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POOR PERFORMANCE OF A POPULAR MALARIA RAPID DIAGNOSTIC KIT COMPARED TO MICROSCOPY IN ADULTS *P. FALCIPARUM* INFECTIONS IN NIGERIA

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ABSTRACT

Rapid diagnostic tests (RDTs) have become the mainstay of malaria diagnosis, especially in endemic rural settings where microscopy is difficult to perform. However, the performance of malaria rapid diagnostic tests is infrequently accessed in adults in Nigeria. We therefore accessed the performance of CareStart™ malaria RDT in the routine detection of *Plasmodium* in adults. A total of 134 participants were tested for malaria infection using both microscopy and CareStart™ Malaria Pf (HRP2) Ag RDT (ACCESSBIO, USA) in Owo, Ondo State, Nigeria. The prevalence of malaria was higher based on microscopy (20.1%) compared to RDT (3.7%), with a mean parasite density of 2954.1 parasites per microliter of blood. The sensitivity and specificity of the RDT were 3.70% and 96.26%, respectively, while the positive predictive value and negative predictive value were 20.00% and 79.84%, respectively. Although RDT sensitivity varied significantly with parasite density, it failed to detect malaria infection with high parasitaemia. Our observation suggests possible reduced diagnostic sensitivity of RDT for the diagnosis of *P. falciparum* malaria in adults and the need to support the interpretation of RDT results with microscopy.

Keywords: Malaria, RDTs, Microscopy, Adults, Parasite density, Nigeria

INTRODUCTION

Malaria, caused by *Plasmodium* species of parasites, is an endemic disease in Nigeria. In 2022, Nigeria accounted for 26.8% of the global 608 000 malaria deaths (WHO, 2023; USAID, 2024). Infants, children below 5 years old, and pregnant women are at higher risk of severe infection. Of the five *Plasmodium* parasite species, *P. falciparum, P. vivax, P. malariae, P. ovale*, and *P. knowlesi*, that cause human malaria infections, *P. falciparum* is the deadliest species, posing the greatest threat (Zekar & Sharman, 2023).

With Nigeria bearing 38.4% of global malaria deaths in children, children below five years of age and pregnant women are the primary targets of prevention and control strategies. However, recent data showed that the prevalence of malaria may be higher than described in adults (Bouyou-Akotet et al., 2014). Compared to the higher-risk groups, reports on malaria management among non-pregnant adults are fewer in Nigeria. This is possibly due to the reduced risk of life-threatening disease in this group, as the risk of malaria decreases with age in people residing in malaria-endemic areas because of sequentially acquired immunity (Bouyou-Akotet et al., 2014; Doolan et al., 2009; Färnert et al., 2015).

Prompt diagnosis and treatment of malaria have been shown to reduce disease burden and prevent deaths. Therefore, the WHO has recommended that all alleged cases of malaria infection should be validated through either blood microscopy or rapid diagnostic testing (WHO, 2023). Microscopy has remained the gold standard for malaria testing and diagnosis. However, it has limitations, as it requires trained personnel, and the reliability of the result depends on the skill of the personnel. Also, microscopy often takes time, depending on the locality, which may result in a treatment delay. The detection limit is also a concern, as asymptomatic individuals with lower levels of parasitaemia may remain undiagnosed (WHO, 2024). On the other hand, the use of RDT testing has significantly increased across sub-Saharan Africa. Rapid diagnostic tests are designed to identify parasite antigens using an immunochromatographic strip where the blood is placed on one end and the results are analyzed by bands on the strip surface (Wilson, 2012). The majority of the commercially available RDTs target the *Plasmodium* histidine-rich protein (HRP) 2 (pHRP-2) antigen. Although RDTs provide a lot more advantages than microscopy in terms of cost, ease of usage, and field deployment in low-resource and hard-to-reach settings, false negatives are getting more frequent due to changes in the parasites involving the deletion of the pHRP-2 gene and the prozone phenomenon in patients with high parasitaemia (Wilson, 2012; Mbanefo & Kumar, 2020; Gillet et al., 2011). The changing epidemiology of the parasite poses a problem for the use of RDTs. Additionally, it can lead to false positives because the pHRP-2 antigen can persist in the bloodstream up to 30 days post-treatment and elimination of an active infection. The variation in performance of different RDT brands reduces the reliability of the method (Wilson, 2012). In Nigeria, poor storage conditions pose a threat to the authenticity of medical products, including malaria prevention tools and drugs. The quality and efficacy of interventions are frequently compromised by these unfavorable storage conditions, which impedes the advancement of malaria control initiatives (NMEP, 2021).

