *dhps* haplotype VAGKGS (431Val-436Ala-437Gly-540Lys-581Gly-613Ser) was present in four countries.

Interpretation In seven African countries, evidence of a significant reduction in parasite carriage among children receiving seasonal malaria chemoprevention was found 2 years after intervention scale-up. Combined resistance-associated haplotypes remained rare, and seasonal malaria chemoprevention with sulfadoxine–pyrimethamine and amodiaquine is expected to retain effectiveness. The threat of future erosion of effectiveness due to *dhps* variant haplotypes requires further monitoring.

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Introduction

The burden of morbidity and mortality due to malaria continues to challenge health systems throughout Africa. In 2012, WHO recommended seasonal malaria chemoprevention in areas of high seasonal malaria transmission in the Sahel subregion of Africa to clear parasites and prevent infection.[1] This intervention is delivered to all children aged 3–59 months in the community, regardless of malaria status, as a single dose of sulfadoxine–pyrimethamine and three daily doses of amodiaquine, monthly for up to 5 months during the short transmission season. Meta-analysis of clinical trial data provides an estimated mean decrease in clinical malaria episodes per child per year of 75% with seasonal

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Research in context

Evidence before this study

We sought detailed molecular studies of resistance to
sulfadoxine–pyrimethamine and amodiaquine in *Plasmodium
falciparum* in populations implementing seasonal malaria
chemoprevention. We searched PubMed using the following
text: (seasonal malaria chemoprevention) AND (*Plasmodium
falciparum*) AND (molecular markers) AND (children) AND (drug
resistance). No date or language restrictions were applied. The
search yielded eight studies, of which five were conducted in one
of the seven countries that participated in the Achieving
Catalytic Expansion of Seasonal Malaria Chemoprevention in the
Sahel project, which sought to remove barriers to the scale-up of
seasonal malaria chemoprevention in seven countries in 2015
and 2016. These studies included genotype analyses of
P falciparum isolates from 201 children with fever with a positive
rapid diagnostic test in Niger; 394 men and four women with
fever in Chad; and 1164 children younger than 5 years sampled
cross-sectionally in the community over 3 years of seasonal
malaria chemoprevention implementation in Mali. One study
conducted *k13* and *mdr1* genotyping among 27 children PCR
positive for *P falciparum* DNA receiving seasonal malaria
chemoprevention in Burkina Faso. None of these results were
available before the current study. Our earlier work assessing
dhfr and *dhps* genotypes in 1000 PCR-positive samples from
pregnant women and children with uncomplicated malaria in
Nigeria was available and informed our study design. There were
no population-level studies available that permitted systematic
comparison of parasite genotypes under selective pressure from
programmatic implementation of seasonal malaria
chemoprevention in the Sahel.

Added value of this study

This study provides a comprehensive, high-throughput
assessment of *P falciparum* genotype variation at all four
parasite genes known to contribute to resistance to the seasonal
malaria chemoprevention drugs (amodiaquine and sulfadoxine–
pyrimethamine) across seven countries implementing seasonal
malaria chemoprevention in the African Sahel at the outset of
scale-up in 2015–16. Both the target age group of children
younger than 5 years and older residents who would not have
received the study drugs were sampled. This assessment was
repeated 2 years later in 2017–18, using identical sampling and
genotyping methodologies, permitting direct comparison
between the two sample periods.

Implications of all the available evidence

This study, and previous smaller studies in the region, provide
substantial evidence that seasonal malaria chemoprevention
with sulfadoxine–pyrimethamine and amodiaquine is not
currently under a serious threat from drug-resistant parasites in
these implementation areas. However, the data indicate that
continuing surveillance is needed to guard against future
emergence of resistance to an extent that would threaten the
effectiveness of seasonal malaria chemoprevention. Our data
provide a comprehensive baseline in seven locations across the
regions where seasonal malaria chemoprevention is being
deployed at scale. The surveys could be repeated, using the
same sampling and laboratory methods, to monitor the effect
of seasonal malaria chemoprevention at scale on the
frequencies of markers of resistance and to provide early
warning of loss of effectiveness.

malaria chemoprevention compared with placebo, and a
modest beneficial effect on the prevalence of anaemia.[2]
According to WHO, 13 countries in the African Sahel had
active seasonal malaria chemoprevention programmes
in 2021.[1]

Sulfadoxine–pyrimethamine and amodiaquine are
known to select specific variants of *Plasmodium falciparum*
resistance genes currently circulating in Africa, which
might reduce the effectiveness of seasonal malaria
chemoprevention. These variants can be monitored by
studies of molecular markers. Amodiaquine resistance is
associated with mutations in the *P falciparum* genes
chloroquine transporter (*crt*) and multidrug resistance
gene 1 (*mdr1*).[3] Variant haplotypes of *crt* at codons 72–76,
encoding 72Cys-73Val-74Ile-75Glu-76Thr (CVIET) and
72Ser-73Val-74Met-75Asn-76Thr (SVMNT), and of *mdr1*
(encoding Tyr at codons 86, 184, and 1246 [YYY]) are
associated with amodiaquine resistance in therapeutic
studies.[4] A variety of point mutations in *P falciparum*
dihydropteroate synthase (*dhps*) confer resistance to
sulfadoxine and point mutations in dihydrofolate reductase
(*dhfr*) confer resistance to pyrimethamine. In Africa, the
combined haplotype GE-IRN, comprising mutations in

both *dhps* (encoding 437Gly and 540Glu [GE]) and *dhfr*
(51Ile, 59Arg, and 108Asn [IRN]), is known to be strongly
associated with sulfadoxine–pyrimethamine resistance.[5]
To date, this GE-IRN variant haplotype has been very rare
in west Africa compared to east and southern Africa,[6–10] but
evidence acquired since 2009 suggests that other
haplotypes of *dhps*, in particular 431Val-436Ala-437Gly-
540Lys-581Gly-613Ala (VAGKGA) and 431Val-436Ala-
437Gly-540Lys-581Gly-613Ser (VAGKGS), are emerging in
Nigeria and Cameroon.[8,11]

The widespread deployment of sulfadoxine–
pyrimethamine and amodiaquine for seasonal malaria
chemoprevention might select for resistant parasite
variants, leading to a progressive loss of efficacy of this
chemoprevention. Therefore, it is essential that seasonal
malaria chemoprevention programmes incorporate a
resistance-monitoring component. We provide a com-
prehensive assessment of *P falciparum* resistance
genotypes, and assemble complex multigenic haplotypes
present at baseline in seven countries of the African
Sahel, and again after 2 years of large-scale expansion of
seasonal malaria chemoprevention administration in the
same districts.[12]

