ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 1, pp: (67-72), Month: January - March 2015, Available at: www.researchpublish.com

Diagnosis of Pulmonary Tuberculosis Using Conventional Smear Microscopy and Culture Methods in a Tertiary Care Hospital

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***Abstract*: Tuberculosis, one of the oldest diseases known to affect humans is caused by bacteria belonging to the Mycobacterium tuberculosis complex. Smear microscopy and culture forms the backbone of tuberculosis (TB) laboratory investigations in tertiary healthcare facilities which have a large number of cases and financial constraints. 1030 patients Sputum samples received in Microbiology laboratory in a tertiary care hospital during one year period was performed with Ziehl-Neelsen Acid Fast Staining, grading's were as per RNTCP grading. Among 1030 patients, 130(12.62%) were AFB smear positive. All positive sputum specimens were concentrated by using Petroff's method and inoculated in Lowenstein Jensen medium. The inoculated LJ medium was incubated at 37 C. The results were recorded. Mycobacterium tuberculosis and Non-Tuberculosis Mycobacteria were differentiated based on their colony morphology and other identification tests.**

***Keywords*: Sputum smears microscopy, LJ medium, Conventional diagnostic methods, Mycobacterium tuberculosis, Non-Tuberculosis mycobacteria.**

I. INTRODUCTION

Tuberculosis is a chronic infection caused by Mycobacterium tuberculosis. Infection commonly involves the lungs (Pulmonary TB), though almost any organ can be affected e.g. bones and joints, abdomen, brain, genitourinary system etc (Extra pulmonary TB). Tuberculosis is an important health problem and proper drug therapy is important for not only in the treatment, but also in the prevention of infection[(4).] TB can develop at any point during the progression of HIV infection. The presentation of pulmonary TB depends on the degree of immuno-suppression. At an early stage of HIV infection, the clinical picture resembles the disease in non-HIV-infected adult patient, with no difference in the frequency of smear positive findings. At a late stage of HIV infection the clinical picture resembles primary pulmonary TB: the sputum smear is negative and the chest radiography shows infiltrates without cavities. Cough and haemoptysis are less common in HIV patients than non-HIV patients [(3)]

The current laboratory diagnosis of TB relies on the microscopic identification of acid fast bacilli and the culture of Mycobacterium tuberculosis from various clinical samples although smear examination provides rapid detection its sensitivity is in general very low. On the other hand, cultures provide greatest sensitivity and definite diagnosis, but it takes about 3 to 8 weeks to get a final report and there are numerous examples of TB cases in which the various specimen culture fail to grow Mycobacterium tuberculosis. [(6)]

Currently many patients fail to be diagnosed because TB is not considered or skilled examination of sputum smears is delayed or not performed at all, despite patients repeated attendance at the health facilities.

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II. MATERIALS AND METHODS

Sample collection: Two sputum samples were collected, on-the-spot, early morning specimen from the TB suspected patient.

Preparation of smear: Label a new clean, unscratched slide at one end with the laboratory number. The blood –specked, opaque yellowish portion was used for smear preparation.by using 5mm internal diameter 24 SWG, over an area of approximately 2 by 1 cm. The smear is air dried and fixed by passing the slide through a flame three or four times with the smear uppermost.

Ziehl-Neelsen staining method:

Place the slides on a staining rack with the smeared side facing up. The entire slide was flooded with strong carbol-fuchsin. The slides were heated slowly until it was steaming. Maintained steaming for 5 minutes by using intermittent heating. The slide was rinsed in a gentle stream of running water until all free stain is washed away. Flood the slide with the acid alcohol (3%) as a decolourising agent for 2 to 3 minutes. Rinse the slide thoroughly with water. Drain excess water from the slide. Flood the slides with methylene blue counter stain for 30 seconds. Rinse the slide thoroughly with water, allowed the smear to air dry.

The results were recorded following semi-quantitative method recommended under RNTCP guidelines.

