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Efficacy of Fluorescence Light Emitting Diode (LED) Microscopy for Detection of *Mycobacterium Tuberculosis* in Sputum of Patients Attending a Tertiary Hospital in Osogbo Osun State, Nigeria

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ABSTRACT

For developing countries with a large number of cases and financial constraints, evaluation of rapid and inexpensive diagnostic methods has a great importance for early diagnosis of tuberculosis in order to control the spread of infection. This study was carried out to compare the efficacy of fluorescence Light Emitting Diode (LED) microscopy to bright field microscopy. Three hundred and four (304) sputum samples were collected from suspected cases of Pulmonary Tuberculosis from March, 2015 to February, 2016. These samples were processed and subjected to Auramine-O (AO) and ZN staining for detection of TB as described. Positive smears were graded according to International Union against Tuberculosis and Lung Disease and World Health Organization (IUATLD/WHO). Out of 304 sputum smears, the overall prevalence of *M. tuberculosis* was 8.6%with statistical significant difference (P=0.011) Males had higher prevalence rate of *M. tuberculosis* 7(4.6%) than female 6(3.9%) among 152 patients. Age group 31-45yrs had prevalence rate of 6(3.9%). Bight field and fluorescence microscopy detected 24 (7.9%) and 26 (8.6%) respectively with significant difference (DF = 4, X^2=296.490, P=0.000). This study showed that two positive samples were missed on ZN staining but found to be positive with Auramine O staining. Sensitivity and specificity of fluorescence microscopy were 94.2% and 89.5% while Bright field microscopy had sensitivity and specificity of 91.7% and 86.8% respectively. Reliability of microscopist using Crobauch Alpha was 0.753 (75.3%). Fluorescence microscopy has a better diagnostic value in detecting TB cases and is less time-consuming compared to Bright field microscopy in diagnosing tuberculosis.

KEYWORDS: Tuberculosis, Microscopy detection, Prevalence; Ladoke Akintola University of Technology Teaching Hospital.

1. Introduction

Pulmonary tuberculosis is a lung disease in human caused by a single infectious agent called *Mycobacterium tuberculosis* from inhalation of air droplets. It is estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* with three million people dying from it (Rakesh and Sanda, 2015). It is commonly found in sub-Saharan Africa and Asia. The burden of *Mycobacterium tuberculosis* has been noted in Nigeria and ranks 4th among countries that has 80% of the world's TB cases (Pennap *et al.*, 2009). It shows in Nigeria that Tuberculosis is a major cause of health problem and single largest cause of loss of working hour in productive age group.

Microscopy detection of pulmonary tuberculosis in developing countries with a large number of cases and financial constraints, have been evaluated to be of rapid, simple, cheap, practicable, effective and inexpensive diagnostic methods for sputum examination over culture. This is because culture results are available after a period of three to six weeks because of slow growing of *Mycobacterium tuberculosis* (Vishnu *et al.,* 2014*)*. Microscopy methods of detecting Acid Fast Bacilli from smears made from sputum specimens are processed and subjected into two different staining methods (Ziehl-Nelson and Auramine O) Microscopic examination of Ziehl-Neelsen (ZN) or Auramine stained specimen allows detection of most strains in less than an hour but LED fluorescence microscopy by Auramine O staining method can be examined at lower magnification, lesser time than bright field microscopy by ZN staining (40x vs 100x) (Upasana and Gyaneshwari, 2014) as this will be an advantage to laboratory of low resource setting. This retrospective study was carried out to compare fluorescence microscopy to bright field microscopy.

2. Materials and Methods

The retrospective comparative study was carried out in the Department of Medical Microbiology and Parasitology, Ladoke Akintola University Teaching Hospital, Osogbo. Osun State, Nigeria. This study was carried out for a period of 12 months from March, 2015 to February, 2016. Patients of all age groups of both sexes suspected of pulmonary tuberculosis were included.

