Asian Journal of Research in Biochemistry

Volume 15, Issue 1, Page 23-35, 2025; Article no.AJRB.129518
ISSN: 2582-0516

Antimalarial Susceptibility Profile of *Plasmodium falciparum* Isolated from Human Population in Selected Health Facilities in Keffi Metropolis, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IIN and NYB designed the study. Author IIN performed the bench work. Authors NHI and YI wrote the first draft of the manuscript. Author TE ran the statistical analysis and author IT handled the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrb/2025/v15i1348

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here:
https://www.sdiarticle5.com/review-history/129518

Original Research Article

Received: 10/12/2024
Accepted: 15/01/2025
Published: 18/01/2025

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Cite as: *IN, Igbawua, Ngwai YB, Nkene IH, Yahaya I, Timshana E, and Ibrahim T. 2025. "Antimalarial Susceptibility Profile of Plasmodium Falciparum Isolated from Human Population in Selected Health Facilities in Keffi Metropolis, Nasarawa State, Nigeria". Asian Journal of Research in Biochemistry 15 (1):23-35. https://doi.org/10.9734/ajrb/2025/v15i1348.*

ABSTRACT

Aim: This study was aimed to determine the susceptibility profile of *Plasmodium falciparum* to antimalarial drugs to assess the efficacy of the ones in use.
Study Design: This is a hospital-based descriptive study design.
Place and Duration of Study: Department of Microbiology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, between December, 2023 and April, 2024.
Methodology: A total of 385 blood samples were collected and screened for malaria parasites by microscopy. Species-specific screening was done using Rapid Diagnostic Test (RDT). Stock/working solutions of selected antimalarial drugs were prepared using standard protocols. The parasite cultivation and its susceptibility to selected antimalarial drugs was determined using the WHO protocol. Matured schizonts were quantified. The degree of antimalarial drugs inhibition of scizonts maturation was determined and the drugs 50% inhibitory concentration (IC_{50}) required to prevent parasite schizont maturation indicating resistance was determined.
Results: Malaria prevalence by microscopy was 29.9% (95% CI = 25.34 to 34.71%) and 26.7% by RDT. The RDT had a sensitivity of 89.6% (95% CI = 82.48% to 94.49%), specificity of 100.00% (95% CI = 98.64% to 100.00%), Positive Predictive Value of 100.00% (95% CI = 96.48% to 100.00%); and Negative Predictive Value of 95.8% (95% CI = 92.94% to 97.46%). The accuracy of the RDT was 96.88% (95% CI = 94.62 to 98.38%). Six out of the 35 isolates showed 17.1% resistance to Artemether + Lumafantrine.
Conclusion: Malaria is present in the study population and is resistant to some of the antimalarial drugs in use. The need for periodic antimalarial drugs surveillance to determine the efficacy of drugs in use is highly recommended.

Keywords: Plasmodium falciparum; microscopy; antimalarial; resistance; nasarawa.

1. INTRODUCTION

Plasmodium falciparum is one of the 5 species of malaria parasite infecting humans. Malaria generally, is a vector-borne, obligate, intracellular parasitic disease caused by the protozoan parasite of the phylum Apocomplexa and the genus Plasmodium. It remains a major public health challenge in Sub-Saharan Africa Somé et al. (2018); World Health Organization (WHO, 2020). It is estimated to cause 241 million clinical episodes and 627,000 deaths with an estimated 94% of deaths occurring in the World Health Organization's (WHO's) African Region World Health Organization (WHO, 2021); Omoya and Ajayi (2020). Nigeria has the highest number of global malaria cases with 27 % of global malaria cases in 2019 with the highest number of global malaria deaths which stands at 23% Usman-Yamman et al. (2021). It is also reported that 50% of the Nigerian population suffers from at least one episode of malaria each year and this accounts for over 45% of all out-patient visits and 30% hospitalizations especially among children under 5 years of age Olasehinde et al. (2014); Anyanwu et al. (2017).

