Elucidating the Potential Impact of circulating *Plasmodium falciparum* Dihydropteroate Synthase variants on Sulphadoxine-Pyrimethamine Resistance in Ghana and Across Africa

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Background

- Malaria is still a disease of public health concern globally especially in sub-Saharan Africa
- Resistance to Antimalarial therapy is a major concern for malaria control and elimination
- In Ghana, Sulphadoxine-pyrimethamine (SP) is used as Intermittent Preventive Treatment (IPTp) for pregnant women and for children (IPTc)
- SP works by inhibiting *Plasmodium* dihydropteroate synthase (*Pf*dhps) and dihydrofolate synthase (*Pf*dhfr) interfering with folate synthesis
- Initial data analysis of *Pf*dhps isolates from Ghanaian parasites exhibited a high prevalence of the A581G and A613S in the forest and coastal regions
- This two mutations are associated with resistance to Sulphadoxine and a rare occurrence globally.
- Therefore, there is the need to map out Sulphadoxine resistance and assess how the two mutations are spreading across Ghana and other African countries
- Hypothesis: A581G and A613S mutations are spreading across Ghana and other sub-Saharan African countries and could be one of the driving forces of SP resistance in Africa

Methodology

Data set curation:

- Here, we curated data from the *Plasmodium* Community Project (2020) of the MalariaGen repository. The data included 7,113 samples from 29 countries.
- In addition, new data collected in Ghana from two ecologically distinct zones (Begoro and Cape Coast) between 2014-2017 were analysed. The data included 150 and 181 sequences from Begoro and Cape Coast, respectively.

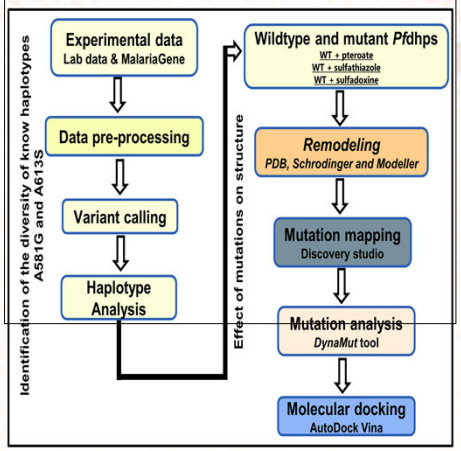

Fig. 1. Schematic flow of procedures applied in this study

Results and Discussion

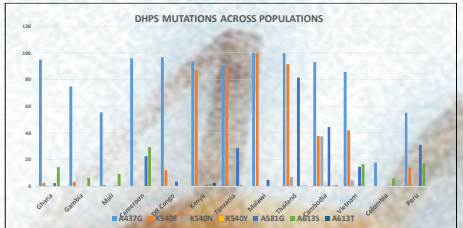

Fig. 2. Global estimates of mutations and their diversified frequencies

- The prevalence of the *Pf*dhps A581G mutation was observed above 20% in Cameroon, Tanzania, Thailand, Cambodia and Peru.
- Mutation A613S showed variable prevalence across most of the sites.
- Sulphadoxine resistant gene marker A437G was shown to be prevalent (almost fixed) in most countries studied.

Table 2. *Pf*dhps haplotypes and their frequencies across Africa

Haplotype		West Africa	East Africa	Central Africa	Southeast Asia	South America
AKAA	Wild-type	4.2 – 42.6 %	0 – 8.9 %	3.3 – 3.8 %	0.2 – 13.9 %	44.8 – 82.4 %
AKAS	Single	0.8 – 2 %	0 – 0.6 %	0 %	0 %	0 %
GKAA	Single	47.5 – 79.1 %	0.4 – 3.3 %	66.7 – 84.7 %	0 – 38.1 %	6.9 – 11.8 %
AKGS	Double	0%	0%	0 – 0.4 %	0%	0%
GEAA	Double	0.7 – 3.0 %	59.5 – 94.9 %	0 – 8.5%	17.2 – 33.0 %	0%
GKGA	Double	0%	0%	0 – 0.3 %	1.3 – 2.0 %	0 – 17.2 %
GKAS	Double	5.2 – 11.3 %	0-1.6%	0 – 7.2 %	0-0.1%	5.9 -17.2 %
GKGS	Triple	0 – 2.1 %	0%	0-21.5 %	0%	0%

- In total, 17 unique haplotypes on the *Pf*dhps locus were identified, but 8 were prevalent.
- A single mutation AKAS, double mutant GEAA, triple GKGS, GNGA were most prevalent.
- The triple mutant haplotype A437G/A581G/A613S were prevalent only in West and Central Africa

