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Research Article

Evaluation of Four Malaria Rapid Diagnostic Test Kits Used at the Enyiresi Government Hospital in the Eastern Region of Ghana

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Microscopic identification of *Plasmodium* spp. is the gold standard for malaria diagnosis. However, malaria rapid diagnostic test kits are also available for prompt diagnosis. This study evaluated four routinely used malaria rapid diagnostic test kits at the Enyiresi Government Hospital in the Eastern Region of Ghana. This cross-sectional study was conducted among 238 patients suspected of malaria. Venous blood samples were collected to identify *Plasmodium falciparum* using microscopic techniques. Further, the performances of four malaria rapid diagnostic test kits, First Response Malaria Ag Pf, Carestart Malaria Pf, SD Bioline Ag Pf, and ABON Malaria Pf, were evaluated using the results from the microscopy as the standard reference. As confirmed by microscopy, 65.5% (156/238) of the patients have falciparum malaria. All malaria rapid diagnostic test kits had sensitivities and specificities over 75% compared to microscopy results as the reference standard. However, the SD Bioline Ag Pf kit recorded the highest agreement with the microscopy (Cohen's kappa = 0.879). All the malaria rapid diagnostic test kits performed quite well and can be used in emergencies. However, results from these kits need to be confirmed by microscopy.

1. Background

In malaria-endemic settings, prompt and efficient diagnosis is required for effective disease management [1]. The World Health Organization (WHO) recommends that all febrile patients in malaria-endemic settings are appropriately diagnosed before commencing any treatment. This recommendation enhances anti-malarial drugs' effective and efficient use and prevents unnecessary treatments that could contribute to drug resistance [2]. In Ghana, an estimated 3.5 million people contract malaria yearly, with most deaths among children under 5 years [3]. Also, *Plasmodium falciparum* malaria remains one of the most significant diseases in Ghana and sub-Saharan Africa, recording a high burden on health services [4].

Worldwide, examining blood film for identifying *Plasmodium* spp. using a light microscope remains the gold standard for confirming malaria in suspected patients [5, 6]. However, in remote rural settings where skilled personnel or microscopes are unavailable, rapid diagnostic test (RDT) kits are recommended to screen for malaria before commencing anti-malarial treatment [7, 8]. Hence, in Ghana, several malaria RDT kits are on the market [9, 10], and the main difference between these malaria RDT kits is the detected antigen. For example, histidine-rich protein 2 (HRP-2) is specific for the detection of *P. falciparum*,

aldolase for the detection of *Plasmodium* spp., and plasmodial lactate dehydrogenase (pLDH) for the detection of either *P. vivax* (*Pv*-pLDH), *P. falciparum* (*Pf*-pLDH), or *Plasmodium* spp. (pan-pLDH) [11].

Some RDT kits show different performances in different geographical settings [12]. There is, therefore, a need to continually evaluate the performance of malaria RDT kits used in primary healthcare in different geographical settings. This study evaluated four routinely used malaria RDT kits at the Enyiresi Government Hospital in the Eastern Region of Ghana using blood film for *P. falciparum* parasites identification as the gold standard.

2. Materials and Methods

2.1. Study Design and Study Site. A cross-sectional hospital-based study was conducted from March to June 2021 among persons suspected of malaria during their visit to the Enyiresi Government Hospital in the Eastern Region of Ghana. This hospital serves the indigenes of the Atiwa District in the Eastern Region.

2.2. Study Population and Sample Size Determination. All persons suspected of malaria visiting the Enyiresi Government Hospital in the Eastern Region of Ghana were considered the study population. The sample size was calculated using a prevalence of malaria reported in Ghana, 41.5% [13], and the Fischer expression for cross-sectional studies [14]. At an 85% confidence level and a 5% margin of error, the minimum sample size required for the study was 202, as illustrated below (1). However, 238 persons suspected of malaria who consented were enrolled, consisting of 84 males and 154 females. The ages of the participants ranged from 2 to 48 years.

$$n = \frac{z^2 \times p\,(1-p)}{m^2} = \frac{1.44^2 \times 0.415 \times (1-0.415)}{0.05^2} = 201.367 \approx 202. \quad (1)$$

Where: n = sample size; $z = z$ -score value (1.44) from a normal distribution at an 85% confidence level; m = margin of error: 5%; p = prevalence of malaria by microscopy: 41.5%.