Methods

Study sites

Community surveys for baseline genotype prevalence estimates were conducted from December 2015 to mid-March 2016, representing the period immediately following the Sahelian malaria transmission season of 2015, to measure the frequency of molecular markers associated with resistance to sulfadoxine–pyrimethamine plus amodiaquine. The surveys were done in the districts of Koupéla (Burkina Faso), Bokoro (Chad), Siguiri (Guinea), Ségou (Mali), and Gaya (Niger), the Anka local government area of Nigeria, and the Upper River region of The Gambia (figure 1), as previously described.[12] Seasonal malaria chemoprevention was implemented in each of the study sites at the time of the baseline survey, with the exception of Upper River Region of The Gambia, which had 1 year of seasonal malaria chemoprevention implementation before the 2016 survey. All seven countries deploy artemether–lumefantrine as the first-line therapeutic antimalarial drug.

Surveys for estimates of post-implementation genotype prevalence were done from December, 2017, to mid-March, 2018, immediately following the 2017 malaria season, in the same districts and using the same survey teams and procedures.[12]

The study was approved by the ethics committee in each country and by the observational ethics committee of the London School of Hygiene & Tropical Medicine (LSHTM; London, UK), as previously described.[12]

Survey design

Each survey included children younger than 5 years, to represent the age group that receive seasonal malaria chemoprevention (children who were aged 3–59 months when cycle one of seasonal malaria chemoprevention would have occurred), and individuals aged 10–30 years, an age group that would not receive seasonal malaria chemoprevention drugs and from whom parasites sampled would therefore represent those circulating in the general population. The target sample size of 2200 in each age group in each country was chosen to have sufficient power to detect important changes in prevalence of markers in the repeat surveys (at least 90% power to detect an odds ratio of 1·4 compared with baseline in the pooled analysis and an odds ratio of 2·5 in each country), and to be able to measure changes over time with adequate precision, between the 2016 and 2018 surveys. In each country, a sample representative of the population in the chosen district, local government area (Nigeria), or region (The Gambia) was selected with a two-stage cluster sample design. In each district, between 18 and 66 clusters (villages) were selected, with probability proportional to estimated population. Compact segment sampling was used to select the survey sample in each cluster. All individuals who slept the previous night in households within the selected segment were eligible for inclusion if they were
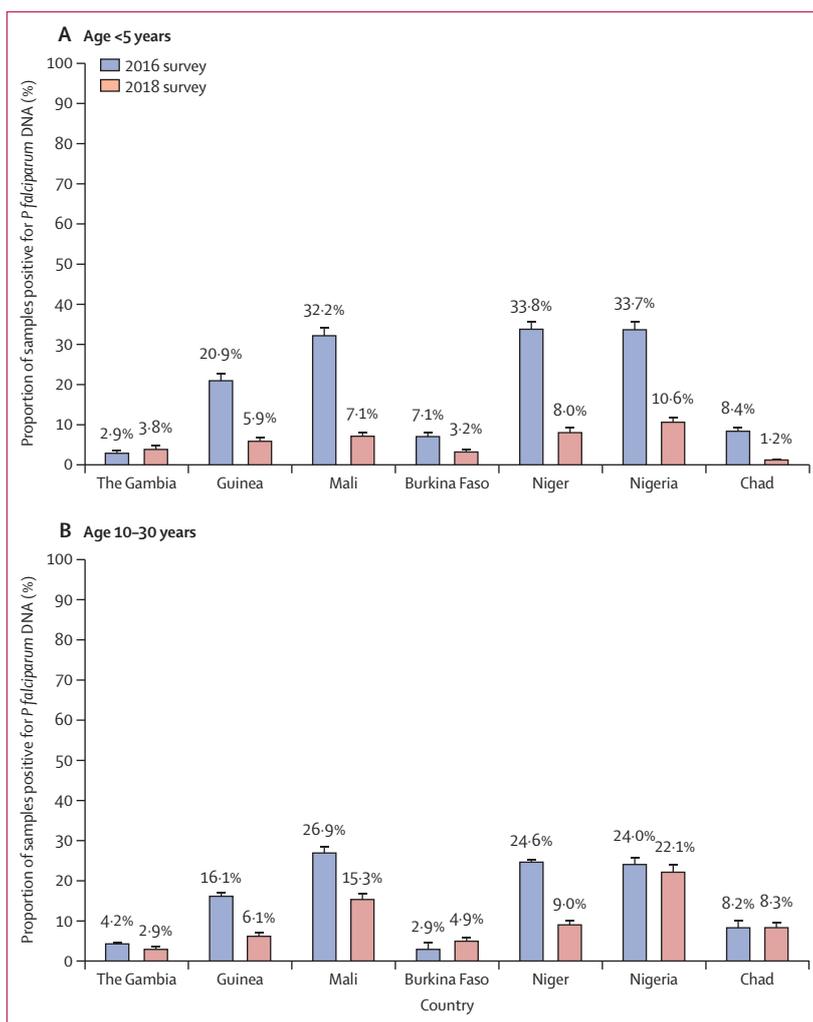
Figure 1: Proportion of participants with *Plasmodium falciparum* parasites detected by qPCR across seven Sahelian countries implementing seasonal malaria chemoprevention with sulfadoxine–pyrimethamine and amodiaquine (2016 and 2018)

Parasite prevalence estimates are shown for children younger than 5 years (A) and individuals aged 10–30 years (B), by country, from the 2016 and 2018 surveys. Error bars indicate the upper limit of the 95% CI for the proportions estimated from the binomial distribution.

within the target age ranges. This method of sampling was chosen to avoid the subjectivity of household listing and to allow easy repetition in future years by sampling the same areas again.[12]

Survey methods

Signed consent was obtained from each adult participant and from the parent or guardian of each child, after explanation of the aims and procedures of the survey. Verbal assent, in addition to parental signatures, was sought and documented from older children identified at the discretion of the field teams. A pre-printed label bearing a barcoded sample number was fixed to the filter paper used to collect a blood sample, and the barcode scanned and linked to the participant's data

using the tablet computer. To avoid any difference in blood sampling procedures, filter paper card and dried blood spot preparation was done in accordance with a single standard operating procedure, which was prepared at the LSHTM laboratory (London, UK) for the purpose of the current study and despatched to each site along with standardised collection materials (appendix p 10).

