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PREVALENCE OF TUBERCULOUS AND NON TUBERCULOUS MYCOBACTERIAL INFECTIONS IN DIFFERENT CLINICAL SPECIMENS OVER A PERIOD OF TWO YEARS.

Microbiology

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ABSTRACT

Introduction: Although the lungs are the most typically affected site in TB (pulmonary TB), organ systems other than the lungs can also be affected. Extrapulmonary TB diagnosis is difficult sometimes because of its paucibacillary nature of samples. Diagnosis of NTM is necessary as to treat these infections different antimicrobial treatment is required. Delayed plus improper diagnosis and treatment is often associated with increased morbidity and mortality. **Material And Method:** All the clinical specimens received were subjected to smear microscopy using the Ziehl–Neelsen (ZN) staining method. Specimens were inoculated into the vials of the BacT/ALERT containing modified Middlebrook 7H9. BacT/ALERT 3D vials were monitored. At the same time these specimens also inoculated on Lowenstein Jensen (LJ) Medium. Cultures with positive growth on the BacT/ALERT 3D or LJ medium were tested for Standard QTBMPT64 Antigen test. **Results:** From January 2022 to December 2023, total 436 samples received. Total 121 samples were Pulmonary including sputum and BAL, rest were Extrapulmonary including Pus, tissue , fluids from various sites. Amongst these samples,114 samples were ZN positive and in 97 samples growth of Mycobacteria seen. Maximum positivity was seen in Pus, BAL/SPUTUM and urine samples. It was found that out of 97 growths 22 (18%) were NTMs, which were negative by MPT64 Antigen and the remaining 75 (82%) were Mycobacterium tuberculosis. **Conclusion:** In pulmonary samples only ZN positivity should not be considered sufficient for treatment. Mycobacteria culture, species identification, and drug-resistance testing should also be done, as NTM are resistant to routine antituberculour drugs and its treatment differs completely. Use of Automated culture methods, Molecular methods like PCR OR NAAT methods can be used for early detection of Mycobacteria and its drug resistance. Newer Techniques like MALDI TOF needs be used for identification of NTM

KEYWORDS

Mycobacterium tuberculosis, Non tubercular Mycobacteria, MALDI TOF, PCR.

INTRODUCTION

Tuberculosis (TB), an airborne communicable disease, before the COVID-19 pandemic, was the world's leading cause of death from a single infectious agent. As per The Global TB report 2022, 10.6 million people fell ill with TB worldwide in 2021[1]. India accounts for 28 per cent of the global TB burden. It also has the highest TBI burden globally[1] .

Although the lungs are the most typically affected site in TB (pulmonary TB), organ systems other than the lungs can also be affected. The World Health Organization (WHO) END TB strategy aims to reduce 95 per cent of TB deaths and 90 per cent of new incident cases of TB by 2035.[2]

Extrapulmonary tuberculosis (EPTB) constituted 16% of the 7.5 million reported TB cases globally. However, these estimates may be the tip of the iceberg, as a considerable proportion remains undiagnosed or not notified. Extrapulmonary TB can involve any organ and can misdiagnosed for other disease, diagnosis also difficult sometimes because of its paucibacillary nature of samples. Non tuberculous Mycobacteria are environmental bacteria and previously considered to cause diseases in immunocompromised or HIV positive indivisuals[3] But nowdays these bacteria are causing both pulmonary and extrapulmonary infections in immunocopmpetent indivisuals too. These bacteria have been reported from sputum, Bronchoalveloar Lavage (BAL), urine, post operative wounds, abscesses.

Nontuberculous mycobacterial rates of infection and disease has significantly increased in recent years and rates vary widely depending on population and geographic location. Prevalence of NTM is unknown in India as NTM disease is not a reportable condition, also many times it is underdiagnosed or mistreated as Tubercular disease. A recent study from central India reported prevalence of NTM increased from 1.0% in 2005 to 3.5% in 2008 and 88.6% of the NTM isolated were clinically relevant.[4]

Diagnosis of NTM is necessary as non tuberculour mycobacteria are resistant to standard anti-tuberculour drugs and to treat these infections different antimicrobial treatment is required. Delayed plus improper diagnosis and treatment is often associated with increased morbidity and mortality.