A total of 304 sputum samples were collected from 152 patients suspected of pulmonary tuberculosis. Two sputum samples were collected from each patient (one spot and one early morning sample) in clean, sterile, heat proof, wide mouth containers. The processing of samples was carried out in a bio safety cabinet for both Z-N staining and Fluorescence Auramine O stain. For Z-N stainig, direct sputum smear was spread evenly over the central area of the slide using a continuous rotational movement of about 20mm by 10mm. The slides were allowed to air dry for about 30 mins and then heat fixed the dried smears. The smears were covered with carbol fuchsin stain and heated until vapour began to rise. Then, heated stain was allowed to remain on the slide for 5 minutes and then washed off with clean water. The smears were then covered with acid alcohol for 2-5mins (3% acid alcohol) until the smears were sufficiently decolorized and then washed with clean water. The smears were then covered with methylene blue stain for 1-2mins after which the stained smears were washed with clean water, the back of the smears wiped and allowed to air dry. For Fluorescence Auramine O staining, air dried smears were flooded with auramine O for 15 minutes then rinsed with clean water. The stained smears were flooded with acid alcohol for 2 minutes, rinsed and then flooded with potassium permanganate for 2 minutes. The slides were then then rinsed with clean water and air dried. Each sample was then subjected to Z-N staining and Fluorescence Auramine O stain.

ZN stained smears were examined by bright field microscope using oil immersion objective (x1000), bacilli appear as pink coloured rods while Auramine O stained smears were examined by LED fluorescence microscope, using 40x objective (x400), bacilli appears bright, glowing yellow against dark background. All the stained smears were examined by trained scientist. The mean time taken to smear examination was approximately 3 minutes by Bright field microscopy while was only about a minute by LED Fluorescence microscopy. The results were graded into negative after examined 300 fields and positive which were recorded as scanty when 1-9 AFB per 100 fields were seen, 1+ when 10-99 AFB per 100 fields were seen, 2+ when 1-10 AFB per field at least in 50 fields, 3+ when more than 10 AFB per field at least in 20 fields which was according to International Union against Tuberculosis and Lung Disease and World Health Organization (IUATLD/WHO) (WHO, 2012).

3. Results

Out of 304 sputum samples obtained from 152 patients that were examined, 24 (7.9%) were positive for TB by Bright field microscopy while 26 (8.6%) were positive for Fluorescence microscopy. Significant statistical difference was observed between the two microscopy methods of detecting TB (DF=4, X^2=296.490, P=0.000) (Table 1).

Of the total number of patients, 73 (48.0%) were males while 79(52.0%) were females. Figure 1 showed that the overall prevalence of

Mycobacterium tuberculosis among patients was 13 (8.6%) of which 7 were males (4.6%) and 6 were females (3.9%). There was significant different between sexes and *Mycobacterium tuberculosis* (DF = 3, X^2 = 11.881, P=0.008).

Table1: Grading result of pulmonary tuberculosis based on WHO grading system (2012)

Grade of sputum smears	**Bright field microscopy**	**Fluorescence microscopy**
Negative	280	278
Scanty	Nil	2
1+	3	3
2+	6	6
3+	15	15
Total	304	304

(DF=4, X^2=296.490, P=0.000)