Blood sample preparation

Finger-prick blood from each participant was applied to filter paper (Whatman 3MM; ThermoFisher Scientific, Waltham, MA, USA) with a barcode attached. Each barcode was linked to a participant identification number, and the linkage list retained by field teams. Dried blood spot samples were attached to individual cardboard covers, assembled in batches of 50–100, and stored in a plastic bag containing silica gel. All dried blood spot samples were subsequently transported to the LSHTM laboratory.

Laboratory methods

DNA was extracted from all samples with use of a previously published protocol using a robotic extraction system.[13] Extracted DNA was stored at –20°C until use.

A modification of a previously published quantitative PCR (qPCR) assay method[14] was used to simultaneously detect *P falciparum* parasites and genotype the *P falciparum crt* locus. Three dual-labelled probes designed to detect three *crt* genotypes at codons 72–76 (encoding Cys-Val-Met-Asn-Lys [CVMNK], CVIET, and SVMNT) were combined with a fourth dual-labelled probe (cy5 reporter) to detect an extraction control target, the human β-tubulin gene.[15] Laboratory isolates 3D7, Dd2, and 7G8 were positive controls for the CVMNK, CVIET, and SVMNT haplotypes, respectively. qPCR amplification was done as a qualitative assay in a single well to maximise throughput in the 72-well rotor of a Rotorgene Q thermal cycler (Qiagen, Hilden, Germany).

All samples confirmed as positive for *P falciparum* DNA by *crt* qPCR were available for genotyping of *mdr1*, *dhfr*, and *dhps*. The three genes were amplified by nested PCR using previously described methods.[7,16,17] Polymorphisms were identified by direct sequencing of amplified products (BigDye Terminator v3.1 cycle sequencing kits and ABI 3730 sequencer; ThermoFisher Scientific) and data analysed using Geneious v10.1.3 (Biomatters, San Diego, CA, USA).

Statistical analysis

In each country and age group, we estimated the prevalence of *dhfr* mutations (the individual mutations 51Ile, 59Arg, and 108Asn, and the combined *dhfr* triple-mutant haplotype IRN); *dhps* mutations (431Val, 436Ala, 437Gly, 540Glu, 581Gly, and 613Ser, and combined *dhps* haplotypes, including VAGKGA and VAGKGS; the two-locus *dhfr* and *dhps* haplotype GE-IRN; *crt* mutations

(74Ile, 75Glu, and 76Thr, and the CVIET haplotype); *mdr1* mutations (86Tyr and 184Tyr, and the YY haplotype); and two-locus haplotype YY-CVIET, comprising mutations in *crt* (CVIET) and *mdr1* (YY); and the combined haplotype YY-CVIET-GE-IRN. We defined a genotype as one or more mutations in a single codon associated with resistance, and a haplotype as a combination of at least one mutation in each of two or more codons of interest in one or more genes.

For each mutation, and each combination of mutations, prevalence in each study year was estimated using a ratio estimator, and the prevalence ratios (fold increase in prevalence from the first to the second survey) and their 95% CIs were estimated with survey Poisson regression, using Stata (version 15; appendix p 2).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

DNA was extracted from 29274 samples (14345 from children younger than 5 years and 14929 from people aged 10–30 years) collected during the 2016 survey, and from 28546 samples (14019 from children younger than 5 years and 14527 from people aged 10–30 years) collected during the 2018 survey (table 1).[12] In 2016, 5130 (17·5%) samples were qPCR-positive for *P falciparum* DNA, with infection detected in 2844 of 14345 samples (19·8% [95% CI 19·2–20·5]) from children younger than 5 years and 2286 of 14929 samples (15·3% [14·7–15·9]) from people aged 10–30 years (appendix p 3). In 2018, 2176 (7·6%) samples were positive, with infection detected in 801 of 14019 (5·7% [5·3–6·1]) samples from children younger than 5 years and 1375 of 14527 (9·5% [9·0–10·0]) samples from people aged 10–30 years (appendix p 3).

Parasite prevalence by country and age group is shown in table 1 and figure 1, reported as a west-to-east transect from The Gambia to Chad. In 2016, parasite prevalence in people aged 10–30 years was lower than in children younger than 5 years in all countries except The Gambia, where seasonal malaria chemoprevention had already started. In 2018, parasite prevalence had decreased among children younger than 5 years in all seven countries, and was lower than the prevalence among people aged 10–30 years in all countries except The Gambia (table 1; figure 1; appendix p 3). Thus, the overall decrease in likelihood of being parasite positive by qPCR from 2016 to 2018 was particularly marked in the age group receiving seasonal malaria chemoprevention (prevalence ratio 0·27 [95% CI 0·24–0·31]) compared with sampled individuals aged 10–30 years (0·62 [0·55–0·71]; $p_{interaction} < 0.001$), providing good evidence of an important parasitological benefit from the implementation of seasonal malaria chemoprevention at scale.

	The Gambia	Guinea	Mali	Burkina Faso	Niger	Nigeria	Chad	Overall
2016								
Age <5 years	51/1752 (2·9%)	418/1996 (20·9%)	704/2189 (32·2%)	161/2281 (7·1%)	725/2146 (33·8%)	601/1786 (33·7%)	184/2195 (8·4%)	2844/14345 (19·8% [13·7])*
Age 10–30 years	85/2012 (4·2%)	305/1893 (16·1%)	585/2176 (26·9%)	65/2275 (2·9%)	570/2317 (24·6%)	495/2062 (24·0%)	181/2194 (8·2%)	2286/14929 (15·3% [10·2])*
2018								
Age <5 years	53/1383 (3·8%)	128/2182 (5·9%)	154/2156 (7·1%)	73/2264 (3·2%)	143/1783 (8·0%)	224/2110 (10·6%)	26/2141 (1·2%)	801/14019 (5·7% [3·2])*
Age 10–30 years	62/2159 (2·9%)	134/2191 (6·1%)	322/2106 (15·3%)	106/2168 (4·9%)	232/2586 (9·0%)	391/1770 (22·1%)	128/1547 (8·3%)	1375/14527 (9·5% [6·7])*
Total	..	..	..	..	..	..	..	7306/57820

Data are n/N (%), representing the number of qPCR positive tests out of the total number of samples for each age group and country. *Data in parentheses are mean [SD] across countries.

