J Genet Resour 2025; 11(1):12-20 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI:* 10.22080/jgr.2025.27936.1409

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Prevalence of *PfATPase6* Gene Mutation for ACT Resistance in *Plasmodium falciparum* Malaria Parasites among Patients with Fever in Osun State Health Facilities, Nigeria

Olufemi Oroge[1], Sunday Olarewaju [2], Adeola Olukosi[2], Akinlabi Rufia[2], Sukurat Oyebanji[2], Ayo Kamilu Fasasi[2], Akinwale John Faniyi[3*] and Monsuru Adeleke[2]

[1] Osun State Malaria Elimination Program, Osun State Ministry of Health, Nigeria
[2] Department of Community Medicine, Osun State University, Osogbo, Nigeria
[3] Research Laboratory, International Foundational Against Infectious Disease in Nigeria, Nigeria

ARTICLE INFO

Article history:
Received 20 November 2024
Accepted 20 December 2024
Available online 15 January 2025

Keywords:
Malaria
PfATPase6 gene
ACT resistance
Plasmodium falciparum

Corresponding authors:
✉ A. J. Faniyi
faniyiakinwale2014@gmail.com

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Malaria remains a significant public health challenge in Nigeria, particularly in Osun State, where *Plasmodium falciparum* is the predominant species responsible for the disease. This research aimed to assess the prevalence of *Plasmodium falciparum* ATPase 6 gene (*PfATPase6*) mutations associated with artemisinin-based combination therapy (ACT) resistance in *P. falciparum* isolates from febrile patients attending health facilities across eight local government areas (LGAs) in Osun State, Nigeria. A cross-sectional study was conducted across eight LGAs in Osun State, where between 36 and 40 febrile patients were randomly selected from each local government area. A total of 315 respondents (175 females and 140 males) participated, with a mean age of 16.7 years. Rapid diagnostic tests (RDTs) for malaria were performed, and samples from positive cases were analyzed for the presence of *PfATPase6* gene mutations using polymerase chain reactions. Out of 293 valid malaria RDTs conducted, 127 (43.3%, CI 37.59%-49.23%) tested positive for *P. falciparum.* The mean age of patients who tested positive was 11.12 years, which was statistically significant ($p < 0.05$). The highest malaria prevalence rate was recorded in Orolu LGA (57.5%), while the lowest was in Osogbo LGA (29.41%). Socio-demographically, malaria prevalence was highest among respondents with no more than primary school education (59.7%). Notably, none of the 127 *P. falciparum* positive samples harbored the *PfATPase6* gene mutation. The study reveals a substantial burden of malaria in Osun State, particularly among younger patients and those with lower educational attainment. However, the absence of *PfATPase6* gene mutations in all positive samples suggests that ACTs remain effective in this region.

Please cite this paper as: Olufemi, O., Sunday, O., Olukosi, A., Rufia, A., Sukurat, O., Fasasi, A., Faniyi, A.J., & Adeleke, M. (2025). Prevalence of *PfATPase6* gene mutation for ACT resistance in *Plasmodium falciparum* malaria parasites among patients with fever in Osun State health facilities, Nigeria. *Journal of Genetic Resources,* 11(1), 12-20. doi: 10.22080/jgr.2025.27936.1409

Introduction

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, with Nigeria bearing one of the highest burdens globally (WHO, 2021). It is the second most common cause of infectious disease-related deaths after tuberculosis. It is also a leading cause of infant and perinatal morbidity and mortality (Schantz-Dunn and Nour, 2009). In school-age children, evidence is unequivocal that malaria is a leading cause of school absenteeism and cerebral impairment (Nankabirwa *et al*., 2014). The World Health Organization (WHO) estimates that Nigeria accounts for nearly 27% of the global malaria cases and deaths, predominantly due to *Plasmodium falciparum,*

the most lethal of the malaria parasites (WHO, 2021). Despite concerted efforts to control and eventually eliminate malaria, the disease continues to pose a significant public health challenge, largely due to the development of drug resistance.