Of the 5 species of malaria parasites infecting humans, *Plasmodium falciparum* is the deadliest World Health Organization (WHO, 2021). This particular virulence is due to its ability to subvert the physiology of its host during the blood stages of its development Igbawua et al. (2024). Malaria treatment and control relies heavily on vector control, administration of antimalarial drugs and recently, the use of malaria vaccines World Health Organization (WHO, 2021). Despite the use of antimalarial drugs in the treatment of malaria there is emergence/spread of Artemisinin-resistance parasites and WHO recommends the use of Artemisinin-based combination therapies (ACTs) as the first-line drugs for the treatment of uncomplicated malaria Igbasi et al. (2019); Eboumbou et al. (2019); Bwire et al. (2020). The emergence of drug-resistant parasites over the last few decades has affected the epidemiology of malaria and options for its treatment and now there is evidence of decreased sensitivity to the Artemisinins Olukosi et al. (2014). The resistance posed by *Plasmodium* species to antimalarial drugs with its attendant consequences entails the need for periodic antimalarial susceptibility testing in order to detect the parasites resistance to antimalarial drugs. This study therefore aims to determine the Antimalarial Susceptibility Profile of *Plasmodium falciparum* Isolated from Human Population in Selected Health Facilities in Keffi Metropolis, Nasarawa State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This hospital-based cross sectional study design was conducted in Keffi, the headquarters of Nasarawa West Senatorial District. Geograpgically, Keffi is located in Nasarawa state which lies between latitudes 8˚51 and 8˚53 North of the equator and longitudes 7˚50' and 7˚51' East of the Greenwich meridian. It is about 128 km away from Lafia, the State capital and 57 km away from Abuja, the Federal Capital Territory of Nigeria. Keffi is the smallest Local Government Area in Nasarawa State, with a total land area of approximately 140 km^2 Akwa et al. (2007); Makut et al. (2022).

2.2 Sample Size Determination

The sample size was calculated with a 95% Confidence Interval (CI) and precision level of 5% using the standard sample size calculation formula as described by Naing et al. (2006).

2.3 Subject Selection (eligibility criteria)

2.3.1 Inclusion criteria

Patients who had clinical symptoms of malaria determined by headache, body aches and febrile condition of 37.5°C and above and have not taken antimalarial drug in the preceding week prior to visitation to the health facility were recruited in the study. Similarly, only consenting Patients residing within the senatorial district and attending the selected health facilities that were enrolled in the study.

2.3.2 Exclusion criteria

Patients residing outside the senatorial district, without clinical symptoms of malaria and have taken antimalarial drug were exempted from the study.

2.4 Sample Collection

A total of 385 blood samples were collected by venipuncture from the study participants at the collection sites namely: South Atlantic Medical Centre, Nasarawa State University, Keffi and Nagari Hospital, Keffi using standard protocol. The collected blood samples were let into labeled EDTA containers and immediately conveyed to Microbiology Laboratory, Nasarawa State University, Keffi for laboratory analysis.

2.5 Laboratory Analysis

2.5.1 Microscopy (the gold standard)

Thin and thick blood films were made on well labeled clean grease free slides, air dried and stained for 10 min using a 1 in 10 dilution of freshly prepared Giemsa stain in buffer water of pH 7.2. The stained dry films were microscopically read by two independent Microscopists and discrepancies (if any) were resolved by a third reader.

2.5.2 Rapid diagnostic test

Whole blood collected from the participants were also tested for malaria parasites using specie-specific random diagnostic test (RDT) (SD bioline®) which test specifically for *Plasmodium falciparum* following manufacturer's instructions.

2.5.3 Determination of RDT diagnostic performance indices

Since it is *P. Falciparum* (the most prevalent specie in the study area) whose susceptibility to antimalarial drugs is to be tested, the need to test the diagnostic performance indices of the RDT for specie specific results became necessary. The diagnostic indices namely: sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the RDT were determined using standard protocol as described by Shreffler and Huecker (2023). Details of these calculations are presented in appendix B.

2.5.4 Performance of the *In vitro* micro-test (Mark III test)

Cultivation and eventual susceptibility testing of Plasmodium parasites to antimalarial drugs was performed on blood samples with mono infection whose level of parasitaemia following parasite density determination was within the acceptable limit of 1000 – 80,000 parasites/µl of blood as described by World Health Organization (WHO, 2001) using the following steps.

2.5.4.1 Preparation of stock/working solutions and dosing of microtitre culture plates

The stock and working solutions of Chloroquine, Artesunate and a combination of Artesunate plus Amodiaquine and Artemether plus Lumefanrine were prepared as earlier described by Ikpa et al. (2009).