Table 1: Prevalence of *Plasmodium falciparum* DNA in 54907 dried blood spot samples collected across a west-to-east transect of seven countries in 2016 and 2018, in children younger than 5 years and in residents of the same compounds aged 10–30 years

The CVIET and YY haplotypes of *crt* and *mdr1* have been previously associated with amodiaquine resistance in Africa.[4,18,19] CVIET showed varying prevalence across the seven countries, fluctuating slightly or decreasing among children younger than 5 years between 2016 and 2018 in three countries, but markedly increasing in prevalence in Burkina Faso, Niger, Nigeria, and Chad (table 2; appendix p 4). Only in Burkina Faso was a marked increase from 2016 to 2018 also observed in the older age group (table 2). These patterns were reflected in the seven-country combined unadjusted prevalence ratios (table 3).

In all countries, in both age groups and in 2016 and 2018, the prevalence of *mdr1* 86Tyr was no higher than 25·6% (seven of 26 isolates, adjusted by survey weights), this value being for children younger than 5 years in Chad in 2018 (table 2). *mdr1* 184Tyr, the wild-type form but associated with the 86Tyr allele in amodiaquine-resistant parasites,[4] fluctuated between 0% (zero of 53 isolates, adjusted) and 47·7% (64 of 134 isolates, adjusted) prevalence across all countries. Although *mdr1* 184Tyr was relatively common, the haplotype comprising *mdr1* 86Tyr and 184Tyr (*mdr1* YY) occurred at a prevalence below 6% in all surveys across both years, and there was no evidence of an increase in prevalence after seasonal malaria chemoprevention scale-up (tables 2, 3).

At baseline across both age groups, the two-locus haplotype YY-CVIET (*mdr1* 86Tyr and 184Tyr and *crt* CVIET), which is associated with resistance to amodiaquine, was not observed in The Gambia, Burkina Faso, or Nigeria, and occurred at a prevalence of only 0·5% (two of 365 isolates, adjusted) in Chad, 0·3% (seven of 1295 isolates, adjusted) in Niger, 1·9% (32 of 1289 isolates, adjusted) in Mali, and 2·0% (16 of 723 isolates, adjusted) in Guinea (figure 2; appendix p 3). In each country, these prevalence estimates generally remained similar or fell after 2 years of seasonal malaria chemoprevention scale-up in both age groups in all seven countries, apart from a rise from 0% (0 of 161 isolates,

adjusted) to 2·0% (two of 73 isolates, adjusted) in children younger than 5 years in Burkina Faso (table 2).

The mutations Asn51Ile, Cys59Arg, and Ser108Asn in the *dhfr* gene, which are associated with pyrimethamine resistance,[20–26] were each common in all seven countries, with overall baseline prevalence estimates of 87·8% (3834 of 4369 single genotype isolates) for Asn51Ile, 87·4% (3668 of 4199 single genotype isolates) for Cys59Arg, and 90·8% (3998 of 4403 single genotype isolates) for Ser108Asn (table 2; appendix p 4). The triple mutation (IRN) was the most common haplotype, with frequency ranging from 59·4% (319 of 601 isolates, adjusted) in participants under 5 years in Nigeria to 98·4% (48 of 51 isolates, adjusted) in the same age group in The Gambia at baseline in 2016. The wild-type Asn51-Cys59-Ser108 haplotype was relatively uncommon, ranging in frequency from 11·2% (70 of 704 isolates, adjusted, in Mali) to 0·8% (two of 184 isolates, adjusted, in Chad). No mutations were observed at *dhfr* codons 140 and 164. The prevalence of the IRN haplotype of *dhfr* appeared higher in most countries in both age groups in the 2018 survey compared with the 2016 survey (prevalence ratio 1·42 [95% CI 0·58–3·46] in children younger than 5 years and 4·01 [1·63–9·83] in those aged 10–30 years, $p_{interaction}$=0·115).

In the 2016 survey, ten amino acid variants were commonly found encoded in the *dhps* gene across the six codons of interest: Ile431Val, Ser436Ala, Ser436Phe, Ser436Tyr, Ser436Cys, Ala437Gly, Lys540Glu, Ala581Gly, Ala613Thr, and Ala613Ser. In addition, six novel *dhps* mutations were discovered at low frequency: Ile470Thr (in Guinea); Gly425Asp, Ile451Met, and Ile141Met (in Niger); Asp575Ala (in Burkina Faso); and Ile466Val (The Gambia). In 2016, the mutant Ala437Gly was present across the combined age groups at high frequency in Burkina Faso (95·5%; 222 of 226 isolates, adjusted), Niger (92·9%; 1154 of 1193 isolates, adjusted), and The Gambia (82·0%; 115 of 135 isolates, adjusted), intermediate in Nigeria (73·1%; 684 of 896 isolates, adjusted) and Guinea

