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Artemether–Lumefantrine Efficacy for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Choco, Colombia after 8 Years as First-Line Treatment

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Abstract. Artemether–lumefantrine (AL) is the first-line treatment for uncomplicated *Plasmodium falciparum* infection in Colombia. To assess AL efficacy for uncomplicated falciparum malaria in Quibdo, Choco, Colombia, we conducted a 28-day therapeutic efficacy study (TES) following the WHO guidelines. From July 2018 to February 2019, febrile patients aged 5–65 years with microscopy-confirmed *P. falciparum* mono-infection and asexual parasite density of 250–100,000 parasites/μL were enrolled and treated with a supervised 3-day course of AL. The primary endpoint was adequate clinical and parasitological response (ACPR) on day 28. We attempted to use polymerase chain reaction (PCR) genotyping to differentiate reinfection and recrudescence, and conducted genetic testing for antimalarial resistance–associated genes. Eighty-eight patients consented and were enrolled; four were lost to follow-up or missed treatment doses. Therefore, 84 (95.5%) participants reached a valid endpoint: treatment failure or ACPR. No patient remained microscopy positive for malaria on day 3, evidence of delayed parasite clearance and artemisinin resistance. One patient had recurrent infection (12 parasites/μL) on day 28. Uncorrected ACPR rate was 98.8% (83/84) (95% CI: 93.5–100%). The recurrent infection sample did not amplify during molecular testing, giving a PCR-corrected ACPR of 100% (83/83) (95% CI: 95.7–100%). No *P. falciparum kelch* 13 polymorphisms associated with artemisinin resistance were identified. Our results support high AL efficacy for falciparum malaria in Choco. Because of the time required to conduct TESs in low-endemic settings, it is important to consider complementary alternatives to monitor antimalarial efficacy and resistance.

INTRODUCTION

Despite the great efforts to control malaria over the last decades, which have reduced its incidence and mortality, malaria remains one of the biggest public health threats in Latin America.[1] Colombia contributes to approximately 15% of malaria cases in the Americas.[1] In 2018, according to the Colombian National Public Health Surveillance System (SIVIGILA), there were 60,385 cases of uncomplicated malaria, of which 29,122 (48.2%) were due to *Plasmodium falciparum* mono-infection.[2]

Early diagnosis and prompt treatment with an effective drug regimen remain important components of malaria control and elimination strategies.[3] In 2001, the WHO formally recommended antimalarial treatment with artemisinin-based combination therapies (ACTs) for the management of uncomplicated *P. falciparum* infection for most malaria-endemic areas of the world.[4] Unfortunately, emergence of artemisinin-resistant parasites and ACT failure has already been reported in Southeast Asia.[5] In South America, Suriname has experienced a considerable increase in post-treatment delayed parasite clearance, a signal of resistance to the artemisinin component of ACTs.[6] Although the presence of *P. falciparum kelch* 13– (*PfK13*-) mutant alleles had been reported in Guyana in 2010,[7] a subsequent therapeutic efficacy study (TES) conducted in 2011–2012 did not detect *PfK13* mutations.[8]

Colombia adopted the use of ACTs in 2006 as its national treatment policy for uncomplicated falciparum malaria, and artemether–lumefantrine (AL) was introduced as the first-line therapy in 2010. Therapeutic efficacy studies assist countries in monitoring for the emergence of drug resistance. The WHO recommends evaluating first-line antimalarial drugs at least every 3 years in low-endemic countries to confirm efficacy.[9] Since the introduction of ACTs in Colombia, studies have shown treatment response rates greater than 95%.[10] Monitoring of drug resistance–associated genes among malaria parasites can be performed during TESs to determine the prevalence of parasites with resistance genotypes, which complements TES findings. Mutations in *PfK13* propeller gene are considered molecular markers of artemisinin resistance in *P. falciparum.*[11] No similar marker has been validated for lumefantrine; however, exposure to this drug seems to select certain alleles in the *P. falciparum* multidrug resistance 1 gene (*Pfmdr1*).[12] The exact role of this gene in lumefantrine resistance in *Plasmodium* remains controversial. In accordance with national and international recommendations, we conducted a TES to assess AL efficacy for the treatment of uncomplicated falciparum malaria in Choco, Colombia. In addition, we aimed to detect the presence of polymorphisms in *PfK13* and *Pfmdr1* genes.

