Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.13.02.Art035

Reliability of Rapid Diagnostic Tests in Diagnosis of Malaria amongst Pregnant Women and Children in Western Equatoria State, South Sudan

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Abstract

Malaria remains a significant contributor to global morbidity and mortality rates especially in Sub-Saharan Africa. Accurate and timely diagnosis of malaria is crucial in enhancing control measures, reducing morbidity, and saving lives. This study aimed to assess the prevalence of Plasmodium spp. infection among high-risk populations, specifically children under five years old and pregnant women, while comparing results between Malaria Rapid Diagnostic Tests (RDT) and microscopy for enhanced diagnostic accuracy, planning and monitoring of malaria treatment and control interventions. A cross-sectional design was employed, involving 964 pregnant women receiving routine antenatal care and 999 under-five children presenting with febrile illnesses at three primary health care centers over a six-month period conducted in two phases. Quantitative data analysis was performed using SPSS version 20.0. Among the pregnant women, RDT indicated an overall malaria prevalence of 14.2%, while microscopy revealed 14.9%; whereas for under-five children, corresponding prevalence rates were 38.0% by RDT and 39.2% by microscopy. The study demonstrated consistent results between the two diagnostic tests across both study groups. The sensitivity and specificity of the malaria RDT were reported as 87% and 99% for the pregnant women group, and 92% and 97% for the under-five children group, respectively. While acknowledging the gold standard status of malaria microscopy, the study underscored the effectiveness of RDTs in diagnosing malaria, supported by their high sensitivity and specificity rates. Consequently, the study advocated for the preferential use of RDTs in resource-limited settings, particularly at primary healthcare facilities, for prompt and accurate case management.

Keywords: *Malaria, Microscopy, Prevalence, Rapid Diagnostic Test, South Sudan.*

Introduction

Malaria remains a significant global health challenge, causing substantial morbidity and mortality worldwide. Timely and accurate diagnosis of malaria is crucial for effective control measures and reducing the burden of this disease. Malaria is a mosquito-borne illness caused by Plasmodium parasites, primarily transmitted through the bites of infected female Anopheles mosquitoes [1]. The global impact of malaria is evident, with approximately 229 million cases and 409,000 deaths reported in 2019 [2].

In the pursuit of malaria control and elimination goals set by the World Health Organization (WHO), many countries are intensifying efforts to combat the disease. However, diagnostic challenges persist, leading to preventable deaths annually, particularly among vulnerable populations such as children and pregnant women. Plasmodium falciparum and *Plasmodium vivax* are the predominant

Received: 13.12.2024 ***Accepted: 08.02.2025*** ***Published on: 30.06.2025***
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species contributing to the global malaria burden.

In Africa, malaria ranks as the second leading cause of death from infectious diseases following HIV/AIDS [3]. WHO defines malaria control as reducing disease burden to a level where it no longer poses a public health threat [4]. South Sudan, an endemic area for malaria, faces a high prevalence of Plasmodium infection nationwide, with children under five years and pregnant women bearing the greatest burden [5, 6].

Various interventions are deployed to combat malaria, including the use of insecticide-treated nets, indoor residual spraying, artemisinin combination therapy, and rapid diagnostic tests [7-11]. Preventive measures targeting pregnant women involve Long-Lasting Insecticide Treated Nets and Intermittent Preventive Treatment with Sulfadoxine-Pyrimethamine, while uncomplicated cases are treated with Artemisinin Combination Therapy using Artesunate and Amodiaquine.

Given the nonspecific symptoms of malaria that overlap with other tropical diseases, reliance on clinical diagnosis alone can lead to the inappropriate use of antimalarials and compromise patient care quality in endemic areas [12-14]. In South Sudan, malaria diagnosis at peripheral health centers primarily relies on Rapid Diagnostic Tests detecting P. falciparum antigens [15].

This study holds particular significance in addressing the challenges associated with malaria diagnosis in low-resource settings, where health workers often resort to clinical judgement due to limited access to reliable diagnostic tools. Such practices not only result in misdiagnosis and unnecessary use of antimalarials but also delay appropriate treatment, increasing the risk of severe complications and mortality [16-18].