Table 1. Illustration of dosed micro-titre culture plate with Chloroquine

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0 µmol
B	0.2											
C	0.4											
D	0.8											
E	1.6											
F	3.2	0	0	0	0	0	0	0	0	0	0	0

Key: well A is the control (drug free well). wells B - F represent Chloroquine concentrations of 0.2; 0.4; 0.8; 1.6 and 3.2 µmol per l blood sample. the concentrations are expressed in µmol/l blood as the malaria parasite shows selective uptake of Chloroquine. The peak plasma level is at 0.8 µmol.

2.5.4.2 Cultivation of parasites and drug susceptibility test in microtiter plates

The parasite cultivation and drug susceptibility of *Plasmodium falciparum* was determined using the WHO standard in-vitro microtest (Mark III) system World Health Organization (WHO, 2001). The deposits of the test wells were harvested and used in making thick films on labeled microscope slides. The well air-dried films were stained with Giemsa solution at a dilution of 1% in buffered water of pH 7.2 for 30 minutes World Health Organization (WHO, 2001). Table 1 illustrates the dosing of the micro-titre plate with Chloroquine. The dosing of the Micro-titre plate with chloroquine is illustrated in Table 1.

2.5.4.3 Examination of the post culture blood slides

The post culture-stained thick blood films were examined microscopically using the 100x objective and the number of schizonts with three or more nuclei out of 200 asexual parasites were counted using digital microscope and compared with the control. The schizont counts were expressed as a percentage of the control as described by Peletiri et al. (2012). The percentage inhibition was then calculated as earlier described by World Health Organization (WHO, 2001). The individual percentage schizont maturation inhibitions were converted to probit, while the various drug concentrations used were converted to log concentration. This was done by feeding the values directly into Microsoft excel. using linear regression lines, their inhibitory concentrations (IC_{50}), was determined. Drug resistant *P. falciparum* parasites were then identified as parasites with IC_{50} values greater than the peak plasma concentration of the tested anti-malaria drugs.

2.6 Statistical Analysis

Data obtained was entered in Microsoft Excel and analyzed to obtain frequency of occurrence and inhibitory concentrations (IC_{50}) which is the concentration at which 50% of parasite growth was inhibited at 95% confidence intervals and 5% precision was determined using linear Regression. Using microscopy as the gold standard, the performance of the Rapid Diagnostic Test (RDT) was compared to it using MedCalc version 22.009 statistical package (MedCalc Software Ltd. Diagnostic test evaluation calculator). Values were considered significant at 95% confidence interval (P= .05).

3. RESULTS AND DISCUSSION

3.1 Prevalence of Malaria

This study examined the *Plasmodium falciparum* susceptibility to selected antimalarial drugs. A total of 385 blood samples were collected during the period of this study. Microscopy detected 115 sample positivity (29.9%) RDT detected 103 (26.7%) positive samples. This result gives a performance rate of 89.6% (103/115) sensitivity of the RDT test method. The prevalence of malaria parasite in the study area is presented in Table 2.

This observed mean prevalence of 29.9% is similar to a study conducted by Metoh et al. (2020) in Cameroon who had (29%) and Igbawua et al. (2024) in Nigeria. It is also closely related to the WHO African regions' current malaria prevalence report of 26.6% World Health Organization (WHO, 2022). The observed prevalence is however lower than reports from other parts of Nigeria namely: 53% in Asaba Ogunfowokan et al. (2020); 64.9% in Kano Oladele et al. (2018) *and 82.7% in* Ondo Omoya and Ajayi (2020). This mean prevalence obtained in this study emphasizes the fact that malaria still remains endemic in the study area and is a public health concern considering all known intervention programs aimed at halting malaria parasite transmission.