2.3. Data Collection and Laboratory Analysis. Data on age and gender, as well as 5 ml of venous blood, were obtained from the study participants after obtaining informed consent. The venous blood samples were collected into K_2EDTA tubes and were used immediately after sample collection. Thick and thin blood smears were prepared from the blood samples, allowed to be air-dried, and stained with 10% Giemsa solution (thin films were pre-fixed in methanol). All slides were observed under oil immersion (100× objective lens) by an experienced microscopist. The limit of detection of malaria parasites by an experienced microscopist is approximately 50 parasites/μL, with a specificity of 99% [15–17].

The blood samples were also evaluated for the presence of malaria parasite antigen using malaria RDT kits, First Response Malaria Ag Pf (Premier Medical Corporation Ltd, Gujarat, India), SD Bioline Ag Pf (Standard Diagnostics, Kyonggi, Korea), Carestart Malaria Pf (Access Bio Inc. New Jersey, USA), and ABON Malaria Pf (ABON Biopharm Company Ltd., Hangzhou, China). All the RDT kits worked on the principle of *P. falciparum* HRP-2 monoclonal antibody binding and were used following the manufacturer's instructions.

Table 1: Prevalence of malaria parasites among the study participants.

Variables	Negative [*n* (%)]	Positive [*n* (%)]	Total [*N* (%)]	*p*-value
Overall	82 (34.5)	156 (65.5)	238 (100.0)	
Age (years)				
<10	15 (18.3)	62 (39.7)	67 (28.2)	0.0009*
10–19	10 (12.2)	8 (5.1)	18 (7.6)	
20–29	18 (22.0)	40 (25.6)	58 (24.4)	
≥30	39 (47.6)	46 (29.5)	95 (39.9)	
Gender				
Males	24 (29.3)	60 (38.7)	84 (35.3)	0.1989
Females	59 (70.7)	95 (61.3)	154 (64.7)	

*Difference calculated by Fisher's exact test (p-value < 0.05).

2.4. Ethics Statement. Ethical approval was sought from the Committee on Human Research, Publications and Ethics (CHPRE) of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology (KNUST), Ghana. Also, informed consent was obtained from the study participants or parents (for participants below 18 years) after explaining the purpose of the study in a language each participant or parent understood. The participants who were positive for malaria parasitaemia were given the appropriate anti-malarial treatment.

2.5. Statistical Analysis. Data were analysed using the Statistical Package for Social Sciences Statistical Software (version 20.0, IBM Corporation, USA). The data were expressed in percentages and frequencies, and Fisher's exact test was used to determine significant differences between the variables. Diagnostic performances of kits were determined by calculating the test sensitivity, specificity, predictive values, and Cohen's kappa using microscopy as the reference standard [18]. The statistical significance was accepted in all comparisons at a p-value <0.05.

3. Results

3.1. Prevalence of Malaria among the Study Participants. The statistical analysis included all 283 persons suspected of malaria enrolled in the study. The study participants were 2–48 years old, and most (39.9%) were 30 years and above. Over half of the study participants were females (Table 1). Overall, 65.5% of the study participants tested positive for malaria by light microscopy. The age group of the participants was significantly associated ($p = 0.0009$) with the presence of the asexual stage of *P. falciparum* in the blood smear, mostly among the participants less than 10 years (Table 1).

