ORIGINAL RESEARCH

Isolation and Characterization of Mycobacterium tuberculosis from Pulmonary and Extra Pulmonary Specimens at a Tertiary Care Centre in Northern Region of India

Shweta Bajpai[1], Divya Srivastava[2], Ritika Tiwari[3], Ramanath K[4*]

[1]Ph.D. Scholar, Department of Microbiology, Index Medical College, Hospital & Research Centre, Malwanchal University Indore, Madhya Pradesh, India.
[2]Tutor, Department of Microbiology, Maharaja Suheldev Autonomous State Medical College, Bahraich, Uttar Pradesh, India.
[3]Tutor, Department of Microbiology, Uma Nath Singh Autonomous State Medical College, Jaunpur, Uttar Pradesh, India.
[*4]Professor, Department of Microbiology, Index Medical College, Hospital & Research Centre Malwanchal University Indore Madhya Pradesh, India

Corresponding Author
Dr. Ramanath K
Professor, Department of Microbiology, Index Medical College, Hospital & Research Centre Malwanchal University Indore Madhya Pradesh, India.
Email: ramanath.karicheri@gmail.com

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ABSTRACT

Background: Tuberculosis, an infectious disease caused by Mycobacterium tuberculosis, remains a major threat to public health. TB is usually divided into two main categories depending on the MTB infection site: pulmonary and extrapulmonary tuberculosis. The elimination of Tuberculosis requires early, rapid and accurate diagnosis and treatment. Acid-fast bacilli microscopy with ZN staining is the most frequent laboratory technique used for its diagnosis. A culture technique using Lowenstein–Jensen medium for mycobacterial growth is being considered as the gold standard method for TB detection and it takes a longer time which is usually 3–4weeks with a high sensitivity.**Methods:**A total of 500 specimens were collected during this study from suspected Pulmonary and Extrapulmonary tuberculosis cases from different wards and outpatient departments Patients with known clinical diagnosis other than the disease, patients already on ATT, and patients with HIV infection were excluded from the study.**Result:**Out of 205 pulmonary and 295 extrapulmonary suspected tuberculosis patients, 32 were positive from pulmonary tuberculosis patients While in extrapulmonary tuberculosis patients 28 were positive.**Conclusion:**The efficiency of MTB culture using the LJ medium a high sensitivity and has been demonstrated to detect MTB when 10 viable bacilli per mL of sputum were present.

Key words: Pulmonary and Extrapulmonary tuberculosis, AFB Staining, Culture.

INTRODUCTION

Mycobacterium Tuberculosis remains a global health burden especially in developing countries.[1] Tuberculosis (TB), caused by acid-fast aerobic bacteria comprising the Mycobacterium tuberculosis complex, is a contagious infection that generally affects the lungs. TB is a communicable infectious disease that is transmitted through cough aerosols, and is characterized pathologically by necrotizing granulomatous inflammation, usually in the lungs[2] The risk factors for TB are: patients infected with HIV, diabetes, alcoholism, leukemia and patients who receive immunosuppressive drugs[3,4] In the absence of an efficient diagnostic modality for Tuberculosis, in the search for a tool that can overcome the available diagnostic tests continues. The elimination of Tuberculosis requires early, rapid and accurate diagnosis and treatment. Acid-fast bacilli microscopy

with ZN staining is the most frequent laboratory technique used for its diagnosis. However, limitations associated with microscopy lead to misdiagnosis.[5] Although the technique is rapid and inexpensive, its sensitivity is variable (20–80 %).[6] Culture is the gold standard technique for diagnosis of TB, which can also provide the testing for drug resistance. But such techniques require complex laboratory infrastructure and takes long time to get results. In fact, the most personnel who need culture to diagnose their Tuberculosis will not have access to the test results in time to save their lives or to avoid transmission to others.[7] A culture technique using Lowenstein–Jensen medium for mycobacterial growth is being considered as the gold standard method for TB detection and it takes a longer time which is usually 3–4weeks with a high sensitivity. The efficiency of MTB culture using the LJ medium has been demonstrated to detect MTB when 10 viable bacilli per mL of sputum were present.[8] Pulmonary tuberculosis and extrapulmonary tuberculosis are the two clinical manifestations of TB. The involvement of Tuberculosis other than lungs is termed as Extra Pulmonary Tuberculosis. Any patient affected with the combination of pulmonary and EPTB is defined as PTB.[9]