Table 2. Prevalence of malaria parasite by Microscopy and RDT

Facility	Microscopy		Prevalence (%)	RDT		Prevalence (%)
	Number collected	Number positive		Number collected	Number positive	
Nagari Hospital, Keffi	155	46	29.7	155	41	26.5
SAPETR, NSUK	230	69	30.0	230	62	26.9
Total	**385**	**115**	**59.7/2 = 29.9**	**385**	**103**	**53.4/2 = 26.7**

3.2 Determination of RDT Diagnostic Performance Indices

The sensitivity, specificity and accuracy of the RDT was determined using standard protocols Shreffler and Huecker (2023). Results shows that the sensitivity of RDT was 89.6% (95% CI = 82.48% to 94.49%) while its specificity was 100.00% (95% CI = 98.64% to 100.00%). The Positive Predictive Value (PPV) was 100.00% (95% CI = 96.48% to 100.00%) while the Negative Predictive Value (NPV) was 95.8% (95% CI = 92.94% to 97.46%). The mean prevalence of malaria using microscopy in this study population was 29.9% (95% CI = 25.34 to 34.71%). The false negative rate was 74.4%. The accuracy of this diagnostic tool was 96.88% (95% CI = 94.62 to 98.38%) (Appendix B) meaning it was fit as a diagnostic tool for this research.

The sensitivity, specificity and accuracy of the RDT obtained in this study shows that the RDT is strongly reliable as an alternative test method of malaria parasite particularly in areas with no electricity, where an experienced Microscopist is not available and where time is of essence in emergency situations. This high rate of specificity and sensitivity of RDT is consistent with literature of other researchers Micheal and Nduka (2016); Mac et al. (2019); Ogunfowokan et al. (2020); Afriyie et al. (2023); Olasehinde et al. (2014).

3.3 Evaluation of *In vitro* Susceptibility of *P. Falciparum* Isolates to Antimalarial Drugs and Determination of the Degree of Inhibition

Out of the 103 *P. falciparum* RDT positive samples, only 35 of the samples were evaluated for antimalarial drugs susceptibility. Some samples were discarded due to failure of schizonts to mature satisfactorily, while others were lost due to poor staining. Results of the schizont maturation counts in Chloroquine and Artesunate (as mono-therapy), then Artesunate + Amodiaquine and Artemether + Lumefantrine as ACTs were determined. Results of the number of schizonts counted per slide per well in all the drugs tested relative to the control well was recorded. The Schizont maturation counts obtained were used in determining the degree of Antimalarial drugs inhibition of Schizont maturation in percentages (%) at different antimalarial concentrations (μM).

The results of the Degree of Antimalarial drugs inhibition of Schizont maturation in percentages (%) against schizont maturation at different antimalarial concentrations (μM) was also recorded. The *in-vitro* response of malaria parasites to various drug concentrations are expressed as the IC_{50} value. This is the concentration of an antimalarial drug that inhibits 50% of schizont maturation as compared with the development in drug-free control wells Peletiri et al. (2012). The drug-resistant *P. falciparum* parasites were identified as isolates with IC_{50} values greater than threshold values for sensitive parasite isolates (Tables 3 - 6). A summary of the threshold values compared were Chloroquine: IC_{50} > 0.8 μM; Artesunate: IC_{50} > 28.6 μM, Artesunate + Amodiaquine: IC_{50} > 5.4 μM and Artemeter + Lumefantrine: IC_{50} > 13.4 μM. The in-vitro susceptibility results are presented (Table 6). From the results, only 6 isolates (17.1%) had values higher than the compared peak plasma level of Artermether (PPL =13.4), an Artemisinin derivative and therefore are resistant. The remaining antimalarial agents had 100% susceptibility.

Table 3. Inhibitory concentration (ic) in micromoles (µm) of chloroquine (ppl = 0.8) against schizont maturation

Isolate	IC_{50}	Remarks*	R^2
1	0.448221	S	0.921151
2	0.403557	S	0.865399
3	0.410732	S	0.947305
4	0.338151	S	0.876581
5	0.343186	S	0.859845
6	0.411174	S	0.957254
7	0.370455	S	0.914332
8	0.360963	S	0.924658
9	0.382063	S	0.929665
10	0.308984	S	0.904816
11	0.399505	S	0.90634
12	0.36535	S	0.950525
13	0.315584	S	0.914843
14	0.331142	S	0.920306
15	0.231309	S	0.934689
16	0.291329	S	0.921563
17	0.377219	S	0.966994
18	0.322195	S	0.898598
19	0.285378	S	0.929886
20	0.327978	S	0.931626
21	0.338698	S	0.928133
22	0.407253	S	0.831309
23	0.670915	S	0.973282
24	0.333329	S	0.894209
25	0.45439	S	0.939497
26	0.49071	S	0.889998
27	0.388292	S	0.923162
28	0.348312	S	0.914716
29	0.42856	S	0.977308
30	0.321035	S	0.891774
31	0.486962	S	0.976902
32	0.399259	S	0.908232
33	0.39755	S	0.917531
34	0.423394	S	0.986692
35	0.430042	S	0.976367

