Genotyping Malaria Drug Resistance

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Background

Resistance to antimalarial drugs challenges our ability to save lives threatened by malaria, and to eliminate the burden that malaria places on individuals and societies. This burden is substantial, with malaria having caused an estimated 228 million cases and 405 000 deaths in 2018 (1). The most current developments in next-generation sequencing technology offer a fresh and efficient method of tracing malaria parasites that are resistant to therapeutic treatment. Our objective was to genotype for the malaria resistance in samples from different countries in the world using bioinformatics tools and prescribe appropriate drugs for each. *Plasmodium falciparum* is the most fatal of all plasmodium species hence our focus. In this study, we were particular about mutations in the following *P. falciparum* genes; Pfcrt, Pfmdr1, Pfdhfr, and Pfdhps, Pfarps10, Pfferredoxin, Pfexonuclease and Pfmdr2.

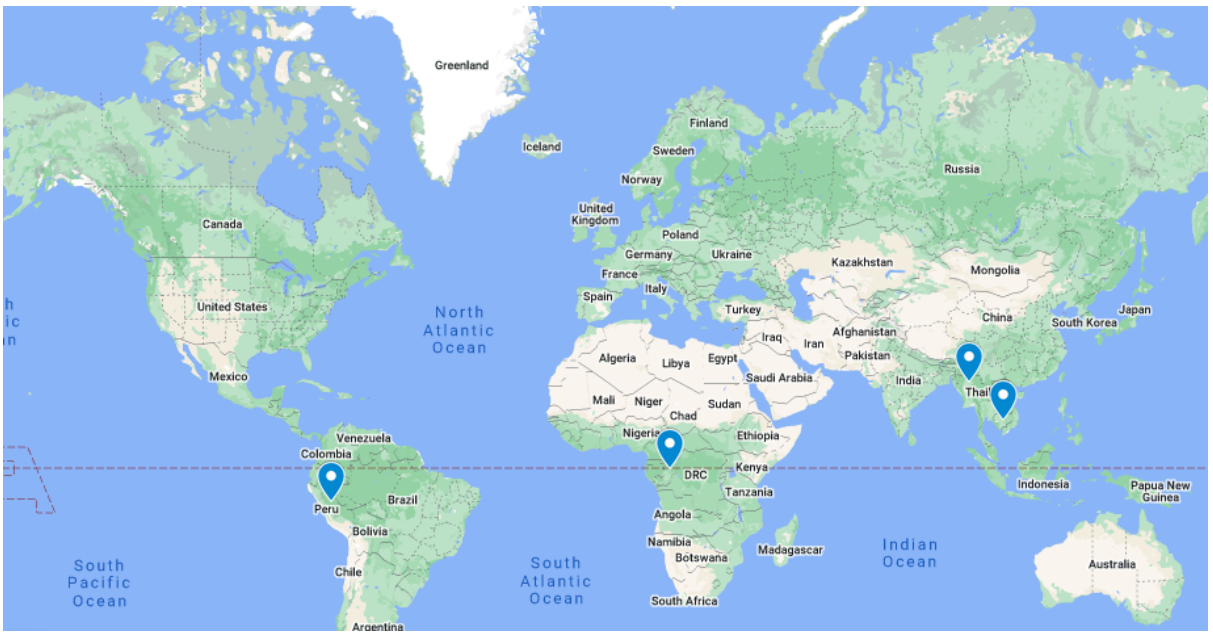

Figure 1. Countries included in the genotyping of malaria drug resistance. Blue pin map: Peru, Congo, Cambodia, Myanmar

METHODOLOGY

Whole genome sequences of plasmodium falciparum were retrieved from the SRA archive of The National Center for Biotechnology Information (NCBI), which is a major resource for bioinformatics tools and services. Five(5) samples each from four countries were used in

this study and they include Myanmar(Asia), Congo(Africa), Peru(South-America) Cambodia(Asia).

Software packages

I. SPAdes 3.15.4.
II. ResFinder 4.1
III. FastP

Analysis

A. Data Collection

The curl command was used to obtain the data from the NCBI on the bash terminal. The metadata containing details of the dataset can be found at;
https://github.com/Praisetechsis/MALARIA-GENOTYPING/blob/main/Project2metadata.tsv

B. Data trimming

The datasets were trimmed using fastp in order to provide clean data for downstream analysis.

C. Genome Assembly

Genome assembly is the process of putting back together a huge number of small DNA sequences to recreate the original chromosomes from which the DNA came. DeNovo genome assembly was done using spades.py on the bash terminal. Reads were assembled into contiguous sequences.

