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Persistence of legacy antimalarial resistance markers in The Gambia from a malaria genomic surveillance study

Mouhamadou F. Diop
Medical Research Council Unit The Gambia
Brandon Nji Amambua-Ngwa
Medical Research Council Unit The Gambia
Martha A. Demba
Muhammed Bah
Benjamin K. Njie
Fatoumatta Cham
Michael Mendy
Fatoumata Bojang
Mamadou O. Ndiath
Anthony Correa
Eyyüb S. Ünlü
Andrew Mains
Nuredin I. Mohammed
Umberto D'Alessandro
Victoria J. Simpson
Shavanthi Rajatileka
Wandifa Samateh
Balla Gibba
Eniyou C. Oriero
Eniyou.Oriero@lshtm.ac.uk

Medical Research Council Unit The Gambia
Alfred Amambua-Ngwa
Alfred.Ngwa@lshtm.ac.uk

Medical Research Council Unit The Gambia

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Abstract

We conducted genomic surveillance of *P. falciparum* in The Gambia (2013–2022) using targeted amplicon sequencing to monitor drug resistance markers and genetic diversity in this low transmission setting. The analysis of 3,624 dried blood spot samples revealed predominantly single-strain infections (70–100%) across most sites, indicating low complexity of infection. Antimalarial resistance markers were highly prevalent; specifically, triple *Pfdhfr* mutations exceeded 90% in frequency. When combined with *Pfdhps* mutations, these formed stable quadruple haplotypes associated with partial Sulfadoxine-pyrimethamine (SP) resistance. Despite chloroquine's withdrawal in 2006, the CVIET resistance haplotype remained at approximately 60% prevalence, effectively splitting the parasite population into two major genetic clusters. Additionally, the *Pfmdr1* Y184**F** mutation—associated with increased lumefantrine tolerance—rose rapidly to over 60% by 2022. While low frequency *Pfkelch13* mutations were detected between 2019 and 2022, none were validated markers for artemisinin resistance. Continued surveillance is crucial for guiding targeted malaria elimination strategies.

Introduction

Antimalarial drug resistance is one of the major biological threats towards malaria elimination, particularly in the African sub-region where over 95% of estimated 246 million cases and 569,000 deaths reported in 2023 occurred [1]. Following the emergence of chloroquine resistance in the 1990s, many African countries, like The Gambia, changed the first line treatment for uncomplicated malaria to sulfadoxine-pyrimethamine (SP). The rapid emergence of SP resistance led to significant increase in malaria morbidity and mortality, until the implementation of artemisinin-based combination therapies (ACTs), mainly artemether-lumefantrine (AL), adopted in The Gambia in 2005 [2, 3]. Together with the implementation of vector control interventions such as insecticide-treated bed nets (ITNs), and the availability of other ACTs, the malaria burden decreased rapidly [4, 5]. However, progress has stalled since 2015, with significant heterogeneity in the malaria burden and the emergence of resistance to both artemisinin derivatives and insecticides. Such heterogeneity in transmission of the deadliest malaria parasite, *Plasmodium falciparum*, can be found across a relatively small spatial distance; in The Gambia, malaria prevalence varies between 1% in the coastal urban Western regions to almost 50% in eastern Gambia, 300Km further inland [6].

In The Gambia, besides ITNs, other malaria interventions such as intermittent preventive treatment in pregnancy with SP and seasonal malaria chemoprevention (SMC) with SP and amodiaquine are implemented, exerting significant drug pressure on the parasite population [7]. With the emergence of artemisinin partial resistance in East Africa [7–9], and the additional threats of the spread of the invasive *Anopheles stephensi* [10–12] and of diagnostic resistance [13], intense molecular and epidemiological surveillance is essential to guide control efforts.

Plasmodium falciparum resistance to chloroquine and other quinolines are associated with mutations in the chloroquine resistance transporter (*Pfcrt*) gene codons 72 to 76, and the multidrug resistance 1

(*Pfmdr1*) gene codons 86, 184 and 1246 amongst others. For SP, associated genetic markers are in dihydrofolate reductase (*Pfdhfr*) gene codons 51, 59 and 108 for pyrimethamine resistance, and dihydropteroate synthetase (*Pfdhps*) gene codons 436, 437, 540, 581 and 613 for sulfadoxine resistance.

In many malaria endemic countries, the withdrawal of chloroquine as first-line treatment resulted in the decline of mutant variants in *Pfcrt* and *Pfmdr1* genes and, in some countries the return of wildtype parasites [14, 15]. However, this reversion seems heterogeneous and at different rates, with the risk that persistence of some alleles could affect ACT partner drugs and those used for chemoprevention, e.g., seasonal malaria chemoprevention using SP and amodiaquine. This has generated concerns over the spread to West Africa of mutations associated with resistance to partner drugs such as piperaquine [16].