The primary aim of this study is to generate epidemiological data on malaria in Western Equatoria State, South Sudan, to inform the National Malaria Control Program's activities and enhance planning and monitoring of malaria interventions. The specific objectives include assessing Plasmodium infection prevalence among children under five and pregnant women at three health centers, comparing the accuracy of Rapid Diagnostic Tests with microscopy, and improving overall malaria diagnosis quality.

The findings of this study are expected to benefit healthcare providers and stakeholders involved in malaria control programs by enhancing the efficiency and accuracy of malaria diagnosis and treatment in South Sudan. Demonstrating the efficacy of Rapid Diagnostic Tests in malaria screening may help reduce overdiagnosis, minimize unnecessary antimalarial use, and improve the identification of alternative causes of febrile illnesses.

Materials and Methods

Study Design and Population

The study was conducted at three Primary Health Care Centres (PHCC) located in different counties within Western Equatoria State, South Sudan with an estimated population of 183,513 people in 2020 according to the DHIS2 statistics. Mundri PHCC is situated in Mundri West County, with an estimated catchment population of approximately 19,500. Mvolo PHCC in Mvolo County serves around 13,000 individuals, while Lakamadi PHCC in Mundri East County caters to about 6,700 residents.

In the first semester of 2020, Mundri PHCC accounted for 37% of Outpatient Department (OPD) consultations and 50% of first Antenatal Care (ANC) visits in Mundri West County. It is staffed with 32 personnel, 17 of whom are qualified. Similarly, Mvolo PHCC reported 24% of OPD consultations and 41% of first ANC visits in Mvolo County, with a staff of 15 individuals, 8 of whom are qualified. Furthermore, Lakamadi PHCC recorded 3,147 OPD consultations and 106 first ANC visits in

the same period, with 15 staff members, 6 of whom are qualified.

The study design employed was a cross-sectional study, with the primary objectives of assessing malaria prevalence in the study areas and comparing the diagnostic reliability and accuracy of malaria using Rapid Diagnostic Tests (RDTs) (specifically a Plasmodium falciparum-based kit.

detecting histidine-rich protein-2) against microscopy. The study focused on populations at higher risk of malaria, including children under five presenting with febrile or acute illnesses, as well as pregnant women attending ANC at various stages of pregnancy across the three PHCCs.

Recruitment of participants occurred over a period of six months, divided into two phases corresponding to different malaria transmission levels: the low or dry season (December 2021 to February 2022) and the high or wet season (March to May 2022). During this period, all eligible individuals accessing any of the study PHCCs were invited to participate in the study. Volunteers were consecutively enrolled until the desired sample size was achieved (refer to Table 1 below).

Table 1. Number of Sought Participants Per Season and PHCC

Health Centre	**Children < 5 years old (N)**		**Pregnant women (N)**		**Total (N)**
	Wet season	**Dry season**	**Wet season**	**Dry season**	
Lakamadi	100	100	100	100	400
Mudri	200	200	200	200	800
Mvolo	200	200	200	200	800
Total	**500**	**500**	**500**	**500**	**2000**

Study Tools & Data Collection

Staff members at the Primary Health Care Centres (PHCCs) recruited participants during routine visits to the Outpatient Department (OPD) for children with febrile or other illnesses, as well as during Antenatal Care (ANC) visits for pregnant women. Trained PHCC staff explained the study's objectives and procedures to participants and, in the case of children under 5, to their guardians. A red information sheet was provided, questions were addressed, and written consent for participation in the study was obtained.

Each participant was allocated a unique study ID/barcode at recruitment, which was recorded on the informed consent form, data collection form, malaria microscopy form, and biological specimens. This ID was used for participant identification while maintaining anonymity and privacy throughout the study. Adult participants and guardians of underage participants were required to sign a written informed consent form. Participants unable to sign but willing to take part used their fingerprint. The consent form template was developed based on guidelines from the Global Health Network (https://tghn.org/).

Sample Collection & Laboratory Methods

Capillary blood samples were collected by finger prick following WHO phlebotomy guidelines. Thick and thin blood films were prepared, air-dried, and stored for microscopy examination according to WHO guidelines [20, 21]. Stained with Giemsa solution, the films were examined under a light microscope by trained technicians following WHO protocols. A positive malaria test was determined if at least one parasite was observed per 100 high power fields, with 200 fields examined before deeming a slide negative.

