Data Article

Data on selected antimalarial drug resistance markers in Zambia

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\textbf{A R T I C L E I N F O}

\textbf{Article history:}
Received 25 September 2020
Revised 8 December 2020
Accepted 9 December 2020
Available online 13 December 2020

\textbf{Keywords:}
Sulfadoxine-pyrimethamine
Mutations
Zambia
\textit{Plasmodium falciparum}
Malaria

\textbf{A B S T R A C T}

This article describes data on selected resistance markers for antimalarial drugs used in Zambia. Antimalarial drug resistance has hindered the progress in the control and elimination of malaria. Blood samples were collected during a cross-sectional household survey, conducted during the peak malaria transmission, April to May of 2017. Dried blood spots were collected during the survey and transported to a laboratory for analysis. The analysed included polymerase chain reaction (PCR) followed by high resolution melt (HRM) for mutations associated with Sulfadoxine-pyrimethamine.
resistance in the *Plasmodium falciparum* dihydrofolate reductase (*Pfdhfr*) and *P. falciparum* dihydropterin reductase synthase (*Pfdhps*) genes. Mutations associated with artemether-lumefantrine resistance in *falciparum multi-drug resistance gene 1* (*Pfmdr1*) were also assessed using PCR and HRM analysis, whereas the *P. falciparum Kelch 13* (*Pfk13*) gene was assessed using nested PCR followed by amplicon sequencing.

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### Specifications Table

| Subject                  | Parasitology                                                                 |
|--------------------------|------------------------------------------------------------------------------|
| Specific subject area    | Antimalarial drug resistance                                                 |
| Type of data             | Table, Image, Chart, Graph, Figure                                           |
| How data were acquired   | DNA was extracted and Polymerase chain reaction followed by high resolution  |
|                          | melt on a LightScanner 384 system (BioFire Diagnostics, Inc., Salt Lake USA) |
|                          | and sequencing using ABI 3500XL Genetic Analyser (Applied Biosystems, Foster, USA) |
| Data format              | Raw                                                                          |
| Parameters for data collection | Genomic DNA was extracted from Dried blood spots collected during the    |
|                          | survey. The extracted DNA was analysed using Photo-induced electron transfer-PCR |
|                          | (PET-PCR) for malaria positivity and species identification. Only Plasmodium |
|                          | falciparum positive samples were analysed for resistance markers             |
| Description of data collection | Data was collected through a household survey from Southern and Western |
|                          | Provinces in Zambia. Sample collected from the survey were used for this analysis. |
| Data source location     | Institution: Ministry of Health-National Malaria Elimination Centre         |
|                          | City/Province: Lusaka/Lusaka                                                  |
|                          | Country: Zambia                                                              |
| Data accessibility       | Repository name: mendeley                                                     |
|                          | Data identification number: DOI:10.17632/zfk9brr5d9.1                       |
|                          | Direct URL to data: https://data.mendeley.com/datasets/zfk9brr5d9.1          |
| Related research article | L. Sitali, M. C. Mwenda, J. M. Miller, D. J. Bridges, M. B. Hawela, A. Hamainza, |
|                          | M. Mudenda-Chilufya, E. Chizema-Kawesha, R. Daniels, T. P. Eisele, A. H. Nerland, |
|                          | J. Chipeta, B. Lindtjørn (2020) Surveillance of antimalarial drugs in Zambia: |
|                          | Surveillance of Molecular Markers for Antimalarial Resistance in Zambia:     |
|                          | Polymorphism of *Pfkelch* 13, *Pfmdr1* and *Pf dhfr*/*Pf dhps* genes, Acta Tropica. 2020 Sept. 105704. DOI https://doi.org/10.1016/j.actatropica.2020.105704 |

### Value of the Data

- This data is important for the monitoring of antimalarial drug resistance.
- This data can guide policy makers on the resistance pattern of the currently used antimalarial.
- The data can be used for further studies on resistance makers especially in systematic review and meta-analysis.
- The data add to the body of information of mutations in the *Pfdhfr* and *Pfdhps* genes of SP, *Pf mdr-1* related to lumefantrine sensitivity and *Pfkelch 13* related to artemether resistance.
1. Data Description

The data set (https://data.mendeley.com/datasets/zfk9brr5d9/1) consist of results obtained from HRM-PCR technic from samples collected from the Western and Southern Provinces. The results were from an analysis of three gene Pf dhfr (51, 59, 108 and 164), Pf dhps (436, 437, 540 and 581) and Pfmdr (86, 184 and 1246). The wild type is indicated as ‘1’, while the mutant ‘0’. The data shows wild type, mutant and mixed infections.