So its need of hour to use newer methods for detection of mycobacteria along with convention methods like microscopy and culture. Also Mycobacterial infections should be rule out from lesions.

In this study we have seen prevalence of Mycobacterial tuberculosis in both pulmonary and extrapulmonary infections and Non Tubercular Mycobacterial infections in different clinical specimens received in our laboratory.

Study Period- Over a period of 2 years from January 2022- December 2023.

Aims And Objectives

- To find out prevalence of Pulmonary and Extrapulmonary Tubercular Infections over a period of 2 years.
- To know prevalence of Non-Tuberculous Mycobacterial Infections in different clinical specimens received in laboratory

MATERIAL AND METHOD

All the clinical specimens received were subjected to smear microscopy using the Ziehl–Neelsen (ZN) staining method.

Sputum samples were decontaminated with the standard N-acetyl-L-cysteine-NaOH method. Specimens were inoculated into the vials of the BacT/ALERT three-dimensional (3D) system (BioMerieux, France) containing modified Middlebrook 7H9. BacT/ALERT 3D vials were monitored continuously with the BacT/ALERT 3D system. At the same time these specimens also inoculated on Lowenstein Jensen (LJ) Medium, and kept for incubation at 37℃ for atleast 60 days.

Samples with positive growth vials were removed from the machine and smears were made from bottle and ZN staining done for confirmation of growth of Acid Fast Bacilli in culture.

LJ bottles were examined regularly for growth. Samples that failed to show any growth after 60 days of incubation were removed and reported as no growth of Mycobacteria.

Cultures with positive growth on the BacT/ALERT 3D or LJ medium were tested for Standard QTBMPT64 Antigen test.

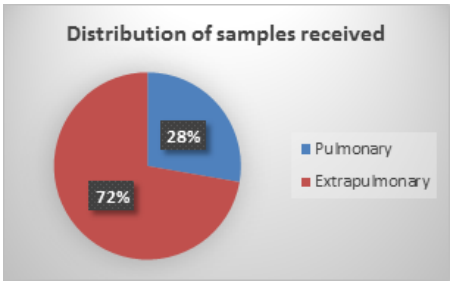
Standard QTBMPT64 Antigen test is a rapid immunochromatographic

immunoassay for the qualitative detection of MPT 64 antigen in the liquid culture or solid culture colonies.

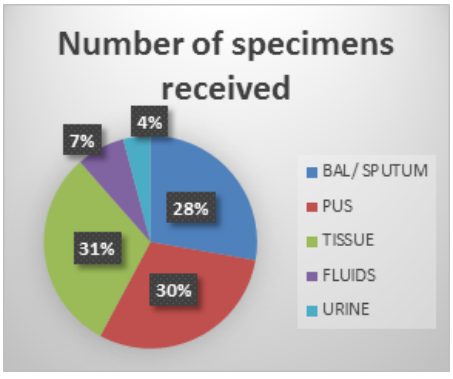
Positive test indicates Mycobacterium tuberculosis infection and those with negative test suggestive of NonTubercular infections and were sent for further identification of species by MALDI TOF.

RESULTS

From January 2022 to December 2023, total 436 samples received.



Total 121 samples were Pulmonary including sputum and BAL, rest were Extrapulmonary including Pus, tissue , fluids from various sites.

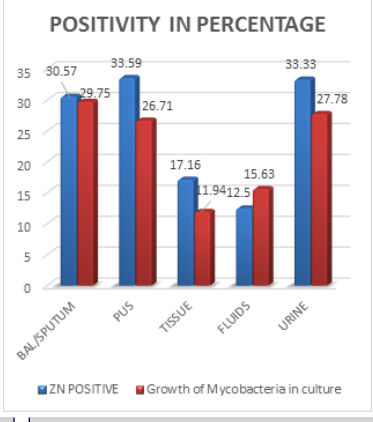


Majority samples received were Tissue samples which were including Endometrial tissue followed by Vertebral discs tissue. Endometrial tissue were sent to rule out infertility due to Mycobacterial infection. Followed by Pus(30%) and BAL/Sputum or Pulmonary (28%). Amongst these samples,114 samples were ZN positive and in 97 samples growth of Mycobacteria seen. Total Number of Different types of samples and its positivity rate is as follows.

