Molecular surveillance for anti-malarial drug resistance and genetic diversity of *Plasmodium falciparum* after chloroquine and sulfadoxine-pyrimethamine withdrawal in Quibdo, Colombia, 2018

Angela Patricia Guerra¹*, Mario Javier Olivera¹, Liliana Jazmín Cortés¹, Stella M. Chenet², Alexandre Macedo de Oliveira³ and Naomi W. Lucchi³

**Abstract**

**Background:** Resistance to anti-malarial drugs is associated with polymorphisms in target genes and surveillance for these molecular markers is important to detect the emergence of mutations associated with drug resistance and signal recovering sensitivity to anti-malarials previously used.

**Methods:** The presence of polymorphisms in genes associated with *Plasmodium falciparum* resistance to chloroquine and sulfadoxine-pyrimethamine was evaluated by Sanger sequencing, in 85 *P. falciparum* day of enrollment samples from a therapeutic efficacy study of artemether–lumefantrine conducted in 2018–2019 in Quibdo, Colombia. Samples were genotyped to assess mutations in *pfcrt* (codons 72–76), *pf dhfr* (codons 51, 59, 108, and 164), and *pf dhps* genes (codons 436, 437, 540, and 581). Further, the genetic diversity of infections using seven neutral microsatellites (NMSs) (C2M34, C3M69, Poly α, TA1, TA109, 2490, and PfPK2) was assessed.

**Results:** All isolates carried mutant alleles for *pfcrt* (*K76T* and *N75E*), and for *pf dhfr* (*N51I* and *S108N*), while for *pf dhps*, mutations were observed only for codon A437G (32/73, 43.8%). Fifty samples (58.8%) showed a complete neutral microsatellites (NMS) profile. The low mean number of alleles (2 ± 0.57) per locus and mean expected heterozygosity (0.17 ± 0.03) showed a reduced genetic diversity. NMS multilocus genotypes (MMG) were built and nine MMG were identified.

**Conclusions:** Overall, these findings confirm the fixation of chloroquine and pyrimethamine-resistant alleles already described in the literature, implying that these drugs are not currently appropriate for use in Colombia. In contrast, mutations in the *pf dhps* gene were only observed at codon 437, an indication that full resistance to sulfadoxine has not been achieved in Choco. MMGs found matched the clonal lineage E variant 1 previously reported in northwestern Colombia.

**Keywords:** Colombia, Molecular markers, Chloroquine, Sulfadoxine-pyrimethamine, Neutral microsatellites, *Plasmodium falciparum*
of low intensity [1]. In addition, a downward trend of malaria cases was observed between 2000 and 2014, which changed in 2015, when a significant resurgence of epidemic transmission in Venezuela led to an increase in number of cases along with increased transmission in endemic areas of Brazil, Colombia, Guyana, Nicaragua, and Panama; as well as outbreaks in countries that were moving towards elimination [2]. In 2019, more than 86% of malaria cases in the Americas, were concentrated in Venezuela, Brazil, and Colombia [3].

Colombia is endemic for *Plasmodium falciparum* and *Plasmodium vivax* and approximately 70,000 malaria cases are reported annually by the public health services, mainly in departments of Choco, and Nariño [4]. Despite the relatively large number of malaria cases reported each year and the use of different anti-malarial treatments in recent decades, little is known about the molecular markers of drug resistance and genetic diversity of *P. falciparum* in the country. Since 2010, the first-line regimen for treating uncomplicated *P. falciparum* malaria in Colombia has been artemether–lumefantrine (AL). Prior to the use of this artemisinin-based combination therapy (ACT), chloroquine (CQ), sulfadoxine-pyrimethamine (SP), and amodiaquine (AQ) were widely used, until high rates of therapeutic failure were reached [5–7]. CQ monotherapy was employed until the early 1980s, but when resistance to this anti-malarial emerged, SP in combination with CQ or AQ became the therapeutic regimens utilized. In 1999, AQ-SP was implemented as the first-line treatment for uncomplicated *P. falciparum* malaria and from 2006, two artemisinin-based combinations, artesunate-mefloquine and AL, were introduced.

