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Clinical, parasitological aspects, and gene detection (Pfk13) of *Plasmodium falciparum* in children aged 6 to 59 months with uncomplicated malaria in Bolenge, Democratic Republic of the Congo

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ABSTRACT

Introduction

Malaria, caused mainly by *Plasmodium falciparum* in sub-Saharan Africa, remains a major public health concern. It typically manifests as persistent fever and high parasitemia. Of particular concern is the emergence of resistance to artemisinin derivatives, the current reference treatment. Such resistance is often linked to mutations in the Pfk13 gene, recognised as a molecular marker for surveillance. However, clinical, parasitological, and Pfk13 gene data on children under five years of age in Bolenge remain insufficient.

Purpose

To describe the clinical and parasitological profiles and determine the prevalence of the Pfk13 gene in children aged 6–59 months with uncomplicated malaria in Bolenge, DRC.

Methods

This descriptive cross-sectional study was conducted between April 2021 and September 2022, using blood samples collected on filter paper during previous research on the therapeutic efficacy of antimalarial drugs in Bolenge (2017). The study involved 90 children selected through convenience sampling. DNA was extracted from filter paper samples and analysed via classic PCR for Pfk13 mutations. Children included had fever ≥ 37.5 °C and *P. falciparum* monoinfection with parasitemia between 2,000 and 200,000 trophozoites/µl. Data were analysed using SPSS v20, with descriptive statistics and 95% confidence intervals reported.

Results

The mean age was 43.5 months. A total of 85.6% presented with fever ranging from 37.5–39 °C. Mean parasitemia was 25,000 trophozoites/µl. The Pfk13 gene was detected in 50% (n = 45) of samples, producing 800 bp amplicons.

Conclusion

These results highlight the need to optimise DNA storage and integrate molecular surveillance of Pfk13 into national malaria control policies in Bolenge. Such integration would strengthen the early detection of potential artemisinin resistance and enable the development of targeted therapeutic strategies.

INTRODUCTION

Background and rationale

Malaria remains one of the most pressing global health challenges. In 2025, the World Health Organization (WHO) estimated over 240 million malaria cases and about 600,000 deaths worldwide, with sub-Saharan Africa bearing the greatest burden. Children under five account for approximately 75% of malaria-related deaths in the region (WHO, 2025). In the Democratic Republic of Congo (DRC), malaria continues to be the leading cause of morbidity and mortality, especially in children under five years of age. In 2024, the National Malaria Control Program (PNLP) recorded 29,123,262 malaria cases in the DRC, including 13,903,437 (47.7%) in children under five, and 21,695 related deaths, of which 15,091 (69%) occurred in this age group (PNLP, 2024).

Uncomplicated malaria is the most common form of the disease in the DRC. It is generally caused by *Plasmodium falciparum*, the most virulent species in sub-Saharan Africa, and the most common clinical symptom remains fever, often with an average temperature above 38 °C (Doudou et al., 2022; WHO, 2022). Diagnosis relies on detecting the parasite in the blood. In the DRC, parasitemia in uncomplicated malaria is generally below 2%, or less than 100,000 parasites/µl, according to PNLP and WHO guidelines. Studies in similar contexts, such as Niger, reported mean parasitemia levels between 2,000 and 5,000 parasites/µl in asymptomatic children. Although a study in Kinshasa confirmed significant parasitemia in children with malaria, precise data on uncomplicated cases remain scarce in the local literature (Doudou et al., 2020; Gbaguidi et al., 2014; WHO, 2022).

Bolenge, in Mongala province of the DRC, is an area of stable, high malaria transmission. Its equatorial climate, with heavy rainfall and constant humidity, favours mosquito breeding. Numerous water collections provide ideal habitats for *Anopheles*, the vector of *Plasmodium falciparum*. According to PNLP and WHO data, malaria transmission is perennial, with peaks during the rainy season. The annual incidence remains high, placing Bolenge among priority zones for malaria control (PNLP, 2024; WHO, 2023). Healthcare infrastructure is severely limited, with few qualified staff and inadequate equipment. The main health facility provides only basic care, insufficient for the population's needs. These gaps mirror broader systemic weaknesses in rural DRC health services (Ministry of Health, 2023; PNDS, 2021). Local studies from 2015–2021 show parasite prevalence above 40% and high parasite loads, confirming intense transmission (Mukadi et al., 2021; Ngweme et al., 2019).