	2016							2018						
	The Gambia	Guinea	Mali	Burkina Faso	Niger	Nigeria	Chad	The Gambia	Guinea	Mali	Burkina Faso	Niger	Nigeria	Chad
Age <5 years														
crt CVIET	49·10%	62·24%	78·76%	29·81%	22·68%	11·64%	55·35%	53·49%	55·62%	51·16%	89·61%	82·86%	74·21%	86·38%
mdr1 86Tyr	0%	15·88%	18·66%	12·40%	7·02%	4·59%	23·78%	7·63%	7·08%	8·16%	11·98%	7·25%	1·45%	25·61%
mdr1 184Tyr	19·97%	40·14%	42·53%	28·74%	28·48%	33·79%	23·14%	0%	34·62%	34·62%	40·01%	13·60%	27·19%	29·02%
dhfr 51Ile	98·43%	86·67%	83·93%	92·86%	94·24%	85·54%	93·99%	95·99%	98·50%	84·22%	83·06%	92·87%	92·33%	88·61%
dhfr 59Arg	100·00%	83·63%	88·19%	98·34%	96·37%	82·13%	85·85%	98·28%	94·50%	86·86%	93·48%	93·57%	85·87%	100·00%
dhfr 108Asn	100·00%	91·44%	89·77%	98·87%	97·45%	81·45%	99·07%	100·00%	99·29%	99·35%	97·36%	96·86%	100·00%	100·00%
dhps 431Val	0%	0%	0·16%	0%	5·02%	4·50%	12·78%	0%	0%	0%	0%	3·59%	17·68%	3·12%
dhps 436Ala	11·06%	44·12%	63·19%	70·52%	61·86%	55·87%	79·01%	66·74%	38·16%	44·58%	55·33%	68·83%	66·22%	78·07%
dhps 437Gly	90·35%	79·17%	63·40%	97·94%	97·25%	75·09%	37·79%	97·70%	85·24%	91·79%	94·44%	99·30%	94·80%	71·34%
dhps 540Glu	0%	2·13%	0·30%	0%	0·39%	0%	0%	0%	3·77%	0%	0%	0·57%	0%	0%
dhps 581Gly	0%	0%	0·16%	1·12%	3·41%	4·68%	8·47%	0%	0%	0·62%	0%	4·47%	14·24%	4·37%
dhps 613Ser	0%	3·73%	9·38%	5·10%	12·29%	10·97%	9·03%	0%	5·59%	4·61%	10·87%	6·34%	22·88%	4·37%
mdr1 YY	0%	4·52%	5·84%	0%	1·01%	0%	0·53%	0%	0·83%	0·57%	2·01%	0%	0·52%	4·20%
YY-CVIET	0%	2·96%	3·49%	0%	0·24%	0%	0·54%	0%	0·92%	0·59%	2·01%	0%	0·53%	0%
dhfr IRN	98·38%	60·93%	75·41%	92·37%	90·91%	59·37%	77·80%	94·27%	93·71%	78·57%	73·89%	88·95%	82·61%	82·58%
dhps GE	0%	1·92%	0·29%	0%	0·38%	0%	0%	0%	3·77%	0%	0%	0·57%	0%	0%
GE-IRN	0%	1·61%	0·33%	0%	0·25%	0%	0%	0%	3·80%	0%	0%	0·61%	0%	0%
YY-CVIET-GE-IRN	0%	0·30%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
dhps VAGKGS	0%	0%	0·19%	0%	0·93%	0·75%	5·39%	0%	0%	0·44%	0%	1·48%	2·72%	14·05%
Age 10–30 years														
crt CVIET	67·63%	61·93%	76·26%	19·51%	18·34%	12·13%	59·88%	63·82%	57·36%	56·89%	58·77%	23·45%	14·86%	29·39%
mdr1 86Tyr	7·67%	14·13%	13·00%	6·05%	9·00%	4·98%	24·01%	1·20%	12·76%	14·43%	8·21%	6·46%	5·76%	9·63%
mdr1 184Tyr	30·87%	34·49%	45·42%	25·47%	29·13%	33·63%	26·51%	0%	47·65%	24·30%	11·47%	7·56%	30·81%	32·90%
dhfr 51Ile	97·56%	88·95%	83·77%	98·25%	92·75%	85·03%	95·91%	100·00%	96·28%	91·60%	96·91%	95·71%	88·28%	89·63%
dhfr 59Arg	96·10%	85·52%	88·46%	100·00%	96·67%	78·05%	89·99%	100·00%	97·07%	92·31%	100·00%	96·99%	95·29%	93·62%
dhfr 108Asn	99·21%	90·26%	88·80%	100·00%	97·21%	85·88%	99·31%	100·00%	99·25%	94·13%	100·00%	98·28%	96·93%	99·21%
dhps 431Val	0%	1·79%	0%	0%	2·31%	3·32%	8·92%	0%	0%	0·33%	0%	4·39%	15·92%	22·52%
dhps 436Ala	14·73%	43·59%	60·31%	79·97%	53·40%	48·95%	72·51%	57·00%	35·01%	62·74%	69·58%	89·52%	76·73%	84·24%
dhps 437Gly	81·54%	71·73%	59·39%	97·46%	96·61%	78·49%	39·79%	92·65%	82·14%	83·66%	71·48%	98·13%	96·39%	43·37%
dhps 540Glu	0%	1·83%	0%	0%	0%	0%	0%	0%	6·07%	0·61%	1·30%	0·83%	0·23%	0%
dhps 581Gly	0%	0%	0·17%	0%	3·65%	3·71%	7·68%	0%	0%	0·33%	0%	5·35%	13·28%	16·55%
dhps 613Ser	0%	4·69%	9·16%	3·28%	9·88%	10·34%	7·68%	1·42%	4·51%	12·17%	4·58%	11·11%	36·38%	18·14%
mdr1 YY	0%	1·98%	2·91%	0%	0·91%	0·30%	0·52%	0%	3·88%	1·59%	0·25%	0·82%	1·26%	2·19%
YY-CVIET	0%	1·36%	1·64%	0%	0·32%	0%	0·53%	0%	0·80%	0·68%	0%	0·42%	0%	0·56%
dhfr IRN	96·07%	61·30%	73·72%	98·08%	88·26%	61·13%	80·34%	100·00%	95·35%	89·16%	96·84%	95·28%	86·69%	85·42%
dhps GE	0%	1·63%	0%	0%	0%	0%	0%	0%	6·07%	0·61%	0·51%	0·83%	0%	0%
GE-IRN	0%	1·45%	0%	0%	0%	0%	0%	0%	6·31%	0·75%	0·53%	0·85%	0%	0%
YY-CVIET-GE-IRN	0%	0·42%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
dhps VAGKGS	0%	0%	0%	0%	0·37%	0·49%	4·81%	0%	0%	0·44%	0%	1·60%	2·72%	14·05%

Prevalence estimates incorporate survey weights as described in the Methods. crt CVIET=crt 72Cys-73Val-74Ile-75Glu-76Thr. YY=mdr1 86Tyr-184Tyr. YY-CVIET=mdr1 86Tyr-184Tyr and crt CVIET. IRN=dhfr 51Ile-59Arg-108Asn. GE=dhps 437Gly-540Glu. GE-IRN=dhfr 51Ile-59Arg-108Asn and dhps 437Gly-540Glu. VAGKGS=dhps 431Val-436Ala-437Gly-540Lys-581Gly-613Ser.