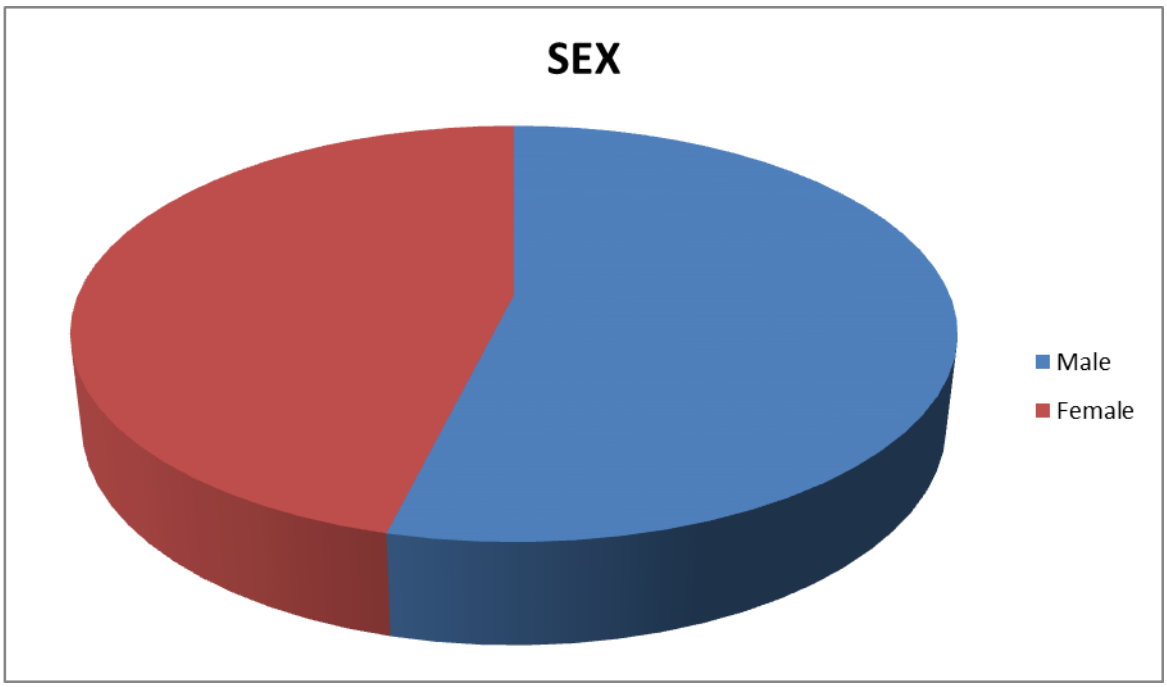

Figure 1: Occurrence of *Mycobacterium tuberculosis* between sexes (DF = 3, X^2 = 11.881, P=0.008)

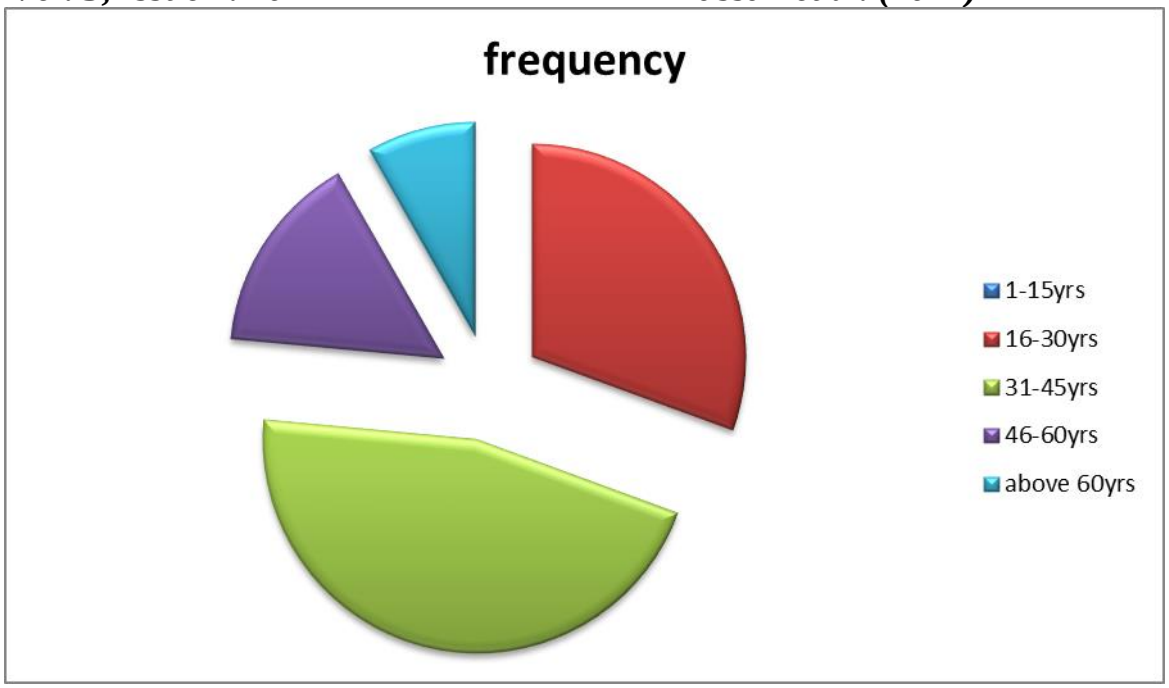

Figure 2: Distribution of *Mycobacterium tuberculosis* among different age groups (DF=8, X^2=3.873, P=0.874).

