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TURBO TALK: Prevalence of pfhrp2/3 deletions in South Sudan: results of a 10-site national survey

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Introduction

Pfhrp2/3 deletions are recognised as a threat to malaria control, particularly in the Horn of Africa, and novel mutations in the *Pfk13* gene (K13 mutations) associated with partial artemisinin resistance have been noted in several countries in East Africa. We conducted a cross-sectional survey in South Sudan to estimate the prevalence of *Plasmodium falciparum* with *Pfhrp2/3* gene deletions and K13 mutations.

Methods

This observational study included patients younger than 15 years with suspected uncomplicated malaria in ten Médecins Sans Frontières (MSF)-supported outpatient facilities in South Sudan. After providing informed consent, each participant provided a capillary blood sample and answered a short questionnaire collecting clinical and demographic characteristics. HRP2 and PfpLDH-based rapid diagnostic tests (RDTs) were performed in parallel, and dried blood spots prepared. All participants with a positive test on either RDT were given malaria treatment. Multiplex quantitative PCR amplified *pfhrp2*, *pfhrp3*, *pfldh* and human tubulin genes simultaneously. Samples with ΔCq (Cq*pfhrp2* – Cq*pfldh* and Cq*pfhrp3* – Cq*pfldh*) values ≥3 were classified as having *pfhrp2/3* deletions. The propeller region of the *pfk13* gene was amplified from HRP2+/pLDH+ and HRP2-/pLDH+ *P falciparum* samples randomly selected from each collection site. Using the standard WHO protocol for *pfhrp2/3* deletions, we targeted enrolling 200 suspected cases per site in order to have at least 80 pLDH-positive cases per site.

Ethics

This study was approved by the South Sudan Ministry of Health Research Ethics Review Board (ERB) (Ref: MOH/RERB/A/32/2023), the MSF ERB (Ref: 2310a), and the Australia Departments of Defence and Veterans' Affairs Human Research Ethics Committee (Ref: 518-23).

Results

From January 22 to March 27, 2024, we enrolled 1842 participants (970 [53%] male and 872 [47%] female; median age 3 years [IQR 1–8]). Overall, 729 (39.6%) of 1842 valid RDT tests were HRP2-positive, with site-specific positivity rates ranging between 12.7% and 65.4 %; and 584 (31.8%) of 1839 valid tests were pLDH-positive, with site-specific positivity ranging between 11.8 % and 55.9 %. 15 (2.6%) of 584 pLDH-RDT-positive samples were HRP2-RDT-negative, with the proportion ranging between 0 and 5.4% by study site. The prevalence of *Pfhrp2/3* deletions causing false-negative HRP2-detecting RDT was 0.35% (95% CI 0.04–1.24; 2/579). Two single amino acid mutations were detected in the propeller domain of the K13 gene: C469Y and R622I, with prevalence of 0.8% (95% CI 0.16–2.28; 3/381) and 0.5% (0.06–1.88; 2/381), respectively.

Conclusion

This is the first large-scale evaluation of *pfhrp2/3* deletions and K13 mutations in South Sudan. The prevalence of *pfhrp2/3* deletions remains below the recommended threshold for changing to pLDH malaria RDTs. Prevalence of K13 mutations was low. While the sampling strategy was not conceived to be nationally representative, study sites were purposefully distributed across the country; therefore, these results are reassuring for the continued use of HRP2 RDTs in South Sudan.

Conflicts of Interest

All authors declare no competing interests.