Sample processing for sputum:

To 5 ml of sputum added 10 ml of 4% sodium hydroxide. Allowed to stand for 15 mins at room temperature with occasional shaking. Centrifuged at 3000rpm for 15 minutes. Poured off supernatant. Added 20 ml sterile distilled water and re-suspend sediment. Centrifuged at 3000rpm for 15 minutes. Decant the supernatant and inoculate the deposit on to 2 slopes of Lowenstein Jensen medium.

Culture examination and identification:

All cultures incubated at 35 to 37°C, examined 48 to 72 hours after inoculation to detect gross contamination. The cultures were examined weekly up to 8 weeks. Culture reports recorded were qualitative (positive or negative) as well as quantitative.

Identification tests:

1. Susceptibility to p-nitro benzoic acid (PNB):

Inoculate with the neat bacterial suspension one slope of LJ medium and one slope of p-nitrobenzoic acid (PNB) at a concentration of 500µg/ml and incubate at 37^{o}C for each set. Read on 28th day. PNB should be kept for reading at 42 day.

2. Niacin test:

To the culture slant add 1ml of sterile distilled water or isotonic saline. Allow the sterile distilled water to remain in contact with the culture medium for 30 minutes at room temperature (20˚-25˚c).Remove the liquid extract from the culture into the sterile screw capped test tube. Centrifuge at 3000 rpm for 15 minutes to obtain a clear supernatant.0.6 ml of supernatant is transferred into another clean sterile test tube. Add one drop of potassium thiocyanate, citrate buffer, chloramine –T solution, para-amino benzoic acid and allow to stand on a test tube rack at room temperature. Observe the colour change from colourless to yellow using a white background. Record the readings immediately and definitely at 15 minutes.

3. Catalase activity at 68°C/pH 7:

A mixture of equal volumes of H_2O_2 and 2% catechol in distilled water is added to 5 ml of the test culture. The test tubes were allowed to stand for few minutes.

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III. RESULT

a) Ziehl-Neelsen staining, 130(12.62%) sputum samples were positive for Acid Fast Bailli among 1030 patients.