Table 2: Prevalence of genetic markers of sulfadoxine–pyrimethamine and amodiaquine resistance among samples PCR-positive for P falciparum DNA in 2016 and 2018 by country

|	Age <5 years			Age 10–30 years		
	Prevalence, 2016	Prevalence, 2018	Prevalence ratio, 2018:2016	Prevalence, 2016	Prevalence, 2018	Prevalence ratio, 2018:2016
crt CVIET	0·422 (0·378–0·467)	0·685 (0·643–0·725)	1·62 (1·43–1·84)*	0·428 (0·385–0·471)	0·364 (0·326–0·403)	0·85 (0·73–0·99)†
mdr1 86Tyr	0·118 (0·102–0·136)	0·066 (0·050–0·087)	0·56 (0·41–0·76)†	0·108 (0·093–0·124)	0·088 (0·072–0·108)	0·82 (0·65–1·04)
mdr1 184Tyr	0·342 (0·317–0·368)	0·263 (0·228–0·302)	0·77 (0·66–0·90)†	0·348 (0·322–0·374)	0·246 (0·216–0·278)	0·71 (0·61–0·82)†
dhfr 51Ile	0·888 (0·872–0·903)	0·914 (0·891–0·932)	1·03 (1·00–1·06)*	0·890 (0·870–0·907)	0·922 (0·902–0·939)	1·04 (1·01–1·07)*
dhfr 59Arg	0·891 (0·873–0·907)	0·905 (0·876–0·928)	1·02 (0·98–1·05)	0·886 (0·859–0·908)	0·954 (0·941–0·965)	1·08 (1·05–1·11)*
dhfr 108Asn	0·915 (0·888–0·936)	0·991 (0·982–0·995)	1·08 (1·05–1·11)*	0·920 (0·900–0·937)	0·973 (0·963–0·980)	1·06 (1·03–1·08)*
dhps 431Val	0·030 (0·023–0·040)	0·064 (0·043–0·093)	2·09 (1·30–3·39)*	0·022 (0·016–0·030)	0·079 (0·062–0·101)	3·66 (2·44–5·50)*
dhps 436Ala	0·591 (0·551–0·630)	0·581 (0·537–0·623)	0·98 (0·89–1·09)	0·531 (0·483–0·579)	0·712 (0·675–0·746)	1·34 (1·21–1·48)*
dhps 437Gly	0·780 (0·741–0·815)	0·928 (0·907–0·945)	1·19 (1·13–1·25)*	0·747 (0·704–0·785)	0·852 (0·824–0·876)	1·14 (1·07–1·22)*
dhps 540Glu	0·005 (0·003–0·008)	0·007 (0·003–0·015)	1·42 (0·58–3·46)	0·002 (0·001–0·006)	0·010 (0·006–0·016)	4·01 (1·63–9·83)*
dhps 581Gly	0·025 (0·019–0·032)	0·056 (0·039–0·079)	2·26 (1·43–3·58)*	0·023 (0·017–0·031)	0·067 (0·052–0·085)	2·96 (2·03–4·33)*
dhps 613Ser	0·091 (0·078–0·106)	0·109 (0·078–0·150)	1·19 (0·82–1·75)	0·082 (0·067–0·100)	0·183 (0·158–0·210)	2·23 (1·76–2·83)*
mdr1 YY	0·023 (0·016–0·033)	0·007 (0·003–0·016)	0·29 (0·12–0·72)†	0·013 (0·009–0·020)	0·015 (0·009–0·023)	1·11 (0·62–1·98)
YY-CVIET	0·013 (0·009–0·020)	0·005 (0·002–0·014)	0·41 (0·15–1·14)	0·007 (0·004–0·012)	0·003 (0·001–0·008)	0·50 (0·20–1·21)
dhfr IRN	0·754 (0·721–0·785)	0·849 (0·818–0·875)	1·12 (1·07–1·18)*	0·752 (0·713–0·788)	0·908 (0·885–0·926)	1·21 (1·14–1·27)*
dhps GE	0·004 (0·002–0·008)	0·007 (0·003–0·015)	1·84 (0·68–4·97)	0·002 (0·001–0·005)	0·010 (0·006–0·016)	4·78 (1·67–13·73)*
GE-IRN	0·000 (0·000–0·003)	0·000 (0·000–0·001)	..	0·001 (0·000–0·004)	0·000 (0·000–0·001)	..
YY-CVIET-GE-IRN	0·001 (0·000–0·004)	0·000 (0·000–0·001)	..	0·000 (0·000–0·003)	0·000 (0·000–0·001)	..
dhps VAGKGS	0·008 (0·005–0·012)	0·003 (0·001–0·012)	0·39 (0·09–1·57)	0·006 (0·003–0·011)	0·027 (0·015–0·047)	4·86 (2·07–11·44)*

Values in parentheses are 95% CIs. These analyses are not survey-weighted or adjusted for clustering. *crt* CVIET=*crt* 72Cys-73Val-74Ile-75Glu-76Thr. YY=*mdr1* 86Tyr-184Tyr. YY-CVIET=*mdr1* 86Tyr-184Tyr and *crt* CVIET. IRN=*dhfr* 51Ile-59Arg-108Asn. GE=*dhps* 437Gly-540Glu. GE-IRN=*dhfr* 51Ile-59Arg-108Asn and *dhps* 437Gly-540Glu. VAGKGS=*dhps* 431Val-436Ala-437Gly-540Lys-581Gly-613Ser. *Statistically significant increase from 2016 to 2018 (lower bound of 95% CI >1). †Statistically significant decrease from 2016 to 2018 (upper bound of 95% CI <1).

Table 3: Overview of change in prevalence of selected markers and haplotypes, for all seven countries combined, from 2016 to 2018

(68·8%; 445 of 585 isolates, adjusted), and a lower frequency in Chad (35·0%; 129 of 341 isolates, adjusted; table 2). However, in 2016, the *dhps* 540Glu mutation was observed in Guinea (2·0%; 13 of 651 isolates, adjusted), Mali (0·2%; two of 1277 isolates, adjusted), and Niger (0·2%; three of 1227 isolates, adjusted), and was not detected in The Gambia, Burkina Faso, Nigeria, or Chad. In the 2018 survey, after 2 years of seasonal malaria chemoprevention scale-up, *dhps* 540Glu was detected in the same three countries and also in Burkina Faso and Nigeria at low prevalence. Of the other mutations, Ile431Val occurred in all countries except Burkina Faso and The Gambia; Ser436Ala and Ala613Ser/Thr occurred in all countries; and Ala581Gly occurred in all countries except The Gambia and Guinea.