Advances in genotyping, particularly the use of targeted amplicon sequencing and barcoding techniques have facilitated rapid and detailed monitoring of resistance mutations in key genes associated with resistance to different antimalarial drugs [17, 18]. Amongst several malaria parasite amplicon sequencing panels, the Malaria Genetic Epidemiology Network (MalariaGEN) SpotMalaria panel has been developed and deployed in Southeast Asia, where is has been useful in detecting the evolution and epidemiology of resistance to artemisinin and ACT partner drugs [19]. The amplicon panel targets most of the validated markers of drug resistance and generates a genetic barcode from 101 single nucleotide polymorphisms (SNPs) [20]. The Genomic Surveillance of Malaria in West Africa (GSM) project is designed to increase the use of this panel for regional high resolution spatial and longitudinal surveillance of *P. falciparum* parasites as West African countries scale up interventions for malaria elimination. This study focuses on The Gambia, where the National Malaria Control Program's (NMCP) plans for pre-elimination require tracking parasites circulating within the country and those imported from across neighboring Senegal. The Gambia shares all its land borders with Senegal, a country that also aims at malaria elimination. Here were explored samples across a decade (2013–2022) from most health districts to elucidate the relationship between malaria transmission, drug resistance, and genetic diversity of malaria parasites within The Gambia.

Results

Sample counts across the country and parasite genetic diversity

Over 12,000 participants were screened across 9 transmission seasons, and 4,815 PCR-positive dried blood spot samples were sequenced. Following filtration for missingness, 3,624 samples (Fig. 1a), with a minimum of 93 high-quality barcode allele calls were retained, allowing for robust downstream analyses. Most of the samples (78.4%) analyzed were collected between 2019 and 2022 [18], with interruptions in field activities in 2020 and 2021 due to restrictions imposed by the COVID-19 global pandemic (Fig. 1b). Complete target haplotype calls at drug resistance loci was obtained for 75.3% of PCR-positive samples;

all retained samples, including mixed-strain infections, were used to assess prevalence of resistance markers and genetic diversity.

The complexity of infection (COI) analysis showed predominantly single-strain infections of *P. falciparum* over time, with a slight upward trend of multiple-strain infections in recent years, dominated by samples from the eastern regions of The Gambia (Figs. 1a & 1c). Spatial trends show overall higher proportions of single-strain infections across all regions of the country, ranging from 70–100% (Table 1; Fig. 1a), with the exception of the referral hospital – Banjul, with 47% single infections.

Table 1
Complexity of *P. falciparum* infections from all the sites across the country (2013–2022)

Location	Region	Multiple Infections (%; n/N)
Brikamaba	Central River	7.89 (3/38)
Janjangbureh	Central River	12.90 (8/62)
Kaur	Central River	8.89 (4/45)
Kudang	Central River	16.67 (1/6)
Kuntaur	Central River	29.63 (24/81)
Sami Karantaba	Central River	26.67 (4/15)
Bansang	Central River	9.65 (11/114)
Keneba	Lower River	11.76 (4/34)
Pakaliba	Lower River	7.87 (7/89)
Soma	Lower River	9.47 (9/95)
Kerewan	North Bank	5.21 (5/96)
Kuntaya	North Bank	4.00 (1/25)
Badja Kunda	Upper River	22.22 (4/18)
Basse	Upper River	11.99 (71/592)
Fatoto	Upper River	27.27 (12/44)
Gambissara	Upper River	16.19 (17/105)
Koina	Upper River	20.00 (6/30)
Sabi	Upper River	19.26 (26/135)
Sotuma Sere	Upper River	27.96 (59/211)
Yorobawol	Upper River	12.11 (31/256)
Banjul	Western 1	52.94 (9/17)
Fajikunda	Western 1	6.54 (21/321)
Brikama	Western 2	4.98 (28/562)
Kafuta	Western 2	4.58 (6/131)
Pirang	Western 2	10.00 (2/10)
Tanjeh	Western 2	0.00 (0/41)

There was a relatively low level of genetic differentiation based on geographic origin, which was largely supported by a neighbor-joining tree. However, a small cluster of divergent parasite isolates comprising mostly of infections from the more urban and coastal region of the country – Brikama, Tanjeh, and Fajikunda was evident (Fig. 2a). PCoA analysis also revealed three groups of divergent isolates, mostly from infection recruited from the coastal regions (Fig. 2b). Further analysis of the PCoA results across temporal and spatial dimensions revealed no significant temporal shifts in genetic diversity or genotype aggregation. Overall, parasite isolates were highly related genetically, with short genetic distances suggestive of clonal expansion in urban centers in western Gambia. This was investigated further to explore genetic relatedness of the parasites in the divergent clusters from the PcoA plot, using algorithms that show identity-by-descent (IBD) (Fig. 2c).

Antimalarial resistance marker frequencies across study sites

The prevalence of mutations in *P. falciparum* genes associated with antimalarial drug resistance varied substantially across study sites, with higher prevalence in some of the regions with higher residual transmission such as the urban coastal Western Regions and the Upper River Region. The mutations observed showed similar temporal trends overall across all drug resistance loci. For the chloroquine resistance locus (*Pfcrt*), no mutation was detected at codons 72 and 73 whereas over 50% infections carried mutant alleles at codons 74–76 as mixed or single genotypes (**Extended data** Fig. 1). Prevalence of the K76**T** mutation peaked in 2015–2016 and then decreased to plateau at around 60% in 2021 and 2022 (Fig. 3a). This mutation is in strong linkage disequilibrium with the variants at codons 74 and 75 driving the high prevalence of the predominant CVIET haplotype for chloroquine resistance (Fig. 3b). At the *Pfmdr1* locus, the highest mutation frequency was observed at codon 184, with 63.6% infections having the mutant allele. The change from predominantly wildtype at this codon to the Y184**F** mutation occurred between 2013 and 2014 and rapidly increased up to 60% in 2022 (Fig. 3a). The prevalence of the *Pfdhfr* mutations at codons 51, 59, and 108 was over 90% throughout the study period while no mutation was observed at position 164. Mutations at codon 437 and 436 of *Pfdhps* were detected in 76.9% and 33.7% of infections, respectively. The combination of the triple *Pfdhfr* mutations with either *Pfdhps* A437G or S436A resulted in the predominant quadruple *Pfdhfr-Pfdhps* haplotype, which determines partial resistance to SP, with overall prevalence of 54.1% (**Extended data** Fig. 1; **Extended data** Table 1). Singletons or low frequencies (≤ 1%) mutations were detected in the propeller domain of *Pfkelch13* gene marker of partial artemisinin resistance, across all study sites from 38 infections sampled between 2019 and 2022 **(**Fig. 3b**)**. A total of 15 non-synonymous *Pfkelch13* mutations occurred as either mono or mixed genotypes (Table 2), none of which are included in the list of WHO-validated or associated candidate genetic markers for partial artemisinin resistance.