*Plasmodium falciparum* resistance to CQ and SP has been associated with point mutations in the chloroquine-resistance transporter (*pfcr* gene) [8], and the dihydrofolate reductase (*pfldhfr*) and dihydropteroate synthase (*pfldhps*) genes [9], respectively. The *pfcr* point mutation K76T is critical to confering CQ resistance, but mutations in other codons are also associated with resistance [8]. Resistance to sulfadoxine-pyrimethamine is associated with the accumulation of mutations at codons 50, 51, 59, 108, and 164 in the *pfldhfr* gene (codon 50 being more relevant in South America) and 436, 437, 540, and 581 of the *pfldhps* gene, respectively [9].

In 2018, a therapeutic efficacy study (TES) was carried out to evaluate the efficacy of AL for the treatment of uncomplicated *P. falciparum* malaria in Quibdo, Choco, together with the evaluation of the confirmed molecular marker of resistance to artemisinin in the *pfk13* gene, and to unconfirmed marker for lumefantrine resistance, *pfmdrl* gene [10]. Although TESs are indispensable to assess the efficacy of anti-malarial drugs, molecular surveillance is helpful to detect the emergence of mutations associated with drug resistance and signal recovering sensitivity to an anti-malarial used in the past. To evaluate if the prevalence of wild-type genotypes of *pfcr*, *pfldhfr*, and *pfldhps* genes has changed over time since their withdrawal in Colombia, molecular testing of samples collected during the 2018 TES was conducted. In addition, the genetic diversity of infections using seven neutral microsatellites (NMSs) loci was assessed.

**Methods**

**Ethics statement**

Samples for this study were obtained from a TES conducted in Quibdo in 2018 [10]; as part of the TES, written informed consent for secondary use of remnant samples were obtained. The TES protocol was reviewed and approved by the Institutional Ethics Committee of the Colombian National Institute of Health (Protocol CEMIN 2-2018, Minute #5 of March 22, 2018) and the PAHO Ethics Review Committee (PAHOERC) (PAHO 2018-04-0029). CDC considered the study a public health evaluation (reference number: 2018-063).

**Study site, sample collection, DNA extraction, and PET-PCR**

Quibdo is the capital of Choco department, located in northwestern Colombia (lat. 5° 41' 32" N, long. 76° 39' 29" W) on the banks of the Atrato river in the Pacific region, at an altitude of 43 m above sea level, the average temperature is 28 °C and annual humidity oscillates between 86 and 88%. The Pacific region is separated from Amazon and Orinoco region by the Andes Mountain range. Quibdo has a population of 116,087, mainly consisting of Afro Colombians (87.5%), of which 65% is settled in the urban area. Quibdo contributes 6 to 11% of the total cases of malaria reported in Colombia.

Samples collected at patient enrollment in the TES were used in this study. Parasite DNA was extracted from dried blood spots collected on filter paper using the QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The *Plasmodium* species was confirmed by a real-time PCR, photo-induced electron transfer polymerase chain reaction (PET-PCR) as previously described [11].

**Sanger sequencing for molecular markers of resistance**

*Plasmodium falciparum* samples were genotyped using PCR or nested PCR followed by Sanger sequencing to evaluate mutations at *pfcr* gene (codons 72–76), *pfldhfr* gene (codons 51, 59, 108, and 164), and *pfldhps* gene (codons 436, 437, 540, and 581). Primer sequences and PCR cycling conditions for each gene are reported elsewhere [12–14]. Briefly, primary PCR was performed with 1 μL genomic DNA, 7.5 μL of 2X master mix (Promega, Madison, WI, USA), and 0.27 μM each primer in 15 μL
final volume for pfhdfr and dhps genes. Conditions for pfcr 
types were the same, except 1.2 μL of DNA and a 
final concentration of 0.33 μM each primer. Nested PCR 
was done for pfhdps and pfcr genes. PCR products were 
purified with ExoSAP-it reagent (New England Biolabs, 
Ipswich, MA, USA) and sequenced using the Sanger 
method (Genewiz INC, NJ, USA). Laboratory P. falciparum 
strains, 3D7 and 7G8, were used as wild-type and 
mutant-type controls, respectively. Sequence analysis 
was performed using Geneious® 7.1.7 software (Biomate 
s, Auckland, New Zealand) using the P. falciparum 
3D7 reference strain for pfcr (PF3D7_0709000), pfhdfr 
(PF3D7_0417200), and pfhdps (PF3D7_0810800) genes. 
Mutant alleles were identified, and the frequency of each 
allele was determined.