Undetected infections in adults can contribute to the persistence of malaria transmission (Okell et al., 2012, Manjurano et al., 2011). In Nigeria, most epidemiological studies have focused on children and pregnant women (Obebe et al., 2020), despite the fact that severe clinical malaria and malarial-related deaths have been reported in adults living in endemic areas (Bouyou-Akotet et al., 2014). Most research on the performance of RDTs has focused on diagnosing malaria infections in children or pregnant women; few studies have examined the performance of RDTs in adults in Nigeria. Therefore, the present study was designed to determine the prevalence of malaria in adults and compare the diagnostic performance of a commercially available RDT kit in Nigeria with microscopy.

MATERIALS AND METHODS

Study Area

Owo, a local government area in the Northern Senatorial District of Ondo State (7° 11' 46" N and 5° 35' 12" E), has a

population of 222,262 people based on the 2006 population census. It is about 150 m above sea level, with an average temperature varying from 18 °C to 31 °C and rainfall over 1500 mm annually (Adewunmi et al., 2018). The town is a center of trade for many agricultural products such as cocoa, yams, cassava, maize, rice, cotton, and teak (Fig. 1).

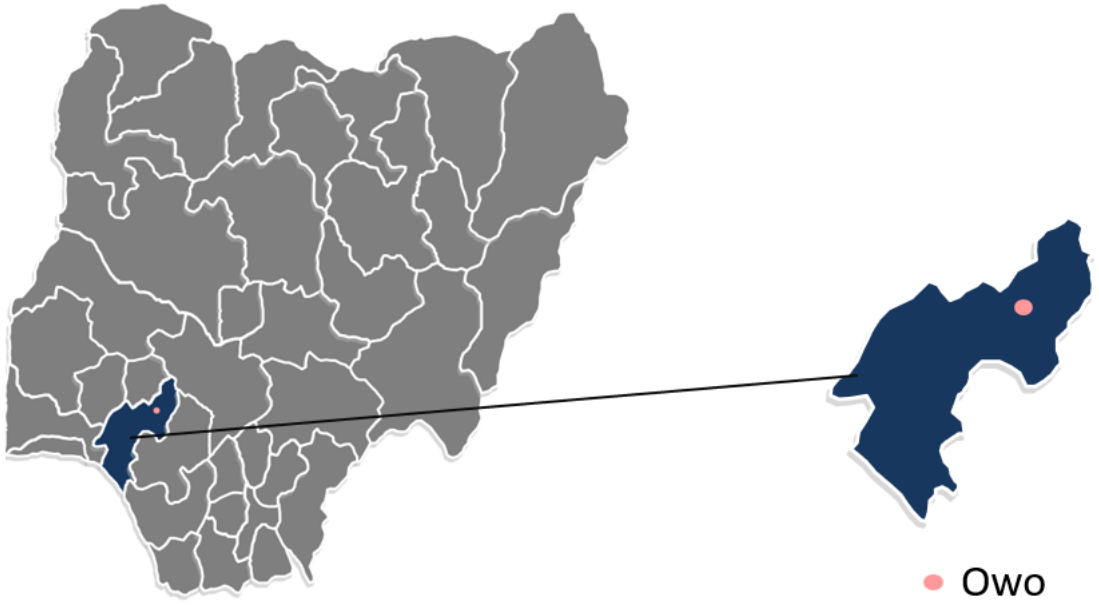

Figure 1: Map of Nigeria showing Owo in Ondo state.

Design and setting of the study

The protocol for the study was approved by the ethical review committee of Adekunle Ajasin University, Akungba Akoko (REF: AAUA/FSC/AEB/19/03/2021). Informed consent was obtained from individual volunteers after explaining the purpose, risks, and benefits of the study. The sample size was calculated using the 90% specificity of Care Start malaria RDT (Abdulkadir et al., 2015; Adebisi et al., 2018) and 0.05 error margin based on the recommendations by Hajian-Tilaki (2014) for determining the sample size in a single diagnostic test. Blood samples were collected from self-recruited voluntary participants for a malaria infection test by microscopy, and demographic characteristics were obtained through the use of questionnaires. Infected participants were notified and advised to obtain Antimalaria treatment at the nearest health centre.

