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ISOLATION AND CHARACTERIZATION OF MYCOBACTERIUM FROM PULMONARY TUBERCULOSISPATIENTS IN TAMIL NĀDU, INDIA

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ABSTRACT

Tuberculosis (TB) is presently recognized as one of the most common opportunistic infections seen in HIV seropositive patients, mostly presenting in the form of pulmonary and extrapulmonary infections. A total of 234 clinically diagnosed and radiologically evident cases suggestive of pulmonary tuberculosis were selected for this study. Sputum sample of each patient was screened for Acid Fast Bacillus (AFB) by smeared and cultured staining. On examination 113 smears were found positive for AFB and 73 smears were negative by concentration method. A total of 186 samples were found to be culture positive and 48 were culture negative, of these 186 stains were identified as *Mycobacterium tuberculosis*, one was identified as *M.avium complex* (MAC). The biochemical tests showed successfully yielded true-negative results for TM cultures and 2 false-negative results for *M.tuberculosis* cultures. The most prominent TM species identified using PCR-were *M. abscessus* (48.7%), *M. kansasii* (15.4%), *M. gordonae* (10.3%), and *M. intracellulare* (7.7%). None of the 186 culture-positive samples collected from patients were identified as containing mixed cultures of *M.tuberculosis* and TM respectively.

KEYWORDS: Tuberculosis, Mycoacterium, biochemical tests, PCR.

INTRODUCTION

Tuberculosis (TB) is a potentially serious infectious disease that mainly affects the lungs. The bacteria that cause tuberculosis are spread from person to person through tiny droplets released into the air via coughs and sneezes ().Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV, the virus that causes AIDS. HIV weakens a person's immune system, so it can't fight the TB germs. In the United States, because of stronger control programs, tuberculosis began to decrease again in 1993. But it remains a concern.[1] Many tuberculosis strains resist the drugs most used to treat the disease.

People with active tuberculosis must take many types of medications for months to get rid of the infection and prevent antibiotic resistance.

Symptoms

Although your body can harbor the bacteria that cause tuberculosis, your immune system usually can prevent you from becoming sick.[2] For this reason, doctors make a distinction between:

Latent TB: You have a TB infection, but the bacteria in your body are inactive and cause no symptoms. Latent TB, also called inactive TB or TB infection, isn't contagious. Latent TB can turn into active TB, so treatment is important.

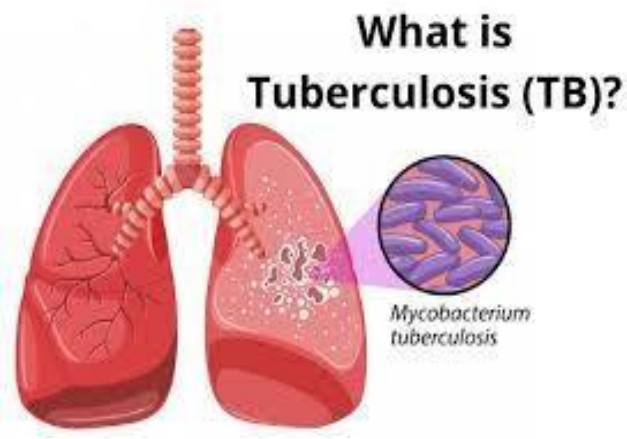
Active TB: Also called TB disease, this condition makes you sick and, in most cases, can spread to others. It can occur weeks or years after infection with the TB bacteria.

Signs and symptoms of TB includes

- Coughing for three or more weeks
- Coughing up blood or mucus
- Chest pain, or pain with breathing or coughing
- Unintentional weight loss
- Fatigue
- Fever
- Night sweats
- Chills
- Loss of appetite

Tuberculosis can also affect other parts of the body, including the kidneys, spine or brain (3).

When TB occurs outside lungs, signs and symptoms vary according to the organs involved. For example, tuberculosis of the spine might cause back pain, and tuberculosis in kidneys might cause blood in the urine.



Causes

Tuberculosis is caused by bacteria that spread from person to person through microscopic droplets released into the air. This can happen when someone with the untreated, active form of tuberculosis coughs, speaks, sneezes, spits, laughs or sings. Although tuberculosis is contagious, it's not easy to catch.[4]