Table 2
Non-synonymous mutations observed at Kelch 13 loci

S/N	Mutation	Type	#samples	Year of occurrence	Site	Region
1	WT P667T	Heterozygous	12	2021	Kafuta, Kerewan	Western 2, North Bank
2	A578S	Homozygous	8	2019, 2020, 2021, 2022	Soma, Brikamaba, Sabi, Sotuma, Pakaliba	Lower River, Central River, Upper River
3	WT A578S	Heterozygous	6	2019, 2020	Bansang, Brikamaba, Sabi	Central River, Upper River
4	H384Q	Homozygous	1	2019	Sotuma	Upper River
5	H560Y	Homozygous	1	2020	Bansang	Central River
6	R528K	Homozygous	1	2020	Bansang	Central River
7	V534L	Homozygous	1	2021	Kafuta	Western 2
8	V589I	Homozygous	1	2022	Koina	Upper River
9	WT A621T	Heterozygous	1	2019	Yorobawol	Upper River
10	WT G392E	Heterozygous	1	2020	Yorobawol	Upper River
11	WT H366N	Heterozygous	1	2019	Yorobawol	Upper River
12	WT K390E	Heterozygous	1	2019	Fajikunda	Western 1
3	WT M608I	Heterozygous	1	2020	Bansang	Central River
14	WT P615L	Heterozygous	1	2019	Fajikunda	Western 1
15	WT T677I	Heterozygous	1	2020	Yorobawol	Upper River

Multilocus haplotype genetic relatedness

Further investigation of pairwise parasite identity across drug resistance loci revealed two major subgroups of multilocus drug resistance backbone mainly separated by chloroquine resistant and sensitive haplotypes, CVIET and CVMNK (Fig. 4a). The largest clade in both the chloroquine resistant and sensitive clusters had the SGKAA haplotype at the *Pfdhps* loci, with the A437**G** mutation. The *Pfdhps*

SGEAA haplotype with the K540**E** mutation was only found in the chloroquine resistant cluster. Based on the genetic barcode, 1.05% of chloroquine resistant were highly related, with pairwise identity-by-descent metrics of at least 0.9 (Fig. 4b). Distinct clusters of highly related chloroquine-sensitive isolates were also identified. The highly related chloroquine-resistant pairs were mostly from infections recruited at the coastal regions of the country. These infections spread across towns as indicated by the IBD connectivity between pairs of infections from different places (Fig. 4c).

Discussion and Conclusion

We report on *P. falciparum* antimalarial resistance mutations and genetic diversity in The Gambia, captured by high throughput targeted next-generation sequencing (NGS) techniques. The data provides valuable insights into potential transmission dynamics of malaria parasites and circulating adaptive mechanisms against antimalarial drugs across time and different regions of the country. As The Gambia combines both drug and insecticidal interventions unevenly to eliminate malaria at local level, spatial surveys and evidence of heterogeneity are useful to pinpoint hotspots of transmission or of antimalarial drug resistance [21]. Our analysis showed, as expected in a low transmission setting, mostly single-strain infections across the study period. Continued surveillance will be important to monitor the trends of infection complexity and to understand the recent modest increase of multiple-strain *P. falciparum* infections especially in the higher transmission regions in the East, against massive focal interventions in the last decade. The eastern end of the Gambia over is 350km within Senegal, bordering the highest malaria transmission regions with that country, from which some infections could have been imported across the porous borders. For Gambia to eliminate malaria, cross-border monitoring of importation and co-coordination of interventions may be imperative.

Genetic diversity analysis showed relatively low genetic differentiation among *P. falciparum* samples across the country. Clustering of the genetic fingerprints of infections by Principal coordinate analysis (PCoA) revealed extensive genomic diversity among *P. falciparum* populations across all regions of The Gambia as expected for most panmictic malaria populations. This indicates that the low transmission over the last decade has not depleted local diversity in circulating clones, and substantial gene flow and admixture among the parasite populations persist across the country, facilitated by human and mosquito movement. However, three small subclusters of parasites comprising of samples from the coastal sites showed evidence of divergence from the main cluster including isolates from all regions. It could be an indication of newly introduced parasites into the population followed by clonal expansion in urban dwellings where inhabitants probably have lower immunity due to low levels of exposure. The high human traffic across the coastal regions of the country buttresses the need for cross-border surveillance to monitor malaria transmission patterns and better deployment of targeted interventions towards malaria elimination. This genetic mixing across regions provides a critical baseline for understanding the persistence and spread of drug resistance markers and their potential to disseminate across local health districts, and geopolitical boundaries.

