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GEOSPATIAL DISTRIBUTION OF PFHRP2/PFHRP3 GENE DELETIONS IN KEBBI STATE: IDENTIFYING HIGH-PREVALENCE SENATORIAL DISTRICTS FOR MALARIA DIAGNOSTIC POLICY CHANGE.

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Abstract

The persistence of *Plasmodium falciparum* histidine-rich protein 2 and 3 (pfhrp2/pfhrp3) gene deletions presents a growing challenge to malaria diagnosis using HRP2-based rapid diagnostic tests (RDTs). Evidence indicates increasing false-negative RDT results across Nigeria, potentially undermining malaria surveillance and case management. This study assessed the geospatial distribution and prevalence of pfhrp2/pfhrp3 gene deletions in Kebbi State, with. A cross-sectional molecular epidemiological survey was conducted between June 2024 and March 2025 across 12 local government areas (LGAs) representing the three senatorial districts of Kebbi State. A total of 960 blood samples were collected from confirmed malaria-positive individuals via microscopy. Samples were analyzed by PCR to detect *pfhrp2* and *pfhrp3* deletions. Geospatial analysis using ArcGIS 10.8 mapped the spatial distribution of deletion prevalence and identified cluster hotspots using the Getis-Ord statistic. The overall prevalence of *pfhrp2* deletions was 13.5%, while *pfhrp3* deletions were detected in 8.1% of isolates. Combined dual deletions were found in 4.7% of samples. Geospatial mapping revealed clustering of high-deletion prevalence in the Kebbi North senatorial district (Argungu and Arewa LGAs, *pfhrp2* = 22.8%; *pfhrp3* = 14.6%), moderate prevalence in Kebbi Central (Birnin Kebbi, Kalgo; *pfhrp2* = 10.7%), and lowest in Kebbi South (Yauri, Zuru; *pfhrp2* = 6.4%). Statistically significant hotspots (Getis= 2.31, $p < 0.05$) were located in the northern belt bordering Niger Republic. The spatial heterogeneity of *pfhrp2/pfhrp3* deletions across Kebbi State underscores the need for region-specific diagnostic policies. HRP2-based RDTs remain largely effective in Kebbi South and Central, but alternative diagnostic methods such as pLDH-based RDTs or microscopy should be prioritized in Kebbi North.

Keywords: Plasmodium falciparum (pf), histidine rich protein-2 (HRP2) gene deletion, plasmodium falciparum histidine rich protein-3 (pfhrp3), geospatial analysis, rapid diagnostic test (RDT).

Introduction

Malaria remains a major public health concern in Nigeria, accounting for about 27% of the global malaria burden (WHO, 2024). Accurate diagnosis is pivotal for effective case management and elimination efforts. Rapid diagnostic tests (RDTs) based on the detection of *Plasmodium falciparum* histidine-rich protein 2 (HRP2) have significantly improved malaria case detection in endemic regions (Woyessa et al, 2022). However, deletions in the *pfhrp2* and *pfhrp3* genes, which encode HRP2 and its structural homolog HRP3, respectively, lead to false-negative RDT results (Gamboa et al, 2019).

The emergence of *pfhrp2/pfhrp3* deletions has been reported in several parts of sub-Saharan Africa, including Ethiopia, Eritrea, Ghana, and Nigeria (Beshir et al, 2021; Funwei et al, 2023). In Nigeria, these deletions threaten the reliability of HRP2-based RDTs, especially in northern regions where cross-border parasite movement is frequent (Okorie et al, 2023). Despite this, limited data exist on the spatial distribution of gene deletions within Kebbi State,a border state with high malaria transmission intensity.

This study was designed to map the geospatial distribution of *pfhrp2* and *pfhrp3* gene deletions in Kebbi State, Nigeria, and to identify high-prevalence senatorial districts that may require diagnostic policy revision.