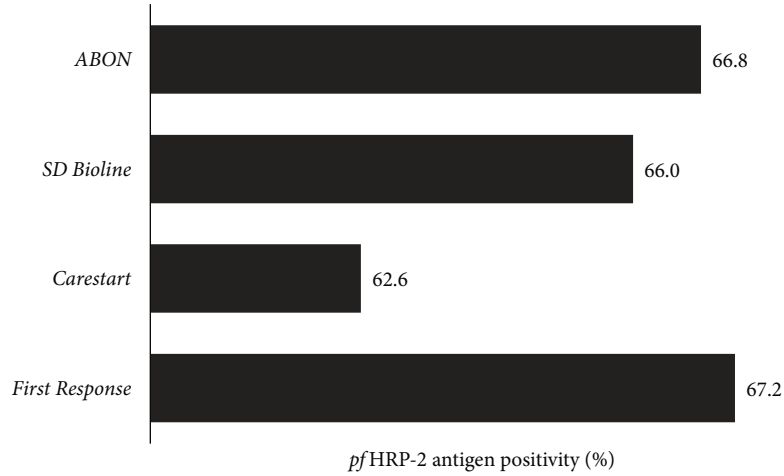

Figure 1: Prevalence of malaria antigens among the study participants.

Table 2: Diagnostic performance of malaria rapid diagnostic test kits.

RDT kits	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	Cohen's kappa
First Response	91.8 (86.2–95.5)	79.3 (68.9–87.4)	89.4 (83.5–93.7)	83.3 (73.2–90.8)	0.718
Carestart	92.3 (87.0–96.0)	93.9 (86.3–98.0)	96.4 (92.3–98.9)	86.5 (77.6–92.8)	0.845
SD Bioline	96.2 (91.8–98.6)	91.5 (83.2–96.5)	95.5 (91.0–98.2)	92.6 (84.6–97.2)	0.879
ABON	93.0 (87.7–96.4)	82.9 (73.0–90.3)	91.2 (85.7–95.1)	86.1 (76.5–92.8)	0.765

CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

Concerning the malaria RDTs, the ABON Malaria Pf kit recorded the highest positive for the *pf* HRP-2 antigen (66.8%), with the least being the Carestart Malaria Pf kit (62.6%) (Figure 1).

3.2. Diagnostic Performance of Malaria Rapid Diagnostic Test Kits. Table 2 shows the diagnostic performance of the malaria RDT kits routinely used at the Enyiresi Government Hospital in the Eastern Region of Ghana. All the malaria RDT kits have sensitivities of over 75%. However, the SD Bioline Ag Pf kit recorded the highest sensitivity (96.2%) and agreement with the microscopy (Cohen's kappa = 0.879) (Table 2).

4. Discussion

Malaria remains a major public concern, with over 90% of global cases occurring in Africa. Among children, the prevalence and mortalities associated with malaria are higher than among adults, and in 2019, about 67% of children died of malaria worldwide [reviewed in [19]]. The falciparum malaria prevalence in this current study was 65.5%, mostly among children under 10 years. This finding agrees with previous studies conducted in Ghana [20, 21] and other parts of Africa, such as Mauritania and the Ivory Coast [22], where a higher falciparum malaria prevalence was reported among children under 10 years. Malaria control interventions are mostly implemented for children under 5 years, which have been reported to be very effective [20, 23]. Hence, with the higher frequency of malaria among children under 10 years in the Enyiresi Government Hospital in Ghana, it will be imperative to scale up these interventions to include children up to 10 years in the study area and Atiwa District.

Studies have shown that many anti-malarial drugs have been misused on patients with non-malarial diseases due to a lack of prompt and accurate laboratory diagnosis [24]. Hence, in malaria-endemic settings, RDT kits are essential in remote areas where microscopes or skilled personnel are unavailable to ensure the effective use of anti-malarial drugs. It has been reported that temperatures in the tropics can denature antibodies in the membrane or damage the nitrocellulose membrane forming the strip of the RDT kits, thereby changing its flow characteristics or causing the antibody to detach from the membrane [25]. Hence, evaluating the performance of these RDT kits in different geographical settings is imperative.