METHODS

Study site and study personnel. We conducted a one-arm 28-day health facility–based TES using the WHO and Pan American Health Organization (PAHO) guidelines for

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antimalarial efficacy monitoring with some modifications.[9,13] The study took place from July 2018 through February 2019 at Ismael Roldan Valencia Hospital and San Vicente Health Center, both health facilities in Quibdo, the capital city of Choco, a department in the rainforest of northwestern Colombia (Figure 1) that has ongoing malaria transmission. The study team, consisting of one physician, two nurses, and two microscopists, conducted patient recruitment and follow-up.

Study population. Patients aged 5–65 years with symptomatic, uncomplicated *P. falciparum* infection who presented at the hospital and met the inclusion criteria were invited to participate in the study. The inclusion criteria included having thick smear–confirmed *P. falciparum* monoinfection, asexual parasite density between 250 and 100,000 asexual parasites/μL, a fever or a history of fever in the previous 24 hours (defined as axillary temperature of ≥ 37.5°C), ability to swallow medications, residence within 45 minutes of enrollment sites, and willingness to attend all seven follow-up visits. Participants were also requested to provide informed consent. Breastfeeding women, pregnant women, or women who tested positive for a pregnancy urine test were excluded. Any person reporting the use of any medication with antimalarial effects in the past 4 weeks, with a history of hypersensitivity to the treatment being offered, or with heart, kidney, and liver diseases, and HIV infection were also ineligible. The required sample size was 78 participants based on the assumption of therapeutic failure of 5.3% (based on a previous study in Colombia)[10] in a population of infinite size with a level of significance of 5%, and a maximum tolerable error of ± 5%. We added 15% to account for loss to follow-up, with a final target of 90 participants. We used EpiData Manager software (version 4.2, EpiData Association, Odense, Denmark) for sample size calculations.

Follow-up. After enrollment, participants were followed up for 28 days. In total, they were seen on eight separate days, starting with the enrollment day visit (day 0) and on days 1, 2, 3, 7, 14, 21, and 28. On the first 3 days of the study (days 0, 1, and 2), participants were seen twice to be able to receive supervised therapy. Of note, the day 3 visit was scheduled 72 hours after treatment initiation, with a tolerance of plus or minus 1 hour. Participants were contacted by phone for visit reminders before scheduled appointments. Additional follow-up visits were done if the participants developed any relevant signs and symptoms of disease progression. Study personnel performed clinical examinations of participants at all visits, together with a thick and thin smear for malaria microscopy. These visits monitored for the presence of a fever (using axillary temperature measured with a digital thermometer), symptoms of severe malaria, and drug adverse reactions. Four drops of blood spots on Whatman 903TM filter paper (GE Healthcare, Cardiff, United Kingdom) (lot 7064416-W152) were collected by finger prick on day 0 and during follow-up in the case of parasitemia on or after day 7.

Interventions. Treatment with AL was provided according to the Colombian national antimalarial treatment guideline, and treatment doses were based on participant weight.[14] The Colombian Ministry of Health and Social Protection purchased and provided quality oversight over the 20-mg artemether and 120-mg lumefantrine (Macleods Pharmaceuticals Ltd., Oxalis Labs, Baddi, India, Lot 17TAI002A, Exp 12/2019). Artemether–lumefantrine doses were administrated twice daily for three consecutive days at enrollment and 8 hours later, and every 12 hours on days 1 and 2. Treatment was administered concomitantly with a dairy product (e.g., milk or yogurt). Participants took all doses under direct observation of study personnel, who monitored for vomiting or adverse events for 30 minutes after administration. A

FIGURE 1. Map of Quibdo, Choco, Colombia.

participant vomiting during this observation period would receive the same dose again with an additional 30 minutes of observation. Study personnel would send participants who had vomited more than once for rescue treatment according to treatment guidelines in Colombia and withdraw that person from the study. In addition, paracetamol was given to all participants with axillary temperature ≥ 38°C.