The frequencies of genetic markers at antimalarial resistance genes showed substantial variability across study sites but exhibited broadly similar temporal trends. The chloroquine resistance CVIET haplotype was present in over half of infection, in contrasts with decrease in the prevalence of this haplotype in other countries following withdrawal chloroquine as first-line treatment at the dawn of the millennium [14, 15]. This persistence of chloroquine resistance could be driven by compensation for fitness loss by other genetic variants that will require whole genome analysis to identify and characterize. Already, the amino acid transporter, *Pfaat1* mutation at codon 258 had been shown to modulate fitness of *Pfcrt* mutants, and the 258L mutant is at high frequencies in The Gambia [22]. Multilocus analysis of the antimalarial resistance backbone highlighted two major parasite clusters separated by the two main chloroquine resistance transporter haplotypes; the resistant CVIET and sensitive CVMNK, each linked to various *Pfmdr1, Pfdhfr* and *Pfdhps* haplotypes. Interestingly, the small number of isolates with *Pfdhps* K540**E** mutation was only observed in the subcluster with chloroquine resistant backbone. *Pfdhps* K540**E** mutation is rare in West Africa and associated with increased resistance to sulfadoxine, and SP treatment in East Africa [23]. The occurrence of multiple resistant variants on the strain could lead to multi-drug resistance. However, this comes with a fitness cost seen as low parasitemia, although these parasites tend to generate more gametocytes and therefore more transmissible [24]. As it may be contributing to the persistence of chloroquine resistant strains and their clonal expansion, this peculiar observation warrants continuous surveillance and further investigation to understand the contribution of these strains to transmission dynamics in the region. The near fixation of *Pfdhfr* triple mutation observed and its combination with either *Pfdhps* A437**G** or S436**A** constituted the predominant quadruple haplotypes, associated with partial SP resistance. These partial resistance markers for SP showed high prevalence and temporal stability, whose consequences for SP-based chemoprevention in SMC and intermittent preventive treatment of malaria in pregnancy (IPTp) remain to be established and monitored.

The primary resistance-associated mutation at *Pfmdr1* (Y184**F**) showed a rapid temporal increase. This mutation has been under selection since the introduction of lumefantrine-based ACTs in Africa. In combination with the wild N86 variant, they are associated with reduced increasing tolerance to drugs like lumefantrine [25]. Thus, continued monitoring of resistance to the long-lasting partner drugs is crucial to maintaining the efficacy of ACTs. The *Pfkelch13* locus, associated with partial artemisinin resistance, was predominantly wildtype, consistent with reports from other parasite isolates from West Africa. While a low frequency of non-synonymous mutations was observed, none of them were among the WHO-validated or candidate markers associated with partial artemisinin resistance. More studies are required on the role of these emerging *Pfkelch13* variants in West Africa parasite genetic backgrounds to pre-empt de novo partial artemisinin resistance as seen for parasites in the East and horn of Africa [26, 27].

In the context of constrained funding and the need to prioritize implementation activities, this study demonstrate that genomic surveillance provides actionable evidence that can be explored in strategically tailoring interventions and efficiently disburse resources to areas that threaten the push for malaria elimination in low burden countries like The Gambia. Genomic surveillance for malaria should be

a critical tool for the Gambian and other regional NMCPs, enabling robust monitoring of regional transmission dynamics and early detection of imported parasite strains.

Study limitations

Most samples analyzed (78.4%) were collected between 2019 and 2022 in sentinel health facilities. Although temporal trends showed similar patterns, the possibility of sampling bias cannot be ruled out since smaller numbers were processed from earlier years (ranging from 29 to 163 samples) with limited geographic coverage (Western and Upper River Regions only). While they contributed to aggregated regional data, the spatial analysis for the sites would have benefited from a larger and comparable sample size across all sites.

Methods

Sample Collection and Ethical Considerations

Sample were collected each year between September to December (2016–2022), covering the malaria transmission season. The samples were obtained from several studies which obtained ethical approval from the Joint Gambian Government/MRCG Ethics Committee (REF: SCC 1629; SCC 1626; LEO26260). Study participants were recruited from a total of 26 sentinel health facilities (including the referral teaching hospital, Banjul) in different regions across the country following written informed consent. After the training by the MRCG team, consenting was done by the health facility staff and the language used for obtaining the consent was documented for each participant. Blood samples were collected by a single finger prick and used to perform a rapid diagnostic test (RDT) and to collect two blood spots on 3mm Whatman filter paper for dried blood spot (DBS) according to the MalariaGEN SpotMalaria protocol [20]. All DBS were placed with desiccant in small ‘ziplock’ sample bags and stored at room temperature or refrigerated cold room prior to parasite molecular detection and genotyping.

Molecular laboratory analysis

DNA extraction and diagnostic PCR was done as previously described [28]. Three 6mm spots were punched from the dried blood spots (DBS) for DNA extraction using the automated QIAcube HT according to manufacturers’ protocols (Qiagen GmbH, Hilden, Germany). Briefly, a punched DBS was incubated in lysis buffer (ATL) for 10 mins at 85ºC in a 96-well 2ml deep well block, Proteinase K was added and the samples were incubated for 1 hour at 56ºC. Buffer AL was then added and further incubated for 10 mins at 70ºC, taking care to spin down with a plate centrifuge in-between steps to remove precipitated droplets. The supernatant was then transferred to a new deep well block, which was set up in the QIAcube HT for automated washing and elution steps. Final elution DNA was done using 100µl of Buffer AE.

