Molecular Surveillance in Rondônia, Brazil: HRP2/3 Deletions, Drug Resistance, and Genetic Diversity in *Plasmodium falciparum* using NGS AmpliSeq.

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ABSTRACT

Introduction: Molecular malaria surveillance is essential to support the Brazilian National Malaria Control Program (NMCP), particularly in near-elimination settings. Deletions in *pfhrp2/3* genes compromise HRP2-based rapid diagnostic tests (RDTs), threatening case detection. Additionally, monitoring drug resistance and population connectivity provides insights into transmission dynamics beyond conventional methods. The Pf AmpliSeq deep sequencing assay offers a cost-effective alternative to whole-genome sequencing (WGS) for integrated genomic surveillance. **Objective:** To assess the prevalence of *pfhrp2/3* deletions, drug resistance markers, and genetic diversity in *P. falciparum* from Rondônia using the Pf AmpliSeq platform. **Methods:** Thirty-eight retrospective samples (2021–2024) were analyzed via multiplexed Pf AmpliSeq. The pipeline included: *pfhrp2/3* deletion detection using normalized read depth (DESeq2) cross-validated by a CNV-based approach; drug resistance genotyping; and population structure analysis (SNP barcoding and *pfcsp* diversity) for comparison with global isolates (Peru, Africa, and SE Asia). **Results:** Samples failing MQ60 filtering (n = 3) were excluded from deletion analyses. Complete *pfhrp2* deletion was rare (2.9%, 1/35). In contrast, *pfhrp3* deletions were detected in 100% of analyzed samples, with no isolate showing full gene coverage. No mutations associated with artemisinin resistance were detected across any of the resistance-associated targets included in the panel. Preliminary analysis of the *pfcsp* gene indicates low genetic diversity. SNP barcoding analyses are underway to investigate the genetic diversity and uniqueness of the Brazilian isolates. **Conclusion:** Pf AmpliSeq provides robust surveillance of diagnostic escape, resistance, and diversity. The high prevalence of *pfhrp3* deletions highlights a vulnerability in local RDTs, supporting NMCP strategies for malaria elimination in Brazil.