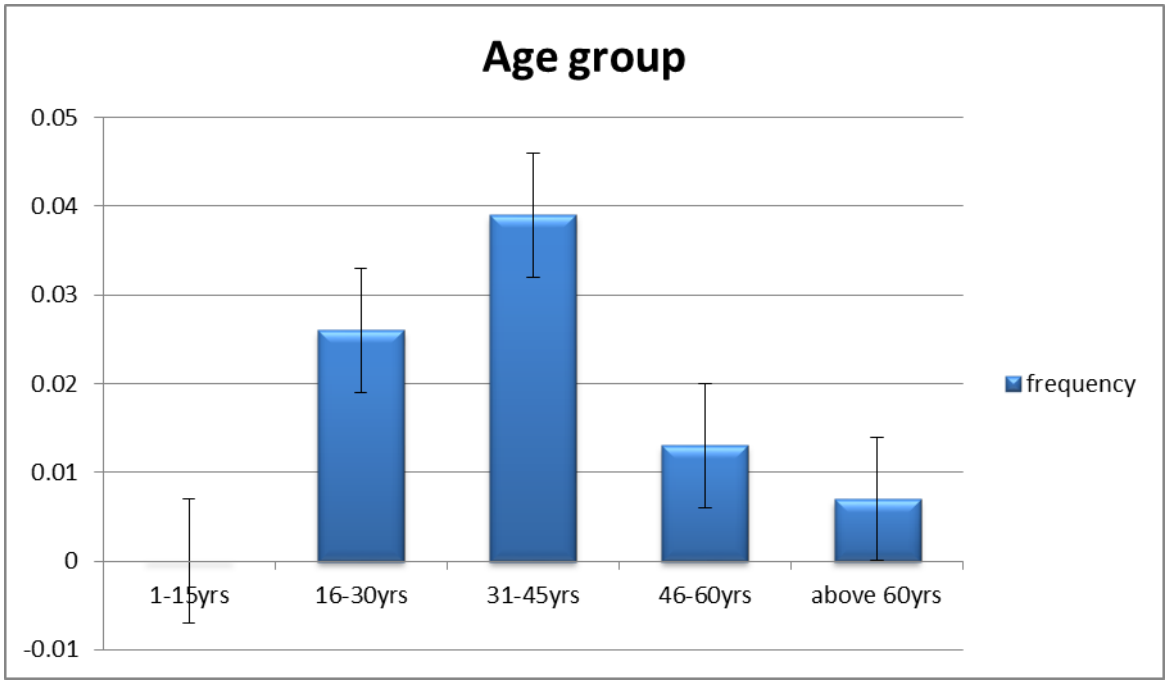

Figure 3: Detection of *Mycobacterium tuberculosis* by microscopy methods

There were more patients in the 31 to 45 years age group; 63 (41.4%) followed by age group 45 to 60; 40(26.3%) and age group 16 to 30; 39(25.7%) while age group 1 to 15 followed by age group 60 and above had less patients; 3 (2.0%) and 7 (4.6%) respectively. The age frequency distribution of *Mycobacterium tuberculosis* are shown in Figure 2 of which age group 31-45 had highest rate of infection 6(3.9%) followed by age group 16-30; 4(2-6%) then age group 46-60; 2(1.3%) while age group 60 years and above had 1(0.7%). *Mycobacterium tuberculosis* was not found in age group 1-15.There was no significant different among the age groups (DF=8, X^2=3.873, P=0.874).

