



Research Article

PREVALENCE OF NON-TUBERCULOUS MYCOBACTERIA IN A TERTIARY CARE HOSPITAL

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Abstract- NTM is increasingly isolated worldwide. Different species are prevalent in different geographic areas around the world¹. Treatment of NTM varies depending upon the species isolated and susceptibility profile of the species. So, it is very important to know which species is prevalent in a specific geographical area. In this study we isolated the various NTM species from the clinical samples received in our laboratory and from our study we found that *M. avium* complex is the most commonly isolated species followed by *M. haemophilicum*.

Key Words- Non-Tuberculous Mycobacteria, GeneXpert,

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Introduction

Mycobacteria other than the *M. tuberculosis* complex and *M. leprae* are known as Non-tuberculous mycobacteria. These are also known as Mycobacteria Other Than *M. tuberculosis* (MOTT). Since about 100 years, NTM has been observed. [2] Non-tuberculous mycobacteria (NTM) are acid-fast bacteria. They are ubiquitous in the environment. NTM can also be present in soil, water sources, and food supplies and they can cause infection [3]. NTM are classified as rapid growing and slow growing species. Similar to TB, NTM can affect any part of the body, most commonly it causes pulmonary infections, lymphadenitis, and skin and soft tissue infections. Various manifestations of the disease and susceptibility pattern of organism depends on both host factors and various characteristics of organisms [4,5]. In most of the lung diseases NTM are an important cause of morbidity and mortality [2].

For the diagnosis of NTM, American Thoracic Society and Infectious Diseases Society of America have developed guidelines, which include various criteria like Clinical findings of the patients, radiographic finding, and microbiologic criteria [6]. NTM causes various pulmonary and extra-pulmonary diseases [4]. Most commonly, NTM causes disseminated disease in immune-compromised individuals [6,7] worldwide, there is considerable increase in prevalence as well as incidence of the NTM [7]. Increasing NTM isolation is very much important as these are both difficult to diagnose and treat and the treatment also varies according to the NTM species involved, its susceptibility profile and the disease site [1].

The aim of the present study is to isolate and identify NTM species prevalent in samples received in our laboratory.

Material and Methods

In the present study, we examined 4236 samples which we sent to our laboratory under RNTCP programme in the duration of June 2015-June 2016. Diagnosis of NTM was done according to the guidelines provided by the American Thoracic Society (ATS)

Sample collection was done according to the RNTCP guidelines. Total 2 samples were collected A and B. and all the samples were received with fully filled

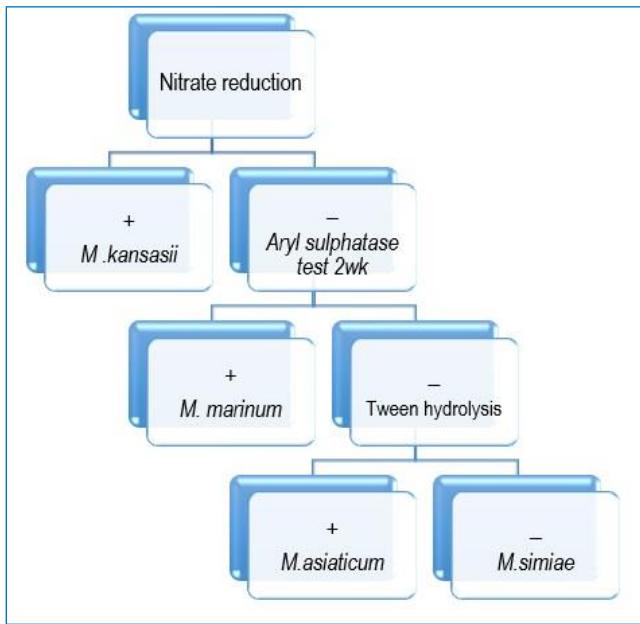
annexure 1. After receiving the samples, those samples were opened in the Bio-safety Cabinets (BSC). Then smears were prepared from the sample and kept inside the BSC to air dry. When the smears were air dried they were stained by ZN stain. For ZN staining we followed the procedure as described in the RNTCP manual. Stained smears were scanned under microscope (100X lens) by using Z technique for the presence of Mycobacteria. Then ZN stain positive samples were processed by Line Probe Assay (LPA) and negative by using Gene Xpert MTB/RIF assay. If specimen was smear positive but molecular test negative then it was suspected as NTM. Second sample (sample B) also processed for NTM and another sample also recalled.