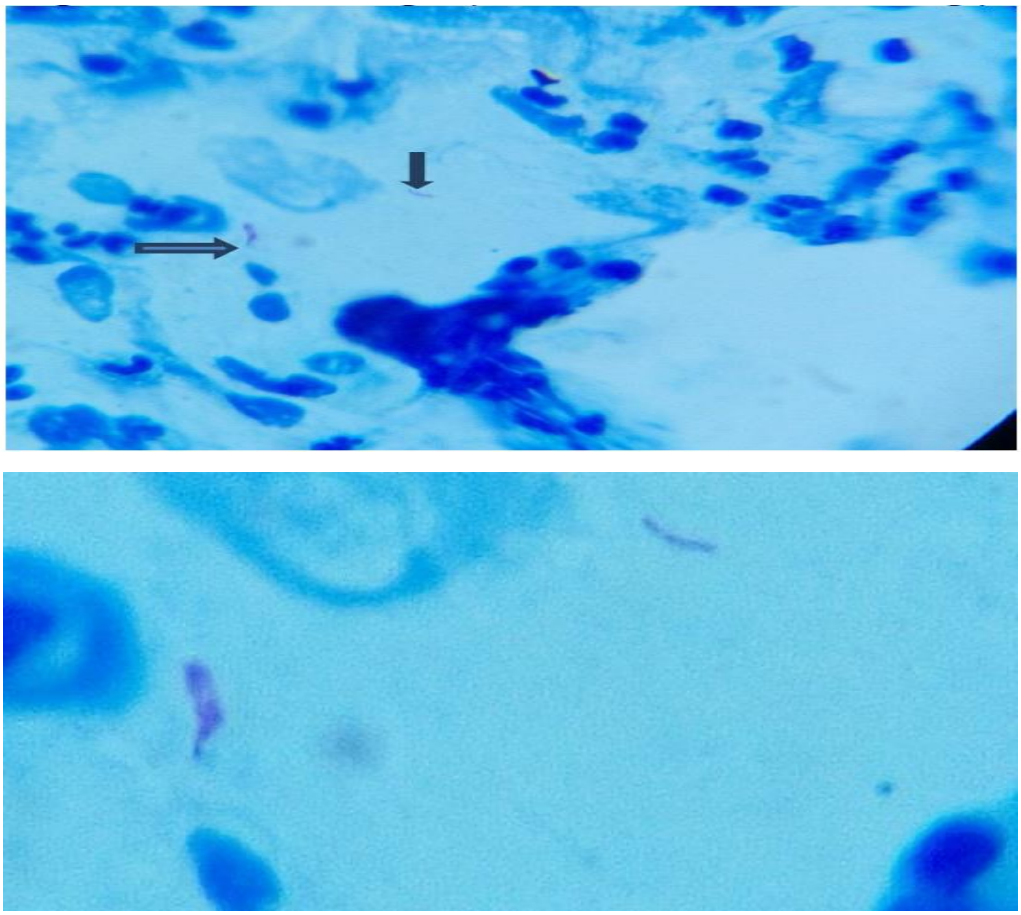

Figure 1: shows slightly curved rods occur singly or in pairs.

b) Culture in Lowenstein Jensen medium:

Among 130 smear positives 64 (49.23%) shows growth in LJ medium.

Based on colony morphology and identification tests,

- Mycobacterium tuberculosis (96.8%)
- Non-Tuberculosis mycobacteria, Photochromogens (3.12%) were identified.

Figure 2: Mycobacterium tuberculosis shows dry, rough, raised, irregular colonies with wrinkled surface.

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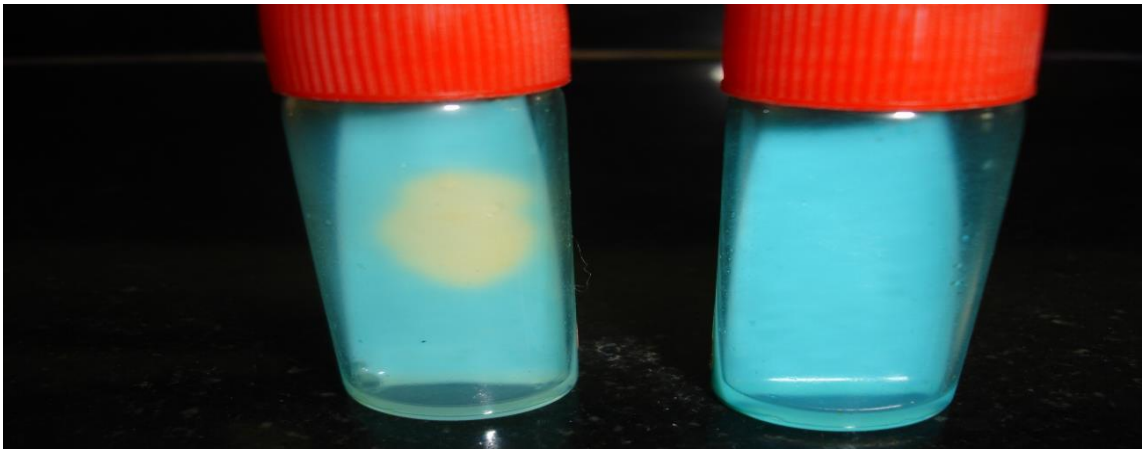
Figure 3: Non-Tuberculosis mycobacteria, Photochromogens shows smooth colonies with no pigment in the dark, become pigmented on exposure to light.

c) Identification tests:

Mycobacterium tuberculosis does not grow on PNB medium. All other mycobacteria are resistant to PNB.