The abbreviations in the dataset are as follows: W-wild type; M-Mutant; I-Isoleucine; L-Leucine; S-Serine; N-Asparagine; C-Cysteine; R-Arginine; E-Glutamic acid; K-Lysine; A-Alanine; G-Glycine; Y-Tyrosine; D-Aspartic acid; F-Phenylalanine; dhfr-dihydrofolate reductase; dhpsdihydroterote synthase; M-Male; F-Female; Haplo-Haplotype

The table in the article (doi:10.1016/j.actatropica.2020.105704) related to this data in brief [1] as supplementary information, shows the frequencies of the Pf dhfr, Pf dhps and Pf mdr1 single nucleotide polymorphisms for the samples from Southern and Western Provinces. The most prevalent mutant alleles observed were: Pf dhps 437G (87.7%), followed by Pf dhfr 59R (81.3%), 511 (66.7%) and 108N (66.8%). The other observed mutant alleles or point mutations were at low frequencies. Mixed alleles were also observed in all the genes with exception isolates from Southern, where Pf dhps 436 and 581 did not have mixed alleles. It is difficult to compare the two provinces as the sample sizes were very different, Southern has a small number of positives samples. For Pf mdr, no mutations were observed in Pfd, where 1 N86Y while Y184F has 33.3% mutations. In the case of Pf kelch 13, out of the 80 sequenced samples, only 3 has mutations (Fig. 1). In Figure the frequency of mutations is shown 4.1%.

2. Experimental Design, Materials and Methods

2.1. Polymerase chain reaction (PCR)

2.1.1. DNA extraction

Genomic DNA was extracted from 6 mm DBS punches, approximately 13.8 μl whole blood, using a Qiagen DNA mini kit (Qiagen, Hilder, Germany) and eluted in 100 μl elution buffer. The puncher was cleaned after every sample by dipping in 70% ethanol and flaming. The extraction process for RDT positive and negative samples was different. RDT-positive samples were extracted individually, while RDT-negatives were extracted in pools of 10, and pools that came out positives were deconvoluted and re-extracted individually. The extracted DNA was stored at 4 °C for immediate analysis.

2.1.2. PET-PCR analysis

Samples were run using PET-PCR and PCR-HRM. PET-PCR was performed on a LightCycler LC 480 real-time PCR machine (Roche, Basel, Switzerland), as described in 2013 by Lucchi et al [2]. To amplify Plasmodium18S ribosomal RNA the primer shown in Table 2 were used. Briefly, 5μl of DNA template was amplified in a 20 μl reaction volume containing 1x of the Taqman 2X environmental master mix (Applied Biosystems, Life Technologies LTD, Warrington, UK) as follows: 95 °C for 15 min, followed by 45 cycles of 95 °C for 20 s, 60 °C for 40 s and 72 °C for 30 s. Samples were tested in duplicate and recorded positive if both duplicate samples had a cycle threshold (CT) value < 40.

2.1.3. HRM analysis for Pf dhfr, Pf dhps and Pf mdr1

Pre-amplification: The PCR-HRM analysis started with a pre-amplification process to enhance the template concentrations. The pre-amplification was performed on all P. falciparum positive samples. A pre-amplification master (PreAmp Master Mix, Life Technologies, Inc, Grand Island, NY, USA) was used with a mixture of primers for the assays that were run. DNA from samples with a CT value of > 35 was pre-amplified in a 10 μl reaction volume and the ones with CT <
Fig. 1. Nucleotide sequence for PfKelch 13 and deduced amino acid sequences, showing the three samples that had mutations.

35, in a 20 μl reaction volume. The following were pre-amplification conditions: pre-incubation 95 °C for 10 min, followed by 14 cycles of amplification for 15 min and annealing for 4 min; and final extension for 15 min. After pre-amplification, the DNA was cleaned using a Zymo kit-ZR-96 DNA sequence Clean-up Kit (Catalogue No. D 4053, Zymo research, Tustin, CA, USA).
**Fig. 2.** *Pfkelch* 13 mutations.