Confirmation of malaria parasite DNA in the samples was done using an ultra-sensitive qPCR protocol targeting the *var* gene acidic terminal sequence (*var*ATS) on the Biorad CFX96 real-time PCR machine [29]. Briefly, 5µl of template DNA was amplified in a 20µl final volume reaction containing 0.4µM *Var* probe, 0.8µM of forward and reverse primers and 1× qPCR Taqman Universal Master Mix (ThermoFischer Scientific, USA). Cycling conditions included a pre-incubation step for 2 mins at 50ºC, enzyme activation and initial denaturation at 95ºC for 10 mins, followed by a two-step amplification reaction (45X) of denaturation for 15 secs at 95ºC and annealing/extension for 1 min at 55ºC. Samples with Ct threshold less than 40 were qualitatively scored as positive and taken forward for targeted amplicon sequencing. The *P. falciparum* reference isolate 3D7, and no-template controls were appropriately included during the DBS punching, nucleic acid extraction and diagnostic PCR to check for cross contamination during sample handling and assay processes.

Prior to library preparation for amplicon sequencing, selective whole genome amplification (sWGA) was performed to enrich for *P. falciparum* DNA using primers selected on the basis of their differential frequency of binding to the desired (*P. falciparum*) and contaminating (human) genomes [30]. 2µl of sWGA material per sample was used for amplicon generation, processed in a 96-well format including positive and negative controls. Amplicons were generated from 136 pre-balanced primer pairs in three panels (GRC1 with 68 targets, GRC2 with 66 targets and SPEC with 2 targets) as described in the *P. falciparum* Amplicon Toolkit Protocols [17]. A Genetic Report Card (GRC) of 36 non-synonymous amino acid changes associated with antimalarial drug resistance and genotype barcode of 101 single nucleotide polymorphisms (SNPs) carefully selected to capture genetic diversity among *P. falciparum* populations was generated for each sample. The amplicons generated from each panel were labeled using unique custom sequencing tags in a 96-well format, bead-cleaned according to manufacturers' protocol (AMPure XP beads, Beckman Coulter, USA) to remove excess primers and primer dimers and subsequently pooled together for sequencing runs in multiplex batches of 96 or 384 amplicon libraries. Quality check on the libraries generated was done using Invitrogen Qubit Fluorometer 2.0 (Thermo Fisher Scientific, USA), Tapestation (Agilent, USA) and library quantitation was done using Kapa library quantification kit (Roche, Switzerland). A final dilution of 8 pMol library was denatured and loaded for paired end deep amplicon sequencing on the MiSeq V2 kit (Illumina, USA). The library preparation and sequencing protocols were developed at the Welcome Sanger Institute and transferred to the Medical Research Council Unit, The Gambia at LSHTM (MRCG) for routine operational runs.

Data Analysis

Custom bioinformatics pipelines developed at the MRCG's Data Science platform were used for amplicon sequence data analyses, using R software environment (Version 4.2.2) (https://github.com/mrcg-mpb/grcMPB_Package). To ensure the integrity and reliability of subsequent analyses, samples with more than 75% missing genotypes and loci with over 80% missing data were excluded. Genetic distances between pairs of infections were calculated using a custom package, which was explored using principal coordinate analysis (PCoA), dendrograms and clustered heatmap to explore population structures. Genetic distances for IBD was calculated using the hmmIBD command-

line tool, which implements a hidden Markov model (HMM) for detecting genomic regions that are identical by descent. Genetic diversity between geographic regions and temporal periods were determined by pairwise populations differentiation expressed as F_{ST}.

The prevalence of each mutation associated with antimalarial resistance was calculated as a fraction of isolates carrying the mutant over the samples genotyped. Haplotype prevalence was determined for each gene and classified as single mutant, double mutant or others combining different alleles at different codons for each of the genes. The frequency of haplotypes was displayed as donut charts on the country map, showing fractions per gene. The country map was drawn using raster package, presenting boundaries between health districts. Temporal trends in allele frequency of key mutations were determined across the study period (2013–2022), representing varying malaria transmission settings and level of development, and displayed as a line plot.

Parasite isolates were classified as either chloroquine sensitive or resistant based on the presence of the chloroquine resistance associated allele Pfcrt-76T. Pairwise relatedness between isolates in these groups was inferred from pairwise identity-by-state (IBS). IBS pairwise matrix was generated based on the number of shared alleles between parasite isolates (1 = match, 0 = mismatch, 0.5 = heterozygous). Network analysis of related clusters of *P. falciparum* parasites were visualized using igraph, tidygraph, and ggraph software packages, at different pairwise IBS thresholds. All codes used in these analyses are available on github (https://github.com/mrcg-mpb/grcMPB_Package).

Declarations

Data availability

All sequences are available on the European Nucleic Acid (ENA) database under study ID PRJEB94802.

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Competing Interests

The authors have none to declare.