Over the past two decades of continued reliance on artemisinin-based combination therapies (ACT) for malaria treatment, there have been observed delays in *P. falciparum* clearance after the administration of complete courses of ACT, and this is described as partial resistance to the ACT. Full resistance to ACT is, however, confirmed when delayed *P. falciparum* clearance occurs in the presence of a validated resistance mutant gene (WHO, 2017). *P. falciparum* Adenosine Triphosphatase 6 (*PfATPase6*) gene mutations, and more recently, *PfKelch13* gene mutations have been identified as markers of ACT resistance (Igbasi *et al*., 2019; Afoakwah *et al*., 2011).

P. falciparum Adenosine Triphosphatase 6 (*PfATPase 6*) is a calcium ATPase involved in calcium ion transport, also called *PfSERCA*, essential for the parasite's calcium homeostasis. A review by Afoakwah *et al.* in 2011 noted about 44 different single nucleotide polymorphisms (SNPs) of the *PfATPase6* gene from 35 countries and that E431K, A623E, S769N, and L263E mutations are the ones that have been associated with artemisinin resistance 6. It was noted that the Single nucleotide polymorphisms of *PfATPase6* linked with artemisinin resistance were rarely observed. However, their prevalence may increase as the pressure for ACT selection increases in the population (Afoakwah *et al*., 2011). It is possible that such paucity in the detection of significant SNPs of *PfATPase6* has contributed to attenuation in its reliance on it as a marker of artemisinin resistance. It is the right time to revisit the relevance of *PfATPase6* mutations as a marker of artemisinin resistance to reduce the potential widespread treatment failures.

In Nigeria, the Federal Ministry of Health, in its National Malaria Drug Policy, adopted ACTs as the treatment of choice for malaria, and till now, there have been situations that suggest possible treatment failure or delayed parasite clearance after completing courses of ACT, making surveillance of drug resistance a public health priority. Osun State, located in the southwestern region of Nigeria, is one of the many areas where malaria is endemic, with frequent outbreaks, especially in the rainy season. Health facilities across the state are often overwhelmed with febrile patients, many of whom are diagnosed with malaria. A study was conducted in Osun State by Nassar *et al.* (2016) on the prevalence of *PfATPase6* mutation in ACT resistance, but zero prevalence was reported (Nassar *et al*., 2016). A similar study was conducted by Tola *et al.* (2020) in both Osun and Lagos but found one S679S mutation of the *PfATPase6* gene (which is not validated for ACT resistance) in 83 samples (Tola *et al*., 2020). However, it remains necessary to continue to understand the role *PfATPase6* plays in the artemisinin mechanism of action and development of resistance and to understand its prevalence, especially in areas of high malaria transmission like Nigeria. Therefore, this study aims to determine the prevalence of *PfATPase6* gene mutations in *P. falciparum* isolates obtained from febrile patients attending health facilities in Osun State, Nigeria.

Materials and Methods

Study design and settings

This cross-sectional study was conducted in selected health facilities across Osun State, Nigeria, between October 2020 and November 2021. Multi-stage random sampling was employed to select the health facilities that served as study centres. The ten local government areas in each of the three senatorial districts in the state were categorized into urban and rural local governments, and one local government area was randomly selected from each of the two categories. At least two primary health centres, one urban and one rural, were thus selected from each of the senatorial districts. Forty persons who presented with fever participated in each of the study centres. This brought the maximum sample size to 240, which was 8 samples less than the calculated sample size. To meet the required sample size, a senatorial district was randomly selected to produce rural and urban local government areas through another random selection to produce one additional facility each in rural and urban South

communities. This took the sample size to 320. The local government areas where the study occurred are Ilesa West, Oriade, Orolu, Osogbo, Ifelodun, Olorunda, Ede South, and Ede North.

Study population and sample size

The study population consisted of persons living within Osun State who presented to the selected public health facilities on account of fever or a history of fever in the 48 hours preceding the presentation. Inclusion criteria include: 1) Patients attending chosen health facilities in Osun State; 2) Patients with fever or a history of fever in the 48 hours preceding presentation; 3) Patients that sign the consent form. Exclusion criteria include: 1) Patients with severe malaria; 2) Patients with obvious concomitant infection.