Neutral microsatellites

All samples were genotyped using a set of seven NMSs to 
identify polyclonal infections. The microsatellite loci 
amplified were on chromosome (chr) 2 (C2M34), chr 3 
(C3M69), chr 4 (Polya), chr 6 (TA1 and TA109), chr 10 
(2490), and chr 12 (PPK2) [15–17]. Amplification prod 
ucts labeled fluorescently with FAM or HEX of the 7 loci 
were followed by fragment electrophoresis on a capillary 
sequencer ABI 3130 xl Genetic Analyzer (Applied Bio 
systems, Foster city, CA, USA) and then fragment sizes 
were scored using GeneMapper® V2.7.0 software 
(Applied Biosystems, Foster city, CA, USA). PCR cycling 
conditions for each locus [15–17] are registered else 
where. Samples that carried a single allele at each locus 
were considered monoclonal infections and, when more 
than one allele was found at any ≥1 locus, they were 
considered polyclonal infections. Presence of alleles (multi 
ple peaks) was evaluated by locus and minor alleles were 
scored if they were more than 33% the height of the peaks 
corresponding to the predominant alleles. Genetic varia 
tion at each locus was quantified by the allelic diversity,

\[ H_e = \frac{n}{\sum p_i^2} \]

where \( n \) is the number of P. falciparum isolates genotyped for a 
particular locus and \( p_i \) is the frequency of the \( i \)th allele. The 
sampling variance of \( H_e \) was estimated as 

\[ \frac{2(n-1)}{n^2} \left( \frac{2}{n^2} \sum p_i^2 - \left( \sum p_i^2 \right)^2 \right) \]

[18]. Infections 
containing more than one allele at any of the seven NMSs 
were not included in the analysis.

Results

Molecular markers of resistance

A total of 88 samples were processed of which 85 could be 
confirmed as P. falciparum by PET-PCR. Among these 
85 samples, sequencing of the pfcr, pfhdfr, and pfhdps 
genes was successful in 72 (84.7%), 79 (92.9%), and 73 
(85.9%) samples, respectively. For the pfcr gene, all iso 
lates carried the K76T and N75E mutant alleles. Only 
two mutant alleles, N51I and S108N, were observed in 
the pfhdfr gene, and for the pfhdps gene wild-type alleles 
prevailed in all codons investigated except in codon 
437, where 32 (43.8%) isolates carried the mutant allele 
A437G (Table 1).

**Table 1** Observed P. falciparum pfcr, pfhdfr, and pfhdps resistance alleles, Quibdo, Colombia, 2018

| Gene   | Polymorphic positions | Genotype | Genotype frequency* |
|--------|-----------------------|----------|---------------------|
| pfcr   | C72S/N73/M74I/N75E/K76T | CV/ME    | 72/72               |
| pfhdfr | N51I/C59R/S108N/I164L | IC/N     | 79/79               |
| pfhdps | A437G/K540/A581G      | AKA      | 41/73               |
| pfhdps | A437G/K540/A581G      | G/K/A    | 32/73               |

*The frequency was calculated only with the samples that amplified
In bold are mutant-type alleles
of the clonal lineage E, which was described earlier in Peru [22] and Ecuador [23]. Eleven (22.9%) isolates had different alleles from those described for Ev1 such as 154 in Poly-α, 160 and 163 in PfPK2, and 80 in 2490. This finding suggests that in Quibdo another variant of the clonal line E circulates, which was named variant 2 (Ev2). This variant was found in 8 isolates (MMGs 5, 6, 7, and 8) and its microsatellite profile was TA1: 172, Polyα: 148, PfPK2: 160/163, TA109: 160, 2490: 74/80, C2M34: 226, and C3M69: 124/140 (Fig. 1).