Laboratory procedures. Training of laboratory staff was carried out over 2 weeks before study initiation. Training covered reagent preparation, Giemsa staining, thick and thin smear preparation, collection of dried blood spots (DBSs) on a filter paper, and data recording. A 2.5–3% Giemsa preparation was used to stain slides for 45–60 minutes. Asexual parasite density, expressed as the number of asexual forms per μL of blood, was calculated by dividing the number of asexual forms by the number of white blood cells multiplied by a white blood cell density of 6,000 per μL. Gametocyte presence was noted, but not quantified.[9] Two microscopists independently read slides. A third independent microscopist reexamined the slides for discordant results (parasite density > 50%, or difference in species or positivity). Final asexual parasite density was the geometric mean of the two closest readings.

Molecular analysis. *Plasmodium* DNA was isolated from filter paper DBSs using the QIA amp DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Before genotyping for resistance molecular markers, molecular confirmation of *P. falciparum* infection was performed using photo-induced electron transfer polymerase chain reaction (PET-PCR).[15] Further testing was performed only on positive samples.

Attempts to differentiate recrudescence from reinfection paired samples from the same participant (day 0 and day of recurrent infection occurring on or after day 7) were conducted with seven neutral microsatellites markers: C2M34 on chromosome (chr.) 2, C3M69 (chr. 3), poly-α (chr. 4), TA1 (chr. 6), TA109 (chr. 6), 2490 (chr. 10), and PfPk2 (chr. 12).[16] Allele size differentiation was carried out by capillary electrophoresis on an Applied Biosystems (ABI) 3130xl sequencer (Foster City, CA) and scored using GeneMarker®V2.7.0 software (State College, PA). Isolates with at least one microsatellite difference in one of the loci would be considered to be from different strains, hence classified as reinfection during follow-up.

Single-nucleotide polymorphisms (SNPs) in the propeller domain of *PfK13* and *Pfmdr1* genes were analyzed using the Sanger sequencing method, as previously described.[7,17,18] Each PCR run included control isolates: strain IPC 3445; MRA-1236 with known *PfK13* polymorphisms (obtained through Biodefense and Emerging Infections Research Resources Repository, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD), 3D7, and 7G8 *P. falciparum* strains. Purified PCR products were sequenced using the Big Dye Terminator Cycle Sequencing Kit, version 3.1 (Carlsbad, CA) and an ABI 3130xl capillary sequencer. Sequence analysis was performed using Geneious 7.1.7 (Auckland, New Zealand).

Outcome measures. Treatment outcomes were assessed on the basis of clinical and parasitological criteria and were classified according to the WHO protocol.[9] In brief, early treatment failure was defined as having signs of severe malaria on days 1–3 with parasitemia, parasite density on day 2 higher than on day 0, parasitemia on day 3 with axillary temperature ≥ 37.5°C, or parasite density on day 3 ≥ 25% of that of day 0. Late clinical failure was defined as parasitemia in the presence of either danger signs/symptoms of severe malaria, or axillary temperature ≥ 37.5°C on any day between days 4 and 28 in participants who did not previously meet the criteria for early treatment failure. Late parasitological failure was defined as the presence of parasitemia on any day between days 7 and 28 in the absence of a fever or any criteria for early treatment failure or late clinical failure. Patients with treatment failures or recurrent infection during follow-up were withdrawn from the study and sent for rescue treatment according to malaria treatment policies in Colombia. Adequate clinical and parasitological response (ACPR) was defined as the absence of parasitemia on day 28 in participants who did not previously meet any of the criteria for treatment failure. Censorship of participants occurred for missed medication dose, missed follow-up visits, more than 1 day of delay for a visit, deviations from the protocol, or development of concomitant disease that interfered with the classification of treatment outcome, including *Plasmodium vivax* infection.