**Table 2**
Primer sequences for species identification.

| Primer name              | Sequence (5’ – 3’)                                                                 |
|--------------------------|------------------------------------------------------------------------------------|
| Original Genus 18sFor    | GCC CTA ACA TGG CTA TGA CG                                                          |
| Original Genus FAM 18sRev| FAM-aggccatagcgcctggCTGCCTCCT TAG ATGTGG TAG CT                                     |
| Falciparum For           | ACC CCTGCGCTG GTG TTT                                                           |
| Falciparum Rev           | HEX-aggccatagcgcctggTGG GCC CCA AAA ATA GGA A                                      |
| *P. vivax* For           | GTA GCC TAAGAAGGC CGT GT                                                          |
| *P. vivax* Rev           | HEX-aggccatagcgcctggCCTGGGG GAT GAA TCT TAC AGC ACT GT                             |
| *P. malariae* For        | AAGGCAGTAACACCAACACGAGTA                                                         |
| *P. malariae* Rev-based on dihydofolate reductase-thymidylate synthase (DHFR-TS) gene | FAM-aggccatagcgcctggTCCCCATGAAGTTATATTCCGGCTC                                      |
| *P. ovale* For           | FAM-aggccatagcgcctggCCACAGATAAGAAGTCTCAAGTACGATTT                                 |
| *P. ovale* Rev           | TGGAGCACTTTTGTGTTGCAA                                                            |

*Note: the lowercase letters represent sequences with non-homology to the template DNA*

**PCR amplification and HRM:** All PCR amplifications were performed on the LightCycler 480 real-time PCR machine. The reaction consisted of 2.0 μL of Lightsscanner Master Mix, 2.5 μL of the pre-amplified template, 0.5 μL of primers and probes (Final primer/probe concentrations for a 5-μL total reaction volume was 0.5 μM excess primer, 0.1 μM limiting primer, and 0.4 μM of the 3’-blocked probe). The list of primers and probes are shown in Table 3. Specific controls for wild type or mutant genes were included for each assay. The amplification conditions were as follows: 95 °C denaturation for 2 min, 50 cycles of 94 °C for 5 s and 66 °C for 30 s, and a pre-melt cycle of 5 s each at 95 °C and 37 °C. The product was heated from 40 °C to 90 °C on the Lightsscanner system and the change in fluorescence was recorded as the samples melted incrementally. The following assays were run Pfdhfr (N51I, C59R, I164L and S108N); Pfdhps (S436F, A437G, K540E/N and A581G); and *Pfmdr* (N86F, Y184F and D1246Y). The annealing temperature for all assays was 66 °C with the exception of two assays S108N and D1246Y that were run at 63 °C [3,4].
Table 3
High resolution melting assays primer and probe sequences.

| Primer | Forward Primer 5'→3' | Reverse Primer 5'→3' | Probe 5'→3' |
|--------|----------------------|----------------------|-------------|
| PfcrT76/K76 | GTAAAGCAGGCCGCTCCTTCCT | CAGGAAAGCAGAATGCTTTTGAAT | GGTAATGCTCTTTAAAGTTTTC |
| Pfbrf5S1C59 | GCCTACTGCCTTA | CCAATAATGTCCTCTTATAATAA | CTGCTGATGTTTTTAAGTTTTC |
| PfbrfI164 | ACATTAACCGCGTTACAATACTTTT | CTGAAAAATACACCATTTA | TTTAATTTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
Zambia Biomedical Research Ethics Committee (UNZABREC) (Zambia) Ref no. 010-05-16. This study was an analysis of samples from a larger study, that was assessing progress made in malaria control in Zambia, thus ethical clearance for the larger study was earlier obtained from UNZABREC, ref no. 007-03-14. Permission to use the Ministry of Health data was obtained from the National Health Research Authority. All data analysed were anonymized. Consent was obtained during the data collection from the participants involved in the study.

Credit author statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

Acknowledgments

We acknowledge the role of PATH and their Malaria Control and Elimination Partnership in Malaria (MACEPA) project in providing laboratory reagents, support in form of staff who assisted in the sample sorting and analysis (Conceptor Mulube, Sandra Chishimba, and Brenda Mambwe), and data extractions (Maya Fraser). We also wish to express our gratitude to the Ministry of Health through the National Malaria Elimination Centre for allowing us to use the samples. We further wish to acknowledge Sara Volkman and Dyann F. Wirth of Harvard University for their roles in optimizing and validating the high-resolution melt assay. Travis Porter from Tulane University helped with data extraction.

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