MATERIALS AND METHODS

All the suspected tuberculosisSpecimens were collected in sterile, leakproof, disposable, and appropriately labelled containers without fixative and placed in bags to contain leakage. In case of delay in transport and processing longer than 1 hour all specimens were refrigerated at 4^0 C[10]

Sample processing - The collected pulmonary and extrapulmonary tuberculosis specimens was processed singly. Each processed specimen was inoculated on solid Lowenstein–Jensen (L–J) slants and incubated at 37 ◦C for up to 8 weeks. Within this period, the media examined weekly for visible bacterial colonies, if there is no visible growth on LJ slant then categorized as culture negative.[11] Processed specimen was also be subjected to AFB Microscopy for all samples and all the smears were stained by the Ziehl Neelsen method and examined with a light microscope and confirmed by Culture method[12]

Specimen Decontamination[13]

All non-sterile samples were processed by using NALC-NaOH procedure todecontaminateandconcentratedtheprocessedsample. OneadvantageofusingNALC-NaOH method was that it contains NALC acts as a very good mucolyticagent used with reduced concentrations of a decontaminating agent (the final NaOHconcentrationinsputumwas1%).Thesterilesampleswerenotrequireddecontaminationso theywere directly usedwiththe liquidmedium.

Ziehl-Neelsen(ZN)Staining

Smear Preparation

A thick mucoid part of sputum was selected to prepare a smear in the form ofsmall concentric circles at the centre of the slide. In case of CSF sample, one loopfulofCSFwasplacedatthecentreofaslidewithnospreadingandallowedtodryinair.One more drop of CSF was placed on the same drop and allowed to air dry. A thirdmoredropwasalsoplacedonsamespotaftercentrifugationofCSFandthenallowedto air dry and fix properly. The smear was allowed to air dry completely at roomtemperature.Afterairdrying,theslidewasfixedbypassingitontheflame3-4times.

Culture on Solid Medium

From the decontaminated sediment inoculation for culture was done on Lowenstein Jensen medium prepared in-house as per manufacturer's instructions using dehydrated media (HimediaM162). Cultures were incubated at 35^0 c in an atmosphere of 5-10% carbon dioxide and high humidity.

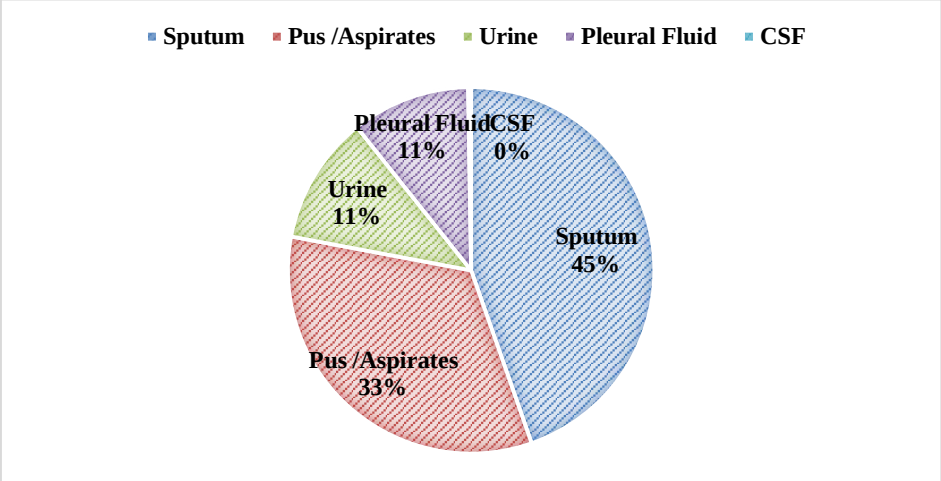
Tube media were incubated in a slanted position with screw caps loose for at least 1 week to allow for the evaporation of excess fluid and the entry of CO_2.

