Genotyping Malaria Drug Resistance

Introduction:

Malaria is an infectious disease that is known to affect humans and other animals. It is caused by microorganisms of the *Plasmodium* group and it is spread exclusively through the bites of infected *Anopheles* mosquitoes. There are five species of *Plasmodium* that can infect and be spread by humans and they are *Plasmodium falciparum* (which causes high number of deaths and severe cases of malaria), *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi* (which rarely causes disease in humans) (1).

The global burden of malaria has increased during the last decade and it continues to cause signifcant morbidity and mortality globally, despite the control interventions (2).

Current malaria control and elimination strategies rely mainly on effective antimalarial drugs. However, drug resistance has become a major threat facing malaria control programs because *Plasmodium falciparum* as a parasite has adapted to anti-malarials and this can be attributed partly to its enormous genetic diversity (3).

Identifying, monitoring and understanding the molecular markers of adaptive mechanisms against anti-malarials are of importance as anti-malarial therapy and prophylaxis have been key in reducing mortality and morbidity from malaria (4).

Although, mutations in genes determining resistance to drugs such as chloroquine have been identified, there is still a need to have full understanding of the resistance mechanisms, and genes that contribute to resistance to many other drugs. The knowledge of the antimalarial resistance genes will help in optimizing anti-malarials usage in medicine.

Aim:

This project aims to identify the presence of one or more of these variants (Pfcrt, Pfmdr1, Pfdhfr, and Pfdhps, Pfarps10, Pfferredoxin, Pfexonuclease and Pfmdr2) in *Plasmodium falciparum* genome sequences from different countries in the world and make drug prescriptions that fit each.

Methodology:

Five (5) Whole Genome Sequences each of *Plasmodium falciparum* from three countries – China-Myanmar border, Malawi, and Vietnam were retrieved from the SRA archive of NCBI (https://www.ncbi.nlm.nih.gov/sra) a public biological database.

The Accession IDs were used to retrieve the datasets and their metadata from the SRA (https://sra-explorer.info/), an open and free access repository of high throughput sequencing data.

The GitHub link to the metadata of the datasets is https://github.com/ruthobaado/Project-Malaria-Drug-Resistance/blob/main/Metadata_samples.tsv.

The script file for the codes used for this project can be accessed via the GitHub link: https://github.com/ruthobaado/Project-Malaria-Drug-Resistance/blob/main/Stage%203.sh

Software packages used for the analysis:

FastP: This was used to trim the adapters in the datasets for downstream analysis

SPAdes 3.15.5: This was used for the genome assembly of the trimmed data.

#Bash scripts were used for the trimming and the genome assembly.

ResFinder 4.1: The contigs.fasta files of the assembled genomes were uploaded to ResFiinder (https://cge.food.dtu.dk/services/ResFinder-4.1/), an open online resource for identification of antimicrobial resistance genes in high-throughput sequencing data and prediction of phenotypes from genotypes.

Bioinformatics web tools and Excel were used to visualize the compiled results from ResFinder (https://bioinformatics.psb.ugent.be/webtools/Venn/.

The image and the summary table can be accessed via the links:

https://github.com/ruthobaado/Project-Malaria-Drug-Resistance/blob/main/venn_result%20(3%20Countries).png,

and

https://github.com/ruthobaado/Project-Malaria-Drug-Resistance/blob/main/Summary-table.txt respectively.

Results and Discussion:

Evidence suggests that *Plasmodium falciparum* has developed resistance against antimalarial medications such as Chloroquine, Sulfadoxine, Pyrimethamine, Mefloquine, Artemisinin and Piperaquine which are as a result of mutation in the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT), dihydropteroate synthase (dhps), dihydrofolate reductase (dhfr), multidrug resistance mutation (PfMDR1), K13 genes and PIP respectively; mutation in the PfCRT genes can also express resistance to Piperaquine (5).

The table comparing the distribution of the resistance genes in the three countries is shown below in Table 1. It was observed that in the samples for each of the countries, there was mutation in most of the genes of interest.

Countries	crt	mdr1	dhfr	dhps	K13	PIP
China-Myanmar	4	3	4	2	1	7
Malawi	1	2	3	1	0	15
Vietnam	7	5	2	1	1	31

Table 1: Plasmodium falciparum resistance gene mutations in China-Myanmar, Malawi and Vietnam.

The prevalent mutations identified in the countries are PIP and PfCRT, which implies that Chloroquine and Piperaquine are not suitable for treatment of malaria in the countries. Since China-Myanmar, Malawi and Vietnam has the highest prevalence of PfCRT and PIP mutations, a bar chart was plotted to show this distribution in Figure 1.

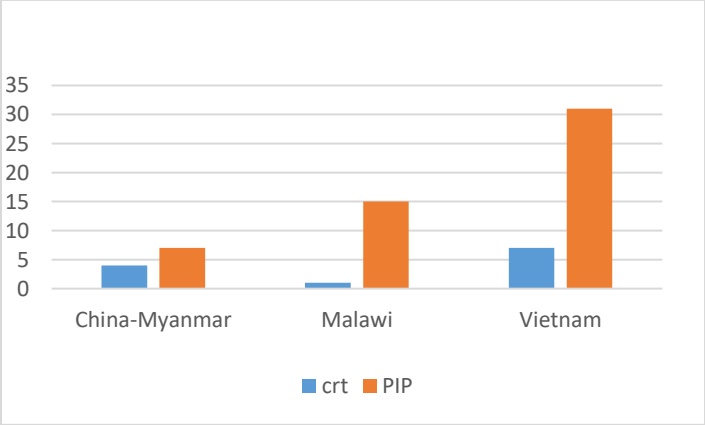

Figure 1: Prevalence of PfCRT and PIP resistance genes in China-Myanmar, Malawi and Vietnam

The recommended drug-based preventive malaria intervention for Vietnam is a combination of Dihydroartemisinin plus Piperaquine, which has proven to be effective in countries like China. Dihydroartemisinin needs to be taken for at least 7 days to achieve a maximum cure rate.

Due to the low prevalence of *dhfr, dhps and K13* mutations in the countries, Sulfadoxine, Pyrimethamine Artemisinin can be recommended intermittently for use, Sulfadoxine can be combined with Pyrimethamine to be more effective. Lumefantrine & Mefloquine can be combined against mutation in the mdr1 gene. Lumefantrine-Artemether (Coartem), Mefloquine-Artesunate, and Amodiaquine-Artesunate are ACT-drug combinations that can be used generally to treat malaria infections especially using Lumefantrine-Artemether in cases of PfCRT resistance (6).

There are options of combining antibiotics with antimalarial therapy such as Clindamycin and Doxycycline. Clindamycin does not directly kill the parasites causing malaria, instead it stops their growth, allowing the body's immune system to destroy them (6).

This study showed that it will be important to ensure that mechanisms are in place to continually monitor the emergence of resistance to these drugs,

References:

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