D. Resistance Genotyping

This was done using ResFinder, a database that captures antimicrobial resistance genes from whole-genome data sets. The contigs.fasta file from the genome assembly was exported to ResFinder.

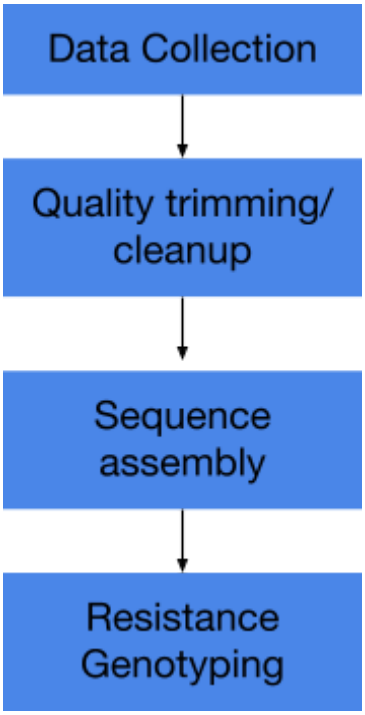

Figure 2. Flowchart for analysis pipeline

RESULT AND DISCUSSION

A major obstacle to effectively treating malaria is the quick emergence of parasite resistance to antimalarial medications (2). Sulfadoxine-pyrimethamine (SP) is an anti-malarial drug formulation approved for intermittent preventive therapy in pregnancy (IPTp). The development of sulfadoxine-pyrimethamine (SP) resistance has been linked to mutations in the dihydrofolate reductase (dhfr) and dihydropteroate synthase (dhps) genes, according to research conducted on the molecular basis of antimalarial drug resistance (3). Chloroquine resistance is mostly brought on by mutations in the Plasmodium falciparum chloroquine resistance transporter (PfCRT) gene. Evidence suggests that altered PfCRT results in chloroquine resistance by allowing for increased efflux of the medication from the digestion vacuole and reducing the quantity of medication available to bind target heme (4). Plasmodium falciparum has developed resistance against all antimalarial medications used to treat it such as chloroquine, sulphadoxine-pyrimethamine, quinine, piperaquine and mefloquine (5). Although the molecular processes underpinning P. falciparum's multidrug resistance are still completely understood, mutations in the Plasmodium falciparum multidrug resistance 1 (Pfmdr1) gene, which encodes a transmembrane homolog of the PGH1 protein, have been linked to the disease (6). The primary pfmdr1 Single Nucleotide Polymorphisms (SNPs) that have been implicated are N86Y, Y184F, S1034C, and N1042D (7).

In the samples obtained from Peru, all five samples had mutations in the DHFR gene, four samples had mutations in the DHPS gene, and one sample had mutation in the MDR gene.

Thus, it is not recommended that SP be used as antimalarial treatments in Peru. In Cambodia, the most prevalent mutation identified in the dataset is in the DHFR gene. As mentioned earlier, mutation in DHFR results in pyrimethamine resistance implying that pyrimethamine is not a suitable drug candidate for treatment of malaria in the country. Fewer cases of DHPS, CRT, MDR, and K13 mutations were also noted. Since mutation in these genes result in resistance to Sulphadoxine, chloroquine, Lumefantrine & Mefloquine, and artemisinin respectively, it is recommended that these drugs be used with caution in Cambodia. A similar pattern to Cambodia was observed in Myanmar. The samples contained mutations in DHPS, DHFR, CRT, and K13 genes which confer resistance to Sulphadoxine, pyrimethamine, chloroquine, and artemisinin.

Table 1: Plasmodium falciparum gene mutation in Peru, Cambodia and Myanmar

Country/Accession no	Mutation	Nucleotide change	Amino acid change	Resistance
CONGO				
SRR13013579	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
PERU				
ERR6226018	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
ERR6226019	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine

	MDR p.Y184F	TAT -> TTT	Y -> F	Lumefantrine, Mefloquine
ERR6226020	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
ERR6226021	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
ERR6226023	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
CAMBODIA				
ERR2172115	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
	DHPS p.S436A	TCT -> GCT	S -> A	Sulphadoxine
	K13 p.C580Y	TGT -> TAT	C -> Y	Artemisinin
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
ERR2172116	No mutations present for Pfcrt, Pfmdr1, Pfdhfr, and Pfdhps, Pfarps10, Pfferredoxin, Pfexonuclease and Pfmdr2			
ERR2172117	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine

	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	K13 p.C580Y	TGT -> TAT	C -> Y	Artemisinin
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
ERR2172118	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
	K13 p.C580Y	TGT -> TAT	C -> Y	Artemisinin
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	N -> I	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
ERR2172118	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
MYANMAR				
SRR5346206	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
SRR5346207	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine

	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
	DHPS p.S436A	TCT -> GCT	S -> A	Sulphadoxine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
SRR5346208	DHPS p.S436F	TCT -> TTT	S -> F	Sulphadoxine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A613T	GCC -> ACC	A -> T	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
SRR5346209	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine
	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine
	K13 p.C580Y	TGT -> TAT	C -> Y	Artemisinin
	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
SRR5346210	CRT p.N75E	AAT -> GAA	N -> E	Chloroquine
	DHPS p.K540E	AAA -> GAA	K -> E	Sulphadoxine
	DHPS p.A581G	GCG -> GGG	A -> G	Sulphadoxine
	DHFR p.N51I	AAT -> ATT	N -> I	Pyrimethamine
	DHFR p.C59R	TGT -> CGT	C -> R	Pyrimethamine

	DHFR p.S108N	AGC -> AAC	S -> N	Pyrimethamine
	DHFR p.I164L	ATA -> TTA	I -> L	Pyrimethamine

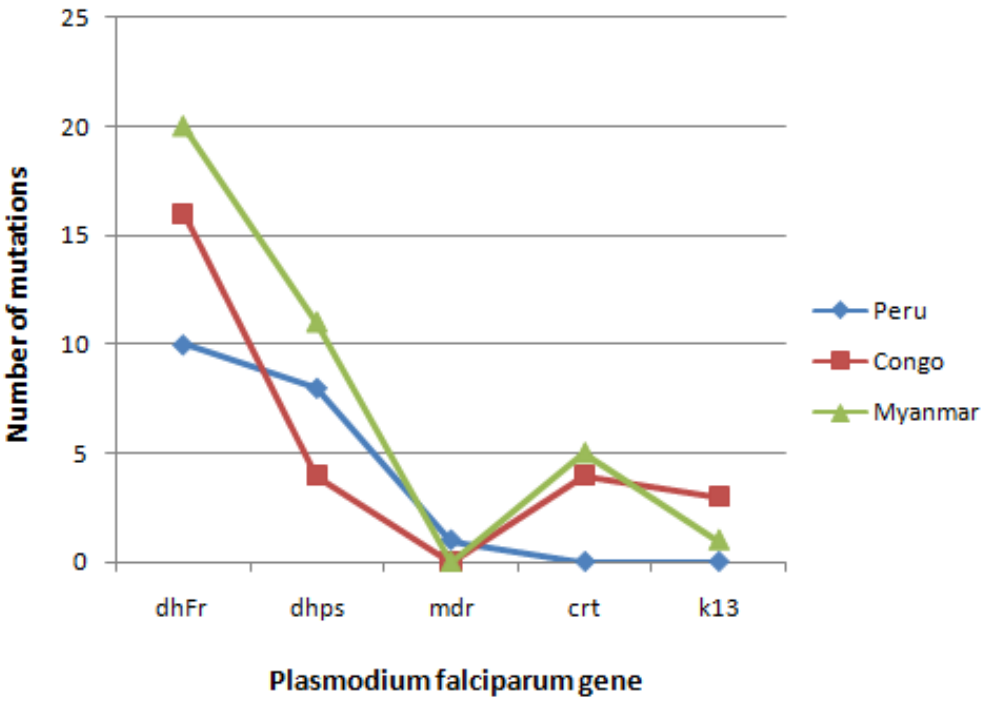

Figure 2. Plasmodium falciparum mutations in Peru, Congo and Myanmar

CODE

The script for the above pipeline are available at:
https://github.com/Praisetechsis/MALARIA-GENOTYPING/edit/main/project2.sh

CONCLUSION

In conclusion, it is evident from this study that alternative treatments are needed for malaria treatments in Congo, Peru, Cambodia and Myanmar due to resistance developed to present treatments. The following choices can be utilized for P. falciparum infections contracted in regions where chloroquine and Sulfadoxine-Pyrimethamine resistance exists. The first choice, assuming it is easily available, is artemether-lumefantrine (CoartemTM). Next,

malarone®, which contains atovaquone-proguanil. Additionally, there is a therapy option that combines quinine sulfate with doxycycline, tetracycline, or clindamycin. Mefloquine can also be a further option. Mefloquine however can cause some side effects thus It is advisable to use only when other options are not available. Due to drug resistance in some areas, mefloquine and lumefantrine are not advised for infections contracted there (8).

This study employed a small sample size since we had a limited amount of time. It is possible that with more time and resources, more mutant genes would be identified in these countries.

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