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Lack of resistance of Plasmodium falciparum to dihydroartemisinin in Uganda based on parasitogolgical and molecular assays

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807

Lack of resistance of *Plasmodium falciparum* to dihydroartemisinin in Uganda based on parasitological and molecular assays

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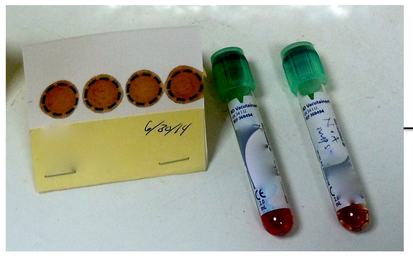
Introduction

- Artemisinin-based combination therapy is now standard treatment for falciparum malaria. However, this regimen is threatened by resistance to artemisinins, manifest as delayed clearance of parasitemia after therapy, in southeast Asia[1].
- Artemisinin resistance in southeast Asia is associated with increased parasitemias in culture, compared to those in sensitive parasites, 72 hours after a 6 hour pulse with 700 nM dihydroartemisinin (DHA), and with propeller domain polymorphisms in the *Plasmodium falciparum* kelch (*K13*; PF3D7_1343700) gene[1,2].
- **Given that artemether/lumefantrine has been adopted as standard therapy for malaria within the last decade in Uganda, we characterized artemisinin sensitivity in fresh *P. falciparum* isolates from Kampala using *ex vivo* ring-stage survival and IC_{50} assays. We also assessed the *K13* gene for polymorphisms.**

Methods



53 fresh *P. falciparum* isolates were collected from patients diagnosed with malaria from May-August 2014, at Mulago Hospital, Kampala.

Parasite IC_{50}s to DHA were determined by a standard 72 h *ex vivo* microplate assay using HRP2 detection[3].

Parasite susceptibility to DHA was assessed in the *ex vivo* ring-stage survival assay as described[2]. Survival rates were expressed as the proportion of parasites in the 6 h, 700 nM DHA-pulsed cultures relative to DMSO controls, at the end of the 72 hour assay. Twelve cultures exposed to DHA were allowed to grow for 30 d to test for recrudescence.

K13 propeller-encoding domains (codons 440-726) were dideoxy sequenced. Polymorphisms in *pfcrt* and *pfmdr1* were assessed with multiplex ligase detection reaction-fluorescent microsphere assays as previously described[4].

Results

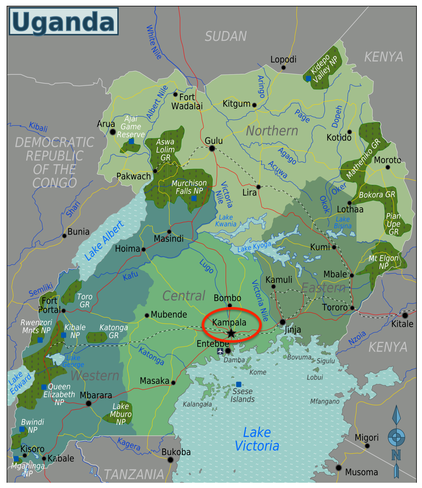


Fig. 1. Study site - Kampala, UG.

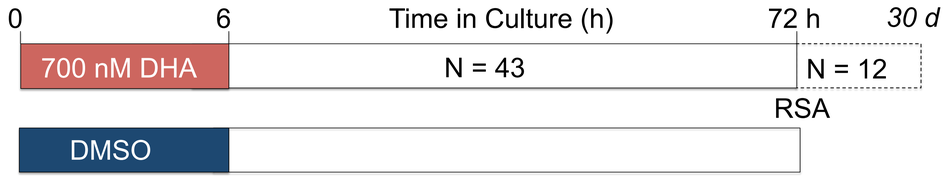


Fig. 2. Ring-stage survival assay.

- **Ring-stage survival was 0% in 40/43 cultures**
- **Ring-stage survival ranged 0.7 - 1.9% in 3/43 cultures**
- **No association with survival and SNPs in *K13*, *pfcrt* or *pfmdr1***
- **Parasites reemerged from 10/12 long-term cultures after DHA pulse**

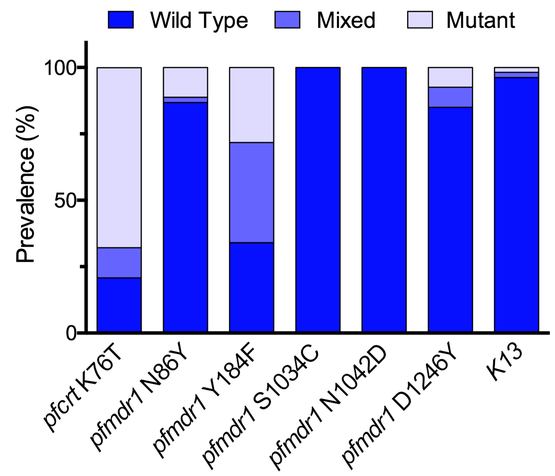


Fig. 3. Prevalences of wild-type, mixed, and mutant sequences at the indicated positions for all 53 isolates.

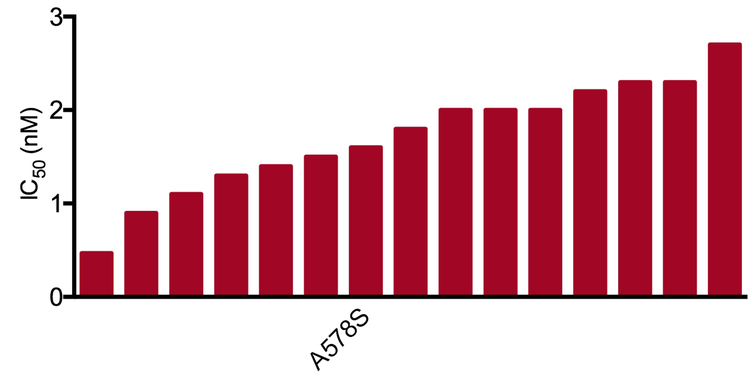


Fig. 4. *Ex vivo* IC_{50} values for DHA from 15 isolates. The geometric mean IC_{50} value was 1.6 nM.

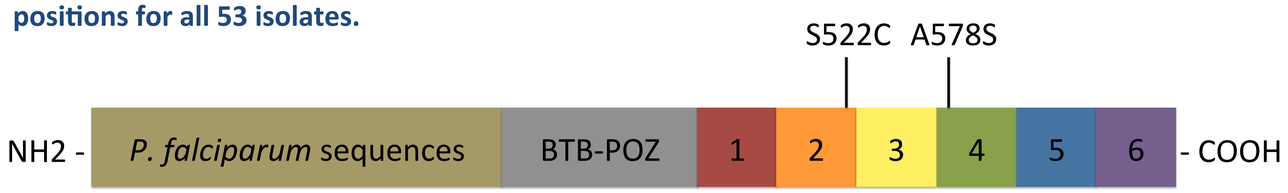


Fig. 5. Two kelch 13 (K13) polymorphisms were detected from our samples. Numbered boxes indicate the six blades comprising the propeller domain of the kelch 13 protein.

Summary and Conclusions

K13 mutations were found in 2/53 parasite isolates from Kampala, but were not mutations associated with resistance in SE Asia.

RSA and IC_{50} data showed that parasites remain highly sensitive to DHA *in vitro.*

The results of this study, as well as findings from other studies[5], suggest that artemisinin resistance is not yet a problem in Uganda. The polymorphic nature of K13 in Africa[6] and altered ACT partner drug sensitivity in Uganda[4] indicate the continued need for surveillance of ACT efficacy in the region.

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