In this current study, the sensitivities of the RDT kits ranged from 91.8% for the First Response Malaria AgPf kit to 96.2% for the SD Bioline Ag Pf kit. In other parts of the world, sensitivities of RDT kits ranging from 96.0% to 97.6% have been reported [26–30], which implies that three of the evaluated RDT kits have lower sensitivities. The specificities of RDT kits in this current study ranged from 89.4% for the First Response Malaria Ag Pf kit to 96.4% for the Carestart Malaria Ag Pf kit, and specificities of RDT kits within the ranges of 87%–100% have also been reported in previous studies [26, 31, 32]. Considering the WHO standard of choosing malaria RDT kits, based on 95% sensitivity [7], the SD Bioline Ag Pf kit is the preferred choice. However, all the malaria RDT kits evaluated (First Response Malaria Ag Pf, SD Bioline Ag Pf, Carestart Malaria Pf, and

ABON Malaria Pf) recorded specificities lower than the WHO recommendation of 97% specificity [7]. Hence, microscopic confirmation of malaria is always required after using RDT kits in primary healthcare settings.

The RDT kits detected malaria in some patients who tested negative by microscopy and vice versa. In this current study, the false positive results of the RDT kits ranged from 3.6% for the Carestart Malaria Pf kit to 10.6% for the First Response Malaria Ag Pf kit. In other parts of the world, false positive results from RDT kits ranging from 2.7% to 15% have been reported [33, 34], implying that all the RDT kits evaluated (First Response Malaria Ag Pf, SD Bioline Ag Pf, Carestart Malaria Pf, and ABON Malaria Pf) have comparable false positive results as in the previous studies. The false positive reactions could be the persistent viable asexual stage parasitaemia below the detection limit of microscopy and the persistence of antigens resulting from sequestration. The negative predictive values were higher for all the malaria RDT kits in this current study, comparable to a previous study in Bangladesh [35]. A higher negative predictive value permits a diagnosis of a negative test as non-malarial patients assertively [36]. The false negative results could be due to the deletion of the *pf* HRP-2 from the parasites [37].

The deletion or mutation in the *pf* HRP-2 has been reported during prolonged *in vitro* culture of asexual *P. falciparum* parasites [38, 39]. However, spontaneous deletion of the *pf* HRP-2 has been reported in clinical settings in Peru and Papua New Guinea [40, 41]. Also, the deletion of the *pf* HRP-2 has been reported in asexual *P. falciparum* parasites in sub-Saharan Africa [42, 43]. In Ghana, *P. falciparum* parasites lacking the exon 2 of the *pf* HRP-2 have been reported in Accra and Cape Coast [43]. However, there is limited evidence of *pf* HRP-2 deletion in the *P. falciparum* parasites from Enyiresi in the Eastern Region of Ghana. Hence, attributing the false negative cases to the deletion of the *pf* HRP-2 is speculative.

5. Conclusion and Recommendation

All the malaria RDT kits, especially the SD Bioline Ag Pf kit, showed reliable performance. At the Enyiresi Government Hospital in the Eastern Region of Ghana, the malaria RDTs evaluated provide rapid results, with less training and skilled personnel to use the test kits. However, some of the disadvantages are the persistence of the *pf* HRP-2 making it difficult to distinguish recently and effectively treated infections from new infections. Concerning cost-effectiveness, using the malaria RDTs at the hospital is cheaper than light microscopy for diagnosing malaria. However, for malaria diagnosis, malaria RDT kits cannot be relied upon alone; hence, microscopic confirmation is always required. We recommend that RDT kits be continually assessed in-house for effective malaria diagnosis.

6. Limitations of the Study

Malaria parasite density could have affected the sensitivities of the RDT kits. However, we did not assess the malaria parasite density during the study.

Data Availability

All the data obtained and analysed are included in this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

SAD conceived and supervised the work, and BYD and RKG conducted the experiments. EAA analysed the data. SAD wrote the manuscript, and CO reviewed the manuscript. All authors read and approved the final manuscript.

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