

Stage-specific transcriptomic responses of *Plasmodium vivax* under chloroquine pressure

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Introduction: Chloroquine resistance (CQR) in *Plasmodium vivax* remains poorly characterized due to the absence of continuous culture systems, stage-dependent drug responses and the lack of validated molecular markers. Roraima is a key malaria hotspot in Brazilian Amazon, however phenotypic data on drug susceptibility in this region are scarce. **Objective:** To establish a baseline phenotypic profile of *P. vivax* susceptibility to antimalarials in Boa Vista (Roraima), and to generate a framework for investigating stage-specific parasite responses to CQ pressure. **Methods:** *Ex vivo* schizont-maturation assays were performed using clinical isolates of *P. vivax* collected in Boa Vista in October 2025 (CEP 91874625.50000.5302 for CQ, amodiaquine, artesunate, mefloquine, lumefantrine, piperazine, pyronaridine and pyrimethamine. DNA and RNA were collected for genomic and transcriptomic analyses. **Results:** Among 35 collected isolates, 22 met inclusion criteria for *ex vivo* assays, and 12 were fully processed so far. CQ exhibited a wide IC₅₀ range (min: 16.3 nM; median: 1,061 nM), with 11 of 12 isolates (98%) presenting values above the resistance threshold (100 nM). In parallel, 11 isolates classified as CQ-resistant were subjected to controlled CQ pressure during *ex vivo* incubation at 20 nM and 100 nM for 20h and 40 h. RNA was collected from these isolates under drug-exposed and drug-free conditions at both time points. Preliminary data revealed no detectable reduction in parasite quantity or alterations in parasite stage in CQ-exposed cultures compared to drug-free controls, consistent with the resistant phenotype of the analyzed isolates, all 11 isolates were classified as CQ-resistant. **Conclusions:** Our findings reveal a high prevalence of CQ-resistant *P. vivax* isolates in Boa Vista. Ongoing expansion of the isolate panel, combined with transcriptomic profiling under stage-specific CQ pressure, will enable insights into the biological mechanisms underlying *P. vivax* CQR.