Rapid Diagnostic Tests (RDTs) for malaria were conducted in the PHCC laboratory for each participant using SD-Bioline Malaria

HRP-II Antigen as per the manufacturer's instructions. Positive results were indicated by the presence of two red lines near specific letters, while a negative result showed one line near 'c' and no line near 't'. An invalid result was identified when the control line was absent. Patients with confirmed malaria infections (both uncomplicated and asymptomatic) based on RDT and/or microscopy results were administered artemisinin combination therapy (ACTs) treatment in accordance with established clinical and laboratory practices.

Data Analysis

Data collection and malaria microscopy forms were created using the EpiData open software and ensured participant anonymity. The data collection form captured information such as date, health center, reason for consultation, personal details (age, sex, residence, parity), specimen collection status, and RDT results (positive/negative). The malaria microscopy form documents malaria microscopy results (Plasmodium species, stages, parasite density). Data was entered into Excel 2013 and analysed using SPSS 20.0 version statistical software.

The questionnaire was administered by trained field enumerators and it was validated by three experts taking into consideration the content of the instrument, clarity, appropriateness of the language and suitability. The number of individuals positive to malaria is the dependent variable, while the test method (mRDT or microscopy) is an independent variables for this study. Data were analysed using frequency and percentages and results presented in tables. Sensitivity was defined as the proportion of true malaria cases (positive blood smears) that were correctly identified by positive RDTs, whereas specificity was the proportion of true negative malaria cases (negative blood smears) that were correctly identified by negative RDTs. Positive predictive value (PPV) was the proportion of true malaria cases (positive blood smears) among the individuals with the positive RDTs. Negative predictive value (NPV) was the proportion of true negative malaria cases (negative blood smears) among the total number of negative RDT tests. Chi-square was used to determine whether there is an agreement between the results of the two tests. $P < 0.05$ was considered statistically significant. Taking blood slide microscopy as the gold standard, the performance of the Rapid Diagnostic Test was compared to it by computing the sensitivity, specificity, the negative predictive value, and the positive predictive value of the test [22].

The agreement between microscopy and RDT was determined by calculating Kappa values with a 95% confidence interval [23] using Epi-info version 6. Kappa values express the agreement beyond chance and a kappa value of 0.21-0.60 is a moderate, a kappa value of 0.61-0.80 a good and kappa > 0.80 an almost perfect agreement beyond chance.

Ethical Considerations

The research protocol received ethical clearance from the Ethical Review and Research Committee of the South Sudan Ministry of Health. Prior to commencing the study, investigators engaged with administrative and health authorities in the study area, informing local chiefs and leaders about the research objectives. Strict confidentiality of participant information was assured. Verbal and written informed consent was obtained from participants during recruitment, and all consent forms were securely stored by the local principal investigator.

Results

A total of 964 pregnant women (phase 1: 430, phase 2: 534) and 999 under-5-year-olds (phase 1: 457, phase 2: 542) were recruited into the study from the 3 PHCCs from December 2021 to May 2022 in 2 phases. Among all the children under-5 years children recruited, 41%

were males and 59% were females. Subsequently, for ease of data analysis, 154 participants with invalid RDT results were excluded from the study, with only 895 pregnant women and 914 under 5 years eventually enrolled.

Noting that almost all the pregnant women that participated in the study were asymptomatic, in phase 1, Fifty-nine (14.9%) were positive for malaria by RDT and 61 (15.4%) by microscopy, while in phase 2, Sixty-eight (13.7%) were positive for malaria by RDT and Seventy-two (14.5%) by microscopy. Hence, the overall prevalence of malaria among all the pregnant women over the 6 months by RDT was 14.2% and by microscopy was 14.9%. See Table 2 below for more details.

Table 2. Results of RDT and Microscopy for Pregnant Women Attending Routine ANC

	Study location	**RDTs**					**MICROSCOPY**		
		Enrolled Particinants	**Positive cases**	**Negative cases**	**Invalid tests**	**% Positive Cases**	**Positive cases**	**Negative cases**	**% Positive Cases**
Phase 1 December 2021 to February 2022	Lakamadi	72	5	67	2	6.9%	5	67	6.9%
	Mvolo	136	26	110	10	19.1%	24	112	17.6%
	Mundri	189	28	161	21	14.8%	32	157	16.9%
	Total	**397**	**59**	**338**	**33**	**14.9%**	**61**	**336**	**15.4%**
Phase 2 March – May 2022	Lakamadi	125	17	108	7	13.6%	19	106	15.2%
	Mvolo	212	29	183	16	13.7%	27	185	12.7%
	Mundri	161	22	139	13	13.7%	26	135	16.1%
	Total	**498**	**68**	**430**	**36**	**13.7%**	**72**	**426**	**14.5%**
	Overall Total	**895**	**127**	**768**	**69**	**14.2%**	**133**	**762**	**14.9%**