*S = Sensitive; R^2 = Regression constant; Peak Plasma Level = 0.8 *The remarks is based on comparison of IC_{50} with peak plasma level of the test drug*

Table 4. Inhibitory concentration (IC) in micromoles (µM) of Artesunate (PPL = 28.6) against schizont maturation

Isolate	IC_{50}	Remarks*	R^2
1	16.8832	S	0.984522
2	15.9056	S	0.898268
3	13.98326	S	0.937982
4	16.26453	S	0.981835
5	13.65754	S	0.946283
6	13.58368	S	0.922245
7	12.22406	S	0.936933
8	15.65312	S	0.980324
9	13.3076	S	0.916627
10	13.52528	S	0.935115
11	13.87395	S	0.901688

Isolate	IC_{50}	Remarks*	R^2
12	12.28914	S	0.920207
13	13.01908	S	0.909576
14	14.53565	S	0.92062
15	13.41887	S	0.899564
16	12.87836	S	0.923775
17	13.40372	S	0.926663
18	14.4696	S	0.892208
19	15.38027	S	0.971749
20	14.33525	S	0.909532
21	53.1706	S	0.990983
22	11.55901	S	0.980328
23	13.7433	S	0.963577
24	10.87019	S	0.876835
25	15.39003	S	0.944705
26	9.86941	S	0.942665
27	16.00334	S	0.964228
28	18.28928	S	0.856908
29	12.90444	S	0.924658
30	10.99708	S	0.919732
31	14.2787	S	0.961162
32	12.75367	S	0.916972
33	13.50535	S	0.894876
34	16.82945	S	0.903506
35	13.11524	S	0.925562

*S = Sensitive; R^2 = Regression constant; Peak Plasma Level = 28.6 *The remarks is based on comparison of IC_{50} with peak plasma level of the test drug*

Table 5. Inhibitory concentration (IC) in micromoles (µM) of Artesunate + Amodiaquine (PPL = 5.4) against schizont maturation

Isolate	IC_{50}	Remarks*	R^2
1	2.721715	S	0.976468
2	2.669114	S	0.886012
3	2.424742	S	0.932864
4	2.472819	S	0.975827
5	2.617441	S	0.946089
6	2.335194	S	0.91584
7	1.965862	S	0.934152
8	2.75843	S	0.977285
9	2.267524	S	0.908006
10	2.355269	S	0.931714
11	2.451442	S	0.897659
12	2.22641	S	0.867787
13	2.264102	S	0.906496
14	2.525778	S	0.916026
15	2.574813	S	0.901851
16	2.130628	S	0.917975
17	2.595759	S	0.927365
18	2.698614	S	0.894182
19	3.136195	S	0.914722
20	2.67671	S	0.885267
21	2.895354	S	0.882319
22	2.759943	S	0.857164
23	2.626389	S	0.838602
24	2.701199	S	0.87689
25	2.846487	S	0.909549

Isolate	IC_{50}	Remarks*	R^2
26	2.471566	S	0.8503
27	2.617588	S	0.889931
28	2.825038	S	0.882251
29	2.362436	S	0.78338
30	2.556836	S	0.876435
31	2.54794	S	0.888508
32	2.553378	S	0.903695
33	2.291745	S	0.884869
34	2.08503	S	0.885096
35	2.546427	S	0.848776

*S = Sensitive; R^2 = Regression constant; Peak Plasma Level = 28.6. *The remarks is based on comparison of IC_{50} with peak plasma level of the test drug*

Table 6. Inhibitory concentration (IC) in micromoles (µM) of Artemether plus Lumefantrine (PPL = 13.4) against schizont maturation