Growth of Atypical mycobacteria No growth of Mycobacterium tuberculosis

Figure 4: Susce ptibility to p-nitro benzoic acid (PNB)

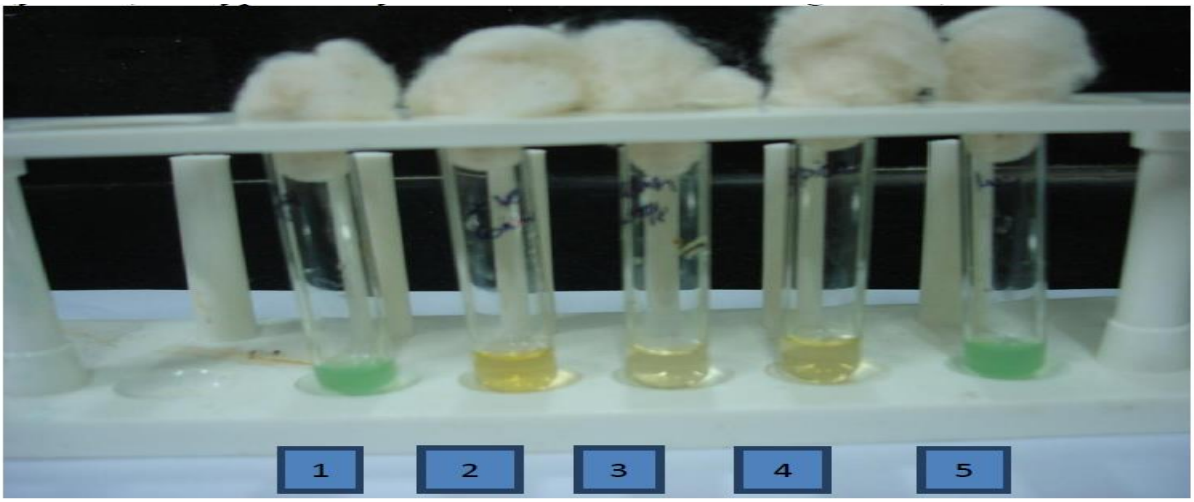


Figure 5: Niacin test: Mycobacterium tuberculosis is niacin positive (yellow), Atypical mycobacteria is niacin negative (no colour change)

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1. Atypical Mycobacterium
2. Mycobacterium Tuberculosis
3. Standard
4. Positive Control (H_{37} Rv Mycobacterium Tuberculosis)
5. Negative Control (Atypical Mycobacterium)