For the under-five children enrolled in the study from the OPD with febrile and other acute symptoms, in phase 1, One hundred and seventy-two (40.4%) were positive for malaria by RDT and One hundred and seventy-nine (42.0%) by microscopy, and in phase 2, One hundred and seventy-five (35.9%) were positive for malaria by RDT and 179 (36.7%) by microscopy. Hence, the overall prevalence of malaria among all the under-5-year-old children over the 6-month period by RDT was 38.0% and by microscopy was 39.2%. See Table 3 below for more details.

Table 3. Results of RDT and Microscopy for Under-5 Children with Acute Illnesses

	Study location	**RDTs**					**MICROSCOPY**		
		Enrolled Particinants	**Positive cases**	**Negative cases**	**Invalid tests**	**% Positive Cases**	**Positive cases**	**Negative cases**	**% Positive Cases**
Phase 1: December	Lakamadi	94	52	42	2	55.3%	53	41	56.4%
	Mvolo	154	76	78	17	49.4%	77	77	50.0%

2021 to February 2022	Mundri	178	44	134	12	24.7%	49	129	27.5%
	Total	**426**	**172**	**254**	**31**	**40.4%**	**179**	**247**	**42.0%**
Phase 2 March – May 2022	Lakamadi	115	46	69	5	40.0%	43	72	37.4%
	Mvolo	204	87	117	33	42.6%	92	112	45.1%
	Mundri	169	42	127	16	24.9%	44	125	26.0%
	Total	**488**	**175**	**313**	**54**	**35.9%**	**179**	**309**	**36.7%**
	Overall Total	**914**	**347**	**567**	**85**	**38.0%**	**358**	**556**	**39.2%**

Table 4. 2x2 Contingency Table of RDTs and Microscopy in the Pregnant Women

RDTs	**Microscopy**		**Total**
	Positive	**Negative**	
Positive	116 (TP)	11 (FP)	**127 (TP + FP)**
Negative	17 (FN)	751 (TN)	**768 (TN + FN)**
Total	**133 (TP + FN)**	**762 (TN + FP)**	**895**

Table 5. 2x2 Contingency Table of RDTs and Microscopy in Under-5 Children

RDTs	**Microscopy**		**Total**
	Positive	**Negative**	
Positive	329 (TP)	18 (FP)	**347 (TP + FP)**
Negative	29 (FN)	538 (TN)	**567 (TN + FN)**
Total	**358 (TP + FN)**	**556 (TN + FP)**	**914**

The table 4 and 5 above are results from the further analysis of the study results by comparing the results from the RDTs and microscopy separately for the 2 study groups. Taking blood slide microscopy as the gold standard, the performance of the Rapid Diagnostic Test was compared to it by computing the sensitivity, specificity, the negative predictive value (NPV), and the positive predictive value (PPV) of the test [24]. On the whole, whether microscopy or RDT was used to diagnose malaria among the respondents, there seems to be no statistically significant differences in the results obtained.

Table 6. Sensitivity, Specificity, Positive/Negative Predictive Values and Accuracy of RDT

		Pregnant women	**Under-5 Children**
Sensitivity	TP/(TP+FN) × 100%	87%	92%
Specificity	TN/(TN+FP) × 100%	99%	97%
PPV	TP/(TP+FP) × 100%	91%	95%
NPV	TN/(TN+FN) × 100%	98%	95%
Accuracy	(TP+TN)/ Grand Total × 100%	97%	95%

The sensitivity and specificity of the malaria RDT for the pregnant women group was 87% and 99% respectively, with corresponding positive and negative predictive values (PPV and NPV) of 91% and 98% (Table 6). Similarly, the sensitivity and specificity of the malaria RDT for the under-5 children group were 92% and 97% respectively, with corresponding positive and negative predictive values that were both 95% (Table 6).