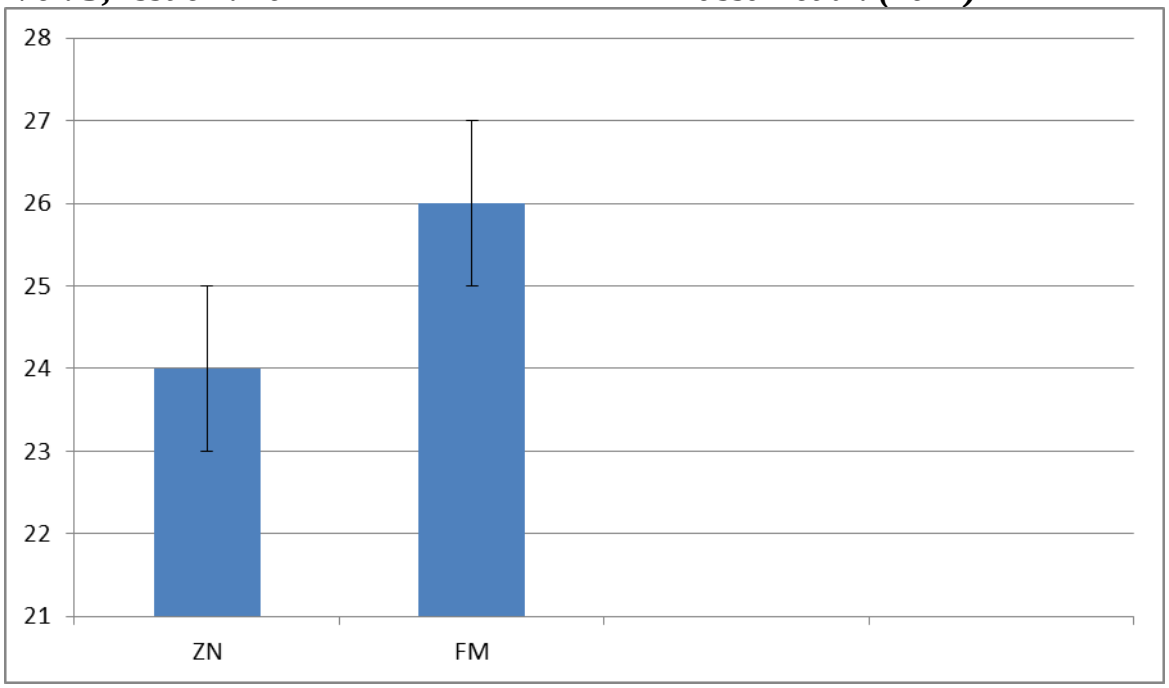

Figure 4: Fluorescence microscopy detected 26 Acid Fast Bacilli using Auramine O stain than Bright field microscopy which detected 24 Acid Fast Bacilli

Two positive samples were missed from Bright field microscopy which was found to be positive with LED fluorescence microscopy. Overall positivity thus increased by 2/304 using LED fluorescence microscopy (0.7%) over the bright field microscopy. There was better sensitivity and specificity of Fluorescence microscopy (94.2% and 89.5%) as compared with the Bright field microscopy (91.7% and 86.8%) in the detection of Acid Fast Bacilli in cases of pulmonary tuberculosis as shown in Table

5. Discussion

The overall prevalence rate of 8.6% *Mycobacterium tuberculosis* was obtained among suspected patients in LAUTECH Teaching Hospital, Osogbo, Osun State, Nigeria in this study. This prevalence rate in this study is higher than 6.9% obtained in Ibadan (Kehinde and Okesola, 2010) but lower than 14.4% obtained in the North-east of Nigeria by Zailani and his co-workers (Zailani *et al.,* 2012). This may be due to duration used for this study and extreme dry weather in northern part of the country which may encourage the spread and inhalation of the causative agent in that region than the south with humid climate. This present study indicated that Males are more infected with higher TB prevalence than their females counterpart with significant difference (DF = 3, X^2 = 11.881, P=0.008) between sexes. Naturally, males are exposed to occupations like okada riding (commercial motor cycling), motorist, carpentry and bricklaying among others that exposed them to dusts and associated pathogens inhalation in this part of country than their female counterpart which might be a reason for this outcome in the present work (Borglorff *et al.,* 2000; WHO, 2004). In addition, this higher rate could be exposure to risk factors such as HIV infection, malnutrition, low standard of living, alcohol intake, smoking and nature of their recreations that predispose the males to TB disease.