MATERIALS AND METHODS

Study Population: The outpatients of (OP) Government Hospital, Chennai, Tamilnadu, India hospital with clinical suspicion of tuberculosis. Study Locale**:** Government Hospital (in and around Chennai). Sample collected as for the NTB guideline, India on December 2022 to February 2023**.** The patient concern form has been received from the electronic registration of the patient. All patients with clinical suspicion of pulmonary /extra-pulmonary tuberculosis. A total of 48 cases were included in this study. For the purpose of comparison, 234 patients diagnosed with non-MDR pulmonary TB who were hospitalized and treated at our hospital during the same period of time were also randomly selected and comprised the control group. For diagnosis of tuberculosis, three specimens of sputum were examined over a period of two days. Specimens were collected in sterile universal containers, which had a fixed label for noting patient's information on the side of the container. The specimen was collected in an aerosol free container. An ideal sample volume was about 5 ml and a minimum of three consecutive sputum specimens were collected. Transportation of the specimen for culture was done in a sterile leak proof container within a period of 3 days. Sputum samples from TB

patients were collected in properly sterilized disposable vials containing 2 ml of N-acetyl pyridinium chloride (CPC) as preservative solution. Condensed moisture observed at the of bottom of culture medium slant were removed partly before inoculation. Two slopes of Lowenstein Jensen medium (LJ) were inoculated in case of conventional cultures. In case of MGIT/BactAlert one LG medium was taken as a backup. Each slope was inoculated with 0.2 to 0.4 ml of the centrifuged sediment, distributed over the surface. Inoculated media were incubated in a slanted position for at least 24 hours to ensure even distribution of inoculum. Tops were tightened to minimize evaporation and drying of media. Following Nitrate reduction test, Aryl Sulphate Test, Tween Hydrolysis test, Niacin Test, Adenosine Deaminase Test and Nucleic acid amplification (NAA) tests or PCR,

RESULT

A total of 234 samples were received and processed in Mycobacteriology laboratory during the study period December 2020 to February 2023. Among them, 186 (79.4%) were culture positive and smear positive. Incidence of non tuberculosis during the two years period was found to be 48 (20.5%) (Table-1 and fig-2). It shows the distribution of Mycobacterium species among the various samples received and were found to be *Mycobacterium tuberculosis* complex.

Fig-3 depicts the distribution of NTM and *Mycobacterium tuberculosis* complex from clinical samples. *M.tuberculosis* were most commonly isolated from sputum samples(85%) followed by broncho alveolar lavage(7%), tracheal aspirate (2%), pus (2%) and tissue (2%). Fig-4 shows the pattern of distribution of NTM from various clinical samples. Growth on Lowenstein Jensen medium with paranitro benzoic acid (PNB) showed 100% positivity.

The obtained 186 (79.4%) smear positive cultures out of 234 different clinical specimens. However, 48 cultures (20.5%) yielded no colonies after being subcultured onto L-J slants; thus, no identification results were obtained for these cultures. We identified 210 L-J culture-positive samples using standard biochemical tests and observed as microscopic observation as *M.tuberculosis* (Fig-5).

129 out of 186 positive TB cases, the positive male percentage was 69.3% and in 3 outof 57 female pulmonary TB the percentage was 30.6% comparatively meaner than male positive (Table-2). Patients from both sexes were taken at random for the study and were between the age group of 10 to above 60 years. 7 out of 26 patients between the age group of 31-40 years

were positive for pulmonary tuberculosis with a positive percentage of 26.92%. Between the age group of 41-50 years, 1 patient was positive for pulmonary tuberculosis with a positive percentage of 25%, and between the age group, 21-30 years 3 cases were positive with a percentage of 30%. In the age group of above 60 years and 10-20 years, no one was found to be a positive patient. Consequently, the age group 21-30 years was the maximum positive for pulmonary tuberculosis with a positive percentage of 30% (Table-3).

The results of the Nitrate reduction test, niacin test, Aryl sulphate test, Tween hydrolysis test, Adenosine Deaminase Tests were compared to the culture results, and the results are summarized in fig-6 to 10. The all tests performed as well as considered as gold- standard biochemical methods. In contrast, the TBc biochemical test successfully yielded true-negative results for NTM cultures and 2 false-negative results for *M.tuberculosis* cultures.

Compared to conventional biochemical methods, the sensitivity of the non tuberculosis test was 98.8%, the specificity was 100%, the positive predictive value was 100%, and the negative predictive value was 95.1%. In contrast, the same four tests performance indexes all were 100% for the NAA method.

In this study, smear negative TB samples of total 186 clinically suspected tuberculosis patients were analyzed for mycobacterial DNA detection in different types of samples. Of the 234 cases, 186 (46.5%) were found positive for TB by PCR method. Different types of respiratory and non respiratory samples showed different positivity rates as described in fig-11. It is observed that 70% of the BAL samples were found TB positive followed by 67.6% TB positive CSF samples while pericardial TB samples were least positive (30%).

The PCR assay is highly sensitive and specific tool available to date for the diagnosis of *M. tuberculosis* in all types of specimens obtained from patients with a clinical suspicion of tuberculosis whether pulmonary or extra-pulmonary and can be reliably used for rapid identification of TB.