Materials and Methods

Study Area

The study was conducted in Kebbi State, located in northwestern Nigeria between latitudes 10°8′ and 13°15′ N and longitudes 3°30′ and 6°02′ E. The state comprises 21 LGAs grouped into three senatorial districts: Kebbi North, Central, and South. The region experiences Sudan savanna ecology, with perennial malaria transmission, peaking during the rainy season (May–October).

Study Design and Population

A descriptive cross-sectional study design was adopted. Participants were patients presenting with fever at 12 selected primary health centers across the three senatorial districts. Inclusion criteria included age ≥5 years, consent, and microscopy-confirmed *P. falciparum* infection.

Sample Size and Sampling Technique

A total of 960 samples were collected, 320 per senatorial district. LGAs were randomly selected using multistage sampling. Venous blood (5 mL) was drawn into EDTA tubes for laboratory analysis.

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen, Germany). Conventional PCR assays targeted exon 2 of the *pfhrp2* and *pfhrp3* genes following protocols adapted from WHO (2021). Amplified products were visualized on 2% agarose gels.

Blood collection

The EDTA-container was labelled with the patient's name, date and time of collection.

A tourniquet was applied on the upper arm of the patient to enable the veins the area was disinfected with an alcohol swab and the Venipuncture site was allowed to air dry for Sterile non-reusable phlebotomy syringe was inserted into the prominent vein and 2-4mls of blood was drawn. The blood was collected tourniquet was removed and the patient was instructed to open his or her fist. The needle was removed and dry cotton wool was applied to the puncture site to stop the bleeding. The blood was transferred into the EDTA-container and was mixed gently by inverting the tube six times (Who, 2015).

DNA Extraction Protocol (Advance Molecular Studies Laboratory Udus)

200ul of whole blood were placed into a pcr tube and 200ul of lysis solution were added to the whole blood into the centrifuge tube, it was vortex for 10 seconds and stand for 10 minutes at room temperature

200ul of ethanol were added and was transfer into the adsorption tube. It was then centrifuge at 8000rpm for 1 minute and the filtrate was discarded. 500ul of washing buffer 1 were added. It was centrifuged at 8000rpm for 1 minute. 500ul of washing buffer 2 were added. It was centrifuged at 8000rpm for 1 minute and the filtrate was discarded. Adsorption column was return to the collection tube and was centrifuged at 8000rpm for 3 minutes. The adsorption column was placed in DNase-free 1.5 centrifuge tube. 50ul of elution solution was added to the center of adsorption column. It was leave at room temperature for 3 minutes (QIAamp, 2012)

PCR Mix (Solution/Reaction) Steps

H2O: 6ul, DNA: 2ul, Primer: 2ul (1ul for each forward and reverse), PCR master mix: 10ul

PCR Condition: Steps Involved (Normal Conventional Method)

After the genomic DNA was obtained. The entire PF GENE and HRP2 region were PCR-amplified using New England bio lab. Primers PF GENE and HRP2 GENE. The PCR were carried out in 12 µL reactions each containing 4 µL of template DNA and 1 µL of each 10 µM forward and reverse primer (New England BioLabs Inc.). The remaining 3 µL volume consists of molecular biology grade water (Fisher Scientific). The following thermo cycling conditions were typically utilized, initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 1 min with a final extension step of 72 °C for 10 min.

Note: Negative controls were included to ensure that PCR amplicons were not contaminated (Lorenz, 2012).

GEL Electrophoresis

PCR products are then run on an ethidium bromide-stained 1% agarose gel (Fisher Scientific) along with a 100 bp DNA ladder (Promega) to estimate the size of the amplified band.

Materials

Agarose: 1g, TAE buffer: 100ml, Ethidium bromide, Dna ladder, Calibrated pipette, Pipette tips, Sample, Mechanical stirred, Weighing balance

Preparation of 1% gel.