Analysis of the combination of mutations at codons 431, 436, 437, 540, 581, and 613 in *dhps* identified 24 distinct haplotypes (appendix pp 8–9). The wild-type haplotype, 431Ile-436Ser-437Ala-540Lys-581Ala-613Ala (ISAKAA), occurred at a baseline prevalence of 12·1% (532 of 4209 isolates, adjusted) in 2016, and the most common haplotypes at both timepoints were 431Ile-436Ser-437Gly-540Lys-581Ala-613Ala (ISGKAA; 44·6% [1834 of 4163 isolates, adjusted] in 2016, 42·3% [590 of 1395 isolates, adjusted] in 2018) and 431Ile-436Ala-437Gly-540Lys-581Ala-613Ala (IAGKAA; 32·7% [1338 of 4141 isolates, adjusted] in 2016, 31·3% [436 of 1395 isolates, adjusted] in 2018). The Lys540Glu substitution was mainly present as

the 431Ile-436Ser-437Gly-540Glu-581Ala-613Ala (ISGEAA) haplotype, except for one isolate of the 431Ile-436Ala-437Gly-540Glu-581Ala-613Ala (IAGEAA) haplotype in Niger in 2018 and an occurrence of the 431Ile-436Ser-437Ala-540Glu-581Ala-613Ala (ISAEAA) haplotype at a prevalence of 0·76% in Burkina Faso in 2018 (appendix p 9), but these haplotypes remained very rare with 0·0–2·1% prevalence in the region as a whole except in Guinea, where the unadjusted prevalence of the Lys540Glu substitution in *dhps* in 2018 was 5·0%. No isolates were found to carry the Lys540Glu and Ala581Gly mutations together, as has been implicated in high-level sulfadoxine–pyrimethamine resistance in east Africa.[21,22] Although there was evidence of an increase in prevalence of *dhps* 540Glu in the 10–30 years age group only, in total 21 of the 2165 evaluable isolates from all ages in 2018 harboured this substitution, representing 0·97% of participants. The *dhps* mutant Ile431Val, mostly encountered in the more easterly countries, occurred as eight different haplotypes of which VAGKGS and VAGKAA were the most common (appendix pp 8–9). VAGKGS and VAGKAA were observed at highest frequencies in Chad, particularly in 2018 (VAGKGS 11·8% [16 of 136 isolates], VAGKAA 5·2% [seven of 136 isolates]). It remains unknown what effect on sulfadoxine–pyrimethamine efficacy, for therapy or chemoprevention, results from these haplotypes that combine variants at codons 431, 437, 581, and 613 of *dhps*.
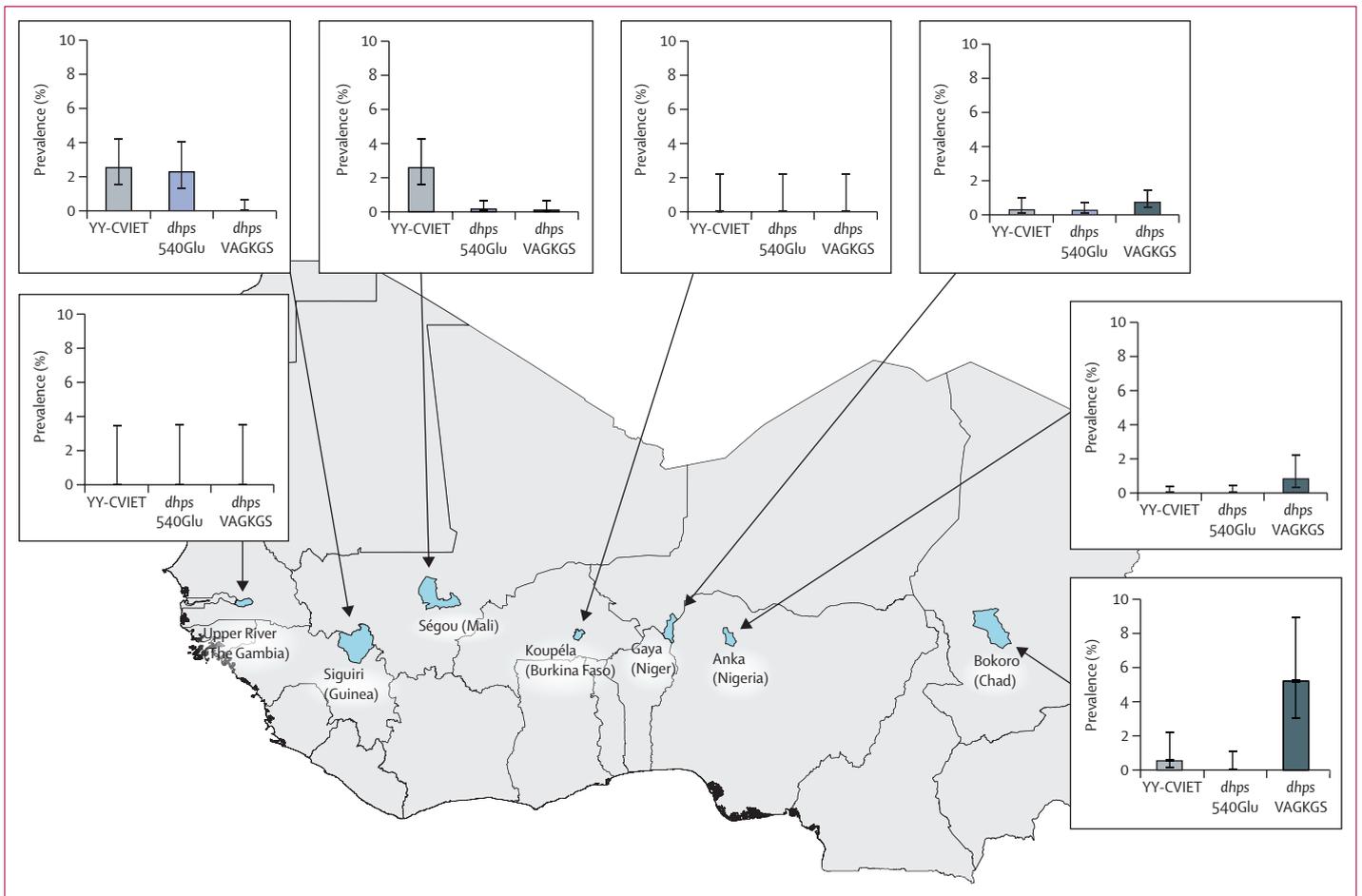
Figure 2: Baseline prevalence (2016) of resistance-associated haplotypes of concern in *Plasmodium falciparum* in both age groups combined across the African Sahel region
YY-CVIET=*mdr1* 86Tyr-184Tyr and *crt* 72Cys-73Val-74Ile-75Glu-76Thr. VAGKGS=*dhps* 431Val-436Ala-437Gly-540Lys-581Gly-613Ser. Graphs show prevalence with error bars indicating 95% CIs.

In the 2016 dataset, 28 (88%) of 32 isolates carrying the *dhps* VAGKGS haplotype also carried the *dhfr* triple mutation IRN, compared with 55% of isolates with the wild-type ISAKAA haplotype in *dhps*, a relative risk of 1·58 (95% CI 1·36–1·85). The 431Val variant was not combined with the 540Glu variant in any of the 7306 isolates analysed.