To determine the hospital fever prevalence of malaria in Osun State, the sample size was calculated using Fisher's formula for estimating simple proportion in a group, with the value of P derived from a study by Jaffu *et al.* (2015), in which the prevalence of *PfATPase6* mutation was found to be 16.2%.

$n= Z^2pq /d^2$

n- is the desired sample size; Z= normal deviate at 95% confidence interval (Z= 1.96); p= a priori estimate of the proportion (p= 0.16); q= 1-p; d= 0.05, with level of significance set at 0.05.

Thus, a minimum of 207 respondents were required. However, the sample size was increased by 20% to 248 to make allowance for non-response or invalid sample collection.

Sample collection and storage

Teaching guides for conducting mRDT and for collecting dried blood spots were developed. The protocol for attending to a potential study subject from the first encounter through the process of clerking, administration of the questionnaire, conduct of mRDT, and collecting a dried blood spot where applicable was also developed and administered to all study participants. A consent form was also developed. At each of the selected health facilities, at least two health workers were trained or retrained on using the questionnaire as appropriate. Six of the facilities used First Response^R mRDT, while two had Care Start^R. DBS were collected on Whatman 3MM-grade filter papers that were cut into quadrants, which were about 2cm in their widest edges and had a cardboard tag for labelling. Only patients with fever were interviewed, and everyone interviewed had an RDT done and dried blood spots collected. Freshly collected blood spots were air-dried for 3 hours using an improvised drying rack. After they were dried, they were put away in an airtight zip-lock bag, which already had a desiccant in it. All was done in adherence to the protocols, which were pasted on the walls close to the testing area. Confidentiality was ensured. Neither their names nor the card/hospital number of participants were taken. Rather, the patients were allotted unique identifiers, the same as on the questionnaire and the dried blood spot papers, and recorded on the RDT cassette. All samples were kept in zip-lock bags and stored in an airtight container. The samples were transported into the laboratory and kept in a freezer at a temperature of -20ºC until they were analyzed.

DNA extraction and PCR-RFLP

For DNA extraction, stock solutions of 1 M of Tris pH 8.0 and 0.5 M of $EDTA_{Na2}$ pH 8.0 were prepared and constituted into Tris-$EDTA_{Na2}$ (TE) buffer at the ratio of 1 ml of Tris base to 0.2 ml of $EDTA_{Na2}$ solution prepared as reported by Panda *et al.* (2019). The paper puncher was rinsed with absolute ethanol before it was used to punch out the dried blood spot from the filter paper. To reduce the risk of cross-contamination, 5 punches were made in a clean 0.5 mm cardboard to clean the cutting edges of the puncher. The puncher was rinsed in a fresh solution of absolute ethanol before it was used to punch out another dried blood spot. The tweezers used to pick the punched-out DBS were also rinsed in absolute ethanol as well. The entire process was repeated for every dried blood spot punched. Each 3mm punched-out DBS disc was placed in a 40µl TE buffer solution in a microtube and incubated overnight at 37ºC. The paper discs were then removed and discarded. The sample solution left was thereafter taken through the NIMR (National Institute of Medical Research) extraction kit to extract the DNA. The buffer solutions were prepared by adding 8mls of absolute ethanol to 32 ml of wash buffer 1 concentrate and 32 ml of absolute ethanol to 8 ml of wash buffer 2 concentrate. About 40 µl of the specimen was added into a microtube, and

250 µl of lysis buffer as well. The mixture was vortexed and then incubated at 56ºC for 10 minutes. This was then centrifuged at 10,000 revolutions per minute (rpm). 100 µl of absolute ethanol was added to the tube, transferred into a spin column, and then centrifuged again at 10,000 rpm for 30 seconds. The flow-through was discarded, and 250 µl of wash buffer 1 was added to the spin column and centrifuged at 10,000 rpm for 30 seconds. The flow-through was again discarded, and 250 µl of wash buffer 2 was added to the spin column and centrifuged at 10,000 rpm for 1 minute. The flow-through was discarded, and the spin column was centrifuged at 12,000 rpm for 3 minutes to ensure no trace of ethanol was left. The spin column was placed in another microcentrifuge tube, and a 50 µl elution buffer was added to the centre of the spin column. This was left to incubate at room temperature for two minutes and again centrifuged at 10,000 rpm for a minute to elute the DNA stored at -20ºC.