No association was observed between the two resistance genotypes (combination of pfcrt, pf dhfr, and pf dhps resistance alleles) and MMGs found in this study; however, parasites with MMGs 2, 3, and 8 carried the CVMET/IKA genotype and parasites with MMGs 4, 7, and 9 harbored CVMET/IKA/GKA. Parasites with MMGs 1 and 6 showed both genotypes (see Additional file 1). When comparing the isolates belonging to Ev1 with the rest of the isolates found in Quibdo, samples shared the same drug resistance genotypes CVMET/ICNI/AKA or CVMET/ICNI/GKA, being more common the genotype CVMET/ICNI/AKA within the variant Ev1 (68.8%). Considering that the samples analysed in a previous report [10] are the same as in this study, Pfmndr1 genotypes NFSDD/NFSDY previously reported [10] were found in both the Ev1 and the remaining isolates; however, genotype NFSDD was detected mostly in Ev1 (25%) and only in isolates with an allele size of 226 bp in C2M34 (see Additional file 1).

**Discussion**

The monitoring of molecular markers of resistance is useful for detecting changes in the parasite genome associated with anti-malarial drugs resistance over time. In this study, only one CQ-resistant genotype (CVMET) was found, in contrast to the three genotypes (CVMET/CVMNT/CVIET) previously reported in samples from the Colombian Pacific [24]. Field isolates carried mutant K76T in pfcrt gene, indicating that, despite the withdrawal of CQ from national treatment guidelines for P. falciparum in 1998, resurgence and re-expansion of wild-type pfcrt P. falciparum has not occurred. A similar situation was reported in a previous study carried out in three Colombian malaria-endemic areas [25] and other countries in the Americas [26, 27]. This finding contrasts with what was observed in some countries of Africa, like Malawi [28, 29], where the recovery of CQ-sensitive wild-type pfcrt P. falciparum occurred in as little as 10 years after the use of CQ was discontinued.

As a result of CQ resistance in Colombia, SP was adopted in 1981; high and moderate levels of therapeutic failure were quickly observed in the Amazon basin [7].

---

### Table 2

Allelic diversity in seven NMS loci and expected heterozygosity from isolates collected in Quibdo, Choco

| NMS Tandem repeats | TA1 ATT | POLYα TAA | PfPK2 AAT | TA109 TAA | 2490 TAA | C2M34 AT | C3M69 TA | Mean No. of allele (± s.d) | Mean (± s.d) |
|--------------------|--------|----------|----------|-----------|---------|---------|---------|---------------------------|-------------|
| No. of detected alleles | 1 | 2 | 3 | 1 | 3 | 2 | 2 | 2.0 (0.57) | 0.17 (0.03) |
| *He: heterozygosity | 0 | 0.04 | 0.33 | 0 | 0.19 | 0.45 | 0.16 | |

*n = 48, monoclonal infections*

### Table 3

Neutral microsatellites multilocus genotypes (MMG) in Quibdo, Colombia (n = 48), 2018

| MMG | Frequency n (%) | Allele size (bp) |
|-----|----------------|------------------|
|     | TA1 Polyα PfPK2 TA109 2490 C2M34 C3M69 | TA1 Polyα PfPK2 TA109 2490 C2M34 C3M69 |
| 1   | 20 (41.7) | 172 148 175 160 74 226 124 |
| 2   | 16 (33.3) | 172 148 175 160 74 236 124 |
| 3   | 1 (2.1)  | 172 148 175 160 75 226 124 |
| 4   | 2 (4.2)  | 172 148 175 160 80 226 124 |
| 5   | 1 (2.1)  | 172 148 160 160 74 226 140 |
| 6   | 2 (4.2)  | 172 148 160 160 80 226 140 |
| 7   | 3 (6.2)  | 172 148 163 160 74 226 140 |
| 8   | 2 (4.2)  | 172 148 163 160 74 226 140 |
| 9   | 1 (2.1)  | 172 154 160 160 74 226 140 |

*n: number of single clone isolates*
and northwestern regions [6], respectively. The analysis of molecular markers of resistance to SP in this study shows a predominance of double mutant (ICNI) for pfdhfr, while mutations in the pfdhps gene were only observed at codon 437 (43.8%), an indication that full resistance to sulfadoxine was not achieved in circulating parasites in Choco. In addition, ACT replaced the combined therapy SP with AQ for uncomplicated P. falciparum malaria in 2008, and since then there has been no selective pressure with SP. Similar resistance profiles were observed in northwest Colombia in previous studies [19, 30, 31]. This likely slow emergence of mutations in pfdhps in parasites from Quibdo could be explained, in part, by the use of multiple drug regimens (SP in combination with CQ or AQ plus primaquine as a gametocytocidal agent) unlike Brazil and the Peruvian Amazon region, where SP monotherapy was used and parasites with double and triple mutations predominate.