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Figures

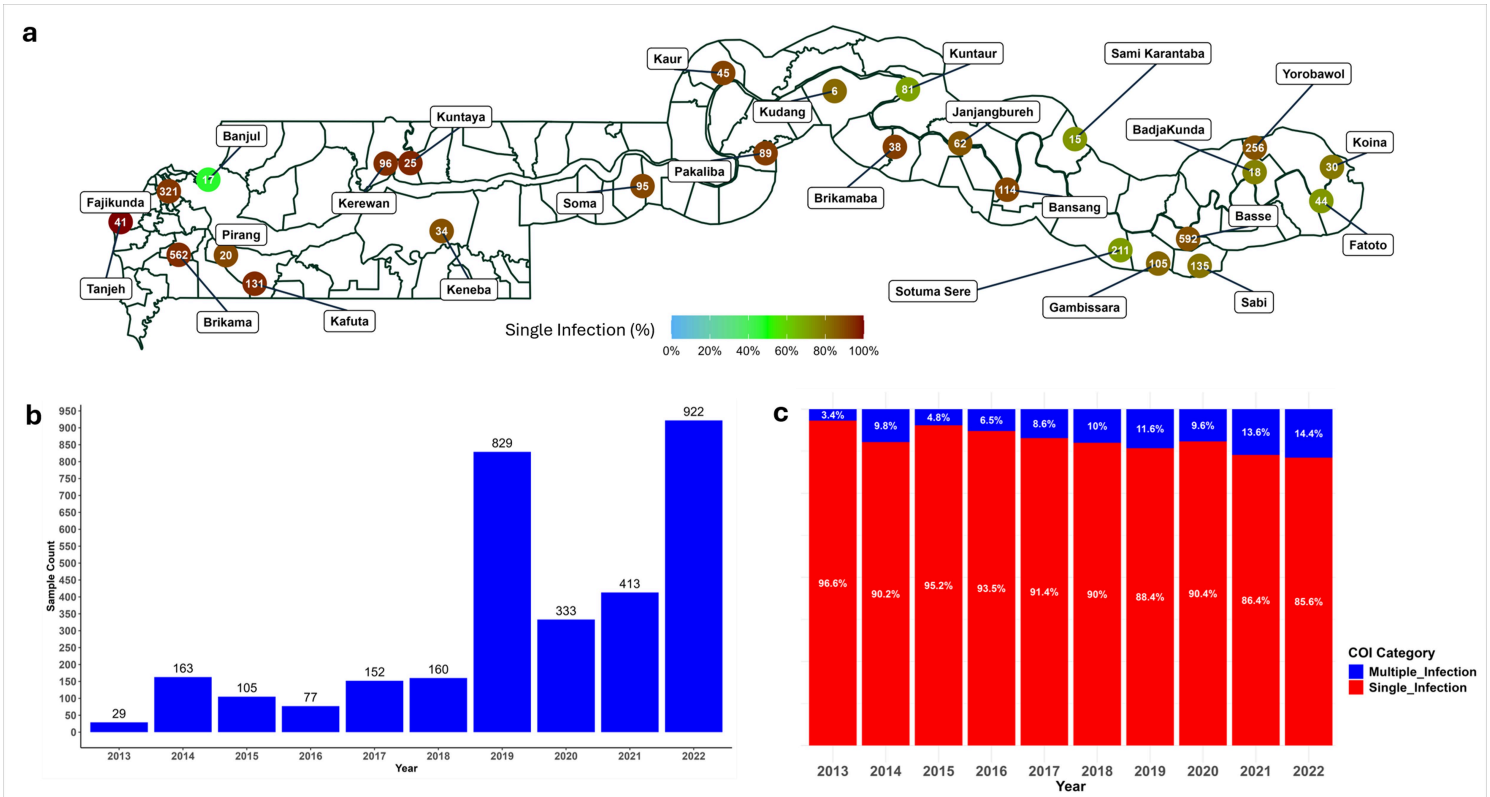

Figure 1

Distribution of sequenced *P. falciparum* samples across the different sentinel health facilities **a)**spatial sample count **b)** number of samples collected by year **c)**complexity of infection (COI) by year.