Culture was done on the LJ media slopes, of which two were kept at 37° C (one wrapped and other unwrapped), one at 45°C and one at room temperature. Samples were simultaneously cultured on the LJ media containing NaCl, PNB, and on the MacConkey agar. All the slopes were observed for the presence of growth every day, till first week and then every week till 8 weeks. Growth on PNB is indicative of NTM. Suspension from the growth was done and it was used for rapid test TB Ag MPT 64 test. For doing this test the suspension was poured in the well of the cassette. And results observed after 20 minutes.

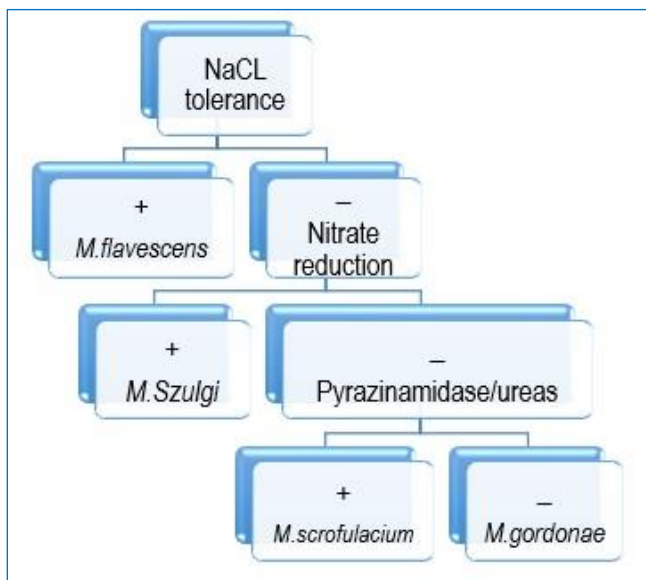
This test is positive in case of *M. tuberculosis* infections and negative in NTM. If growth was seen in the first weeks then it was labelled as rapid growers, if not then slow growers. NTM were again classified into Photo chromogens, Scotochromogen, non-chromogens depending on the pigment production. For species level identification various biochemical tests were done such as nitrate reduction, Aryl sulphatase, catalase at 68°C and semi quantitative catalase test, tellurite reduction, Pyrazinamidase test, growth on PNB, TB Ag MPT 64 rapid test. All these tests were done according to the kit literature of each test. Growth on PNB is indicative of NTM species. TB Ag MPT 64 rapid test is positive in *M. tuberculosis* infections.

We used following flow charts for species level identification.

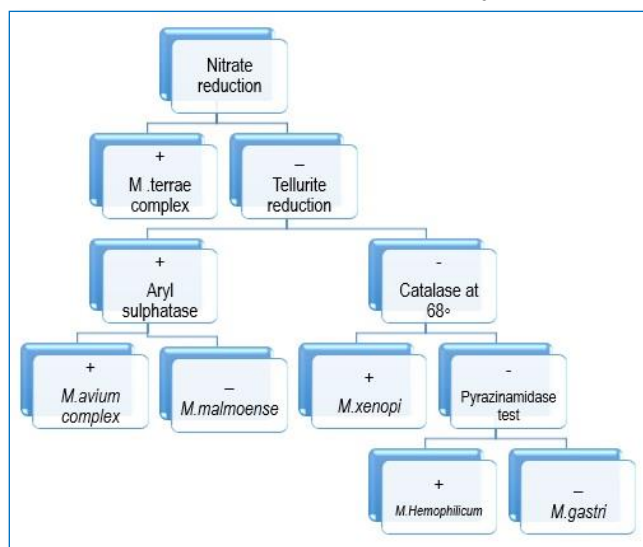
Flow chart 1- for detection of photochromogens



Flow chart 2- for detection of scotochromogen

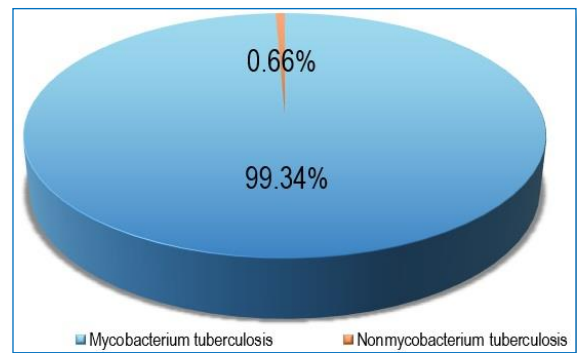


Flow chart 3- for detection of non-chromogens



Results

From the total samples received only 0.66% were found to be of Non-Tuberculous Mycobacteria (NTM), remaining 99.34% were of *Mycobacterium tuberculosis*.

Fig-2 Percentage of *Mycobacterium tuberculosis* and Non-*Mycobacterium tuberculosis*

When we observed the data carefully we found that incidence of infection with NTM is greater in males as compared to females.