DNA amplification was achieved through polymerase chain reaction (PCR) using a TECHNE TC-312 ThermocyclerTM. About 5 µL of DNA was added to the reaction mix to make up a volume of 25 µL. The 696-base pair (bp) fragments of the *PfATPase6* gene were to be amplified with polymerase chain reaction using a TECHNE TC-312 Thermocycler^{TM}. The forward primer used is 7F (5'-AATCACCAAGGGGTATCAAC), while the reverse primer is 8R (5'-ACGTATACCAGCCATATGG). They target a 1771- 2467 bp region, corresponding to the S769N codon of the *PfATPase6* gene.

The process required pre-heating the lid of the Thermocycler to 105ºC. The reaction mix was then taken to 94ºC to achieve initial denaturation for 5 minutes. The samples then went through 35 cycles of 95ºC for a minute, 55ºC for a minute, and 72ºC for a minute. This was followed by a final extension of 72ºC for 6 minutes.

The products of the polymerase chain reaction were analysed for restriction fragment length polymorphism. The 1.5% agarose gel concentration was constituted and stained with 7 µl of ethidium bromide and placed in a Tris Acetate $EDTA_{Na2}$ (TAE) buffer bath on a SCIE-PLAS^{TM} electrophoresis machine. About 5µl of DNA ladder was placed in the first well, and 5µl of the products of PCR for the various samples were added to subsequent wells. The SCIE-PLAS^{TM} electrophoresis machine was run at 100 volts for 1 hour. The gel was then taken out and visualized with an ultraviolet trans-illuminator. A camera mounted on the ultraviolet trans-illuminator took pictures of each session. The presence of amplified *PfATPase6* genes with the S769N mutation in any of the sample wells is expected to be indicated as bands in the 696 bp region.

Data analysis

Data was entered into a backend designed to mirror the questionnaire on the Epi Info 7$^{(R)}$ statistical package. The same tool was used to analyse the data. Socio-demographic characteristics of respondents are presented with frequency tables and charts, and a summary of the collected data is expressed as measures of central tendency and dispersion. Bivariate analysis, such as chi-square, was used to establish an association between categorical variables. The level of significance was set at a p-value less than 0.05.

Ethical approval

Ethical approval to carry out this study on human subjects in Osun State was obtained from the Osun State Ministry of Health Ethics Committee, with reference number OSHREC/PRS/569T/121. Informed consent was also obtained from all participants after the purpose of the study had been explained to them well. Most participants had informed consent explained to them in their mother tongue.

Results

Socio-demographic

Between 36 and 40 respondents were drawn from each of the eight participating local governments and their representative health facilities (Table 1). Although the selection of respondents was made randomly based on their frequency of presentation, there were 175 females (55.6%) and 140 males (44.4%), and a female-to-male ratio of 1.3:1. In three of the local government areas, namely Ede South, Ifelodun, and Ilesa West, there was an equal distribution of 20 male and 20 female respondents in each local government. The mean

age for anyone to present with fever in the facilities is 16.7 years, 11.3 and 21.2 years for males and females, respectively, and the mean age to be confirmed to have malaria was 11.1%.

