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The yield of Auramine O staining using led microscopy with bleach treated sputum samples for detection of pulmonary tuberculosis at St. Peter tuberculosis specialized hospital, Addis Ababa, Ethiopia

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ABSTRACT

Background: Smear microscopy is the mainstay for diagnosis of Tuberculosis (TB) in Ethiopia. This technique; however, is insensitive to detect Mycobacteria from most clinical specimens. Currently, light emitting diode (LED) fluorescence microscope is advocated to be used in high Tuberculosis (TB) burden settings by World Health Organization (WHO). However, the utility of this method is not evaluated for bleach treated sputum samples in Ethiopia.

Objective: The objective of the study is to evaluate the diagnostic importance of Auramine O (AO) staining in direct and concentrated sputum against conventional Zehil-Neelsen (ZN) and culture from the sputum samples of suspected pulmonary tuberculosis patients.

Methods: A cross-sectional study was conducted on 346 adult new pulmonary TB suspected patients at St. Peter's Specialized Hospital, Addis Ababa, Ethiopia. Three sputum samples (spot-morning-spot) were collected in sterile cups for direct Zehil-Neelsen and AO staining. Morning sputum samples were used for Mycobacterial culture on Mycobacterial Growth Indicator Tube (MGIT) 960. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were evaluated against the gold standard culture method. Data were analyzed using STATA version 13.0. All statistical tests were considered as statistically significant if the two sided P-value was < 0.05.

Results: Bleach treated sputum samples with AO staining yielded more cases as compared to direct ZN and direct AO by 6.3% and 11.5%, respectively. The sensitivity of concentrated AO and direct AO were remarkably high as compared to conventional ZN (71.8% vs. 44.5% and 62.7% vs. 44.5%). The concentrated sputum with staining of AO had a high rate (18.6%) of detecting scanty graded smears as compared to conventional ZN method.

Conclusions: Our findings indicated that the concentrated sputum with AO staining yielded high rate of sensitivity (71.8%) as compared to the conventional ZN method (44.5%). Moreover, the concentrated sputum with AO staining had superior ability in detecting scanty graded smears compared to the conventional ZN method. Therefore, it is recommended to utilize AO staining with LED microscopy for better diagnosis of Acid Fast Bacilli (AFB) from TB suspected cases and patients with pauci-bacillary TB in Ethiopia.

1. Background

Globally, TB (Tuberculosis) is a curable infectious disease which remains to be the leading cause of morbidity and mortality. Mycobacterium tuberculosis (M. tuberculosis) complex (including M. bovis, M. africanum, M. canetti and rarely M. microti) are among the dominant species which cause severe illness [1–3].

Regardless of achieving a treatment success rate of all forms of TB (>90%), one-third of the cases remain undetected [4]. Even though advancement in TB diagnostics had existed, only 57% of pulmonary

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cases were reported to WHO as bacteriologically confirmed TB cases. Early, accurate and rapid diagnosis of TB is critical for reducing TB transmission and incidence rates [5]. Smear microscopy is the sole method used for TB diagnosis in most laboratories in developing countries. It relies exclusively on the detection of AFB in sputum or other body fluids since its first discovery. However, the greatest challenge in establishing quality of TB diagnosis by sputum microscopy is its low sensitivity [6]. The sensitivity of sputum microscopy with staining ZN ranges from 18% up to 43% for a single smear as compared to gold standard culture [7–8]. A given ZN stain could reveal AFB only if and only if the sample contains greater than 10,000 bacilli per mL. The sensitivity of smear microscopy is even lower in pediatric and Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Diseases Syndrome (AIDS) patients who usually present as pauci-bacillary form [9–11].

Culture is a more sensitive method for TB diagnosis as compared to smear microscopy which is essential to test for drug resistance; however, it has its own limitations as it requires bio-safety facilities and additional costs. Additionally, culture can take weeks to reveal the results because of the slow growth rate of TB bacilli [12].

So far, efforts have been made to maximize the yield and sensitivity of smear microscopy which change in specimen collection, processing, and techniques [5,7,13]. Thus, developing countries, which have a high load of TB cases and financial constraints, need to have rapid and inexpensive diagnostic methods which could have great diagnostic importance. Sputum samples which have been treated with sodium hypochlorite (NaOCl) and stained with ZN have an increased level of sensitivity for direct microscopic technique [7,14,15].

Ultimately, to the best of our knowledge, the study is different from other studies due to the fact that it compares simultaneously direct smear, concentrated smear samples and culture for evaluating the validity of diagnostic techniques. Moreover, limited information exists in regards to the use of sodium hypochlorite concentration and staining with AO by using LED microscopy for addressing the gaps in lower case detection of pulmonar TB in Ethiopia. Therefore, the objective of study was to evaluate the diagnostic importance of AO staining in direct and concentrated sputum against conventional ZN and culture from the sputum samples taken from pulmonary tuberculosis suspected patients.

2. Materials and methods

2.1. Study design, area and population

A hospital based cross-sectional study was conducted at St. Peter TB Specialized Hospital from May 2013 to December 2015. The hospital is a public facility under the Ethiopian Federal Ministry of Health (FMOH) which is situated in Addis Ababa, the capital city of Ethiopia [4]. It is a TB referral center for Addis Ababa and other regional states. The hospital gives regular health services for inpatient and ambulatory patients through providing TB diagnosis, treatment and monitoring services.

2.2. Inclusion of the study subject

New pulmonary TB suspects, who fulfilled TB symptoms and had a productive cough for two weeks or more, were included.

3. Data collection methods

3.1. Socio-demographic and clinical information

Semi structure questionnaire was prepared to collect socio-demographic and clinical data by the attending physician for all patients who have fever, night sweats, productive cough, loss of weight and appetite, chest pain and hemoptysis. The physicians were oriented about the questionnair and supervised by the principal investigator.

3.2. Chest radio graphy

Chest radiography was taken for 85% of suspected cases in anterior-posterior view by X-ray technician and were read and reported by the radiologist. The X-ray was reported as normal, upper lobe infiltrations (bi-lateral or uni-lateral right), cavitations, patchy and nodular shadows around the cavity.

3.3. Sputum collection and processing

Ten ml of sputum samples (spot specimen on the first day, one early morning and one spot specimen on the second day) were collected from each study subject in clean, sterile, leak-proof and wide-mouth containers. Two sets of direct smears (one for ZN and the other for AO staining) were prepared from purulent part of sputum on sterile and frosted end microscopic slide. Both smears were dried and heat fixed. For concentration method, about l-2 mL of sputum samples were transferred to 50 mL screw-capped Falcon tube and mixed with an equal volume of 5% of NaOCl. The mixtures were incubated at room temperature for 10 min and vortexed at regular intervals. Then, equal amount of distilled water was added and centrifuged at 3000 g for 15 min. The supernatant was discarded and the pellets were re-suspended in a few drops of the remaining fluid. Two sets of smears were prepared from fairly thick smears of suspended sediment. The smears were air-dried and heat fixed.

For all Mycobacterial culture, morning samples were used and processed. Equal amount of Sodium Hydroxide (NaOH) and N-Acetyl-L-Cysteine (NALC) solutions were added to a volume equal to the quantity of sputum in 50 mL Falcon tube and then vortexed and incubated for 15 min. The tube was filled with sterile Phosphate Buffer Solution (PBS) at pH 6.8 and concentrated by centrifugation at a speed of 3000 g for 15 min. The tubes were allowed to sit for 5 min to settle aerosols. The supernatant decanted and the sediment was re-suspended with 1–2 mL PBS.

3.4. Staining, examination and grading by ZN and AO methods

For ZN, the dried slides were placed on a staining rack and stained with 0.3% of carbol fuchsine, heated gently until steam rose, left for 5 min, washed with a gentle stream of water and flooded with 3% acid-alcohol for 1 min. Then, it was washed and flooded with 1% of methylene blue for 1 min. The slides were air dried and examined for the presence of AFB under oil immersion objective for viewing at least 100 fields. AFB is seen as bright pink to red, beaded or barred forms, whereas the tissues cells, debris and other organisms were stained blue. The results were graded and recorded as per the guidelines of International Union Against Tuberculosis and Lung Disease (IUATLD) scales. Smears were interpreted by two laboratory technologists, and all smear results were recorded before culture results were available. Similarly, for AO staining (both direct and concentrated), slides were placed on a staining rack and stained with AO solution for 20 min. At the same time, slides were rinsed with water and flooded with 0.5% of acid-alcohol for 3 min following rinsing with water and counter staining with 1% of KMnO4 solution for 1 min. AO stained slides were examined by LED microscopy at 40 X objectives with a minimum of 40 fields. The tubercle bacilli were seen as yellow luminous organisms in a dark field, and the results were graded and recorded as per the guidelines of IUATLD scale.

3.5. Mycobacterial culture and examination

The processed sputum samples were inoculated into a Mycobacterial Growth Indicator Tube (MGIT) broth (which contained 7 mL of modified middle brook 7H9 broth base supplemented with 0.8 ml of a mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin). After that, MGIT broths were incubated at

37 °C in the BACTEC MGIT 960 system as described [16].

The plates were examined when MGIT broths flagged as positive at the end of the incubation period. All broth cultures, which were flagged by the MGIT 960 as positive, were removed and a portion was stained for the presence of AFB by using ZN method. Once confirmed as positive for AFB, the Capilia TB assay was performed for rapid identification of M. tuberculosis complex [17].

3.6. Data processing and statistical analysis

Data were analyzed using STATA Version 13.0. Descriptive statistics was used for computing continuous variables. Categorical variables were quantified by proportions. Sensitivity, specificity, positive predictive value and negative predictive value for the diagnostic tests were calculated against gold standard (culture). All statistical tests were considered as statistically significant if the two sided P-value was < 0.05.

3.7. Quality control

Internal Quality Control (IQC) was performed continuously to ensure precise and reliable operation by the BACTEC MGIT instrument tubes. Known TB positive and negative sputum samples were used for ZN and AO staining. Specimens were handled with extreme caution by adhering to procedures and recommendations for controlling excessive media contamination. Culture tubes were inspected after 24 h to identify contaminated tubes. MGIT tubes, which were identified as contaminated, were removed and the contents were transferred into 50 mL Falcon tube and decontaminated with 4% of NaOH solution. The procedure was repeated through inoculating into a fresh MGIT tube by placing it in the MGIT instrument and following for growth.

4. Results

4.1. Socio-demographic and clinical characteristics

A total of 346 pulmonary TB suspected patients were enrolled during the study period. The average mean value of age of the study subjects was 29.5 years within the range of 15–80 years. Male patients accounted 243(70.2%). The majority of TB suspected patients (32.3%) was within the age group of 25–34 years. Most of the suspected cases (301(87%)) were living in urban areas (Table 1).

Clinical signs and symptoms were collected from all suspected TB patients. Among these, 344(99.4%) had a productive cough. Chest pain was observed in 213(61.5%) of study participants and hemoptysis in 22(6.4%). Constitutional body symptoms such as night sweats occurred in 294(85%), weight loss 150(43.3%) and fever 323(93.3%) of the suspected cases.

4.2. Chest radiography findings

From the total TB suspected patients, 142(41%) of the cases had abnormal chest X-ray findings. The most common reported chest X-ray findings were cavities 69(20%), nodules 37(10.8%), upper lobe infiltrations in 14(4%) and patchy 6(1.7%). Among suspected TB patients, 33(9.6%) were diagnosed with pneumonia.

Among the 72 TB cases who were found positive by direct AO staining, 69 (20%) of them had abnormal X-ray findings. Similarly, among the 90 TB cases whose sputum were concentrated by bleach and stained by AO, 85(24.6%) of them had abnormal X-ray findings (Table 2) (Table 3).

4.3. Yield of pulmonary TB cases

Among the diagnostic tools, bleach treated sputum samples yielded more cases as compared to direct ZN and direct AO staining. Rate of detection by culture method was 110 (31.8%) among suspected cases. The detection rate by direct ZN method was lower (50(14.5%)) as compared with direct AO (72 (20.8%)) and concentrated AO method (90(26%)). The incremental yield of direct AO and concentrated AO methods against ZN method accounted 6.3% and 11.5%, respectively (Table 1).

4.4. Performance of diagnostic tools

The performance of different diagnostic tools against culture method (gold standard) for TB diagnosis is presented in Table 4. The sensitivity and specificity of direct ZN method was 44.5% and 99.6%, respectively. The sensitivity of direct AO was 62.7% which produced high yield by 18.2% as compared to direct ZN method. The specificity of direct AO (98.7%) was comparable with the specificity of direct ZN (99.6%).

Concentration of sputum by bleach with AO staining had further increased the sensitivity up to the level of 77.3%; this showed an increase in sensitivity by 32.8% and 14.6% as compared to direct ZN and AO staining, respectively. The specificity of concentrated sputum was 97.8%; this was comparable with direct ZN (99.6%) and AO staining (98.7%).

The agreement of direct ZN, direct AO and concentrated AO against the gold standard was 95.8% ($K = 0.67$; $P = 0.000$). Similarly, the agreement between concentrated sputum with AO staining against gold standard was 91.8% ($K = 0.73$; $P = 0.000$) (Table 4).

4.5. Grading of AFB smear by different staining techniques

Smears with scanty results were highly (18.6%) detected by concentrated sputum which were stained by AO as compared to direct ZN (8%) and AO (15.3%) (Table 5). Smears, which were graded as +1, constituted of 36%, 34.7% and 15.2% for direct ZN, direct AO and concentrated AO staining, respectively. While, smears which were graded as +2, were made up of 24%, 27.8% and 26.7% for direct ZN direct AO and concentrated AO staining, respectively. Almost 40% of concentrated AO stained smears were graded as +3 followed by direct ZN (32%) and direct AO (22.2%) (Table 5).

5. Discussion

In developing countries, direct ZN staining is the mainstay of TB diagnosis and a key technique in Directly Observed Treatment Short-course (DOTS) strategy. However, this technique is challenged by its very low sensitivity. To detect AFB by this technique, its concentration should be presented at least 5000 bacilli/mL in a given the sputum sample [18]. Alternatively, culture is the most sensitive method and is the gold standard for the detection of M. tuberculosis complex which could detect up to 100 bacilli/mL of sputum [19]. Unfortunately, this technique can take several weeks to yield the results. Moreover, the routine implementation of culture is costly and technically demanding in resource-limited countries. Therefore, there is a need for a simple and inexpensive staining technique, which provides higher yield and valid alternative methods as compared with the existing techniques.

On the other hand, WHO recommends the use of the GeneXpert which is an automated cartridge-based molecular test, as a primary test to increase TB detection and improve diagnosis of rifampicin (RIF) resistance in pulmonary and extra-pulmonary TB (EPTB) specimens. However, the GeneXpert sensitivity for TB detection is inadequate when few bacilli are present in the specimens, particularly in vulnerable groups such as HIV infected patients, children and in extra-pulmonary TB samples [1].

The sensitivity of direct staining with AO (62.7%) and the concentrated sputum by bleach (71.8%) had higher sensitivity in detecting AFB as compared to the conventional ZN (44.5%) technique. A similar study which was conducted in Tanzania (8) revealed that AO stained

Table 1
Socio-demographic characteristics, clinical findings and yield of AFB results by different diagnostic tools among TB suspected cases at St. Peter specialized Hospital, 2015, Addis Ababa, Ethiopia.

Variables		Yield of AFB			
		ZN (N = 50)(%)	Direct AO (N = 72)(%)	Concentrated AO(N = 90)(%)	Culture (N = 110)(%)
Sex					
Male (n = 243)		38(76)	54(75%)	78(86.7)	82(74.5)
Female (n = 103)		12(24)	18(25)	22(24.4)	28(25.5)
Total		50	72	90	110
Age groups (in years)					
15–24		13(26)	22(30.6)	24(26.7)	30(27.3)
25–34		15(30)	23(31.9)	26(28.9)	34(30.9)
35–44		5(10)	7(9.7)	13(14.4)	14(12.7)
45–54		5(10)	7(9.7)	9(10)	12(10.9)
55–64		7(14)	9(12.5)	13(14.4)	15(13.6)
>65		5(10)	4((5.6)	5(5.6)	5(4.5)
Total		50	72	90	110
Clinical presentations					
Fever	Yes	41(82)	62(86.1)	76(84.4)	99(90)
	No	1(2)	2(2.8)	0	1(0.9)
Chest pain	Yes	40(80)	60(83.3)	72(80)	97(88.2)
	No	2(4)	4(5.6)	4(4.4)	3(2.7)
Hemoptysis	Yes	6(12)	10(13.9)	10(11.1)	15(13.6)
	No	36(72)	54(75)	66(73.3)	85(77.3)
Night sweat	Yes	42(84)	64(88.9)	76(84.4)	99(90)
	No	0	0	0	1(0.9)
Weight loss	Yes	36(72)	54(75)	63(70)	80(72.7)
	No	6(12)	10(13.9)	13(14.4)	20(18.2)

N = Number; AFB= Acid Fast Bacilli; ZN = Ziehl-Neelsen; AO= Auramine O.

with direct and bleach concentration had higher sensitivity as compared with conventional ZN staining.

Our findings suggested that the optimum detection of AFB was best achieved by the application of AO staining followed by examination with LED microscopy. The use of LED microscopy greatly improves the diagnostic value of the sputum smear especially in patients with a low density of bacilli which are likely to be missed on ZN stained smears. LED microscope has several advantages over the existing conventional microscope, and it is currently being advocated for by WHO to be evaluated and used in TB high burden settings [20]. Another advantage of AO staining by using LED microscopy is that its simplicity as compared to ZN staining. Furthermore, the biggest advantage of using LED microscopy is that the smear can be examined at a lower magnification as compared to conventional ZN (40 X vs. 100 X). This could decrease the time for examination of slides by 75% as compared to ZN method [21]. Hence, this microscope would have tremendous benefit for overburdened laboratory systems in many low resource settings.

Processing of sputum samples by sodium hypochlorite with centrifugation could lead a higher yield by concentrating the bacilli. Moreover, the concentration of samples is important in special scenario such as smear negative TB cases with high clinical index suspicion and HIV positive cases. Similarly, a review conducted on sputum processing methods of 14 studies (culture used as the reference standard) showed the importance of sputum processing by centrifugation [20], which increased the yield of sensitivity by 18% as compared with direct ZN method. The major advantage of concentration of sputum by sodium hypochlorite is to obtain the higher density of bacilli per field and leaving free field for bacterial detection by reduction of debris [22]. This facilitates the examination of the slides and decreases the time required for detection. Sodium hypochlorite is cheap and available as household bleach, and also inactivates HIV and M. tuberculosis which could reduce the rate of nosocomial infections in laboratory workers [23].

The study also showed that the sensitivity of AO staining method was remarkably high as compared to the sensitivity of conventional microscopy (ZN) in diagnosing pulmonary tuberculosis. Moreover, sputum concentration followed by AO staining significantly increased the detection rate of AFB from 44.5% up to 71.8%. This study also

Table 3
Detection rate of pulmonary tuberculosis by different diagnostic tools.

Diagnostic tools	Outcomes	Frequency (%)	Detection rate	95% CI
TB culture	Positive	110(31.8)	31.8%	27.2 – 36.4
	Negative	236(68.2)		
Direct ZN	Positive	50(14.5)	14.5%	11 – 17.9
	Negative	296(85.5)		
Direct AO staining	Positive	72(20.8)	20%	16.5 - 24.9
	Negative	274(79.2)		
Sputum concentration and staining with AO	Positive	90(26)	26%	20.2 – 29.5
	Negative	256(74)		

TB= Tuberculosis; CI= Confidence Interval; ZN = Ziehl-Neelsen; AO= Auramine O.

Table 2
Chest radiograph findings in pulmonary tuberculosis suspected patients (N = 346).

Variables	DAO			CAO		
	Positive (%)	Negative(%)	Total (%)	Positive (%)	Negative (%)	Total (%)
Abnormal	69(19.9)	3(0.9)	72(20.8)	85(24.6)	5(1.5)	90(26)
Normal	73(21.1)	201(58.1)	274(79.2)	57(16.4)	199(57.5)	256(74)
Total	142(41)	204(59)	346(100)	142(41)	204(59)	346(100)

DAO = Direct Auramine O staining; CAO= Concentrated with bleach and stained by AO.

Table 4
Comparison of direct and concentrated AO stained smears against ZN staining and culture method (N = 346).

Diagnostic tools	Outcomes	Culture		Total	Culture as gold standard
		Positive (N = 110)	Negative (N = 236)		
Direct ZN (N = 50)	Positive	49	1	50	Sn = 44.5%, Sp = 99.6%
	Negative	61	235	296	K = 0.51 (95% CI = 0.41 – 0.61)
Direct AO (N = 72)	Positive	69	3	72	Sn = 62.7%, Sp = 98.7%
	Negative	41	233	274	K = 0.67 (95% CI = 0.58 – 0.76)
Concentrated AO (N = 90)	Positive	85	5	90	Sn = 77.3%, Sp = 97.8%
	Negative	25	231	256	k = 0.73 (95% CI = 0.64 – 0.80).

ZN = Zehil-Neelsen; AO = Auramine O; Sn = Sensitivity; Sp = Specificity; K = kappa value; CI = Confidence Interval; N = Number.

Table 5
Distribution of quantified smear results by different techniques according to (IUATLD/WHO) scale.

Grades	Staining techniques (%)		
	Direct ZN (N = 50) (%)	Direct AO (N = 72) (%)	Concentrated AO (N = 90) (%)
Scanty	4(8)	11(15.3)	18(20)
+1	18(36)	25(34.7)	15(16.7)
+2	12(24)	20(27.8)	23(25.5)
+3	16(32)	16(22.2)	34(37.8)
Total	50(100)	72(100)	90(100)

ZN = Zehil-Neelsen; O = Auramine O; N = Number.

revealed that the specificity of the AO staining was not changed as compared to ZN method regardless of sodium hypochlorite concentration. A similar study by Bahado et al. (2006) reported that sputum concentration and staining by AO demonstrated higher sensitivity as compared to conventional ZN method [24]. Likewise, a review done by Steingart et al. (2006) also indicated that using AO staining increases the sensitivity of AFB detection as compared to conventional ZN method without apparent loss of its specificity [20].

According to IUATLD/WHO scale, quantification of smear results indicated that smear results with scanty grade were more prevalent in AO staining as compared to direct ZN method. Smears graded as scanty were more prevalent in concentrated smears stained with AO (18.6%) as compared to the conventional ZN method. Similarly, 92% of these scanty results were positive by the reference culture method. A study conducted in Kenya revealed that 15% of the positive smears were missed by using ZN stained method, which contained low density bacilli [25]. Besides, study other study indicated that AO staining could pick up a greater number of pauci-bacillary cases as compared to ZN staining [26]. High number of scanty grade findings in the present study may be due to high number of TB and HIV co-infected patients.

The study also found that false positive results were reported in both directs 4(1.1%) and concentrated sputum 7(1.9%). This may be due to inorganic materials which absorb fluoro-chrome stains which may mistakenly be identified as AFB [27]. Other reasons may be related to the co-infected patients with HIV who are on INH prophylaxis, which may have adversely hindered the bacilli's ability to grow, or more importantly in this case, the bacilli may have been killed by the excessive decontamination procedure. Furthermore, certain non-tuberculosis organism may also be detected by AO staining [28,29].

This study had some limitation. The samples used for direct smear microscopy were not quantitated as it might increase the chance of the comparability againts the treated samples for LED microscopy.

6. Conclusions

The conventional diagnostic method which is being implemented for TB detection in Ethiopia is AFB staining with ZN. However, utilization of this method is compromised by lesser sensitivity while TB culture is considered applicable only in some regional laboratories and hospitals due to logistic and infrastructure barriers. This study indicated that direct and sputum concentration with AO staining yielded a high rate of sensitivity as compared to conventional ZN. Sputum concentration with AO staining is superior in detecting scanty graded smears as compared to conventional ZN method. Therefore, it is recommended to utilize AO staining with LED microscopy for better diagnosis of Acid Fast Bacilli (AFB) from TB suspected cases and patients with pauci-bacillary TB in Ethiopia.

CRediT authorship contribution statement

Nebiyu Gizaw: Visualization, Writing - original draft, Formal analysis. **Adugna Abera:** Formal analysis, Writing - original draft. **Solomon Sisay:** Formal analysis, Writing - original draft. **Kassu Desta:** Formal analysis, Writing - original draft. **Saskia Kreibich:** Formal analysis, Writing - original draft. **Lisa Gerwing-Adima:** Formal analysis, Writing - original draft. **Solomon Gebre-Selassie:** Formal analysis, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no any competing interests.

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Ethic and consent

The research protocol was approved by Institutional Review Board (IRB) of Department of Microbiology, Immunology and Parasitology (DMIP), Addis Ababa University. Support letter was obtained from St. Peter Tuberculosis Hospital. The purpose of the study was clearly explained to each study participants. Written and oral consents were obtained from the subject prior to enrolment. The results of the study were communicated to the responsible physicians if they had been confirmed of having TB. Treatment was given free of charge for patients who were positive for TB as per the national treatment guideline for TB.

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