Detection of malaria infection

For the detection of malaria parasites by microscopy, thin and thick blood films were prepared from venous blood, stained with Giemsa stain, and examined under the microscope at 40x and 100x by two experienced medical laboratory scientists according to the WHO malaria microscopy standard operating procedure (WHO, 2016). *Plasmodium* parasites were counted per 200 white blood cells, which were used to estimate the parasite density per microliter of blood. Thin films were examined to confirm the malaria species identified on the thick film. CareStart™ Malaria pf (HRP2) Ag RDT (ACCESS BIO, USA, LOT Mo20B68, Year of manufacture 2020) was used for detecting malaria infection in situ according to WHO guidelines.

Data Analysis

The collected data was analyzed in Microsoft Excel (version 2019). Sensitivity, specificity, and positive and negative predictive values were calculated for *P. falciparum* at a 95% CI in the Excel software.

RESULTS AND DISCUSSION

A total of 134 adults, comprising 126 males and 8 females, were tested for malaria infection by both microscopy and RDT. The mean age of the participants was 34.3 ± 11.1. The prevalence of malaria by microscopy and RDT was 20.1% and 3.7%, respectively. *P. falciparum* species was the only species identified in infected individuals (Table 1).

Table 1: Baseline characteristics of study population

Parameters	
	Mean (SD)
Age	34.3 (11.1)
Parasite Density	2954.1 (1656.4)
Gender	**Frequency**
Male	126
Female	8
Malaria Infection	**Prevalence**
Microscopy	20.1%
RDT	3.7%

A higher prevalence was observed with microscopy (20.1%) compared to RDT (3.7%). Compared to previous studies in Ondo State, the 20.1% prevalence of malaria infection based on microscopy was lower (Omoya & Ajayi, 2020; Simon-Oke et al., 2023). The lower infection rate could be due to the dry season of the year during which the study was conducted. The incidence of malaria varies with the seasons (Reiner et al, 2015), which is typical of many infectious diseases. Climatic factors such as temperature and rainfall influence malaria vector abundance and host behaviour (Amare et al., 2022). Based on previous reports, malaria incidence increases during the wet rainy season due to the presence of mosquito breeding grounds and the rise in mosquito populations (Simon-Oke et al., 2023).

The sensitivity and specificity of RDT were 3.70% and 96.26%, respectively. Malaria RDT detected 3.7% out of the 20.1% that were truly positive for malaria and 79.8% out of the 79.9% that were truly negative. The CareStart™ malaria RDT recorded 26 false negatives (positive predictive value, 20.00%) and only four false positives (negative predictive value, 79.84%) with a diagnostic accuracy of 77.61% (Tables 2 and 3).

Table 2: Comparison of malaria test results for Microscopy and RDT

	Microscopy n (%)		Total
	Positive	**Negative**	
RDT			
Positive	1 (20.0)	4 (80.0)	5 (100)
Negative	26 (20.2)	103 (79.8)	129 (100)
Total	27 (20.1)	107 (79.9)	134 (100)

Table 3: Sensitivity and Specificity of Microscopy and RDT methods

Statistic	**Value**	**95% CI**
Sensitivity	3.70%	0.09% -18.97%
Specificity	96.26%	90.70% - 98.97%
Positive Likelihood Ratio	0.99	0.12 - 8.51
Negative Likelihood Ratio	1	0.92 - 1.09
Positive Predictive Value (*)	20.00%	2.83% - 68.22%
Negative Predictive Value (*)	79.84%	78.48% - 81.15%
Accuracy (*)	77.61%	69.61% - 84.36%

The sensitivity and specificity values were lower compared to similar studies in other parts of Nigeria and Africa that recorded higher sensitivity and specificity for CareStart™ (Abdulkadir et al., 2015; Adebisi et al., 2018; Sheyin & Bigwan, 2013; Bamou et al., 2021) except for Abdulkadir et al. (2015) and Adebisi et al. (2018) who recorded lower specificities than those obtained in this study. The positive and negative predictive values vary with disease prevalence (Monaghan et al., 2021). The relatively low positive and high negative predictive values obtained in this study are similar to the report by Dozie (2020), who observed lower sensitivity in SD Bioline malaria HRP-2 RDT in adults, suggesting that RDT alone is not sufficient for diagnosing malaria in adults. The low sensitivity recorded for the CareStart™ RDT in this study is far lower than the WHO recommendation of 95% sensitivity for malaria RDT (WHO, 2010).