Discussion

High prevalence of malaria in study areas in Western Equatoria State, South Sudan, was

revealed by the study without any significant statistical difference between the dry and rainy seasons. This implies that malaria transmission is persistently high all year round in these regions. Overall prevalence of malaria among the asymptomatic pregnant women group by RDT was 14.2% and by microscopy was 14.9%, while in the symptomatic under-5 children group, the prevalence by RDT was 38.0% and by microscopy was 39.2%. This is further evidence for all stakeholders to urgently strengthen the malaria control program in South Sudan with emphasis on evidence-based and innovative approaches in both prevention and treatment.

Use of long-lasting insecticidal mosquito nets and/or indoor residual spraying for vector control, prompt access to diagnostic testing for suspected cases, and treatment of confirmed cases with efficient artemisinin-based combination therapy are the interventions currently advised by the World Health Organisation (WHO) for the control of malaria [25]. Intermittent preventive treatment during pregnancy and early childhood is another option that is suggested for particular high-risk groups in regions with high transmission.

A progressive change from a "one size fits all" approach to targeting malaria control measures to specific populations and/or areas for maximum efficiency has been made in response to the evolving epidemiology of malaria. Seasonal malaria chemoprevention (SMC), which is consistent with this strategy and supported by new data, is a recommended supplementary intervention against Plasmodium falciparum malaria [26]. SMC has been shown to be effective, cost-effective, safe and feasible for preventing malaria among children under 5 years of age in areas with highly seasonal malaria transmission. A complete treatment course of Sulfadoxine–Pyrimethamine (SP) plus Amodiaquine (AQ) should be given to children aged 3–59 months at monthly intervals, beginning at the start of the transmission season, up to a maximum of four doses during the malaria transmission season (provided both drugs retain sufficient antimalarial efficacy). SMC could be a useful intervention in South Sudan in tackling the burdens of malaria in the country.

This study also evaluated the performance of malaria rapid diagnostic test using malaria microscopy as a gold standard in the diagnosis of malaria in both asymptomatic pregnant women group and symptomatic under-five years children group attending 3 Primary Health Care Centre (PHCCs) in Greater Mundri, South Sudan. The high sensitivity (87% in pregnant women and 92% in under-5 children group) and specificity (97% in under-5 children and 99% in pregnant women group) results reported in this study showed the reliability of malaria RDT in malaria diagnosis [27], especially in rural areas and where there is no expert malaria microscopist to carry out proper malaria diagnosis.

In order to further support the above deductions, the study revealed that Kappa statistic of RDTs was 0.89 indicating a near perfect agreement when compared with the results from malaria microscopy. Therefore, reliability of RDT in the study was good. Thus, with the results from this study it may suffices to state that the use of RDT would be a favored and effective alternative diagnostic tool especially in poor resources settings because of the beneficial effect of testing and treating more clients for malaria within a short time, since the RDT is unlikely to miss out the infected and non-infected individuals.

The high PPV of RDT (91% in pregnant women and 95% in the under-5 children group) shown in this study suggest that any individual with positive RDT test would probably have malaria with respect to malaria microscopy. Although, predictive values rely more on disease prevalence in a given population, they give practical usefulness on test information. According to the result in this study, any individual who will be tested with positive result using this RDT is highly likely to be

malaria positive. It therefore means that every positive result should be considered with no doubt be treated immediately. In essence, it will reduce over treatment that leads to emergence of antimalarial resistance.

Identical study revealed that the sensitivity, specificity, PPV and NPV of RDT at 95% CI was 97.6%, 97.4%, 91.0% and 99.3% respectively [28]. A Similar study, where SD Bioline Pf antigen HRP II was used, have sensitivity, specificity, PPV, and NPV of 100%, 98%, 88%, and 100%, respectively, among symptomatic children aged < 5 years in a hospital in a mesoendemic area in Markafi in Kaduna state, Nigeria [29].