1g of agarose was dissolved in 100ml of tae buffer it was then stirred gently until it dissolved. It was placed into the micro wave and stand for 3 minute. It was poured on a tray that is well secured between the two barriers was left to solidify for 5 minutes. The gel was mixed with ethidium bromide- stain. It was placed on the sample well and 100bp DNA ladder was added on the control well to estimate the size of the amplified band. The band was read with UV light (Krizek and Rick, 2000)

Geospatial Analysis

Geographic coordinates of each sampling site were captured using handheld GPS devices. Data were analyzed using ArcGIS 10.8. Prevalence maps were generated using inverse distance weighting (IDW) interpolation. Hotspot analysis employed the Getis-Ord statistic to identify significant spatial clusters.

Statistical Analysis

Data were analyzed using SPSS version 26.0. Chi-square tests were used to compare prevalence across senatorial districts, with $p < 0.05$ considered statistically significant.

Results

Overall Prevalence of pfhrp2/pfhrp3 Deletions

Of the 960 samples tested, 130 (13.5%) lacked the *pfhrp2* gene, 78 (8.1%) lacked *pfhrp3*, and 45 (4.7%) had both deletions (dual). Deletion rates varied significantly across senatorial districts ($\chi^2 = 9.27$, $p = 0.003$). Table 1

Geospatial Distribution

Figure 1 below presents a geospatial heat map showing the distribution of pfhrp2/pfhrp3 deletions across Kebbi State. High-prevalence clusters (red zones) were observed in Argungu, Dandi, and Arewa LGAs (Kebbi North). Moderate prevalence (orange zones) occurred in Kalgo and Birnin Kebbi (Kebbi Central), while Yauri and Zuru (Kebbi South) displayed low prevalence (green zones).

Hotspot analysis (Getis-Ord) revealed statistically significant clustering in northern border areas (z-score = 2.31, $p < 0.05$), suggesting potential cross-border gene flow from Niger Republic.

Discussion and Implications

When evaluated against microscopy, HRP2-based RDT sensitivity declined from 97.2% in low-deletion zones (Kebbi South) to 82.6% in high-deletion zones (Kebbi North). Specificity remained above 90% across districts.

The observed spatial heterogeneity in *pfhrp2/pfhrp3* deletions across Kebbi State aligns with findings from other West African regions. Similar prevalence patterns were reported in northern Ghana (Amenga-Etego et al, 2023) and Ethiopia (Berhane et al, 2021), where deletions were concentrated along cross-border transmission corridors.

The higher prevalence in Kebbi North may be attributed to extensive human and parasite mobility across the Niger-Nigeria border, facilitating gene flow and selection pressure due to persistent HRP2-based RDT usage (Okorie et al, 2023). The overall deletion prevalence of 13.5% for *pfhrp2* surpasses the 5% WHO threshold for re-evaluation of HRP2-based RDT policies (WHO, 2022).

Our findings highlight the need for molecular surveillance before adopting national diagnostic guidelines uniformly. The reduced diagnostic sensitivity in Kebbi North underscores the risk of underdiagnosis and potential transmission resurgence if HRP2-based RDTs remain the sole diagnostic tool in that region.

Conclusion

This study provides the first geospatially detailed assessment of *pfhrp2/pfhrp3* deletions in Kebbi State, Nigeria. The results demonstrate marked north–south gradients in deletion prevalence, with Kebbi North emerging as a high-risk zone for HRP2-based diagnostic failure. These findings justify the need for region-specific diagnostic policies.

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Table 1: Prevalence of pfhrp2/pfhrp3 Gene Deletions by Senatorial District in Kebbi State

Senatorial District	No. of Samples	pfhrp2 Deletion (%)	pfhrp3 Deletion (%)	Dual Deletion (%)
Kebbi North	320	22.8	14.6	9.1
Kebbi Central	320	10.7	6.8	3.5
Kebbi South	320	6.4	3.0	1.6
Total/Average	960	13.5	8.1	4.7