Temporal evaluation of diagnostic resistance (*pfhrp2/3* gene deletions) in Ethiopia: 2007 - 2021

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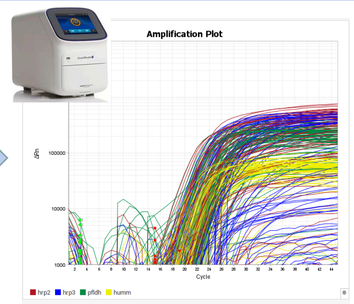
Background

- Malaria is consistently a high cause of morbidity and mortality worldwide. In Ethiopia, 68% of the population is still at risk of malaria with the parasite *Plasmodium falciparum* being the most common.[1]
- Rapid Diagnostic Tests (RDTs) are a primary intervention for malaria prevention. These tests function by targeting the antigen, histidine-rich protein 2 (HRP2) and histidine-rich protein 3 (HRP3).
- Recent evidence suggests that mutations within *pfhrp2* and/ or *pfhrp3* genes has emerged and has been spreading across Africa.[1]
- Deletions are detrimental as they lead to potential false-negative results.
- False-negative malaria RDTs are especially important to Ethiopia as they compromise the country's goal of malaria elimination by 2030.[2]

Hypothesis and Aims

- **Hypothesis:** The prevalence of *pfhrp2/3* deletions may have increased in Ethiopia over time.
- **Aims**: (1) Determine the *pfhrp2/3* deletion prevalence at ten sites across Ethiopia (2) Compare *pfhrp2/3* deletion rates across time and various demographics (i.e. age, sex, etc.) over the past two decades

Methods

Dried Blood Spot (DBS) Punching → **DNA Extraction** → 

Multiplex qPCR using QuantStudio5 ThermoCycler → **Data Analysis in R Studio and QGIS**

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References:
1. World Health Organization (WHO). World Malaria Report 2022. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022. Published 2022. Accessed February 17, 2023.
2. Feleke SM, Reichert EN, Mohammed H, et al. Plasmodium falciparum is evolving to escape malaria rapid diagnostic tests in Ethiopia. *Nat Microbiol*. 2021;6(10):1289-1299. doi:10.1038/s41564-021-00962-4
3. Statement by the Malaria Policy Advisory Group on the urgent need to address the high prevalence of pfhrp2/3 gene deletions in the Horn of Africa and beyond. Published May 28, 2921. Accessed April 20, 2023. https://www.who.int/news/item/28-05-2021-statement-by-the-malaria-policy-advisory-group-on-the-urgent-need-to-address-the-high-prevalence-of-pfhrp2-3-gene-deletions-in-the-horn-of-africa-and-beyond.

Funding: Thrasher Research Fund
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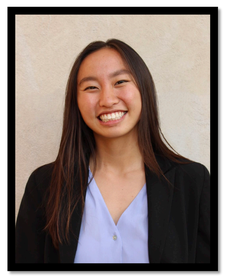
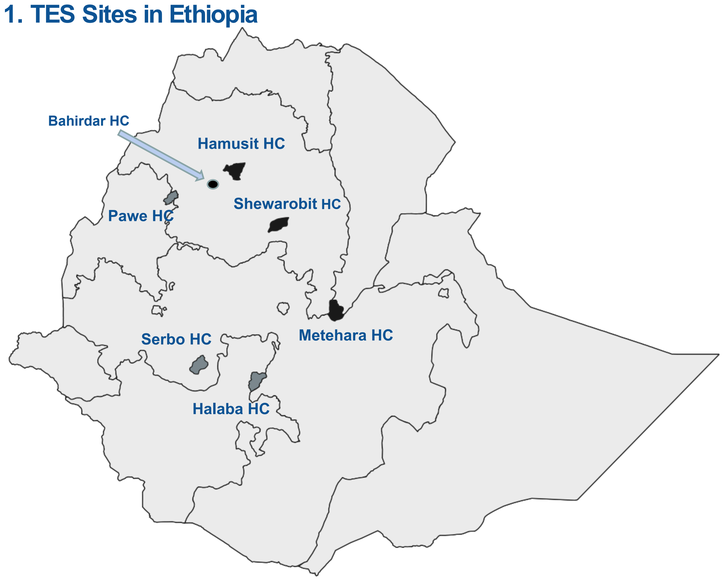