As the parasite has evolved and resistance has emerged, therapeutic protocols have had to be adapted. Currently, four artemisinin-based combination therapies (ACTs) are recommended in the DRC: Artemether-Lumefantrine (AL), Artesunate-Amodiaquine (ASAQ), Dihydroartemisinin-Piperaquine (DHP), and Artesunate-Pyronaridine (AP). However, only AL and ASAQ are made available free of charge in public facilities (Mvumbi Makaba, 2017).

Since 2008, the emergence of artemisinin-resistant strains in Southeast Asia has raised serious concerns. This phenomenon is manifested by a slowdown in parasite clearance, despite correctly administered treatment, of artemisinin derivatives alone or in combination with a partner molecule (Bamadio, 2012).

The *kelch13* (PfK13) gene is a major molecular marker for artemisinin resistance in *Plasmodium falciparum*. Its propeller domain (KRP), made of six β-sheet blades in a helical structure, plays a crucial role in protein interactions and parasite survival. Mutations are classified into surface clusters, affecting protein–protein interactions, and buried clusters, impacting structural stability. Small-angle X-ray scattering (SAXS) analyses reveal a hexameric cauldron-like structure of PfK13. Key mutations such as R539T and C580Y change its conformation without breaking the hexamer, impairing endocytosis (Birnbaum et al., 2022; Siddiqui et al., 2022; WHO, 2023). These alterations disturb interaction with PfPI3K, a regulator of infected red blood cell degradation (Mbengue et al., 2021). As a result, artemisinin's pharmacological action is slowed, enhancing parasite survival (Mbengue et al., 2021; Uwimana et al., 2020; WHO, 2023).

In Rwanda, a study conducted in May 2023 detected PfK13 in 89.8% of samples. The mutations 561H, 675V, and 469F were most frequent, highlighting the worrying spread of artemisinin resistance in the region (van Loon et al., 2023). A study conducted in the DRC between 2020 and 2021 on the therapeutic efficacy of ACTs found the PfK13 gene in

95% of samples, with two WHO-validated resistance mutations detected: R561H in Kabondo/Kisangani/Tshopo and P441L in Rutshuru/Nord-Kivu, near the borders with Uganda and Rwanda (Moriarty et al., 2021).

Because of these findings, PfK13 polymorphism is now a key indicator for monitoring the emergence and spread of resistant strains. The World Health Organization formally recommends its detection as part of epidemiological surveillance programmes (WHO, 2023).

The overall aim of our study was to describe the clinical and parasitological characteristics of uncomplicated malaria in children aged 6 to 59 months managed in Bolenge (DRC), and to detect the presence of the *Plasmodium falciparum* PfK13 gene as a marker of molecular resistance to artemisinin derivatives.

METHODS

Type of study and sampling

This was a cross-sectional, descriptive study of children aged 6 to 59 months with uncomplicated malaria in Bolenge, Equateur Province, Democratic Republic of Congo. Non-probability convenience sampling of 90 children was employed. Data were obtained from retrospective biological samples preserved on Whatman Grade GB003 filter paper, collected in 2017. These consisted of clinical, parasitological, and molecular analyses.

Nature, setting, and period of the study

The samples were drawn from the national database on the evaluation of the efficacy of ACTs, covering the period from April 2021 to September 2022, a total of 17 months.

Inclusion criteria

Children aged 6 to 59 months were included if they met the following criteria:

- Fever ≥ 37.5°C
- Parasitemia between 2,000 and 200,000 trophozoites/µl
- *Plasmodium falciparum* monoinfection confirmed by RDT, thin smear, and thick drop
- Parental or guardian informed consent obtained.

Parameters of interest

The study covered several categories of data:

i. **Sociodemographic parameters:** age, sex, and inclusion.
ii. **Clinical and parasitological parameters:** body temperature measured at the time of diagnosis and parasitemia rate expressed as trophozoites per microliter of blood.
iii. **Molecular parameters:** DNA extraction from stored blood samples, amplification, and identification of the *Pfk13* gene by conventional polymerase chain reaction (PCR).