In these samples received ZN Positivity and Growth in culture as follows.

	Samples received	Acid fast bacteria in ZN stain	Growth of Mycobacteria in culture
BAL/SPUTUM	121	37 (30.57%)	36 (29.75%)
PUS	131	44 (33.59%)	35 (26.71%)
TISSUE	134	23 (17.16%)	16 (11.94%)
FLUIDS	32	4 (12.5%)	5 (15.63%)
URINE	18	6 (33.33%)	5 (22.5%)
Total	**436**	**114 (26.14%)**	**97 (22.25%)**

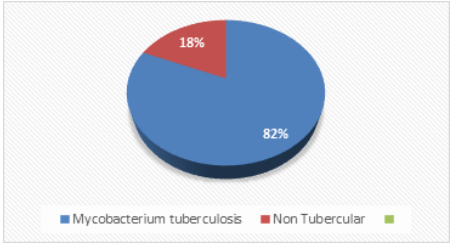
Positivity percentage in different specimens are as follows;



Maximum positivity was seen in Pus, BAL/SPUTUM and urine samples. Positivity in fluids samples was low.

Those samples having growth were tested by Standard QTBMPT64 Antigen test . it is rapid chromatographic immunoassay for qualitative detection of MPT 64 antigen in liquid or solid culture growth specimen. Using this test we differentiated in Mycobacterium tuberculosis infection and Nontuberculour infections.

It was found that out of 97 growths 22 (18%) were NTMs, which were negative by MPT64 Antigen and the remaining 75 (82%) were Mycobacterium tuberculosis.



Amongst Atypical Mycobacteria majority bacteria were Mycobacterium abscessus, other Mycobacteria were Mycobacterium xenopi, M. avium, M. chelonae, M. chimera.

DISCUSSION

In present study total 436 samples received. Majority of samples received were Extrapulmonary that is 72% which includes pus, tissue, fluids and urine.

In our study out of 436 specimens total 114 were positive for acid fast bacilli by ZN staining (26.14%), and 97 (22.25%) positive for growth of Mycobacteria in culture. This is similar to a study from Delhi where 25.6% samples were positive by smear microscopy (ZN and/or fluorescent) and 131 (30.7%) specimens were culture positive.[5]

In one more study results are similar where 20.52% were positive by smear microscopy[6] and Culture positivity rate in this study was 22.27%.

Positivity by ZN staining in pulmonary specimens is 30.57% and in extrapulmonary samples is 24.44%.

Out of total Mycobacterial growth , growth in pulmonary specimens is 37.11% and Non pulmonary specimens growth is 62.89%. In a study by Sarwat et al Growth was 50.98% were from pulmonary specimens and 49.01% from extrapulmonary[6].

We differentiated growth of Mycobacteria and NTM by using Rapid MPT 64 AG Test, so as per this test , In present study total growth of M.tuberculosis is 82% and Non tuberculour mycobacterial growth is 18%,

This is very similar to a study by Sarwat et where M.TB was the predominant isolate as 84.31% followed by NTM as 15.68%[6]. This was also seen in other study by VP Nayeedu as 98.53% and 1.46% of Growth of MTB and NTM respectively.[7]

CONCLUSION

In pulmonary samples only ZN positivity should not be considered sufficient for treatment. Mycobacteria culture, species identification, and drug-resistance testing should also be done, as NTM are resistant to routine antituberculour drugs and its treatment differs completely.

As Extrapulmonary Mycobacterial infections remain to be undiagnosed most of the times, thorough investigations including Mycobacterial staining and Culture by both methods ie.Conventional and Automated are need to considered on priority as High prevalence is seen in Non HIV seropositive patients also.

Use of Automated culture methods, Molecular methods like PCR OR NAAT methods can be used for early detection of Mycobacteria and its drug resistance. Newer Techniques like MALDI TOF needs be used for identification of NTM. These advanced methods can reduce turn around time significantly for Slow growing NTM OR MDR TB. This will help in early initiation of therapy as well as targeted therapy as per

isolate and its sensitivity pattern.

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