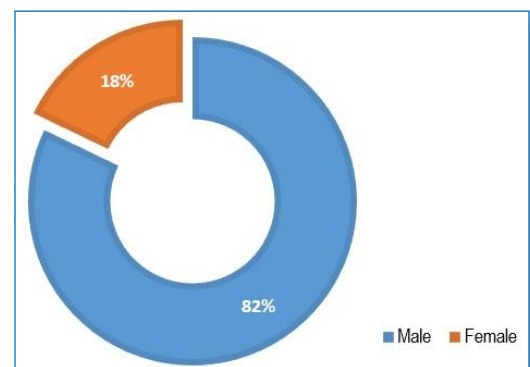


Fig -1 Sex-wise distribution

Most common age group affected was 41-50 years.

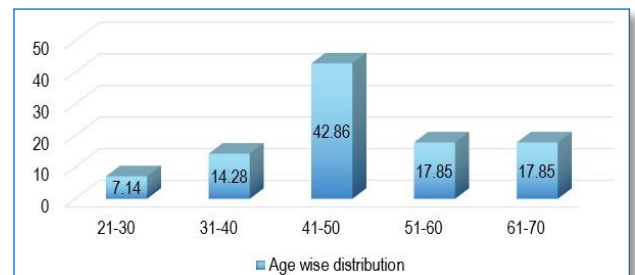


Fig 3- Age wise distribution

Following chart shows the percentage of various types of NTM, observed in our study.

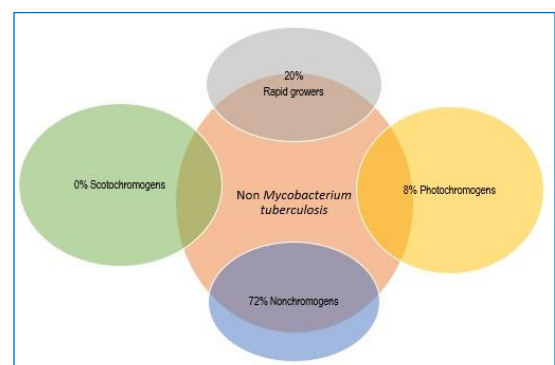


Fig 4- Percentage of various non tuberculous mycobacteria isolated

The chart below shows that *M. avium* complex is the most commonly isolated species followed by *M. hemophilicum*, *M. terrae* complex, *M. gastri*, *M. malmoense*

Discussion

NTM incidence and prevalence is increasing now a day. It is very important in NTM cases to identify various species as treatment according to the species identified differs. For this reason, identification is very important.

Most of the time NTM infection is confused with *M. tuberculosis* infection clinically therefore to patients infected with NTM, treatment of *M. tuberculosis* is given and due to wrong treatment patients may develop drug resistance. To avoid all these consequences microbiological identification of NTM to species level is very important. In the present study we had identified various isolates and found that *M. avium* complex is most commonly isolated followed by *M. haemophilicum*, *M. terrae* complex, *M. gastri*, *M. malmoense*.

The following table shows comparison of various studies with our study:

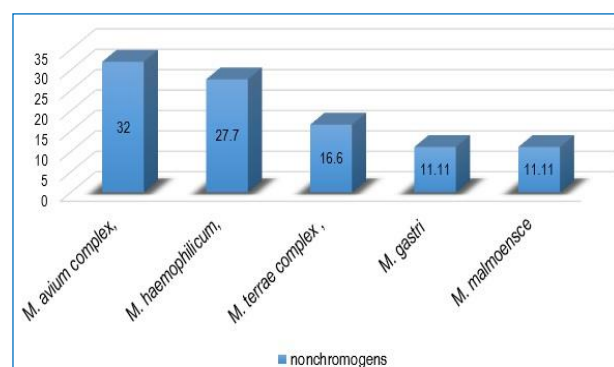


Fig 5- Nonchromogens

Table-1 Comparison of various studies

	Ahmed <i>et al.</i> , 2013	Myneedu V.P., <i>et al.</i> , 2013	Marios Panagiotou, <i>et al.</i> , 2014	Mohammad nasiri, <i>et al.</i> , 2015	Maurya A.K., <i>et al.</i> , 2015	Our study
Sample tested	25955	15581	-	-	756(EPTB)	4236
NTM isolates	104	60(0.38%)	-	-	62(27.4%)	28(0.66%)
Predominant age group	40-60	-	≥60	Older age	-	40-50
Most common NTM species	<i>M. Avium</i> complex	<i>M. simiae</i>	<i>M. Avium</i> complex	<i>M. