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Assessment of Malaria Microscopic Diagnosis Performance of Laboratory Professionals in Addis Ababa's Public Health Facilities

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Abstract: Cross-sectional study was conducted using panel blood films and questioner to assess detection & identification performance of laboratory professionals' and identify factors affecting the performance of malaria microscopic diagnosis. Study participants had 91.7% (95% CI: 89.96 – 93.44) agreement for detection of malaria parasites, 67.63% (95% CI: 64.91 – 70.35) species identification agreement for *Plasmodium falciparum*, 5.08% false positive and 21.04% false negative results. Correct species identification percentage for *Plasmodium falciparum* were 60.9% (510), *Plasmodium vivax* 59.17% (371) and Mixed (*Plasmodium falciparum* and *Plasmodium vivax*) 25% (53) were also identified in the study. In addition, sensitivity 94.69% (95% CI: 93.02 – 96.36) and specificity of 79.71 (95% CI: 75.22 – 84.2) were calculated from panel blood film results. The most frequent type of misdiagnosis was 85(40.09%) mixed BFs diagnosed as *Plasmodium vivax*, 67 (31.6%) mixed BFs as *Plasmodium falciparum* and 218(26%) *Plasmodium falciparum* BFs as *Plasmodium vivax*. Moreover, only 18(8.5%) laboratory professionals were participated in external quality assessment. From multiple logistic regression analysis training was the major factor for species identification percent agreement performance improvement of laboratory professionals. It showed statistical significance with p-value < 0.05 and untrained laboratory professionals were 64% less likely to perform ⩾ 85% agreement of species identification. Training of laboratory professionals on malaria microscopic diagnosis help to improve the accuracy and reliability of reported results. This will help to provide the right and recommended medication and patient management.

Keywords: Malaria, Microscopy, Diagnosis, Performance, Laboratory Professionals, Addis Ababa & Public Health Facilities

1. Introduction

Microscopy of giemsa stained both thick and thin blood film (BF) is the gold standard and preferred option for diagnosis of malaria [1]. Parasitological testing is the only way to diagnose malaria accurately in febrile patients. If malaria is suspected on clinical grounds, it is mandatory to obtain the laboratory confirmation for the presence or absence of malaria parasites. According to 2011-2015 national strategic plan 100% of suspected malaria cases are expected to be diagnosed using microscopy or rapid diagnostic test (RDT) within 24 hours of fever onset. Proper diagnosis provides accurate and reliable result and is used for optimal treatment and save lives [1, 2, 3].

Malaria microscopic diagnosis (MMD) is better than clinical diagnosis for the proper management of malaria cases and other febrile illnesses. Misdiagnosis is the major challenge for the diagnosis of malaria using microscopy. This is due to the competency ability of laboratory professionals and lack of adherence to malaria microscopy standards [3]. Malaria treatment based on the laboratory diagnosis is the most preferred option and has its own advantage than the presumptive diagnosis. Misdiagnosis of malaria infection facilitates further transmission of malaria to the community

and cause misuse of anti-malaria drugs. Moreover, unable to identify patients not having malaria infection will lead to the exclusion of other options for febrile illness diagnosis and treatment. Since early 2010, World Health Organization(WHO) has recommended prompt parasitological confirmation by microscopy or RDT for all patients suspected of having malaria infection, before treatment is started [4, 5].

MMD performance depend on the competency of laboratory professionals, quality of reagents, training status on MMD, adherence to the standards, participation to external quality assessment(EQA) and microscope functionality. Panel testing or proficiency test is one type of EQA method. It is used to assess the performance of laboratory professionals on malaria parasite detection and identification using prepared known panel BFs. Panel BFs is prepared in a set of containing both positive and negative BFs according to the standards. Panel BFs species is detected and identified by qualified skilled laboratory professionals [3, 5].

Addis Ababa is located in a highland which is assumed to be malaria free. However, the city hosts peoples from different parts of the country and abroad. Currently the movement of people to and fro the city increases due to different reasons. In addition, the residents of the town have a chance of going to the malarious area and also peoples from the malarious area coming to the city.

Laboratory professionals working in Addis Ababa's health facilities are not exposed to positive malaria parasite BF readings frequently. So their malaria parasite detection and identification skill my decline over time, thus assessing their competency skill is a key point to identify major gaps affecting their performance. Clinicians in malarious areas may treat false negative cases. But since Addis Ababa is malaria free area the chance of false negative cases to be treated is low. The residents of the city are less immune to plasmodium species and if a patient is misdiagnosed as negative then its effect will be dangerous. For these reasons efficient MMD service is required to provide accurate and reliable laboratory result to febrile patient(s).

2. Methods

2.1. Study Area and Population

Addis Ababa is the capital and largest city of Ethiopia. The city divided in to ten sub cities and lies at an altitude of 2,300 meters above sea level. During the study period 67 Health Centers and 6 Hospitals owned by Addis Ababa regional health bureau giving malaria microscopy diagnosis service. Two hundred eleven laboratory professionals were participated during the data collection period, November 2013 to February 2014, from 47 Health Centers and 3 Hospitals.

2.2. Study Design

Cross sectional study design was conducted in public health facilities of Addis Ababa.

2.3. Data Collection

Laboratory professionals at the study health facilities were provided set of panel blood films. Each BF had both thick and thin BF on the same slide, the thin BF fixed with absolute methanol and stained with giemsa stain working solution. The panel BFs were prepared, fixed, stained and mounted according to WHO BF preparation standards. Study participant laboratory professionals were examined BFs using microscope found in their health facilities and reported the finding on provided reporting format. The report of panel BFs results were included the type of malaria parasite diagnosed (species) and stage for those positive BFs. We were used set of panel BFs for each laboratory professionals. Each set of panel BFs were consisted of four *Plasmodium falciparum*, three *Plasmodium vivax* and one Mixed (*Plasmodium falciparum* and *Plasmodium vivax*) infections with parasite densities ranging from 5440 – 23,160 parasites/μl and two negative BFs. A total of 211 laboratory professionals were examined 2096 BFs; 838(40%) were *Plasmodium falciparum*, 627(30%) *Plasmodium vivax*, 212 (10%) Mixed (*Plasmodium Falciparum* and *Plasmodium vivax*) and the rest 419 (20%) negative BFs. After completion of malaria microscopy diagnosis, interview based on prepared study questioner were filled and the finding recorded accordingly. Observations were also one way of data obtaining procedure during questioner assessment of the laboratory professionals.

3. Results

3.1. Characteristics of Laboratory Professionals Participated in the Study

A total of 211 laboratory professionals were participated in the study: 98(46.44%) female, 130(61.61%) diploma, 81(38.39%) BSc and above and 43(20.38%) trained on MLD (See Table 1).

Table 1. *Characteristics of laboratory professionals participated in the study.*

Factors		Frequency	Percent
Sex	Female	98	46.44%
	Male	113	53.56%
Age	20-27	139	65.88%
	28-54	72	34.12%
Service Year	<5Years	118	55.92%
	≥5Years	93	44.08%
Training on MLD	No	168	79.62%

Factors		Frequency	Percent
Education Level	Yes	43	20.38%
	Diploma	130	61.61%
	BSc and above	81	38.39%
Marital Status	Never Married	155	73.46%
	Ever Married	56	26.54%
SOPs	Used	42	19.9%
	Not Used	127	60.2%
	Not Available	42	19.9%
National EQA Guidelines	Not Available	211	100%
Job Aid	Not Available	211	100%
Type of Blood film Prepared	Only Thin Film	67	31.8%
	Only Thick film	73	34.6%
	Both thin and thick on the same slide	71	33.6%
IQC for Giemsa stain	No	126	59.7%
	Yes	85	40.3%
EQA Participation	No	193	91.5%
	Yes	18	8.5%

3.2. Performance of Laboratory Professionals

Among the total examined BFs 85 false positive, 89 false negative, 1588 true positive, 334 true negative and 654 species misdiagnosis were detected and identified. Study participant laboratory professionals were scored 91.7% (95% CI: 89.96 – 93.44) agreement for detection of malaria parasite, *67.63% (95% CI: 64.91 – 70.35)* species identification agreement for *Plasmodium falciparum*, 5.08% false positive and 21.04% false negative results. Moreover, their sensitivity and specificity were 94.69% (95% CI: 93.02 - 96.36) and 79.71% (95% CI: 75.22-84.2) respectively.

3.3. Species Identification Performance of Laboratory Professionals

According to national treatment guidelines species identification is the key for accurate treatment provision for malaria infected patients. Generally, study participant laboratory professionals in Addis Ababa were identified correctly malaria species in 60.5% (1268) (95% CI: 60.41-60.59) BFs. Correct species identification for mixed BFs were 25% (53) (95% CI: 24.94-25.06) and it was a very minimum diagnostic result compared to other species (See Table 2).

Table 2. *Correct species identification performance of laboratory professionals in Addis Ababa.*

Species	Total BF Examined	Correctly Identified	Percent correctly Identified (95% CI)
Plasmodium Falciparum	838	510	60.9% (60.76 - 60.96)
Plasmodium Vivax	627	371	59.17% (59.09 - 59.25)
Mixed (*Plasmodium Falciparum* & *Plasmodium Vivax*)	212	53	25% (24.94 - 25.06)
Negative	419	334	79.71% (79.65-79.77)
Total	2096	1268	60.5% (60.41 – 60.59)

3.4. Misdiagnosis

Misdiagnosis is an error encountered by the laboratory professionals during MMD. There were three types of misdiagnosis; species misdiagnosis, false negative and false positive. In the study 39.5%(828) misdiagnosis were reported. The most frequent type of misdiagnosis was 40.09% (85) mixed species reported as *Plasmodium vivax* and 26% (218) *Plasmodium falciparum* as *Plasmodium vivax* (See Table 3).

Table 3. *Misdiagnosis type and performance of laboratory professionals in Addis Ababa.*

Misdiagnosis Type		Frequency	Percent Misdiagnosis
Species Misdiagnosis	Pf as Pv	218	26%
	Pf as Mixed	73	8.7%
	Pv as Pf	159	25.36%
	Pv as Mixed	52	8.29%
	Mixed as Pf	67	31.6%
	Mixed as Pv	85	40.09%
False Negative	Pf as Neg	37	4.4%
	Pv as Neg	45	7.18%
	Mixed as Neg	7	3.3%
False Positive	Neg as Pf	36	8.59%
	Neg as Pv	49	11.7%
	Neg as Mixed	0	0%
	Total	828	39.5%

Note: Pf – *Palasmodium falciparum*, Pv- *Plasmodium vivax*, Mixed – *Pf & Pv and* Neg – Negative for malaria parasite.

3.5. Sensitivity and Specificity

Sensitivity and specificity tells the ability of laboratory professionals to detect malaria parasites from positive blood films and absence of malaria parasite from negative blood films respectively. Trained laboratory professionals had better performance of sensitivity and specificity 97.38 *(95% CI: 94.49-100)* and 90.79 *(95% CI: 83.97 – 97.43)* respectively (See Table 4).

Table 4. *Sensitivity and specificity performance of laboratory professionals in Addis Ababa.*

Category		Number of Lab Professionals	Total BF Examined	Sensitivity *(95% CI)*	Specificity *(95% CI)*
Training Status	Trained	43	430	97.38 *(94.49 - 100)*	90.79 *(83.97 - 97.43)*
	Untrained	168	1666	94 *(92.05 – 95.95)*	76.88 *(71..59 - 82.17)*
Education Level	Bsc	80	791	97 *(95.19 - 98.81)*	86.71 *(80.37 - 93.03)*
	Diploma	131	1305	93.3 *(72.88 - 77.72)*	75.48 *(69.44 - 81.52)*
Service Year	≥5 Years	94	931	95.84 *(93.7 - 97.98)*	84.95 *(78.98 - 90.92)*
	<5 Years	117	1165	93.78 *(91.32- 96.24)*	75.54 *(69.07 - 82.01)*

3.6. Factors Affecting Performance of Malaria Microscopy Diagnosis

Binary logistic regressions analysis was done by taking percent agreement as dependent variable (outcome) and sex, age, service year, training on malaria laboratory diagnosis, educational level and marital status taken as independent variables. Factors which show statistical significance by binary logistic regression were training on malaria laboratory diagnosis and educational level with P-value < 0.05. In the multiple logistic regression analysis training was the only factor which shows statistical significance with p - value of 0.01. Trained laboratory professionals had better performance than untrained laboratory professionals. Untrained laboratory professionals were 64% less likely to perform ≥ 85% species identification agreement for *Plasmodium falciparum* (AOR - 0.36 (95% CI: 0.16-0.78)) (See Table 5).

Table 5. *Multiple logistic regression analysis for factors affecting species identification percent agreement performance of Laboratory Professionals.*

Factors	Species Identification Percent Agreement for Pf		COR (95%CI)	AOR** (95%CI)	P-value*
	<85%	≥85%			
Sex					
Female	75	23	0.89(0.47-1.67)	0.90(0.46-1.78)	0.77
Male	84	29	1	1	
Age					
20-27	105	34	0.97(0.50-1.88)	1.53(0.68-3.46)	0.31
28-54	54	18	1	1	
Service Year					
<5 years	94	24	0.59(0.32-1.11)	0.73(0.33-1.60)	0.43
≥5 years	65	28	1	1	
Training on MLD					
No	135	33	0.31(0.15-0.63)	0.36(0.16-0.78)	0.01
Yes	24	19	1	1	
Educational Level					
Diploma	105	25	0.48(0.25-0.90)	0.66(0.33-1.34)	0.25
BSc and Above	54	27	1	1	

Key: * Significance for p ≤ 0.05 ** Adjusted for Sex, Age, Service year, Training on MLD and Educational Level.

4. Discussion

The study identified that, MMD performance of laboratory professionals in Addis Ababa were 91.7% (95% CI: 89.96 – 93.44) agreement for detection of malaria parasite and *67.63% (95% CI: 64.91 – 70.35)* species identification agreement for *Plasmodium falciparum. In addition,* 67 (31.6%) mixed BFs diagnosed as *Plasmodium falciparum*, *218*(26%) *Plasmodium falciparum* BFs as *Plasmodium vivax and 11.7% (49)* negative BFs were diagnosed as *Plasmodium vivax*. Detection was higher (percent agreement - 91.7%) than identification (species identification percent agreement for *Plasmodium falciparum- 67.63%).* So the study identified that there was higher performance problem on species identification.

Misdiagnosis results cause provision of incorrect anti-malaria medication for the patient due to species misdiagnosis and false positive results. Moreover, false negative results like *Plasmodium falciparum* were reported as negative in 4.4% (37) and mixed as negative in 3.33% (7) BFs cause malaria infected patients might not getting proper medication, worsen the disease condition of the patient and facilitate further transmission of malaria parasite in the community. Similar condition indicated from the study conducted in Uganda on malaria laboratory diagnosis and treatment [12, 13].

Misdiagnosis were higher among non-trained than trained laboratory professionals. From the study conducted on misclassification of plasmodium infection by microscopy, post training performance of malaria parasite species

identification was increased compared to pre-training. This showed that training brought a remarkable change in species identification [15]. Trained laboratory professionals' performance was better than non-trained laboratory professionals on MMD. This was also seen in other studies on MMD performance assessment [6, 7, 8, 9, 10, 11]. From the study conducted in Kenya misdiagnosis were reduced by providing training, sensitivity improved by a mean of 14% (CI 9–19%) from 77% baseline (CI 73–81%), while specificity improved by a mean of 17% (CI 11–23%) from 76% (CI 70–82%) baseline [8]. In addition, from the study conducted in Uganda, after training sensitivity improved from 84% to 95% and specificity improved from 87% to 97% [14, 15]. Similarly, the study identified that trained laboratory professionals had higher sensitivity and specificity (97.38% & 94%) than untrained laboratory professionals (90.7% & 76.88%) respectively.

The quality of BF preparation was one factor for the performance improvement of MMD [14, 15]. The recommended BF preparation for diagnosis of malaria parasite is doing both thin and thick BFs on the same slide using 2µl and 6µl whole blood respectively. The study was identified 31.8% (67) laboratory professionals were prepared only thin BF, 34.6% (74) only thick BF and 33.6% (71) thick and thin BF on the same slide. Moreover, BFs performed by 89.1% (1880) laboratory professionals were not met the quality of good BF for MMD.

Internal quality control, used to check the quality of giemsa stains, was performed by 40.3% (85) laboratory professionals. In addition, only 8.5% (18) laboratory professionals were participated in EQA, which is a very minimal number. EQA for malaria microscopy diagnosis used to identify deficiencies and take corrective action. It is the key for the performance improvement of the accuracy and reliability of reported results in MMD. Moreover, EQA should be supported by the availability and usage of quality reagents and documents like standard operating procedures, guidelines and job aids [16, 17, 18, 19, 20].

5. Conclusions and Recommendation

Misdiagnosis is the key cause for misusage of anti-malaria medications, to become worse the disease condition of malaria infected patients and allow further transmission of the parasite in the community. Misdiagnosis of malaria parasites was higher among untrained than trained laboratory professionals. Training on MMD and adherence to the standards improve performance of laboratory professionals in order to obtain accurate and reliable results. This will bring remarkable change to provide the right and recommended anti-malaria medication for better management of malaria parasite infected patients, minimize further transmission of the plasmodium species in the community and identify patients not infected with malaria parasites in order to consider other febrile illnesses differential diagnosis and treatment.

Abbreviations

BF - Blood Film
EQA - External Quality Assessment
MLD - Malaria Laboratory Diagnosis
MMD - Malaria Microscopic Diagnosis
RDT - Rapid Diagnostic Test
WHO – World Health Organization

Authors Contributions

LD being the principal investigator, developed the study protocol & tools, done the data management and wrote the first draft manuscript. HN assisted with the study design and protocol development, and helped to draft the manuscript. LD and HN contributed to data analysis and interpretation. Both authors read and approved the final manuscript.

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Ethics Approval and Consent to Participate

The study had ethical approval from University of Gondar and Addis Continental Institute of Public Health ethical review board. Permission were asked and permitted from Addis Ababa Regional Health Bureau in order to conduct the study based on the ethical review board approval. In addition, written consent was obtained from all study participant laboratory professionals.

Consent for Publication

Not applicable.

Availability of Data and Material

All data and materials used for the study available and kept in safe place confidentially.

Competing Interests

The authors declare that they have no competing interests

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