DNA extraction and amplification

DNA extraction

DNA was extracted from blotting papers collected in Bolenge and stored in the Faculty of Medicine's molecular biology laboratory. Extraction was performed using the Qiagen® kit, according to the manufacturer's protocols (Maiga Souleymane, 2019). The quality and concentration of the DNA obtained were assessed spectrophotometrically (Nanodrop®), before amplification under the conditions described by Benoit-Vical et al. (2016).

Amplification

PCR mixes were prepared according to the protocol shown in **Table 1**.

Table 1:
Mix preparation

Component	Volume (µl)
$MgCl_2$ (25 mM)	5
Forward primer (25 µM)	1
Reverse primer (25 µM)	1
Taq polymerase (5 U/µl)	0.2
Distilled water (PCR-grade)	27.4
PCR mix	**40**

Sample DNA + mix, template DNA: 10 µl, giving a total reaction volume of 50 µl.

Table 2:
Protocol and cycles for the Pfk13 gene

Steps	Temperature (°C)	Time	Number of cycles
Initial denaturation	95	3′	-
Denaturation	95	30′′	40
Hybridization	60	30′′	
Final elongation	72	5′	

Steps 2–4 were repeated for 40 cycles.

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The primers (forward and reverse) used are shown in Table 3.

Table 3:
Pfk13 gene amplification primers

Names	Sequences
K13F	5'-CTAGGTGTTGGATG-3'
K13R	5'-TTATGAGAAATCAAAGTCTTTGGGT-3'

PCR detection and analysis

The PCR reaction was carried out in the central veterinary laboratory. Amplified products were subjected to migration on 2% agarose gel, stained with ethidium bromide (0.5 µg/ml), and visualised under ultraviolet transillumination (280–320 nm).

Statistical analysis

Data were entered in Excel and analysed using SPSS v20.0. Analyses were conducted with a 95% confidence interval. The Chi-square test was applied for categorical variables, with a significance threshold of $p < .05$. Results were presented in tables and figures, with frequencies and proportions calculated in relation to the presence of the *Pfk13* gene.

Ethical considerations

The data used were derived from a previous study on the therapeutic efficacy of ACTs. The research protocol was validated by the Ethics Committee of the Kinshasa School of Public Health (Ref: ESP/CE/049/2016). Parental or guardian consent was duly obtained.

RESULTS

Sociodemographic data

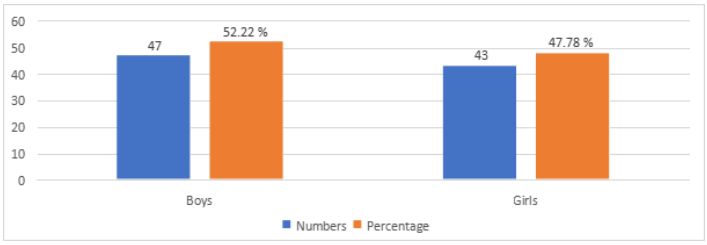
The sample analysed consisted of 90 children aged 6 to 59 months, diagnosed with uncomplicated malaria in Bolenge. All participants were included on the basis of informed consent provided by their parents or guardians.

Table 4:
Age distribution

Age range (months)	Number	Percentage (%)
Less than 10	5	5.6
11–25	25	27.8
26–35	27	30.0
36–45	27	30.0
46–55	6	6.7
56–59	0	0.0
Total	**90**	**100**

Sixty percent of participants were aged 26–45 months. The average age of the sample was 43.5 months. There was no significant sex difference, $p = .677$.

Figure 1:
Gender distribution



Of the children included, 47 (52.2%) were male, 95% CI [41.9%, 62.5%], and 43 (47.8%) were female, 95% CI [37.5%, 58.1%], giving a sex ratio of 1.5 in favour of boys. The difference was not statistically significant, $p = .677$.