Data analysis. Data were double-entered into the Microsoft Excel database (Microsoft Corporation, Redmond, WA) developed by the WHO.[19] In addition, a second database containing detailed patient and laboratory information was developed using EpiInfo for Windows (CDC, Atlanta, GA). These data were also double-entered. Data cleaning and analysis were performed using R, version 2.15.1 (the R Foundation for Statistical Computing, Vienna, Austria). We calculated ACPR rates using uncorrected and PCR-corrected per-protocol analysis, and also uncorrected and PCR-corrected per Kaplan–Meier methodology. As customary, the per-protocol analysis excluded censored participants, and Kaplan–Meier analysis included them up to their day of censorship. We used χ^2 tests for comparing participants' characteristics. Statistical significance was set at < 0.05. The geometric mean of asexual parasite density readings were calculated using the R "DescTools" package.[20,21] Intermicroscopist agreement was measured using Shrout and Fleiss interclass correlation in the R "psych" package.[22,23]

Ethical approvals. All participants older than 18 years provided written informed consent before enrollment in the study, and, for participants < 18 years old, the primary caregivers provided written permission for their participation, and verbal assent from the participant was pursued. The study was reviewed 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 (PAHO 2018-04-0029). CDC considered this study a public health evaluation (reference number: 2018-063).

RESULTS

Enrollment. Of the 6,043 patients presenting with a fever at study enrollment facilities from July 2018 through January 2019, 434 screened positive for *P. falciparum* (Figure 2). Of these patients, 259 were evaluated for study participation; 91 met the inclusion criteria and were conditionally included. Three participants were later excluded. Eighty-eight patients were ultimately enrolled. Participants' follow-up ended in February 2019.

Baseline characteristics. The median age of the participant was 27.5 years (range: 5–65 years), and 26.1% of participants were 17 years old or younger (Table 1). The majority of participants were male (63.6%) and of African descent

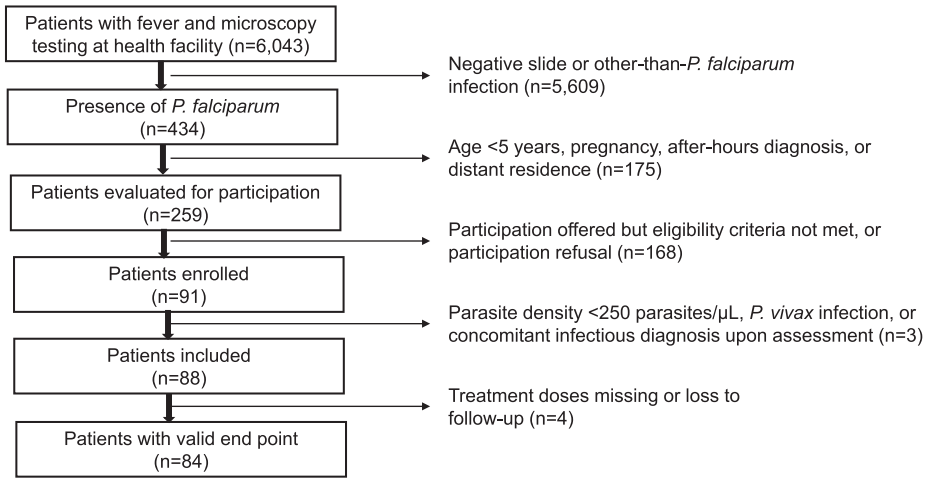

FIGURE 2. Screening, enrollment, and follow-up of study participants, Choco, Colombia, 2018–2019.

(95.5%). The hospital accounted for 84.1% of recruited participants, and 87.5% of participants were covered under the government-subsidized health insurance program. All participants reported having had fever in the last 24 hours, 84.1% reported having had fever for the past 1–4 days, and 15.9% had fever lasting 5–10 days. At enrollment, 14 participants (15.9%) had axillary temperature of ≥ 37.5°C. The median duration of symptoms was 3 days (range: 1–17 days). Other than fever, which was an inclusion criterion, the most commonly reported symptoms were headache (96.6%), chills (90.9%), and sweating (79.5%).

Side effects and patient follow-up. Adverse events reported during AL treatment included nausea, abdominal pain, and vomiting. Nausea was reported by 12 participants on day 1, five on day 2, and four on day 3. Only one participant vomited after having received a dose of medication. This participant subsequently tolerated the second dose. No participant developed signs of severe malaria after enrollment.

TABLE 1

Characteristics of participants at enrollment, Choco, Colombia, 2018–2019 (*N* = 88)

Variable	Value
Age (years), median (range)	27.5 (5–65)
5–17, *n* (%)	23 (26.1)
18–65, *n* (%)	65 (73.9)
Male, *n* (%)	56 (63.6)
Patient with government-subsidized health insurance, *n* (%)	77 (87.5)
African descent, *n* (%)	84 (95.5)
Temperature ≥ 37.5°C on day 0, *n* (%)	14 (15.9)
Duration of symptoms (days), median (range)	3 (1–17)
Duration of fever (days), median (range)	3 (1–10)
1–4, *n* (%)	74 (84.1)
5–10, *n* (%)	14 (15.9)
Most common symptoms, *n* (%)	
Headache	85 (96.6)
Chills	80 (90.9)
Sweating	70 (79.5)
Bone or muscle pain	61 (69.3)
Nausea	46 (52.3)
Asexual parasite density on day 0 (parasites/μL), geometric mean (range)	3,527 (330–30,923)
Gametocytes present on day 0, *n* (%)	11 (12.5)

Four participants were censored from the study: two failed to take all treatment doses and two could not be reached for follow-up visits. Therefore, the per-protocol analysis considered 84 participants who completed the 28-day follow-up.

The geometric mean of asexual parasite densities at baseline was 3,527 parasites/μL (range: 330–30,923) (Table 1). The proportion of participants with asexual parasitemia declined from 90.9% on day 1 to 9.3% on day 2. Among parasitemic patients, the mean asexual parasite density also dropped from 118 parasites/μL (95% CI: 83–168) on day 1 to 30 parasites/μL (95% CI: 15–62) on day 2. By day 3, 72 hours after treatment initiation, asexual parasites were not detected in any participant (Table 2).

Gametocytes were detected in 11 participants (12.5%; 95% CI: 6.4–21.3) at enrollment; this proportion peaked on day 2 at 20.9%, and subsequently declined to 1.2% by day 28 (Table 2). The proportion of patients with gametocytes at baseline was significantly greater ($P = 0.004$) among participants who reported fever lasting ≥ 5 days (58.8%; 95% CI: 32.9–81.6) than among participants who reported < 5 days of fever (19.7%; 95% CI: 11.2–30.9).

Double reader concordance, defined as agreement in positivity ascertainment and species determination with parasite density difference ≤ 50% between readers, of the 704 slides examined from all patient visits reached 100%. Intermicroscopist agreement between the first and second

TABLE 2

Asexual parasite density and gametocyte presence by day of follow-up, Choco, Colombia, 2018–2019 (*N* = 88)

	Asexual parasite presence and parasite density (geometric mean, 95% CI)*		Gametocyte presence
Day	Positive, *n* (%)	Parasites/μL (95% CI)	*n* (%)
Day 0	88 (100)	3,527 (2,757–4,512)	11 (12.5)
Day 1	80 (90.9)	118 (83–168)	17 (19.3)
Day 2†	8 (9.3)	30 (15–62)	18 (20.9)
Day 3	0 (0)	0	16 (18.6)
Day 7‡	0 (0)	0	12 (14.3)
Day 14	0 (0)	0	5 (6.0)
Day 21	0 (0)	0	2 (2.4)
Day 28	1 (1.2)	12 (NA)	1 (1.2)

NA = not available.
* Geometric means include only microscopy-positive samples.
† Two patients were excluded from denominator; see text for details.
‡ Two additional patients were excluded from denominator; see text for details.