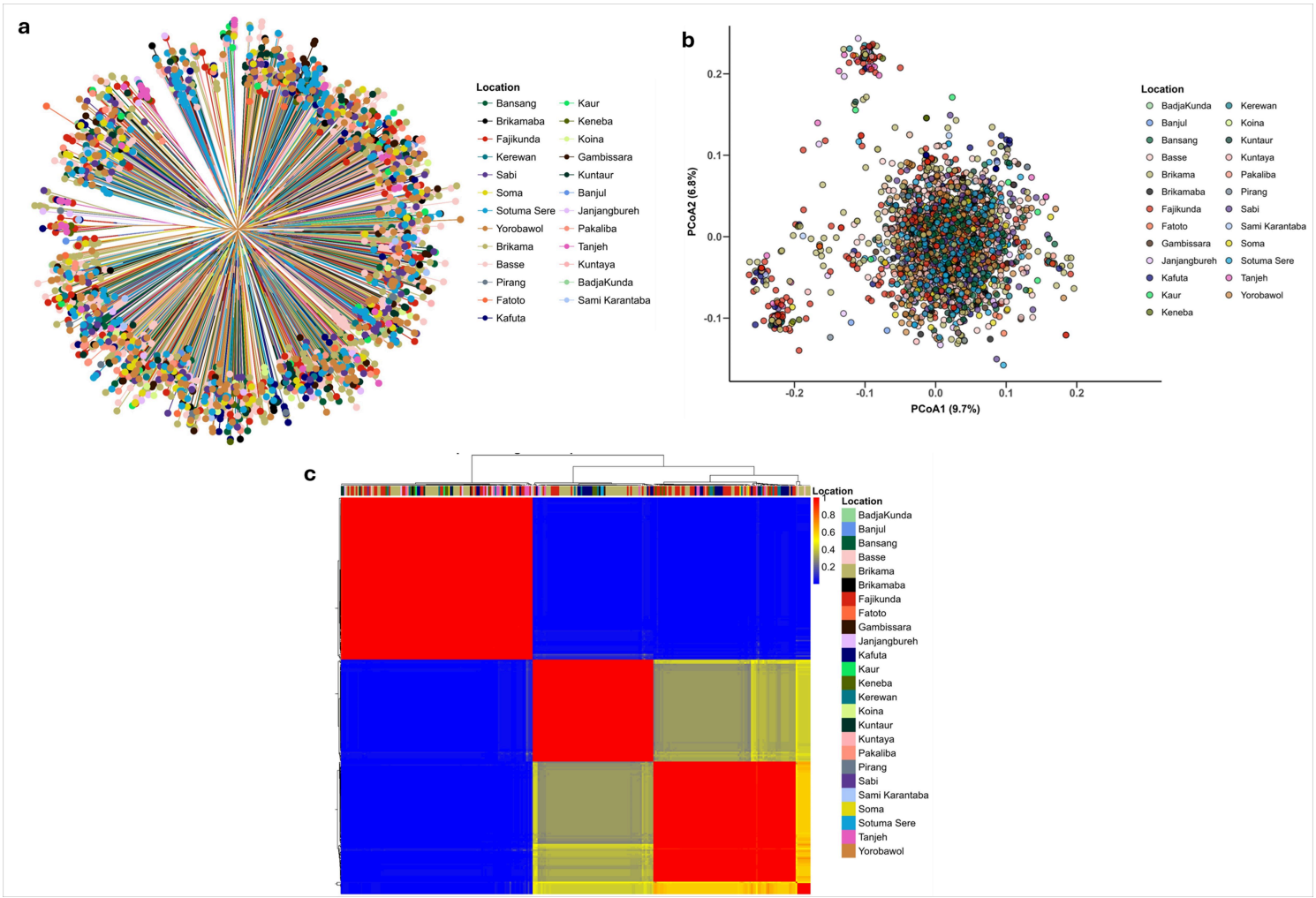

Figure 2

Genetic diversity of *P. falciparum* across health districts in The Gambia shown as, **a)** Neighbor-joining tree **b)**Principal coordinate analysis (PCoA) plot and **c)** Identity-by-descent (IBD) heatmap of the three clusters of divergent samples determined by PcoA of genetic barcode.