Clinical and parasitological data

Clinical data

The mean temperature at diagnosis was 38.25°C, with most children (85.6%) presenting with a fever between 37.5°C and 39°C, 95% CI [38.15°C, 38.35°C]. Extreme temperatures were rare.

Table 5:
Body temperature

Temperature (°C)	Number	Percentage (%)
< 36	1	1.1
36–37.5	6	6.7
37.5–39	77	85.6
> 39	6	6.7
Total	**90**	**100**

Parasitological data

The majority of parasitemia values observed were between 10,001 and 40,000 trophozoites/µl, representing 44.4% of cases, 95% CI [34.1%, 54.7%]. The mean parasitemia was 25,000 trophozoites/µl.

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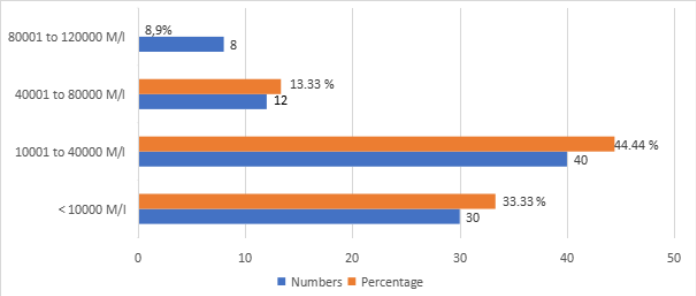
Figure 2:
Parasitemia distribution



Molecular data: Plasmodium DNA extraction

Table 6:
Concentration of extracted DNA

	Value
Average concentration (ng/µl)	47.2
A260/280	1.94

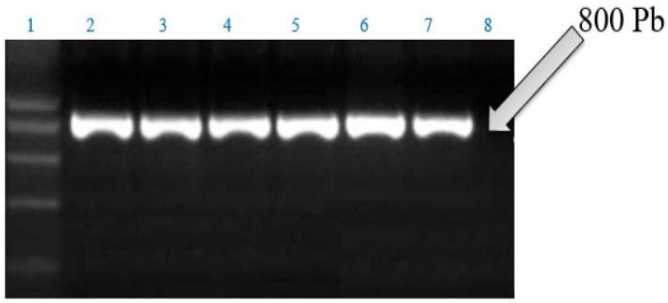
The 90 samples analysed yielded an average DNA concentration of 47.2 ± 7.8 ng/µl. The average A260/280 ratio observed was 1.94, indicating high-quality DNA suitable for molecular analysis.

PCR amplification of the Pfk13 gene

Conventional PCR performed on the 90 DNA samples stored at −20°C for six months resulted in 50% amplification of the *Pfk13* gene (n = 45/90), 95% CI [39.7%, 60.3%]. The expected amplicon size was 800 bp, with a 200 bp target sequence used for identification.

This partial amplification rate suggests possible DNA degradation or variable technical efficiency of amplification after prolonged storage. Other factors, such as parasite load or extraction quality, may also have contributed. No amplification signal was detected in the negative controls, confirming the absence of cross-contamination. A few initially negative or questionable samples that failed the internal positive control were reanalysed, and their results were confirmed as negative.

Figure 3:
Migration image on agarose gel



1. Molecular weight marker
2. Positive samples
3. Negative sample

DISCUSSION

Accumulated experience in malaria-endemic countries shows a consistent trend: whenever a new antimalarial drug is widely used, *Plasmodium falciparum* eventually develops resistance mechanisms (Nag, 2019). This reality underlines the crucial importance of monitoring these resistances, notably through the analysis of molecular markers such as the *Pfk13* gene, which was targeted in our study.

We conducted our research on samples from 90 children aged 6 to 59 months diagnosed with uncomplicated malaria in Bolenge, with the main objective of assessing the clinical and parasitological characteristics and identifying the *Pfk13* gene of *Plasmodium falciparum*. This gene, strongly linked to resistance to artemisinin derivatives, plays a strategic role in surveillance programmes.

The majority of the children included were between 26 and 45 months old (60% of the sample), with an average age of 43.5 months. This profile is slightly older than those reported in some studies conducted in Mali, such as by Soumare (2012), who observed a predominance of children between 24 and 59 months (55.9%), and Coulibaly (2012), who reported 41.5%. This difference could be linked to the size of our sample or the particular vulnerability of children aged 2 to 4 years in our study region.