The frequency of mutations associated with resistance to CQ (K76T 100%) [25, 32, 33] and SP (N51I/ S108N 93–100% and A437G 65–70%) [19, 30, 31] has remained constant in northwest Colombia for at least 10 years since introduction of ACT, suggesting the fixation of resistant alleles [12, 34] in Choco. This situation could be explained by the strong selective pressure exerted by the drugs that fixed the mutant allele in this population, considering that the frequency of these mutant alleles is 100% and it has prevailed unchanged over the last decade in this part of the country. Other possible reasons for fixation of these mutant alleles include CQ and SP drug pressure not allowing for expansion of sensitive strains. Although CQ and SP are no longer used to treat P. falciparum malaria in Colombia, CQ constitutes the first-line regimen for vivax malaria and self-medication with CQ for non-laboratory confirm malaria has also been observed in 32% of symptomatic individuals [35]. In addition, the prevalence of CVMET over time may be because this haplotype, like SVMNT found in Papua New Guinea and Brazil, has no fitness disadvantage.
over the sensitive one even in the absence of drug pressure [36]. Finally, it is likely that no wild-type populations, able to replace the resistant populations, were left in Colombia after the development of resistance.

When comparing the number of alleles per microsatellite locus against collected samples in Quibdo during 2001–2007 [19] and in Turbo during 2002–2008 [20], a town located in Antioquia department in northwestern Colombia, more alleles were detected at that time (six and seven for Polya and six and nine for PPK2). Higher diversity values (expected heterozygosities) were found in both studies, 0.915 for the first, and 0.35 to 0.18 for the second, compared to 0.17 in the present study. These results point to a progressive reduction in the level of genetic diversity in *P. falciparum* from Quibdo, as well as a decrease in the frequency of multiple infections. These findings suggest the circulating *P. falciparum* population have a more clonal composition because these populations have undergone bottlenecks possibly caused by actions of national malaria control programme or interventions focused on improving access to timely diagnosis and adequate treatment, and the coverage in the use of insecticide-treated bed nets such as World Fund Malaria Project implemented in Colombia between 2010 and 2015. Additionally, alleles 172 bp in TA1 and 160 bp in TA109 seem to be fixed in parasites from Quibdo.

Of the 48 isolates analysed for NMS, 77.1% shared the same microsatellite profile of the clonal lineage E* v1*, which was described in the neighbouring department of Antioquia [21], and 16.7% showed a new variant of the clonal lineage E, named E* v2* (Fig. 1). This variant is characterized by having a genetically identical set of markers, but variable at others, with common ancestors occurring more than 15 years ago, according to allele sizes reported in a study carried out in Quibdo during 2001 and 2006 [19].

When comparing the haplotypes found in Quibdo with other haplotypes reported in Colombia, it is evident that the clonal lineage E variant 1 predominates in this town and it is characteristic in this area of the country; this same variant was reported in samples from Antioquia collected in 2012, indicating that in the northwestern zone of Colombia there is a predominance of variants derived from genetic lineage E. The genetic lineage Bv1 predominates in the Amazonas department [21], variant previously observed in Peru [22], and the genetic lineage F [21] in Nariño department (southwestern Colombia) (Fig. 1). The foregoing allows us to suggest that specific genetic lineages predominate in the different endemic areas of malaria in Colombia.

**Conclusions**

Overall, the frequency of anti-malarial drug resistance genotypes and NMS multilocus genotypes was studied in northwestern Colombia to explore possible genomic adaptations in the parasite in response to changes in the anti-malarial policy and genetically characterize *P. falciparum* populations. Monitoring of molecular markers of anti-malarial drugs resistance should be considered as part of regular in vivo efficacy trials in malaria-endemic countries to timely detect emergence of polymorphisms conferring decreased susceptibility to anti-malarials, especially since self-medication, the use of subtherapeutic doses, gold mining activities associated with malaria outbreaks, and human mobility and migration are common in Colombia.