186 (81.4%) were culture positive for *M. tuberculosis*, and 39 (18.6%) were culture positive for TM. The most prominent TM species identified using PCR- were *M. abscessus* (48.7%), *M. kansasii* (15.4%), *M. gordonae* (10.3%), and *M. intracellulare* (7.7%). None of the 186 culture-positive samples collected from patients were identified as containing mixed cultures of *M. tuberculosis* and TM.

Table 1: Incidence of Mycobacterium tuberculosis.

Total no ofcases	M.TB (+)	Positive (+)	M.TB (-)	Negative (-)
234	186	79.4%	48	20.5%

Table 2: Sex with distribution of Mycobacterium tuberculosis.

Sex	Total no .of (+) case	%
Male	129	69.3%
Female	57	30.6%

Table 3: Age-wise distribution of Mycobacterium tuberculosis infection.

Age	Total no of case (N-50)	Pulmonary $(+)^{ve}$ TB (N-11)	$(+)^{ve}$ Percentage	Pulmonary $(-)^{ve}$ TB	$(-)^{ve}$ Percentage
10 – 20	40	0	0	40	72.72
21 – 30	50	15	30	35	70
31 – 40	26	7	26.92	19	73.07
41 – 50	20	5	25.00	15	75.00
51 – 60	10	0	0	10	0
>60	0	0	0	0	0

Fig-1:Biosafety Cabinet

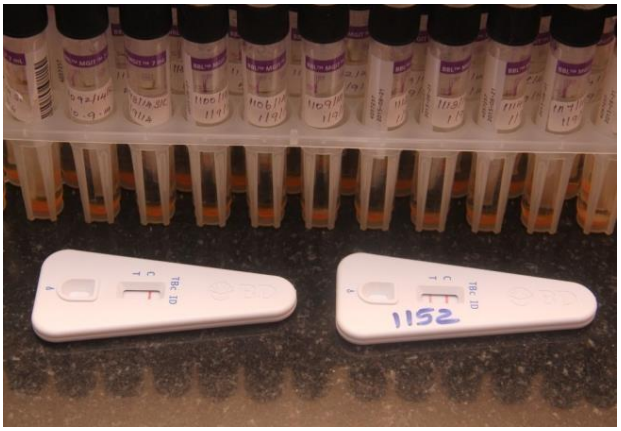


Fig-2:TBc ID test for 186 patients

Fig-3: Growth on LJ with PNB

Fig-4: Growth on 5% NaCl

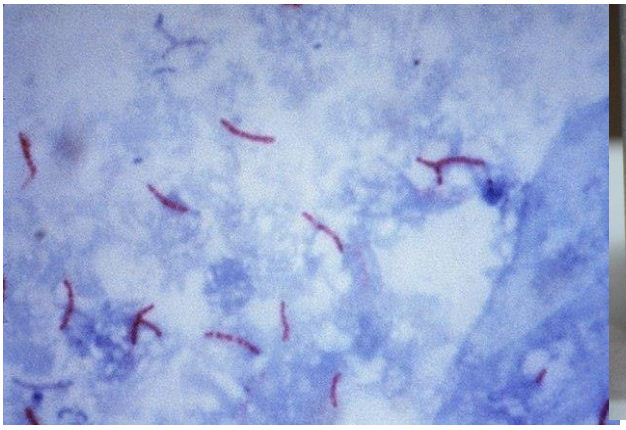


Fig-5: Microscopic observation of M.tuberculosis.

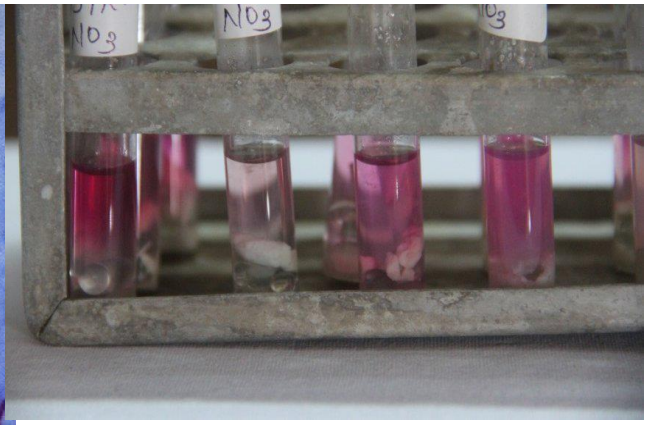


Fig-6: Nitrate reduction test.

Fig-7:Tween hydrolysis test.

