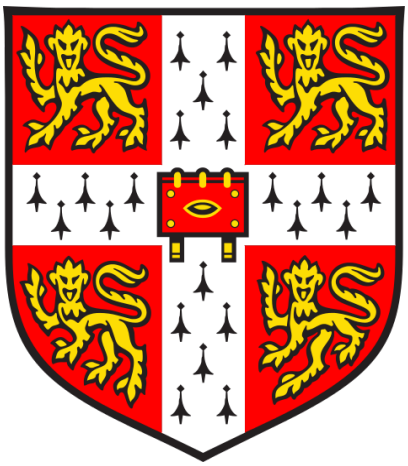
Role of *Plasmodium falciparum* genetic backgrounds in tolerance to antimalarial-resistance



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Declaration

I declare that this thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically stated in the text. It does not exceed the prescribed 20,000-word limit for the School of Biological Sciences Degree Committee.

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Abstract

Malaria remains a global public health burden with the highest mortality rate amongst vector-borne diseases, affecting mostly children under five years of age making it the leading cause of child deaths. There has been significant reduction of the malaria burden as a result of global efforts in implementing control interventions to address malaria morbidity and mortality especially since the introduction of artemisinin. However, the burden of malaria remains high in many endemic areas despite the substantial decline in global spatial distribution and burden since 2000, with over 90% of people within sub-Saharan Africa residing in endemic areas. There has been increasing reports of *Plasmodium falciparum* tolerance to several partner drugs currently in use in artemisinin-based combination therapies in certain endemic countries which has been associated with certain genetic backgrounds.

In this study, the impact of resistance-associated mutations on parasite resistance and fitness, and how the genetic background of the parasite affects these phenotypes was investigated. In the first research chapter, the growth phenotypes of a panel of barcoded *P. falciparum* parasites were measured in parallel using barcode sequencing (BarSeq). These barcoded parasites cover different *P. falciparum* strains from different geographic locations, and are grown *in vitro* in competition with each other in the presence of antimalarial compounds. BarSeq was then used to measure the different phenotypes to antimalarials based on the genetic background. Another chapter in the study established the impact of potential antimalarial compounds on genetically modified *P falciparum* parasites harbouring *Pfkelch13* mutations. These experimental compounds were chosen because of their known activity on human *Kelch*-like ECH-associated protein1 (Keap1), and were tested in drug response assays and established to have activity against parasites. Another element in this

chapter established the generation of CRISPR plasmids for editing the *Pfkelch13* gene. These individual donors were deconvoluted from a complex pool of plasmids with a common pDC2 backbone that encoded all 64 possible codons at the critical position 580 of *Pfkelch13* that is the site of the most prevalent artemisinin-resistance variant. The isolation of each possible individual donor was carried out and sub-pools of plasmids were generated. This was done to facilitate future work to examine which amino acid may be more efficient in replacing cysteine at position 580 and the impact of parasite genetic background on the outcome of the allele.

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