There are several factors that could be responsible for this observation. Firstly, it could be due to manufacturing issues, as considerable variations have been reported in the manufacturing quality of RDTs (WHO, 2010).

Secondly, poor packaging and improper storage during distribution and prior to usage can contribute to the low performance, especially in tropical regions. Malaria RDTs are sensitive to heat and humidity, and this may affect performance (McMorrow et al., 2011). Thirdly, there is current evidence that the deletion of *hrp2* genes in parasite populations could result in false negatives during the diagnosis of *P. falciparum* infections using HRP2-based RDTs (Lee et al., 2006). Recent reports have confirmed the deletion of the *hrp2* genes in *P. falciparum* populations in Nigeria, along with decreased sensitivity to RDTs (Oreh et al., 2022). Additionally, genetic variation in the *Pf*HRP2 amino acid sequence has been observed among parasites isolated from different geographical regions, resulting in false-negative results from RDTs (Mouatcho & Goldring, 2013).

The parasite density ranged from 1040 to 9600 parasites per microliter of blood, with a mean and standard deviation of 2954.1 and 1656.4 parasites per microliter of blood, respectively (Table 1). Only one sample with parasitaemia ranging between 2000 and 4000 parasites per microliter of blood was detected with CareStart™ malaria RDT (Table 4). The inability of the RDT to detect *P. falciparum* despite the high levels of parasitaemia could be due to the prozone effect. The prozone effect is described as false-negative results due to an excess of antigens or antibodies in immunological reactions (Gillet et al., 2009). In high parasitaemia levels, the high antigen concentrations could result in the blockage of all available binding sites for both the detection and capture antibodies, hindering the formation of the antigen-antibody complex and failing to generate an immunochromatographic test line. In addition, the quality of the RDTs could influence the chances of a prozone phenomenon resulting in false negative test results (Luchavez et al., 2011).

Table 4: Parasite Density in relation to the performance of RDT

Parasites/µL	**Microscopy**	**RDT**	
		Negative	**Positive**
0	107	103	4
<2000	9	9	0
2001-4000	13	12	1
>4000	5	5	0

The age of patients has been shown to affect the RDTs' sensitivity and specificity, causing an over- or underdiagnosis of malaria (Mouatcho & Goldring, 2013). A significant variation in the sensitivity of the ParaSight F test kits was observed among different age groups (Mouatcho & Goldring, 2013). In Tanzania, a decrease in sensitivity was observed for Paracheck-Pf RDT in older age groups (Abeku et al., 2008). In Nigeria, the sensitivity of CareStart™ RDT was observed to decline with age (Mac et al., 2019). Although we did not conduct malarial testing in children using the same RDT as at the time of conducting this study, other studies that have used CareStart™ RDT for malaria testing in children recorded higher sensitivities compared to this study (Abdulkadir et al., 2015; Adebisi et al., 2018; Sheyin & Bigwan, 2013; Bamou et al., 2021). Recent studies suggest that malaria should be monitored in the adult population (Bouyou-Akotet et al., 2014), hence the need for a choice of diagnostics.

Our findings point to the possibility of a lower diagnostic sensitivity for P. falciparum malaria in adults when utilizing PfHRP2-detecting RDTs, as well as the necessity of using microscopy to support the interpretation of RDT data. Further studies are required to determine the performance of rapid diagnostic test kits in adults in Nigeria, as well as a more comprehensive investigation of the age of the patient as a contributor to false negative tests. Such studies can provide valuable information on the reliability of RDTs.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Study protocol was approved by the ethical review committee of Adekunle Ajasin University, Akungba Akoko (REF: AAUA/FSC/AEB/19/03/2021). Informed consent was obtained from participants.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this manuscript

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