Table 1. Demographic characteristics of study participants

LGA	Ede North (N=40)	Ede South (N=40)	Ifelodun (N=40)	Ilesa West (N=40)	Olorunda (N=36)	Oriade (N=40)	Orolu (N=39)	Osogbo (N=40)	Total (N=315)
Gender									
Male	14 (35.00%)	20 (50.00%)	20 (50.00%)	20 (50.00%)	19 (52.78%)	14 (35.00%)	20 (51.28%)	13 (32.50%)	140 (44.44%)
Female	26 (65.00%)	20 (50.00%)	20 (50.00%)	20 (50.00%)	17 (47.22%)	26 (65.00%)	19 (48.72%)	27 (67.50%)	175 (55.56%)
Tribe									
Yoruba	38 (95.00%)	40 (100.00%)	40 (100.00%)	39 (97.50%)	34 (94.44%)	35 (87.50%)	39 (100.00%)	39 (97.50%)	304 (96.51%)
Igbo	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	1 (02.50%)	1 (00.32%)
Hausa	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	2 (05.66%)	2 (00.61%)	0 (00.00%)	0 (00.00%)	4 (01.27%)
OM	2 (05.00%)	0 (00.00%)	0 (00.00%)	1 (02.50%)	0 (00.00%)	3 (01.57%)	0 (00.00%)	0 (00.00%)	6 (01.90%)
Age (year)									
< 5	17 (42.50%)	12 (30.00%)	25 (62.50%)	15 (37.50%)	6 (16.67%)	14 (35.00%)	16 (41.03%)	15 (37.50%)	117 (37.14%)
5-15	2 (05.00%)	16 (40.00%)	4 (10.00%)	7 (17.50%)	18 (50.00%)	13 (32.50%)	7 (17.95%)	3 (07.50%)	76 (24.13%)
> 15	21 (52.50%)	12 (30.00%)	11 (27.50%)	18 (45.00%)	12 (33.33%)	13 (32.50%)	16 (41.03%)	22 (55.00%)	122 (38.73%)
Educational Status									
Primary	9 (22.50%)	22 (55.00%)	20 (50.00%)	15 (37.50%)	16 (44.44%)	22 (55.00%)	21 (53.85%)	6 (15.00%)	131 (41.59%)
Secondary	11 (27.50%)	9 (22.50%)	10 (25.00%)	13 (32.50%)	16 (44.44%)	9 (22.50%)	8 (20.51%)	22 (55.00%)	98 (31.11%)
Tertiary	14 (35.00%)	4 (10.00%)	3 (07.50%)	12 (30.00%)	3 (08.33%)	5 (12.50%)	6 (15.38%)	9 (22.50%)	56 (17.78%)
Vocational	1 (02.50%)	4 (10.00%)	1 (02.50%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	0 (00.00%)	6 (01.90%)
Nfe	5 (12.50%)	1 (02.50%)	6 (15.00%)	0 (00.00%)	1 (02.78%)	4 (10.00%)	4 (10.26%)	3 (07.50%)	24 (07.62%)

OM= Other Minorities; Nfe= No formal education

The proportion of the different tribes presenting at the facilities aptly describes the study location as 96.56% of the respondents were of the Yoruba ethnic extraction. The other minority ethnic groups were mainly Hausa and Igbo. Respondents who presented the most with fever were those with no more than primary school education (42%). The proportion grew smaller with increasing education, with secondary education contributing 31% (6% plus 25% for junior and senior secondary education, respectively) and 17% having tertiary education. Similarly, those who possess, at most, a primary school education had the highest proportion of confirmed malaria cases at 59.7% (55.5% had primary education and 4.2% had no formal education), followed by secondary education at 30.3%, and those who had tertiary education comprised 10.1% of positive cases. This picture mirrors the prevalence of malaria in education as reported by the Nigeria Demographic and Health Surveys for 2018 (Rosenthal *et al*., 2019). Out of the three hundred fifteen (315) respondents who presented with fever, two hundred and two hundred ninety-three (293) malaria RDT tests were validly conducted, with results documented in all the eight centres where the study took place. Of these, 166 (56.7%) were negative, and 127 (43.3%) were positive (Table 2). The mean age to test positive for malaria in Osun State is 11.12 years, and this is statistically significant ($p< 0.05$) (Table 3).