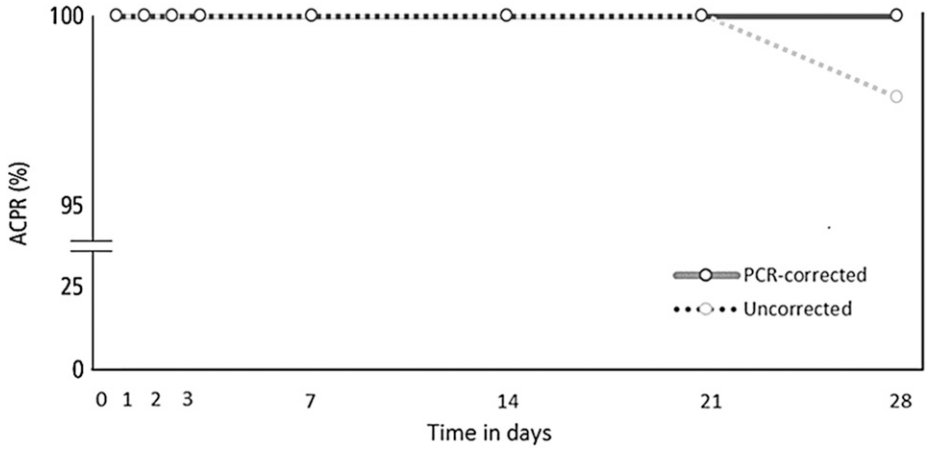

FIGURE 3. Uncorrected and polymerase chain reaction– (PCR)-corrected adequate clinical and parasitological response (ACPR) Kaplan–Meier survival curves, Choco, Colombia, 2018–2019 (N = 88).

parasite readings was high, with single-score intra-class correlation of 99.9% ($P < 0.001$). As such, third readings were not required for any sample. Review of 10% of slides randomly selected by the Colombian National Institute of Health staff during the supervisory visits did not detect any discrepancy with speciation or parasite density estimation performed by study microscopists.

Therapeutic efficacy. Only one patient met the case definition of treatment failure. This patient was classified as a late parasitological failure as he did not develop fever but had asexual parasite density of 12 parasites/μL on day 28. Therefore, the uncorrected ACPR rate was 98.8% (95% CI: 93.5–100) in the per-protocol analysis and 98.8% (95% CI: 91.8–99.8) in the Kaplan–Meier analysis (Figure 3). Likely because of the low parasite density, repeated attempts at PCR amplification for *P. falciparum* of the sample from this recurrent infection were negative, so microsatellite genotyping could not be performed to differentiate between recrudescence and reinfection for this participant. Thus, this patient was excluded from the PCR-corrected analysis, resulting in a per-protocol ACPR rate of 100% (95% CI: 95.7–100) and a Kaplan–Meier estimate of 100%.

Resistance markers. Among the 88 patients included in the study, PET-PCR yielded positive results in 96.6% (85/88) of the samples. For the *PfK13* gene, 95.3% (81/85) of the PET-PCR–positive samples were successfully sequenced, and all but one of them carried wild-type *PfK13* alleles. The only mutation detected in the *PfK13* was the A504**D** polymorphism. For the *Pfmdr1* gene, codons 86 and 184 were successfully genotyped in 95.3% (81/85) of PET-PCR–positive samples, codons 1034 and 1042 in 83.5% (71/85), and codon 1246 in 81.2% (69/85). The prevalence of each allele identified is shown in Table 3. In addition, haplotypes were constructed for the 68 samples that had complete allele information for codons 86, 184, 1034, 1042, and 1246. Two haplotypes were found, N**F**S**D**D and N**F**S**DY**, with a frequency of 23.5% (16/68) and 76.5% (52/68), respectively.