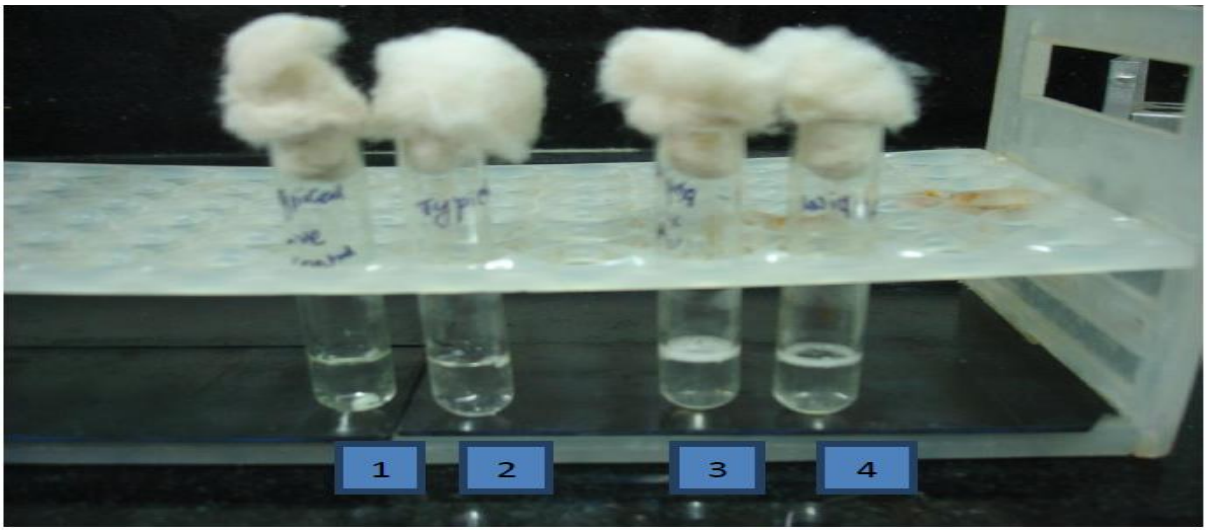

Figure 6: Catalase activity at 68°C/pH 7: Mycobacterium tuberculosis shows weakly positive, Atypical mycobacteria shows effervescence which is strong catalase production[(1)].

1. Control (Atypical Mycobacterium)
2. Test Strain (Atypical Mycobacterium)
3. Control (H_{37} Rv Mycobacterium Tuberculosis)
4. Test Strain (Mycobacterium Tuberculosis)

IV. DISCUSSION

Tuberculosis, one of the oldest diseases known to affect humans is caused by bacteria belonging to the *Mycobacterium tuberculosis complex*. The disease usually affects the lungs, although in upto one third of cases other organs are involved. If properly treated, virtually all cases tuberculosis caused by drug susceptible strains is curable. If untreated, the disease may be fatal within five years in more than half of the cases. Transmission usually takes place through the air borne spread of droplet nuclei produced by patients with infectious pulmonary tuberculosis[(2).] A widely used diagnostic tool for tuberculosis is Ziehl-Neelsen sputum staining, Sputum grading is a significant method of estimating the risk of transmission.

TB is a major public problem, majority of TB cases occur in low and middle income countries [(10)]. In high TB burden countries, infrastructure for the diagnosis is not adequate. ZN staining is the only diagnostic technique. In spite of new technologies such as TB culture, Line probe Assay and Gene X pert.

An estimated 1.1 million (13%) of the 8.6 million people who developed TB in 2012 were HIV-positive. About 75% of these cases were in the African Region. Globally in 2012, an estimated 450 000 people developed MDR-TB and there were an estimated 170 000 deaths from MDR-TB[(11)].

AFB microscopy is believed to be the most practical and fastest technique in establishing a diagnosis of pulmonary TB, especially in developing countries where most of the TB cases live[(7)].

Studies have shown that direct smear microscopy is highly specific in settings where TB is more prevalent (Albert, 2004; Suarez et al., 2002). Though AFB microscopy is simple, inexpensive and provides rapid result, it has some limitations. The threshold for detection of AFB in sputum samples under optimal conditions is between 10^4 and 10^5 bacilli per ml. The sensitivity and specificity of AFB microscopy is low when compared to culture method. Sensitivity is even more reduced if samples are of poor quality [(3,4)].

Microscopy clearly has many advantages when it comes to speed and feasibility, and if sensitivity could be improved it has the potential to become an even more valuable tool for National TB Control Programmes (NTPs) around the world. In the last decade many researchers have suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with chemical reagents and then concentrated by centrifugation or sedimentation prior to acid-fast staining [(6)].

In this Petroff's method has been found to increase the sensitivity of microscopy substantially [(8)]. However, it requires some level of staff training, increases time needed for diagnosis, and requires some level of biosafety arrangement to ensure the security of the lab personnel.

V. CONCLUSION

Smear AFB microscopy and the conventional culture methods for identification of Mycobacterium tuberculosis is feasible, the gold standard method for a tertiary care hospital to diagnose pulmonary tuberculosis. Smear Microscopy to start DOTS for treatment of the TB patients and to follow-up their course of treatment, culture isolate further used for drug sensitivity and also to detect MDRTB.

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