**Abbreviations**

ACT: Artemisinin-based combination therapy; AL: Artemether–lumefantrine; AQ: Amodiaquine; CQ: Chloroquine; DNA: Deoxyribonucleic acid; He: Heterozygosity; MMG: NMS multilocus genotypes; NMS: Neutral microsatellites; PCR: Polymerase chain reaction; PET-PCR: Photo-induced electron transfer-PCR; SP: Sulfadoxine-pyrimethamine; TES: Therapeutic efficacy study.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12936-022-04328-x.

**Additional file 1: Table S1.** Alleles found in samples from Quibdo, 2018. *Pfmdr1* data is already published (10). Tabular data with the alleles found in *crt, dhfr, dphs*, and *mdr1* genes and 7 neutral microsatellites in 42 samples from Quibdo, Colombia, 2018. Red letter indicates a mutant allele. N/A indicates no allele amplification at that locus.

**Acknowledgements**

We would like to thank the managers of the malaria control programme in Choco, and staff and participants for their collaboration in this study.

**Author contributions**

APG performed molecular assays, conceived and wrote the first draft of the article. APG and NWL analysed and interpreted the microsatellites and molecular markers data. SMCh and AMO made important contributions on the initial draft and assisted in development of the final manuscript. SMCh, AMO, MJO, NWL, LJC contributed to the analysis, content, and writing of the manuscript. All authors read and approved the final manuscript.

**Funding**

Funding to carry out the molecular study was partially provided by the U.S. Agency for International Development (USAID) through the Latin American and the Caribbean Regional Malaria Programme, and by the Colombian National Institute of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Availability of data and materials**

Database is available in supplementary information.

**Declarations**

**Ethics approval and consent to participate**

The TES protocol was reviewed and approved by the Institutional Ethics Committee of the Colombian National Institute of Health (Protocol CEMIN 2-2018, Minute #5 of March 22, 2018) and the PAHO Ethics Review Committee.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Grupo de Parasitología, Instituto Nacional de Salud, Bogotá, Colombia. 2Insti-
tuto de Investigaciones en Ciencias Biomédicas, Universidad Ricardo Palma, Lima, Perú. 3Malaria Branch, Division of Parasitic Diseases and Malaria, Center
for Global Health, Centers for Disease Control and Prevention, GA, Atlanta, USA.

Received: 22 March 2022 Accepted: 18 August 2022
Published online: 28 October 2022