Isolate	IC_{50}	Remarks*	R^2
1	5.343815	S	0.967485
2	7.34989	S	0.895837
3	6.439249	S	0.93801
4	5.776725	S	0.972794
5	6.398987	S	0.946283
6	6.001331	S	0.918683
7	4.162396	S	0.929576
8	18.92401	R	0.9836
9	3.876319	S	0.88074
10	5.206609	S	0.9268
11	5.154025	S	0.88869
12	15.46259	R	0.9618
13	4.61932	S	0.89526
14	5.307944	S	0.903753
15	4.978081	S	0.88822
16	3.976024	S	0.907145
17	5.28862	S	0.920274
18	5.417809	S	0.878258
19	4.744729	S	0.857728
20	15.82783	R	0.9630
21	6.942021	S	0.900459
22	15.47544	R	0.9872
23	7.454756	S	0.957972
24	6.407866	S	0.940827
25	5.614074	S	0.998137
26	5.334651	S	0.929233
27	6.515576	S	0.954357
28	16.32995	R	0.9822
29	6.005842	S	0.895429
30	7.266943	S	0.992582
31	5.71134	S	0.928737
32	15.380554	R	0.9812
33	6.285105	S	0.92894
34	5.419045	S	0.930512
35	5.110199	S	0.926922

*S = Sensitive; R = Resistant; R^2 = Regression constant; Peak Plasma Level = 13.4. *The remarks is based on comparison of IC_{50} with peak plasma level of the test drug*

Table 7. *In vitro* resistance profile of *Plasmodium falciparum* isolates to selected antimalarial drugs

Antimalarial drugs	No cultured	No susceptible n (%)	No resistant n (%)
Chloroquine	35	35(100)	0(0)
Artesunante	35	35(100)	0(0)
Artesunate + Amodiaquine	35	35(100)	0(0)
Artemeter + Lumefantrine	35	29(82.9)	6(17.1)

The in-vitro susceptibility of *P. falciparum* isolates to antimalarial drugs in this study revealed that of the 4 studied antimalarial drugs tested, 6 out of the 35 isolates (17.1%) had an IC_{50} that exceeded the peak plasma level (PPL) concentration of 13.4 μM for Artemether plus Lumefantrine (Coartem). The PPL concentration of a drug in the bloodstream higher than the expected or desired therapeutic range could pose the risk of toxicity as the body may not be able to handle the excess drug and continuous use may cause potential side effects. The 6 isolates were therefore considered resistant to Arthemeter + Lumefantrine. This could be as a result of continuous prescription of the drug posing the challenge of drug pressure on the study population which lead to the resistance observed.

A summary of the in-vitro resistance profile of *Plasmodium falciparum* isolates to selected antimalarial drugs in this study is presented in Table 7.

It has been documented that the removal of drug pressure exposes resistant parasites to increased competition leading to a decline in the frequency of resistance conferring mutants (Shafik et al., 2020). Chloroquine, whose usage was discontinued as a result of mutation and subsequent resistance was seen to be susceptible in this research. This probably may be as a result of reduction in its use resulting to clearance of the resistance genes (*P.fcrt*, *P. fmdr-1*). This result of the return of Chloroquine-susceptible *Plasmodium falciparum* parasites is reported in other studies Mohammed et al. (2013); Mekonnen et al. (2014). This result is also consistent with World Health Organization (WHO, 2001) who reported 100% susceptibility of *Plasmodium falciparum* isolates to Artesunate, an Artemisinin derivative. This research result however differs from reports of other researchers who reported a high percentage of resistance in the use of antimalarial drugs such as Artesunate, Chloroquine and Artesunate + Amodiaquine Mekonnen et al. (2014); Olukosi et al. (2014); Jimoh (2016).

4. CONCLUSION

The prevalence of malaria obtained in this study indicates that malaria is still endemic in Keffi metropolis. The performance of the RDT kit was superb and this makes it a valuable tool where facilities for microscopy are not available and immediate time for diagnosis is of essence. The endemicity of malaria in the study population could be as a result of failure to adhere to preventive/control measures. It could also be as a result of antimalarial drug abuse and misuse whose consequence is drug resistance hence the persistence of the parasite in the study area. I therefore recommend adherence to preventive/control measures as well as correct use of antimalarial drugs only when prescribed by Professionals. The need to also explore the reintroduction of Chloroquine in the treatment of malaria may also be considered since it it cheap, effective, readily available and a quick parasite clearance antimalarial drug. Considering the fact that overuse of the ACTs (a WHO recommended 1st line drug for malaria treatment) may exert drug pressure which may lead to resistance, the need for periodic antimalarial drugs surveillance for introduction of new drugs and to determine the efficacy of other antimalarial drugs in use is therefore highly recommended.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The ethical approval was obtained from the Research and Ethical Committee of the Health Services Department, Nasarawa State University, Keffi, Nigeria (NSU/NAS/MSc/MEM/019/15/16).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