The study showed that malaria infections can be asymptomatic or subclinical, as 38.0% (347 out of 914 enrolled pregnant) were tested positive for malaria with RDTs. Pregnant women are at high risk of developing severe malaria, hence, It is important to identify these cases early and institute treatment before the disease progresses to a more serious condition. With this finding, it may be sufficient to recommend that malaria RDT should be included as part of the routine testing for pregnant women during ANC visits. There is need for more researches in this area for it to be acceptable in the global and national ANC guidelines. Microscopy may be more sensitive than RDTs If performed correctly by an experienced malaria microscopist using a standard external quality assessment (EQA) program for malaria microscopy feedback system [30]. However, some of its limitations include the requirement for specialized training, inability to function in areas with lack of power supply, and time-consuming preparation of stained blood smears for microscopic examination, particularly in emergency situations and environments with a high volume of cases. A study in Angola further revealed that poor performance of regular malaria microscopy was attributed to a number of issues, including the high volume of thick blood smears to be read on a daily basis, a lack of competent microscopists, supervision, and infrastructure maintenance [31].

Equations

The reliability of RDT was compared with that of Microscopy in both study group using the 2x2 contingency tables 4 and 5 above. The reliability (repeatability) of RDT means that the result of the test is replicated if repeated. Cohen in 1960 proposed the Kappa (*k*) statistic to calculate the reliability of a test. The formula for Cohen's kappa is calculated as:

$$Kappa\ k\ = ((po - pe))/\ (1 - pe).$$

where: Po: *Proportion of observed agreements,*

Pe: *Proportion of agreements expected by chance*

The Kappa statistic was therefore calculated as 0.87 and 0.89 for the under-5 children and pregnant women groups respectively. Possible values for kappa statistics range from −1 to 1, with 1 indicating perfect agreement, 0 indicating completely random agreement, and −1 indicating "perfect" disagreement. Landis and Koch (1977) provide guidelines for interpreting kappa values, with values from 0.0 to 0.2 indicating slight agreement, 0.21 to 0.40 indicating fair agreement, 0.41 to 0.60 indicating moderate agreement, 0.61 to 0.80 indicating substantial agreement, and 0.81 to 1.0 indicating almost perfect or perfect agreement [32].

Conclusion

Besides, certain challenges arise in providing the facility for accurate microscopic diagnosis of malaria at the rural communities. This is the setting where the malaria menace threatens the life of the pregnant women and the unborn babies. It can strongly be asserted that the findings of this study were good to suggest the adoption of the RDT in the parasitological confirmation of malaria diagnosis. As stated by WHO, this will, with some degree of certainty, help to rule out malaria in pregnant women. [33]. Since the introduction of malaria microscopy at PHCCs and lower-level facilities is challenged by lack of material (electricity,

microscope, reagents and consumables) and human resources (excessive workload of laboratory staff) in South Sudan, it will be highly recommended to promote the confirmation of malaria diagnosis majorly with RDTs.

Investment in malaria microscopy is still worthwhile preferably in secondary and tertiary health facilities. However, this will require training and retraining in malaria microscopy, with set-up of a robust internal and/or external quality controls including continuous supervision of each laboratory staff involved in performing this laboratory investigations. This study demonstrated the efficacy of the malaria RDT as an alternative to microscopy for the detection and management of both asymptomatic and symptomatic patients with febrile illness in South Sudan. Despite the fact that malaria RDT has various advantages over microscopy in terms of diagnosing malaria, malaria microscopy is still the gold standard in the field. The findings of this study are insufficient to support the claim that malaria RDT should be implemented at all levels of healthcare, but they should be used to advocate for its promotion at the primary healthcare level. The Plasmodium species cannot be distinguished by malaria RDT, and parasitemia cannot be measured. However, malaria RDT can be used to enhance the standard of case management of malaria and proper treatment of confirmed cases while preventing the indiscriminate provision of anti-malarial medications to patients who test negative for malaria.

Conflict of Interest

There is no conflict of interest regarding the research presented in this paper for publication. I affirm that no financial or personal relationships have influenced the research outcomes, interpretation of data, or presentation of findings. This work has been conducted with the highest standards of integrity and objectivity, and all authors have disclosed any potential conflicts of interest to ensure transparency and credibility in the publication process.

Acknowledgements

We express our deepest gratitude to all individuals who contributed to the successful completion of this research project. Special thanks to Valentina Mangano and other team members from University of Pisa, Italy, for their invaluable guidance and support throughout the study. We also extend our appreciation to the participants involved in this research who generously shared their time and interest, without whom this research would not have been possible. We acknowledge the funding support provided by Doctors with Africa CUAMM that enabled us to carry out this research effectively. Our sincere thanks to the staff and personnel of the three health facilities involved in the research for their assistance and cooperation. Lastly, Thank you to everyone involved in this project for their contributions and commitment.

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