Males accounted for 52.2% of cases, with a sex ratio of 1.5. These figures are consistent with other surveys carried out in Africa, such as in Bamako by Soumare (2020), who reported 50.9% with a sex ratio of 1.04 (World Health Organization [WHO], 2020). Similarly, Mutombo et al.

(2013) in Lubumbashi also observed a slight predominance of boys (51.6%) with a sex ratio of 1.06. The Malian Ministry of Health likewise found 55.6% male cases, which is also close to that of Diarra in Mali in 2021 (Coulibaly, 2012).

Not surprisingly, fever was the most frequent clinical sign. Most children had a temperature between 37.5°C and 39°C (85.6%), with an average of 38.25°C. These results are comparable to those observed in several studies in African capitals, including Kinshasa, DRC (Doudou et al., 2020), Rwanda (Uwimana et al., 2020), and Mali (Soumare et al., 2021). Mutombo (2018) in Likasi found 100% fever, while Sanogo (2021) in Mali reported fever as the most frequent reason for consultation (82.23%). Coulibaly (2012) reported an even higher proportion, with fever as the main reason for consultation in 97.1% of children. Our more detailed categorisation according to fever intensity may explain these nuances.

Parasitemia was high, averaging around 25,000 trophozoites per microlitre of blood. Almost 44% of children had parasitemia between 10,001 and 40,000/µl. These figures are higher than those reported by Sanogo (2021) in Mali, who found parasite densities ranging from 1,600 to 80,000 trophozoites in 66.6% of cases, and Mukomena et al. (2016) in Lubumbashi, who found that 88.4% of children had parasitemia between 200 and 2,000, while 12% of schoolchildren had levels above 2,000. Studies in similar settings, such as Niger, reported average parasitemia between 2,000 and 5,000 parasites/µl in asymptomatic children (Gbaguidi et al., 2014). The differences may reflect that Bolenge is an area of high malaria transmission. Furthermore, average parasitemia in uncomplicated malaria cases in the DRC varies depending on several factors, and precise data remain limited in local scientific literature (Doudou et al., 2020; Gbaguidi et al., 2014).

DNA extracted from blotting papers revealed a mean concentration of 47.2 ± 7.8 ng/µl, with an A260/280 ratio of 1.94, indicating good concentration. These data are higher than the standard values provided by the manufacturer QIAGEN® (34.0–40.0 ng/µl; Jena Bioscience, 2015), confirming the effectiveness of this medium in resource-limited settings (Adawaye et al., 2013).

Conventional PCR amplified the *Pfk13* gene in 45 of 90 samples (50%). This rate is lower than that reported in comparable studies: 95% in the DRC (Moriarty et al., 2021), 89.8% in Rwanda (van Loon et al., 2023), 95.6% in Togo (Dorkenoo et al., 2016), and 88.2% in a meta-analysis covering nine African countries (Schmedes et al., 2018). Discrepancies may be attributed to differences in protocols, inclusion criteria, sampling and storage conditions, as well as study-site factors. In our case, a prolonged two-week thermal excursion of extracted DNA may have contributed to reduced amplification rates.

This study has several limitations, including the use of convenience sampling, a retrospective study design, and the absence of *Pfk13* mutation sequencing. In addition, suboptimal DNA storage conditions may have impacted amplification efficiency. These limitations underscore the need to strengthen sentinel surveillance and integrate comprehensive whole-gene sequencing in future research. Such approaches will improve understanding of parasite resistance dynamics and facilitate the detection of WHO-validated resistance-associated mutations.

CONCLUSION

Fever predominates alongside high parasitemia. Improved DNA preservation methods are needed to optimise *Pfk13* detection. Whole-gene sequencing will further enable the detection of resistant mutations. This surveillance must be integrated into national policies to strengthen malaria control in Bolenge.

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Ethical Approval: The research protocol was validated by the Ethics Committee of the Kinshasa School of Public Health (Ref: ESP/CE/049/2016).

Conflicts of Interest: None declared.

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