References
1. Padilla-Rodríguez JC, Olivera MJ, Ahumada-Franco ML, Paredes-Medina AE. Malaria risk stratification in Colombia 2010 to 2019. PLoS ONE.
2021;16:e0247811.
2. WHO. World malaria report 2019. Geneva, World Health Organization.
2019. https://www.who.int/publications/i/item/world-malaria-report-
2019. Accessed 3 Nov 2021.
3. WHO. World malaria report 2020. Geneva, World Health Organiza-
tion. 2020. https://www.who.int/publications/i/item/9789240015791.
Accessed 3 Nov 2021.
4. Ferro C, Sastoque LA. Informe del evento malaria, Colombia 2019.
Instituto Nacional de Salud. http://www.ins.gov.co/busrador-eventos/
Informesdeevento/MALARIA%20PE%20III%202019.pdf. Accessed 9 Nov
2019.
5. Osorio LE, Giraldo LE, Grajales LF, Arriaga AL, Andrade AL, Ruebush TK,
et al. Assessment of therapeutic response of Plasmodium falciparum to
chloroquine and sulfadoxine-pyrimethamine in an area of low malaria
transmission in Colombia. Am J Trop Med Hyg. 1999;61:968–72.
6. Blair S, Carmona-Fonseca J, Piñeros JG, Ríos A, Alvarez T, Alvarez G, et al.
Therapeutic efficacy test in malaria falciparum in Antioquia, Colombia.
Malar J. 2006;5:14.
7. Osorio L, Perez LDR, Gonzalez U. Assessment of the efficacy of antimal-
arial drugs in Tarapaca, in the Colombian Amazon basin. Biomedica.
2007;27:133–40.
8. Bray PG, Martin RE, Tilley L, Ward SA, Kirk K, Fidock DA. Defining the role
of PfCRT in Plasmodium falciparum chloroquine resistance. Mol Microbiol.
2005;62:323–31.
9. Gregson A, Plowe CV. Mechanisms of resistance of malaria parasites to
antifolates. Pharmacol Rev. 2005;57:117–45.
10. Olivera MJ, Guerra AP, Cortes LJ, Nortz RJ, Padilla J, Novoa J, et al.
Artemether–lumefantrine efficacy test for the treatment of uncomplicated
Plasmodium falciparum malaria in Choco, Colombia after 8 years as first-
line treatment. Am J Trop Med Hyg. 2020;102:1056–63.
11. Lucchi NW, Natayanian J, Karel MA, Xayavong M, Knulst L, DaSilva AJ,
et al. Molecular diagnosis of malaria by photo-induced electron transfer
fluorogenic primers: PET-PCR. PLoS ONE. 2013;8:e56777.
12. Griffith SM, Mixson-Hayden T, Siddaran S, Collom AM, Mixson-Hayden T, Vinayak
S, et al. pfmdr1 amplification and fixation of pfcr chloroquine resistance
alleles in Plasmodium falciparum in Venezuela. Antimicrob Agents Chem-
other. 2010;54:1572–9.
13. McColloM AM, Poe AC, Hamel M, Huber C, Zhou Z, Shi YP, et al. Antifolate
resistance in Plasmodium falciparum: multiple origins and identification of
novel dhfr alleles. J Infect Dis. 2006;194:189–97.
14. Vinayak S, Alam MT, Mixson-Hayden T, Collom AM, Sem R, Shah NK,
et al. Origin and evolution of sulfadoxine resistant Plasmodium falciparum.
PLoS Pathog. 2010b;6:e1000830.
15. Anderson TJ, Su XZ, Bockarie M, Logog M, Day KP. Twelve microsatellite
markers for characterization of Plasmodium falciparum from finger-prick
blood samples. Parasitology. 1999;119:113–25.
16. Nair S, Williams JT, Brockman A, Paiphun L, Mayxay M, Newton PN, et al.
A selective sweep driven by pyrimethamine treatment in Southeast Asian
malaria parasites. Mol Biol Evol. 2003;20:1526–36.
17. Roper C, Pearce R, Bredenkamp B, Gumede J, Drakeley C, Mosha F, et al.
Antifolate antimalarial resistance in southeast Africa: a population-based
analysis. Lancet. 2003;361:1174–81.
18. Nash D, Sair, Mayxay M, Newton PN, Guthmann JP, Nosten F, et al.
Selection strength and hitchhiking around two anti-malarial resistance
genes. Proc Biol Sci. 2005;272:1153–61.
19. Corredor V, Murillo C, Echeverry DF, Benavides J, Pearce RJ, Roper C, et al.
Origin and dissemination across the Colombian Andes Mountain range
of sulfadoxine-pyrimethamine resistance in Plasmodium falciparum.
Antimicrob Agents Chemother. 2010;54:3121–5.
20. Chenet SM, Taylor JE, Blair S, Zuluaga L, Escalante AA. Longitudinal
analysis of Plasmodium falciparum genetic variation in Turbo, Colombia
implications for malaria control and elimination. Malar J. 2015;14:363.