Study Design and Results

Fig 1. TES Sites in Ethiopia

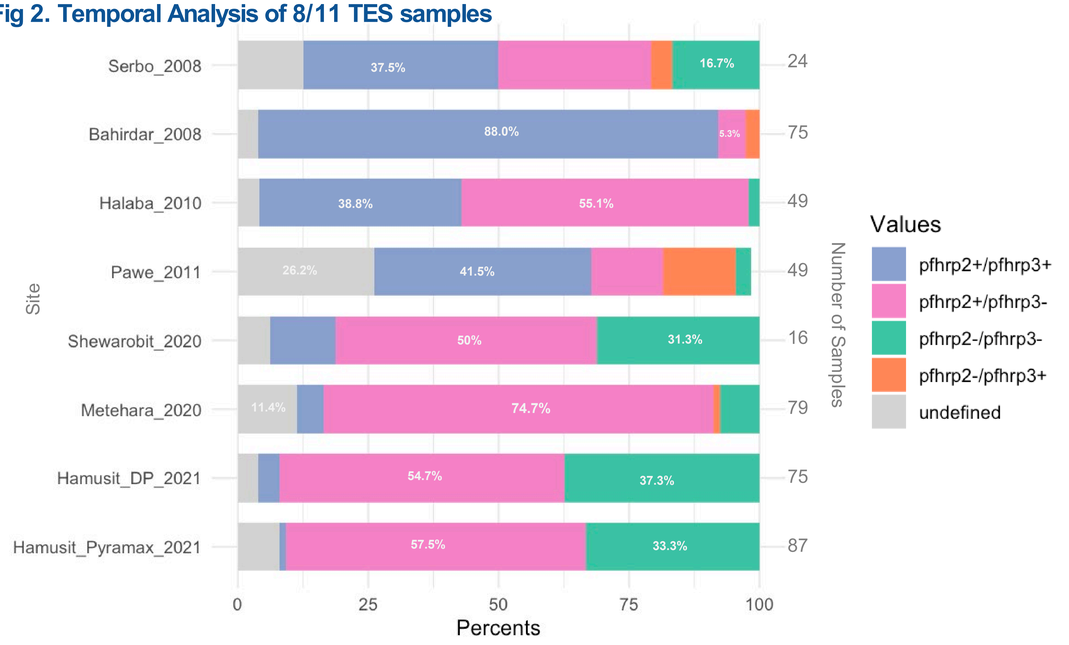


Summary of Site and Participant Data

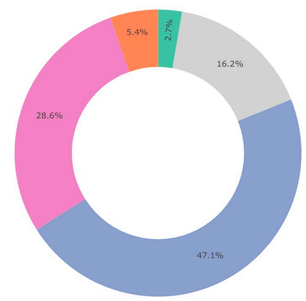
Year	Study Site	Participants, n	Age, Median years (range)	Sex: Male, n (%)	Sex: Female, n (%)
2007	Wendogenet	50	15 (1-74)	25 (50.0)	25 (50.0)
2008	Serbo	24	NA	NA	NA
2008	Bahirdar	75	18 (2-58)	NA	NA
2010	Halaba	49	5 (0-45)	26 (53.1)	23 (46.9)
2011	Pawe	49	15 (2-60)	32 (65.3)	17 (34.7)
2011	Shile	10	10.5 (2-18)	7 (70.0)	3 (30.0)
2019	Metehara	79	13 (0.8-65)	49 (62.0)	30 (38.0)
2019	Shewarobit	16	20.5 (7-60)	7 (43.8)	9 (56.3)
2020	Secha	50	14 (2-73)	34 (68.0)	16 (32.0)
2021	Hamusit Pyramax	87	28 (18-76)	66 (75.9)	21 (24.1)
2021	Hamusit DP	75	18 (2.4-63)	48 (64.0)	27 (36.0)

Table 1. Characteristics of study participants in TES 2007-2021

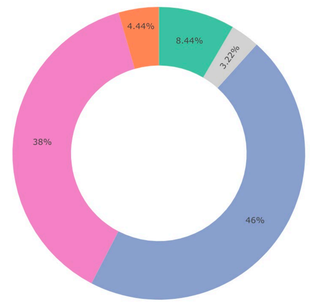
Fig 2. Temporal Analysis of 8/11 TES samples



Temporal Analysis of Biological Sex Data Across All Sites



Pre 2019 Demographic Data



Post 2019 Demographic Data

Conclusions

- **Results demonstrate expansion of *pfhrp2-/3- and pfhrp2+/pfhrp3-* gene deletions across North Central Ethiopia.**
- The data **supports recommendations for changing *pfhrp2/3*-based rapid diagnostic methods** that are used by the National Malaria Control and Elimination Program in Ethiopia.
  - WHO recommendations suggest to change diagnostic method if local prevalence rates report ≥ 5% *pfhrp2/3* deletions.[3]
- Additional genomic studies may be required to investigate the genetic bases of these deletions.