We thank God Almighty for the protection and all the Staff in the Microbiology Laboratory, Nasarawa State University, Keffi for the cooperation during the bench work. No funding was received from any donor.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX A

Calculations for the accuracy of RDT as a diagnostic method:

The Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, False Positive rate, False Negative rate and Accuracy/efficiency of the RDT test method was calculated as follows:

$Sensitivity \ = \frac{TP}{TP + FN} x\ 100 = \frac{103}{103 + 12} x\ 100 = \frac{103}{115} x\ 100$ Sensitivity therefore = 89.6%

$Specificity \ = \frac{TN}{TN + FP} x\ 100 = \frac{270}{270 + 0} x\ 100 = \frac{270}{270} x\ 100$ Specificity therefore = 100%

$Positive\ Predictive\ Value \ = \frac{TP}{TP + FP} x\ 100 = \frac{103}{103 + 0} x\ 100 = \frac{103}{103} x\ 100$Positive Predictive Value therefore = 100%

$Negative\ Predictive\ Value \ = \frac{TN}{TN + FN} x\ 100 = \frac{270}{270 + 12} x\ 100 = \frac{270}{282} x\ 100$Negative Predictive Value therefore = 95.8%

$False\ Positive\ rate \ = \frac{FP}{FP + TN} x\ 100 = \frac{0}{0 + 270} x\ 100 = \frac{0}{270} x\ 100$ False Positive rate therefore = 0.0%

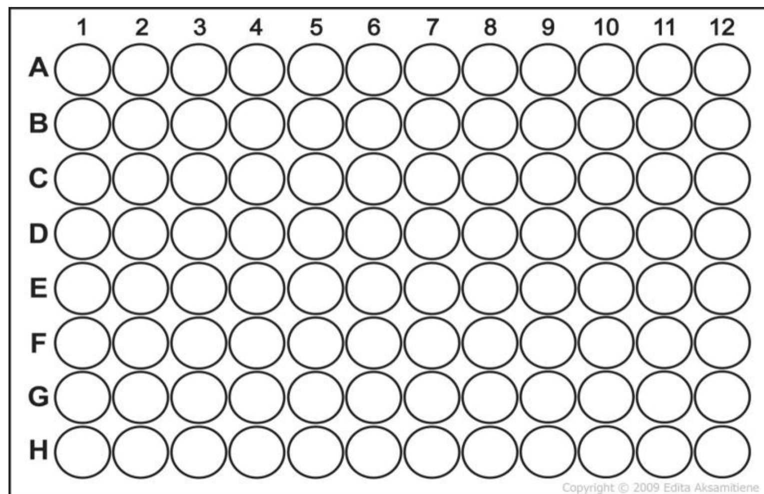
$False\ Negative\ rate \ = \frac{FN}{FN + TP} x\ 100 = \frac{12}{12 + 103} x\ 100 = \frac{12}{115} x\ 100$ False Negative rate therefore = 10.4%

$$\frac{Accuracy}{efficiency} of\ the\ test \ = \frac{TP\ +\ TN}{TP\ +\ FN\ +\ FP\ +\ TN} X\ 100 = \frac{103\ +\ 270}{103\ +\ 12\ +\ 0\ +\ 270} X\ 100 = \frac{373}{385} X\ 100 = \mathbf{96.9}\%$$

$$While\ the\ Prevalence\ of\ malaria\ (using\ microscopy) = \frac{TP\ +\ FN}{TP\ +\ FP\ +\ FN\ +\ TN} X\ 100 = \frac{103\ +\ 12}{103\ +\ 0\ +\ 12\ +\ 270} X\ 100 = \frac{115}{385} X\ 100 = \mathbf{29.9}\%$$

APPENDIX B

Layout of a 96 well Microtitre culture plate:



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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/129518