21. Dorado DJ, O’koth SA, Montenegro LM, Diaz G, Barmwell JW, Udhayakumar
V, et al. Genetic characterisation of Plasmodium falciparum isolates with
deletion of the pfrp2 and/or pfhrp3 genes in Colombia: the Amazon
region, a challenge for malaria diagnosis and control. PLoS ONE.
2016;11:e0163137.
22. Grifﬁng SM, Mixson-Hayden T, Siddaran S, Alam MT, McColloM AM,
Cabezas C, et al. South American Plasmodium falciparum after the malaria
eradication era: clonal population expansion and survival of the fittest
hybrids. PLoS ONE. 2011;6:e23486.
23. Sánz FE, Morton LC, O’koth SA, Valenzuela G, Vera-arias CA, Wélez-Alvarez
E, et al. Clonal population expansion in an outbreak of Plasmodium falciparum
on the northwest coast of Ecuador. Malar J. 2015;14:497.
24. Echeverry DF, Holmgen G, Murillo C, Higuera X, Björkman A, Gil JP, et al.
Polymorphisms in the pfcr and pfmdr1 genes of Plasmodium falciparum and
in vitro susceptibility to artemether and desethylmefloquine. Am J Trop Med Hyg.
2007;77:1034–9.
25. Aponte S, Guerra AP, Álvarez-Larrotta C, Bernal SD, Restrepo C, González,
et al. Baseline in vivo, ex vivo and molecular responses of Plasmodium falciparum
to artemether and lumefantrine in three endemic zones for malaria in Colombia.
Trans R Soc Trop Med Hyg. 2017;111:71–80.
26. Arüspide N, Hijar-Guerra G, de Mota D, Diaz-Correa CE, Veloz-Pérez R,
Gutiérrez S, et al. Mutant alleles associated to chloroquine and
sulfadoxine-pyrimethamine resistance in Plasmodium falciparum of the
Ecuador-Peru and Ecuador-Colombia borders. Rev Peru Med Exp Salud
Publica. 2014;31:282–7.
27. Valenzuela G, Castro LE, Valencia-Zamora J, Vera-Arias CA, Rohrbach P,
Saénz FE. Genotypes and phenotypes of resistance in Ecuadorian Plomodi-
fumum falciparum. Malar J. 2019;18:415.
28. Mita T, Kaneko A, Lum JK, Bwijjo B, Takechi M, Zungu IL, et al. Recovery of
chloroquine sensitivity and low prevalence of the Plasmodium falciparum
chloroquine resistance transporter gene mutation K76T following the
discontinuation of chloroquine use in Malawi. Am J Trop Med Hyg.
2003;68:413–5.
29. Laruffer MK, Takala-Harrison S, Dzinjalamala FK, Stine OC, Taylor PE, Plowe
CV. Return of chloroquine-susceptible falciparum malaria in Malawi was
a reexpansion of diverse susceptible parasites. J Infect Dis. 2010;202:801–8.
30. Galindo JA, Knudson A, Nicholls RS, Guerra AP. Point mutations in dihy-
drofolate reductase and dihydropteroate synthase genes of Plasmodium
falciparum from three endemic malaria regions in Colombia. Biomedica.
2010;30:56–64.
31. Hernández DC, Guerra AP, Cubunhua ZM, Nicholls RS, Barrera SM.
Haplotypes associated with resistance to sulfadoxine-pyrimethamine in
Plasmodium falciparum in two malaria endemic locations in Colombia.
Infect Genet Evol. 2013;18:1183–90.
32. Restrepo-Pineda E, Arango E, Maestre A, Do Rosário VE, Cravo P. Studies
on antimalarial drug susceptibility in Colombia, in relation to Pfmdr1 and
Pfcr. Parasitology. 2008;135:547–53.
33. Restrepo E, Carmona-Fonseca J, Maestre A. Plasmodium falciparum: high
frequency of pfcr point mutations and emergence of new mutant
haplotypes in Colombia. Biomedica. 2008;28:523–30.
34. Pacheco MA, Foero-Perla DA, Schneider KA, Chaverro M, Gamarro A,
Figueras L, et al. Malaria in Venezuela: changes in the complexity of infection
reflects the increment in transmission intensity. Malar J. 2020;19:176.
35. Diaz G, Lasso AM, Murillo C, Montenegro LM, Echeverry DF. Evidence of
self-medication with chloroquine before consultation for malaria in
the Southern Pacific Coast Region of Colombia. Am J Trop Med Hyg. 2019;100:66–71.

36. Mehlotra RK, Fujioka H, Roepe PD, Janneh O, Unsos LM, Jacobs-Lorena V, et al. Evolution of a unique *Plasmodium falciparum* chloroquine-resistance phenotype in association with pfcrt polymorphism in Papua New Guinea and South America. Proc Natl Acad Sci USA. 2001;98:12689–94.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.