Going by the initial analysis (Table 2), the local government area with the highest positive results was Olorunda at 64.86%. However, after correcting for the last ten fever cases in Olorunda, which were recruited into the study only after they had been confirmed positive, the

proportion came down to 51.85%, which provides a more accurate representation of the malaria prevalence among fever cases in the area, reducing potential overestimation caused by selective recruitment. Therefore, the highest prevalence rate of malaria cases was recorded in Orolu Local government area, with a value of 57.50%. Osogbo local government area has the lowest malaria test positivity rate (29.41%). The total facility fever prevalence for malaria is 41.41%. Children aged four had the highest frequency of confirmed malaria cases. Twenty of them participated, and fifteen (75%) of them were positive.

Occurrence of *PfATPase6* gene mutation

None of the 127 samples that tested positive for *Plasmodium falciparum* was found to harbour the S769N mutation in the *PfATPase6* gene (Table 3, Occurrence of PfATPase 6 Gene mutation by Local Government Area).

Gel electrophoresis evaluation

Gel electrophoresis was conducted to analyze the presence of the *PfATPase6* gene mutation, specifically the S769N codon, in *P. falciparum* positive samples. The electrophoresis process involved running PCR-amplified DNA fragments through an agarose gel stained with ethidium bromide, followed by visualization under an ultraviolet transilluminator to detect DNA bands. Amplified fragments of 696 bp showed S769N SNP in the PfATPase 6 gene. However, none of the 127 *P. falciparum*-positive samples showed bands at the expected 696 bp position with restriction site changes, indicating the absence of *PfATPase6* mutations in all tested samples. The clear bands in the control lanes confirm successful DNA amplification and electrophoresis accuracy, validating the findings. These results strongly suggest that *PfATPase6* mutations associated with ACT resistance are currently not present in Osun State. This reinforces the continued efficacy of artemisinin-based combination therapies (ACTs) in treating malaria within the region.

Discussion

Malaria remains a significant public health concern in Nigeria (Dawaki *et al*., 2016), with Osun State exhibiting a state-level prevalence of 27.7%, according to the NDHS 2018. While population-based studies such as the NDHS provide valuable prevalence data, facility-based studies, like the one presented here, offer insights into the proportion of fever cases confirmed as malaria. As expected, the hospital-based malaria prevalence in this study was higher than population estimates, with 41.41% of fever cases testing positive for malaria. This aligns with the general understanding that individuals presenting with fever are more likely to test positive for malaria compared to the general population. Notably, the highest prevalence was observed in rural Local Government Areas (LGAs), consistent with previous findings that rural dwellers have higher malaria prevalence rates due to increased exposure to malaria vectors and limited access to healthcare (Rosenthal *et al*., 2019). Comparative studies in Nigeria have shown varied prevalence rates, with Celina *et al*. (2017) reporting a 32.1% prevalence in Benue State and Nwaneli et al. observing a 23.3% prevalence in Anambra State, both below 50% (Aju-Ameh *et al*., 2017; Nwaneli *et al*., 2020). Conversely, Jeremiah *et al*. (2019) reported a higher prevalence of 56.8% in Nasarawa State (Jemimah *et al*., 2019). These discrepancies likely reflect regional differences in malaria transmission intensity and healthcare access. Furthermore, this study found that only 15.0% of respondents underwent malaria testing before taking artemisinin-based combination therapy (ACT), echoing findings by Omale *et al*. (2021) in Ebonyi State, where 15.7% of caregivers requested malaria tests before treatment (Omale *et al*., 2021). Given the declining trend in malaria prevalence, as seen in national surveys (Nigeria and Health Surveys, 2018), the low rate of diagnostic testing before treatment suggests that many individuals taking ACTs may not have malaria. This highlights the need for greater public awareness and adherence to malaria testing guidelines before treatment to prevent unnecessary drug use and encourage the investigation of other causes of fever.

Age was also a significant factor in malaria prevalence in this study, with children under five years, particularly those aged four, showing the highest frequency of confirmed cases. This finding is consistent with previous research indicating that younger children, particularly

those who have lost maternal antibodies, are at higher risk of malaria (Rosenthal *et al*., 2019). However, contrary to our findings, Kevin *et al*. (2024) reported a reduced risk of febrile malaria in children older than three years in areas of moderate to high transmission (Kimenyi *et al*., 2024). These variations may be due to differences in local malaria transmission dynamics and the protective effects of acquired immunity, which are influenced by age and parasite exposure (James and Santos, 2023).