Cultures were examined weekly for growth. Contaminated cultures were discarded. Most isolates were appeared between 3-6 weeks; a few appeared after 7-8 weeks of incubation.

When the growth appeared, the rate of growth, pigmentation and colonial morphology were recorded. The typical colonial appearance of M. tuberculosis is shown in fig.no.3.3.

RESULT

Distribution of all TB samples represented which showed maximum proportion of sputum followed by pus / aspirates, urine, pleural fluid and CSF respectively showed in graph no.1.



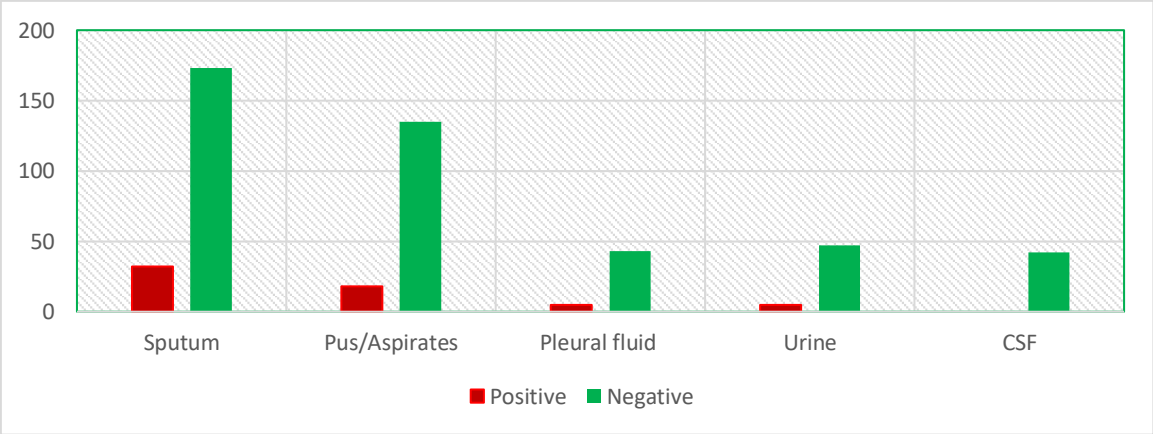
Graph no. 1.1 - Distribution of the Specimens According to their Type

Total no of 500 samples were collected in which 60 were showed positive for tuberculosis out of 500 samples 173 were taken from pulmonary and 267 were taken from extrapulmonary tuberculosis. Out 173 pulmonary tuberculosis suspects 32 showed culture positive while 28 suspects were showed culture positive in extrapulmonary tuberculosis showed in table no.1.

Table.1-Distribution of Culture Positives in Pulmonary and Extra Pulmonary Samples

Specimens	Negative	Positive	Total
Pulmonary	173	32	205
Extrapulmonary	267	28	295
Total	**440**	**60**	**500**

Distribution of both PTB & EPTB positive samples represented by table in which showed 15.60 % of sputum followed by 11.76% of pus aspirates, 10.42 %of pleural fluid and 9.62 % of urine respectively showed in graph no.2



Graph no. 2- Distribution of M. Tuberculosis Complex Detected by Different Specimens Among Pulmonary and Extra-Pulmonary Tuberculosis

representing gender wise distribution of TB positive samples which showed maximum contribution of males than females. Among 500 samples 319 were male and 181 were female. In which 43 out 319 males were positive for tuberculosis while 17 females were positive for tuberculosis out of 181 showed in table no.2

Table.2- Gender Wise Distribution of Suspected TB Patients

Gender	Positive	Negative	Total
Male	43	276	319
Female	17	164	181
Total	60	440	500