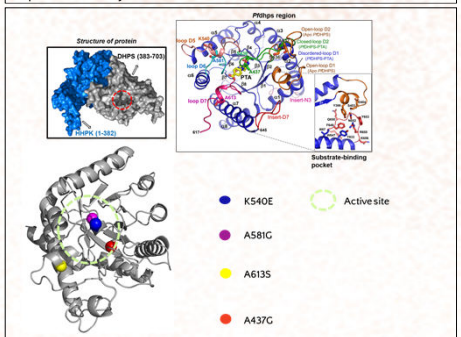

Fig. 3. A descriptive structure of *Pf*dhps and mutant location

- A581G occupies crucial loop structure at the active site in close proximity to A437G and K540E (Fig. 3)
- A613S occurs on alpha helical structure at the entrance of the active site of the protein(Fig. 3)
- Their positions may have a vital effect on sulphadoxine binding

Table 2. Effect of mutations on the stability of *Pf*dhps structure

Mutation	Physiochemical changes	Location on structure	Amino acid changes		
A581G	Hydrophobic to hydrophobic	Buried	Large to small	Destabilizing	0.31
A613S	Hydrophobic to polar	Surface	Small to large	Destabilizing	0.31
A437G	Hydrophobic to hydrophobic	Surface	Large to small	Destabilizing	0.66
K540E	Basic to polar	Surface	Small to large	Destabilizing	0.37
A437G, A613S				Stabilizing	-0.06
A437G, A581G				Destabilizing	
A437G, A581G, A613S				Destabilizing	
A437G, A581G, K540E				Destabilizing	

- Change in physicochemical properties could affect active site changes
- mutations were predicted to destabilize the active site
- The stabilization could result in conformational changes which may affect the binding of compounds

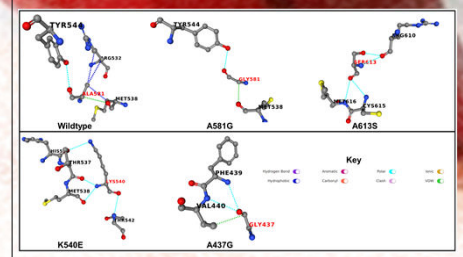

Fig. 5. Drugs and their Binding Energy Profiles in the presence of mutations

- The presence of mutations caused the loss of molecular interactions surrounding the mutant position
- Hydrogen bonds were misplaced indicating major structural and functional recognition

Table 3. Drugs and their Binding Energy Profiles in the presence of mutations

Proteins	Binding energy	Proteins	Binding energy
WT	-9.3	K540E	-9.3
A581G	-9.3	A437G,A613S	-9.0
A613S	-9.4	A437G_A581G	-8.9
A437G	-9.4	A437G, A581G, A613S	-7.8
		A437G, A581G, K540E	-7.9

- Single mutants were predicted to not significantly decreased binding affinity
- Double and triple mutants significantly decrease binding affinity to sulfadoxine

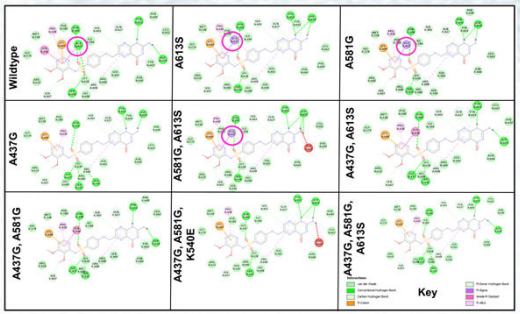

Fig. 6. Molecular Interaction profiles of sulphadoxine in wildtype and mutant proteins

- In WT, sulphadoxine made several hydrogen, Pi- and hydrophobic bonds with active site residues crucial for drug recognition and catalysis
- The presence of mutations resulted in change in interactions stabilizing sulphadoxine
- Sulphadoxine formed unfavorable interactions with Tyr663 could resulted in steric clashes

Conclusion

- A581G and A613S mutations are quickly emerging in Ghana and throughout Africa thus increased surveillance is duly recommended
- These mutations occurred on the same haplotype of genetic makers A437G and K540E
- A581G and A613S are located on beta and loop region respectively closer to the entrance of the active pocket of the *Pf*dhps protein
- Their presence destabilizes neighboring residues through the loss of molecular interactions
- Initial docking resulted in the loss of molecular interactions between the drug and the binding pocket of the protein
- Unfavorable bonds could suggest steric hindrances between the drug and the residues in the presence of mutations
- Overall, structural analysis suggest potential effect of mutations on drug binding and structure
- As future work, running dynamic simulations could additionally highlight some resistance mechanisms