DISCUSSION

We found that, over almost a decade after introduction in Colombia, AL remains highly efficacious for the treatment of uncomplicated *P. falciparum* infection. The uncorrected and PCR-corrected ACPR rates demonstrated in our study were 98.8% and 100%, respectively, which is well above the level (90%) at which the WHO recommends changes in treatment policy.[3] Adequate parasite clearance, demonstrated by parasitemia negative in 90.9% of participants by day 2 and in 100% of participants by day 3, provided reassurance of efficacy of the artemisinin component in AL. In addition, AL was very well tolerated by participants, with none experiencing serious adverse effects and only one participant vomiting after taking a dose. The efficacy rates from our study are comparable to previously reported ones in Colombia. In 2008, a randomized clinical trial with two treatment arms, artesunate plus amodiaquine and AL, reported ACPR rates of 100% and 99%, respectively.[10] In 2007–2008, ACPR rates with AL at days 28 and 42 were 98.7% and 97.5%, respectively, in a study conducted in Tumaco, Nariño Department on the Pacific coast of Colombia.[24]

Contrary to previous studies in Colombia, which experienced challenges recruiting and retaining participants,[25] our study was able to reach the desired sample size in less than 8 months, and our loss to the follow-up rate was limited to only four participants in the 28-day follow-up. Reliance on two enrollment sites that screened patients presenting with fever for malaria testing contributed to successful patient recruitment. High participant retention was likely because of the considerable effort made by our personnel to assure that no participant missed a scheduled appointment. Study personnel contacted participants several times by text message and

TABLE 3

Prevalence of *Pfmdr1* alleles in samples at enrollment, Choco, Colombia, 2018–2019

Pfmdr1 codons	*Pfmdr1* alleles detected*	Prevalence (%) (*n*/*N*)†
86	N86	100 (81/81)
184	184**F**	100 (81/81)
1034	S1034	100 (71/71)
1042	1042**D**	100 (71/71)
1246	D1246	23.2 (16/69)
1246	1246**Y**	76.8 (53/69)

n = number of samples with a specified allele; *N* = number of samples with a valid result.
* Mutant codons in bold.
† Each sample yielded one single allele per codon analyzed.

phone for appointment reminders, and, when necessary, visited participants at home. Last, intense staff training with ongoing supervision and technical support also played a role in the high data quality achieved, as demonstrated by high concordance in laboratory readings.

Gametocytemia has an important role in malaria infection of mosquitoes,[26,27] with opportunity for subsequent transmission to humans.[28] Similar to a systematic review of 121 studies, which found a 12.1% prevalence of gametocytemia among patients initiating antimalarial treatment,[29] gametocytemia prevalence at enrollment in our study was 12.5%. Nonetheless, gametocyte clearance was slow; the day 7 gametocytemia prevalence was 14.3%, higher than that in a similar study conducted in the Brazilian Amazon (8.9%).[30] For patients without contraindications, the WHO recommends a single low dose of primaquine as a gametocytocide for patients with falciparum malaria.[31] Brazil, Venezuela, and Peru have adopted the use of this primaquine dose for uncomplicated *P. falciparum* infection in its national treatment guidelines, whereas Colombia recommends it only in areas targeted for rapid transmission reduction.[14,32]

Our study found that 15.9% of participants took 5 days or more to seek care, estimated by the interval between the onset of fever and presentation at a healthcare facility for evaluation. We also noted that ≥ 5 days of fever before diagnosis was associated with gametocytemia. Delays between the onset of malarial symptoms and presentation at a healthcare facility for treatment play an important role in maintaining malaria transmission,[33,34] as persons who delay treatment remain infectious for longer periods of time.[26] These findings underscore the need for strategies to increase community awareness of the importance of prompt diagnosis and treatment for malaria.