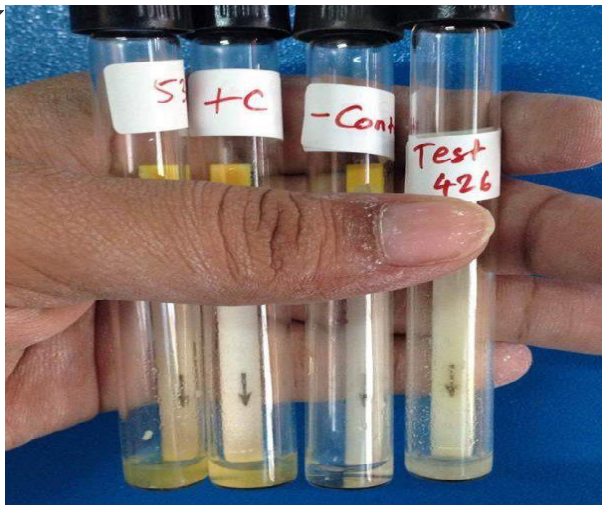


Fig-8:Niacin test.

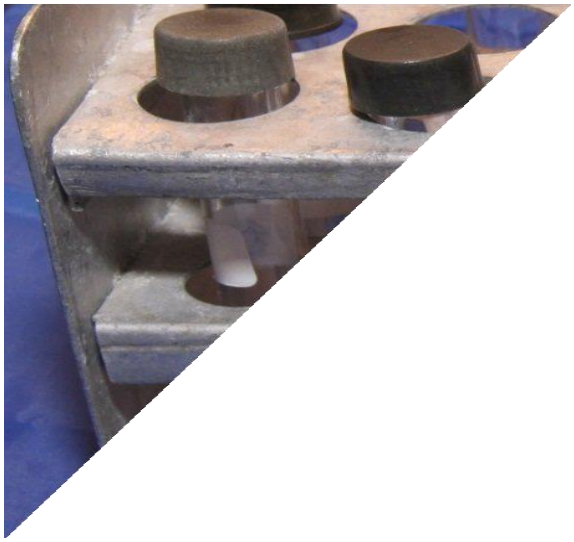


Fig. 9: Aryl Sulphate Test.

Fig. 10: Adenosine Deaminase Test.

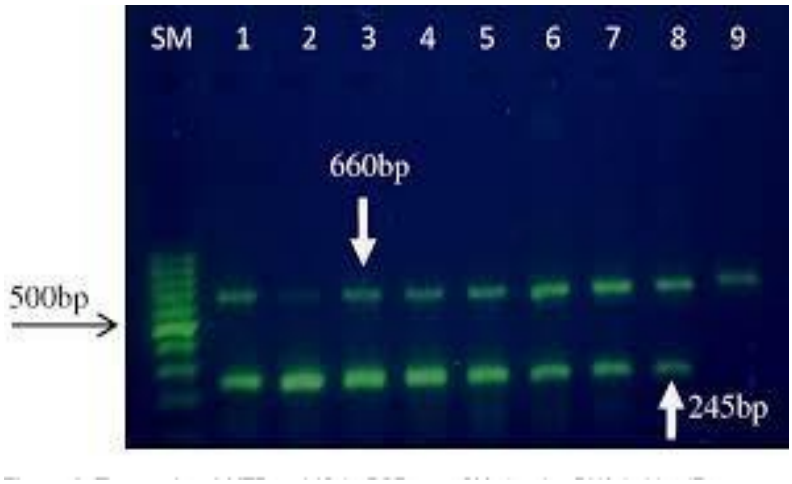


Fig. 11: PCR amplication test for M.tuberculosis.

DISCUSSION

A total of 234 samples were received and processed in Mycobacteriology laboratory during the study period December 2020 to February 2023. Among them, 186 (79.4%) were culture positive and smear positive. Incidence of non tuberculosis during the two years period was found to be 48 (20.5%). The distribution of NTM and *Mycobacterium tuberculosis* complex from clinical samples. *M.tuberculosis* were most commonly isolated from sputum samples(85%) followed by broncho alveolar lavage(7%), tracheal aspirate (2%), pus (2%) and tissue (2%). We identified 210 L-J culture-positive samples using standard biochemical tests and observed as microscopic observation as *M.tuberculosis.* 129 out of 186 positive TB cases, the positive male percentage was 69.3% and in 3 out of 57 female pulmonary TB the percentage was 30.6% comparatively meaner than male positive. In contrast, the TBc biochemical test successfully yielded true-negative results for TM cultures and 2 false-negative results for *M.tuberculosis* cultures. 186 (81.4%) were culture positive for *M. tuberculosis*, and 39 (18.6%) were culture positive for TM. The most prominent TM species identified using PCR- were *M. abscessus* (48.7%), *M. kansasii* (15.4%), *M. gordonae* (10.3%), and *M. intracellulare* (7.7%). None of the 186 culture-positive samples collected from patients were identified as containing mixed cultures of *M. tuberculosis* and TM.

CONCLUSION

This study concludes that the biochemical tests and PCR assay is highly sensitive and specific tool available to date for the diagnosis of *M.tuberculosis* in all types of specimens obtained from TB patients with a clinical suspicion of tuberculosis whether pulmonary or extra-pulmonary and can be reliably used for rapid identification of TB.

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