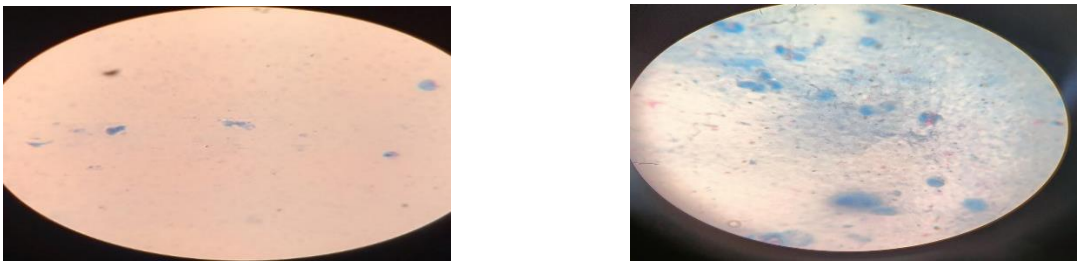
Out of 60 tuberculosis positive patients 19 were suffering from Cough and Fever, followed by 17 had Fever and Night sweats, 10 had Cough, Fever and Night sweats, 7 were suffering from Fever and Weight loss, 5 were had Cough, Fever and Loss of appetite and 2 were suffering from evening rise of temperature showed in table no.3

Table-3 Clinical Signs and Symptoms in Suspected Tuberculosis Patients

Sr. No	Characteristics features	Present	Absent
1	Cough, Fever	19	41
2	Fever, Night sweats	17	43
3	Cough, Fever Night sweats	10	50
4	Fever Weight loss	7	53
5	Cough, Fever Loss of appetite	5	55
6	Evening rise of temperature	2	58

Identification of M. tuberculosis in microscope by ZN staining

Out of 500 pulmonary and extrapulmonary tuberculosis specimens 4 were showed negative for AFB staining (fig.no.1). Followed by 56 were showed positive for AFB staining (fig.no.2). Among positive AFB 31 smear found positive from pulmonary specimens and 25 smear were found positive from extrapulmonary specimens.



The visual identification of M. tuberculosis in microscope by ZN staining shown MTB negative in fig no - 1 and MTB positive in Fig no-2

Culture Identification of M. tuberculosis by Lowenstein Jensen's Medium

Out of 500 pulmonary and extrapulmonary tuberculosis specimens 60 were showed culture positive (fig.no.3). and 440 were showed negative for culture (fig.no.4). Among culture positive specimen 32 were from pulmonary specimens and 28 were found culture positive from extrapulmonary specimens.

M. tuberculosis culture identification by LJ Medium shown MTB positive in fig no -3 and MTB negative in Fig no-4

DISCUSSION

Our study describing the sign and symptoms of tuberculosis. there have also been some similar studies by Disha Arora et al followed by Luxia Kong et al and Yehia Kadhim Jabber Benellam et al respectively, in this some of which match our study and some show higher percentages showed in table no.4.

Table.4 – Comparison of Sign and Symptoms MTB from Various Studies

Disha Arora et al (2020)[14]	Fever, cough chest pain weight loss, loss of appetite, dyspnoea
Luxia Kong et al (2020)[15]	Fever, cough, weight loss, dyspnoea, Haemoptysis, night sweats
Yehia Kadhim Jabber Benellam et al (2022)[16]	symptoms suggestive of TB based on clinical, pathological, or radiological evidence of TB
Present Study	**Fever, cough, fever with cough weight loss, loss of appetite, night sweats, Evening rise of temperature**

In our study prevalence of different diagnostic methods for pulmonary as well as extrapulmonary tuberculosis showed some similar rate in compare

with Geeta et al and Bajrami et al while Elbrolosy et al showed high prevalence rate showed in table no.5.

Table. 5- Prevalence of Different Diagnostic Methods (AFB Smear & Culture in Pulmonary & Extrapulmonary tuberculosis Samples of Various Studies

Study	Positive By Microscopy	Positive By Culture
Geeta et al 2015[17]	21/227 (9.25%)	20/227 (8.81%)
Bajrami et al 2016[18]	17/116 (14.65%)	28/116 (24.13)
Elbrolosy et al 2021[19]	152/582 (53.90%)	160/582 (56.73%)
Present Study	**56/500 (11.2%)**	**60/500 (12%)**

CONCLUSION

The study validates the findings of previous studies which states that solid culture is a better and Gold Standard method for rapid diagnosis for the isolation of M. tuberculosis complex from both pulmonary as well as extra pulmonary specimens.

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