No evidence was found for the presence of mutations associated with resistance to artemisinin in the propeller domain of the *PfK13* gene. Our findings were similar to other studies conducted in Colombia between 2011 and 2015, in which the presence of wild-type alleles was reported.[25,35] By contrast, Guyana reported the presence of the C580**Y-**mutant allele in 2010.[7] Our results show for the first time in Colombia the presence of a polymorphism in the *PfK13* gene in field samples; however, this SNP has not been associated with resistance to artemisinin as of yet.[36]

The *Pfmdr1* haplotypes found in this study had already been found in Colombia,[37] even before the use of AL. We observed a higher prevalence of the N**F**S**DY** triple-mutant haplotype that has been reported in Colombia.[25,35] In 2015, the prevalence of this haplotype in Choco was 25.3%[35] and, in our study 4 years later, it reached 76.5%. Although, the 1246**Y** mutation might indicate selection by lumefantrine, the N**F**S**DY** triple-mutant haplotype was not associated with therapeutic failure in our study. No clear association between these common *Pfmdr1* SNPs and treatment failures has been found in South America to date.

The high inter-microscopist concordance in parasite speciation and parasite density estimation in our study highlights the importance of intense training before TES initiation because there is often high variation in microscopy readings in routine malaria diagnosis laboratories.[38,39] This high concordance was probably because of the quality of laboratory training given to study microscopists because, like in most of the Americas, this study took place in an area with transmission of both *P. vivax* and *P. falciparum.* We also interacted often with the laboratory staff of the health facilities that served as enrollment sites and staff at the regional reference laboratory. We believe our interactions served to foster collaboration between staff of the Colombian National Institute of Health and of participating facilities, providing an opportunity for improvements to be made in malaria diagnosis in the region.

We recognize TESs are the gold-standard methodology for antimalarial efficacy monitoring and policy decision-making. However, TESs have not been carried out at the WHO-recommended interval, 3 years for low to very low endemic countries, in Colombia.[9] Therefore, it becomes critical to seek and implement complementary alternatives to monitor the efficacy of antimalarial treatments in areas where it is difficult to enroll enough patients to conduct a TES with adequate sample size. A possible alternative is to carry out molecular surveillance of recognized antimalarial resistance genes together with an assessment of parasite clearance on day 3 among patients who received antimalarial treatment under supervision. This alternative would be logistically easier than a TES because patients would require only 3 days of follow-up. This strategy, although not yet validated or formally recommended, would allow malaria programs to estimate the existing WHO indicators for artemisinin resistance, clearance of day 3 parasitemia and presence of molecular markers.[40]

Our study has some limitations. First, our findings are not generalizable to other regions of Colombia or surrounding countries. In addition, our sample captured only persons who sought healthcare services at two clinics in the region. It is possible that persons with malaria in the region did not seek care at these clinics; this is especially true for higher income populations, who tend to seek services from private healthcare providers. This is evidenced by the high proportion of study participants on the government-subsidized health insurance program (87.5%), which covers approximately half of the Colombian population. Last, we were unable to determine whether the one case of late treatment failure was the result of reinfection or recrudescence. Attempts to amplify the sample associated with this case were unsuccessful likely because of the participant's low parasite density, presence of PCR inhibitors, or even a false-positive result on the microscopy reading, despite the suitable limit of detection of the PCR technique (3.2 parasites/μL).[15] However, classifying this case as either recrudescence or reinfection would not have changed our conclusions significantly.

This study supports continued use of AL for treatment of uncomplicated *P. falciparum* in Colombia but indicates the need to improve timeliness of patient healthcare-seeking behavior. Given the observation of delayed gametocyte clearance, our results warrant the adoption of low-dose primaquine as a national policy for its gametocytocidal effect as recommended by the WHO and already in place in other countries in the region. In addition, the possibility of migration from neighboring malaria-endemic countries with unknown antimalarial resistance profile underscores the need for continued monitoring of antimalarial efficacy and resistance, possibly adding molecular markers to surveillance systems to allow for the detection of drug-resistant alleles in Colombia.

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