P falciparum genomes simultaneously harbouring specific variants of *crt* (CVIET), *mdr1* (YY), *dhps* (GE haplotype), and *dhfr* (IRN haplotype) are likely to be resistant to both sulfadoxine–pyrimethamine and amodiaquine and could potentially affect the efficacy of sulfadoxine–pyrimethamine plus amodiaquine administered for seasonal malaria chemoprevention. Each of these variants occurred in our study sites, and evidence for the selection of multilocus resistance was examined by comparing the abundance of parasites bearing a combination of these in 2018 with the baseline data collected 2 years earlier. In 2016, the theoretically highly resistant multilocus haplotype YY-CVIET-GE-IRN was found in Guinea only, in one (0·3%, adjusted) of

1996 isolates from participants younger than 5 years and one (0·4%, adjusted) of 1893 isolates from people aged 10–30 years. In 2018, this combination was not detected in either age group (table 2), and thus no evidence was found of an increase in prevalence of YY-CVIET-GE-IRN after 2 years of seasonal malaria chemoprevention deployment (table 3).

Discussion

Mutations at loci within *crt* and *mdr1* and within *dhfr* and *dhps* are associated with the susceptibility of *P falciparum* to amodiaquine and sulfadoxine–pyrimethamine, respectively.[3,4,18–26] This study reports on two large-scale surveys of qPCR-confirmed *P falciparum* carriage and molecular markers of resistance to sulfadoxine–pyrimethamine and amodiaquine across seven countries in sub-Saharan Africa before (in 2016) and after (in 2018) implementation of seasonal malaria chemoprevention through Achieving Catalytic Expansion of Seasonal Malaria Chemoprevention in the Sahel (ACCESS-SMC), a programme that sought to remove

barriers to the scale-up of seasonal malaria chemoprevention in seven countries in 2015 and 2016. Our findings indicate a substantial decrease in parasite carriage from 2016 to 2018 among children younger than 5 years, the recipients of seasonal malaria chemoprevention, in all six of the countries that had no previous seasonal malaria chemoprevention deployment in the study areas. In The Gambia, where seasonal malaria chemoprevention was already in place before 2015, parasite carriage remained low and stable in this age group. This finding confirms a substantial parasitological benefit, and is consistent with the evidence that, when implemented at scale in The Gambia, Guinea, Mali, Burkina Faso, Niger, Nigeria, and Chad, seasonal malaria chemoprevention was 88·2% effective at preventing clinical malaria within 28 days of administration of seasonal malaria chemoprevention with sulfadoxine–pyrimethamine and amodiaquine.[12]

Our genotyping analysis of over 7000 *P falciparum* isolates collected in the two surveys found no evidence of an immediate threat to seasonal malaria chemoprevention from parasite genotypes conferring multidrug resistance after 2 years of implementation, because parasites carrying the theoretically highly resistant four-locus haplotype YY-CVIET-GE-IRN were extremely rare in 2016 (and occurred in Guinea only) and completely absent in 2018, but some indicators of concern were identified. Amodiaquine effectiveness is likely to remain acceptably high for the immediate future because, despite a moderate-to-high prevalence of the CVIET haplotype of *crt,* there was no significant accompanying prevalence of the YY haplotype of *mdr1,* known to be necessary for clinically relevant resistance to amodiaquine in Africa.[4,18] The SVMNT allele of *crt,* which mediates amodiaquine resistance in Asia and the Americas, was not detected.[3] Similarly, although the *dhfr* IRN haplotype at codons 51, 59, and 108 was very common across all study sites, the high-level resistance allele with an additional mutation at codon 164 was not observed. The *dhps* locus was the most variable, with highly complex patterns of amino acid substitution at more than six positions, generating 24 distinct haplotypes. We described six novel variant positions in *dhps*: Ile141Met, Gly425Asp, Ile451Met, Ile466Val, Ile470Thr, and Asp575Ala. These rare, emerging mutations have, as yet, no known effect on sulfadoxine susceptibility. However, the key substitution Lys540Glu, which appears to be a principal mediator of sulfadoxine–pyrimethamine treatment efficacy,[27] remained almost absent apart from in Guinea, and was never combined with Ala581Gly, known to be a highly resistant form in east Africa, suggesting no immediate threat to sulfadoxine–pyrimethamine effectiveness. Of most concern was the emergence in four countries of *dhps* haplotypes that comprise some combination of the Ile431Val, Ala437Gly, and Ala581Gly mutations, either as VAGKGS or VAGKAA, both previously identified in parasites originating from Nigeria.[8,28]

Limitations to the generalisability of our study include uncertainty as to any possible confounding effect of different transmission intensities across the region on parasite carriage rates, and also the relatively short time interval to capture the emergence of evidence of genetic selection due to seasonal malaria chemoprevention in the parasite population. Furthermore, there is a lack of evidence concerning the effect of the emerging VAGKGS and VAGKAA haplotypes on sulfadoxine–pyrimethamine effectiveness, either for therapy or chemoprevention, making the impact of our findings unclear. Finally, as our data are now 4 years old, repeat surveillance of these haplotypes is urgently needed as seasonal malaria chemoprevention implementation continues throughout the Sahel.

The confirmation that complex *dhps* haplotype variants are emerging in Niger and Chad, two countries with relatively few studies of genetic markers of resistance, underlies the need to continue molecular monitoring of parasite genotypes in all jurisdictions implementing seasonal malaria chemoprevention. The absence of the *dhps* Lys540Glu mutation in these areas confirms the predominance of West African parasite genotypes across our expansive transect, despite the proximity of countries such as Sudan, immediately to the east, where the Lys540Glu mutation is present.[29] Data published in 2021 from the region broadly concur with our findings.[30,31] However, a potential threat that the *dhps* VAGKGS haplotype might increase in prevalence, moving westwards across the Sahel, remains. Focused cohort studies to ascertain the effect of these variants on seasonal malaria chemoprevention efficacy are required.

In conclusion, our analysis of 57 666 blood samples collected across seven countries in the Sahel region of Africa before and 2 years after the implementation of seasonal malaria chemoprevention at scale provides an important overview of the parasitological effect of the intervention. We found strong evidence of a reduction in *P falciparum* carriage rates in the age group receiving the intervention, but no evidence of an imminent threat from parasite genomes harbouring multilocus mutations conferring multidrug resistance. Some genotypes of concern were identified, particularly at the *dhps* locus, and continued monitoring is essential to maintain and protect the effectiveness of seasonal malaria chemoprevention in the long term.

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