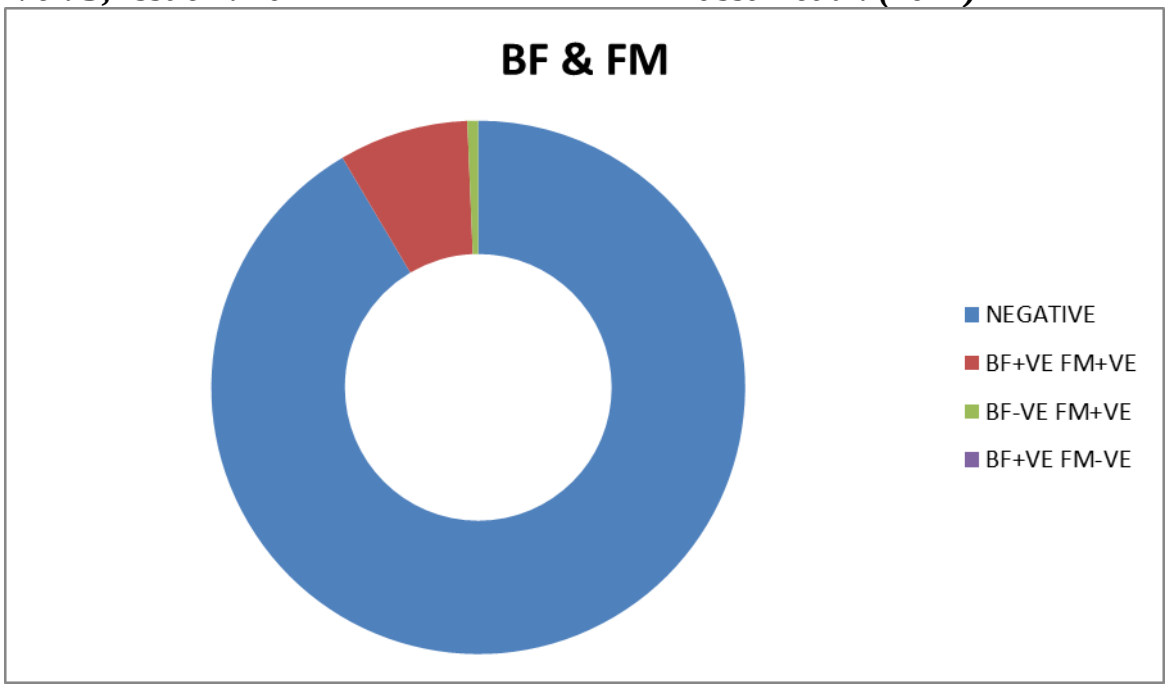

Figure 5: Fluorescence microscopy had more positive predictive value (PPV) than Bright field microscopy

Table 2: Sensitivity and Specificity of Bright field microscopy using Fluorescence microscopy as gold standard.

	Bright field microscopy (95% C.I)
Sensitivity	96%
Specificity	99.%
Positive Predictive Value	92.3%
Negative Predictive Value	99.6%

This study support the result obtained by Kehinde and Okesola (2010) who reported that males had higher prevalence than females and other earlier researchers (Ba and Rieder, 1999; Desai *et al.*, 2009; Ejikeme and Godwin, 2010; WHO, 2014). In contrast to study by Zailani *et al*. (2012) who found the prevalence rate of TB not to be statistically significant difference between sexes.

This study established that tuberculosis caused public health problem among productive age group who are more vulnerable to the tuberculosis infection due to lack of health education and poverty as a result of highest rate of infection obtained among age group 31-45years with no significant difference between age groups (DF = 4, X^2=0.893, P=0.926) for *Mycobacterium tuberculosis*, This finding was similar to the findings of several other workers (Singh and Parija, 1998; Prasanthi and Kumari, 2005; Uzoewulu *et al.*, 2014).

Based on this study, LED fluorescence microscopy is reliable and faster than Bright field microscopy in the diagnosis of pulmonary tuberculosis as it was depicted that LED fluorescence microscopy detected more acid fast bacilli than Bright field microscopy with statistical significant difference (DF = 4, X^2=296.490, P=0.000). This result was in accordance with other workers (Hung *et al.*, 2007; Hooja *et al.*, 2011; Khatun et al., 2011; Zailani *et al*., 2012; Upasana and Gyaneshwari, 2014).

LED fluorescence microscopy is more sensitive and specific (94.2% and 89.5%) in the detection of AFB in cases of pulmonary tuberculosis compared to bright field microscopy (91.7% and 86.8%) in the current work for this locality. This result may due to high detection of bacilli load 10^4 by fluorescence microscopy than bright field microscopy (10^5). This study is in accordance with the work carried out by other researchers (Small and Pai, 2010; Farjana *et al.*, 2011; Kumar *et al.*, 2012; Zailani *et al.*, 2012; Rakesh and Sanda, 2015).

We here by recommended that continuous surveillance of active tuberculosis should be sustained in the state and consolidated with replacement of Bright field microscopy with LED fluorescence microscopy in our peripheral laboratories, DOT clinics or centers.

6. Conclusion

Fluorescence microscopy has a better diagnostic value compared to bright field microscopy in detection of suspected cases of pulmonary tuberculosis in this study. LED fluorescence microscopy is less time consuming, sensitive and improved diagnostic value in patients with a low density of bacilli as observed in our study. We concluded that pulmonary tuberculosis is being actively transmitted in this locality and the use of LED fluorescence microscopy for prompt diagnosis will go a long way in arresting the spread of the disease among the populace.

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