Table 2. Test positivity and negativity rates by Local Government

LGA	Percent Posit	Percent Negat
Orolu	57.50%	42.50%
Olorunda	51.85%	48.15%
Ede South	44.12%	55.88%
Ilesa West	43.24%	56.76%
Oriade	39.47%	60.53%
Ede Noth	33.33%	66.67%
Ifelodun	32.35%	67.65%
Osogbo	29.41%	70.59%
Total	41.41%	58.59%

LGA= Local government area; Percent Posit= Percentage Positivity; Percent Negat= Percentage Negativity.

Table 3. Occurrence of *PfATPase6* gene mutation by local government area

LGA	NPC	N. *PfATPase6*	P. *PfATPase6*
Ede Noth	13	0	0
Ede South	15	0	0
Ifelodun	11	0	0
Ilesa West	16	0	0
Olorunda	24	0	0
Oriade	15	0	0
Orolu	23	0	0
Osogbo	10	0	0
Total	127	0	0

LGA= Local government area; NPC= Number of positive cases; N. *PfATPase6*= Number containing *PfATPase6* gene mutation; P. *PfATPase6* = Prevalence of *PfATPase6* gene mutation.

Educational level was inversely correlated with malaria prevalence, with those having no more than primary education showing the highest prevalence, while the lowest prevalence was observed among individuals with tertiary education. This supports previous large-scale surveys in Nigeria, which consistently show that lower education levels are associated with higher malaria prevalence (Nigeria Demographic and Health Surveys, 2018). The likely explanation is that more educated individuals are better able to access and utilize preventive measures, such as insecticide-treated nets (ITNs). In this study, 48.3% of respondents reported ITN use, a figure similar to that found by Orji *et al*. (2018) in Ebonyi State (Ouji *et al*., 2018). However, barriers to ITN use remain, with 31% citing lack of access and 30% citing hot weather as reasons for non-use. These findings are consistent with those of Ezeigbo *et al*. (2016) and Ajegena and Oti (2020), who identified similar barriers to ITN utilization (Ezeigbo *et al*., 2016; Ajena and Oti, 2020). Regarding the assessment of *P. falciparum* samples for the *PfATPase6 gene mutation*, no mutations were detected, consistent with previous findings in Osun State (Nassar *et al*., 2016; Tola *et al*., 2020). This absence of mutations suggests that current artemisinin-based therapies remain effective in the region. Nevertheless, ongoing surveillance is essential to monitor the emergence of drug resistance, particularly with the advent of triple ACTs as a potential countermeasure against ACT resistance (Van Der Pluijm *et al*., 2020).

Conclusion

This study highlights the significant burden of malaria among febrile patients in Osun State, Nigeria, particularly among younger individuals and those with lower educational attainment. The findings reveal that while the prevalence of malaria remains high, there is currently no evidence of *PfATPase6* gene mutations associated with artemisinin-based combination therapy (ACT) resistance in the sampled population. This suggests that ACTs continue to be an effective treatment option for *P. falciparum* infections in the region. However, the study also underscores the ongoing risk of malaria transmission and the importance of maintaining vigilance in monitoring for potential drug resistance. The absence of *PfATPase6* gene mutations provides a positive outlook for current malaria treatment regimens but should not lead to complacency. Continuous monitoring and research are essential to ensure the effectiveness of ACTs is sustained over time.

Author's contributions

OO and **OS** Study concept and design; **OO** and **AO** Acquisition of data; **AR** and **OS** Data analysis and interpretation; **AJF**, **AF** and **MA** Drafting of the manuscript; **OO** and **OS** Critical

review of the manuscript for the important intellectual concept; **AJF**, **OS, MA** and **OO** Administrative, technical and material support.

Data availability

The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available for confidentiality and ethical reasons.

Funding

No funding was received during this project.

Conflicts of interest

The authors declare no conflict of interest.

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