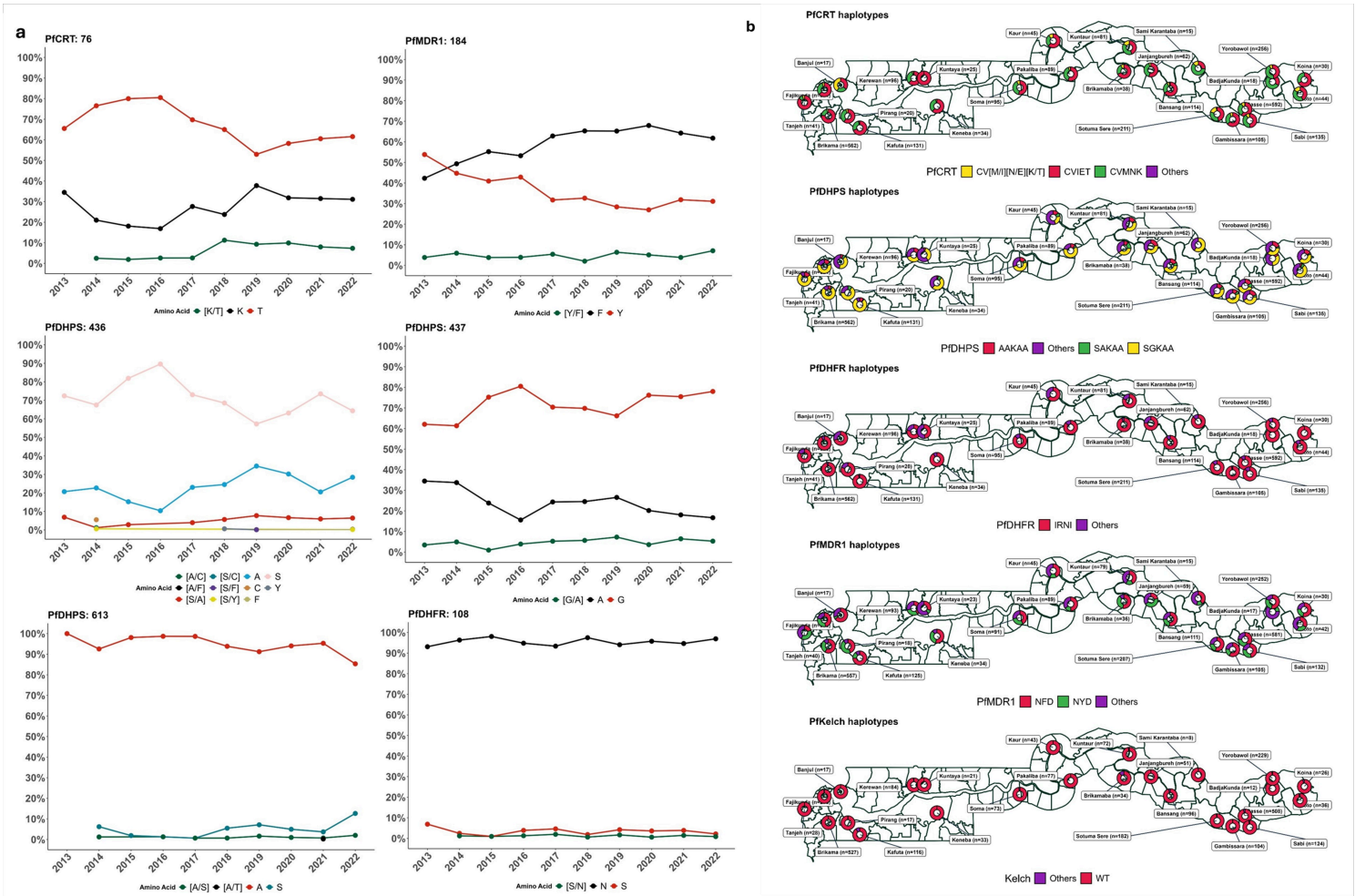

Figure 3

Mutation prevalence at antimalarial drug resistance loci showed as **a)** temporal plots and **b)** spatial haplotype plots.

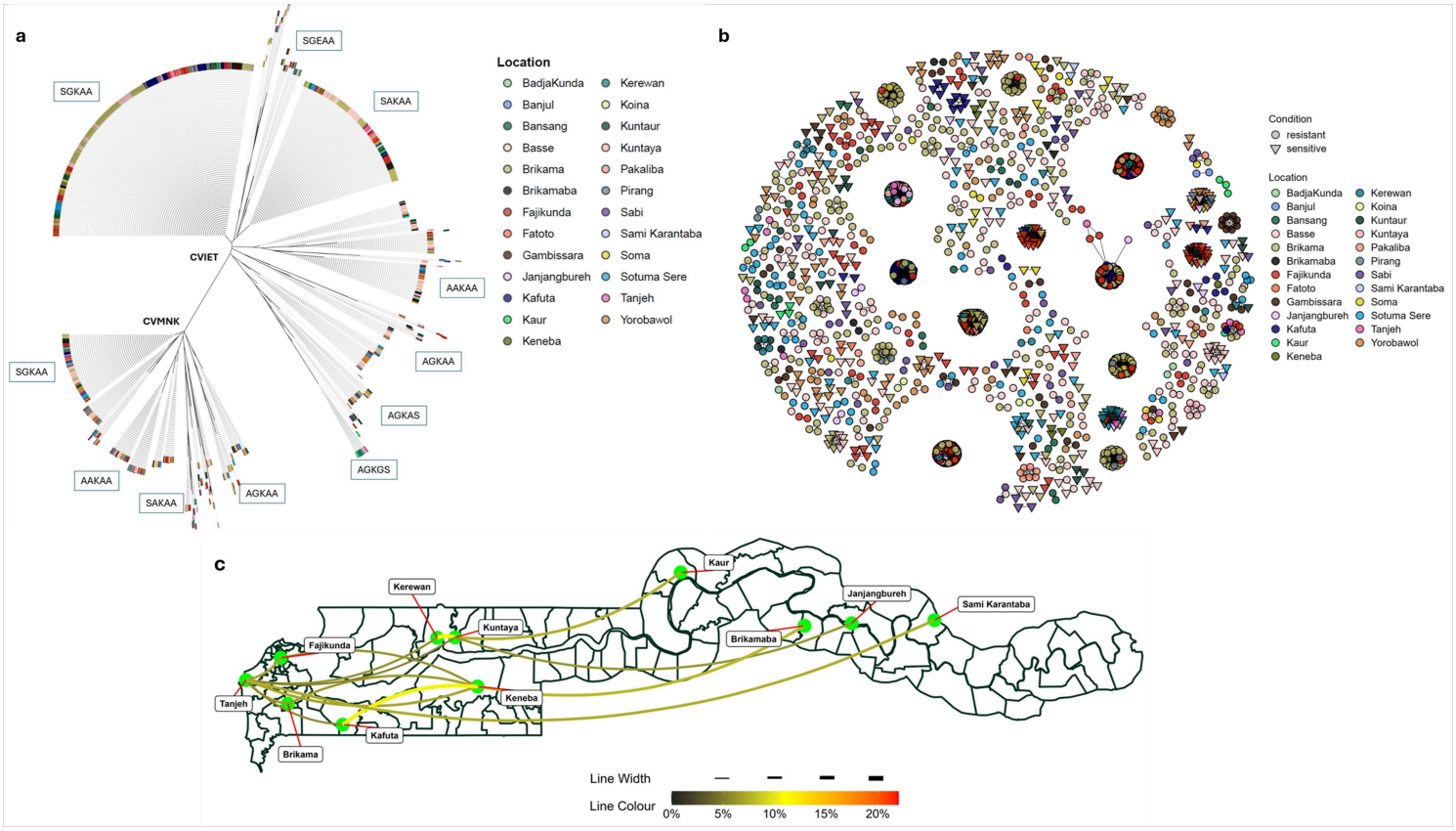

Figure 4

Parasite genetic relatedness shown from **a)** a multilocus drug resistance haplotype tree **b)**Identity-by-descent (IBD) network plot at chloroquine resistance loci (pairwise IBD threshold ≥0.9) and **c)** IBD connectivity plot for chloroquine resistant parasites. The legend color gradient and thickness represent the fraction of pairwise chloroquine resistant infections with IBD³0.9 between pairs of sampling sites.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Extendeddata.docx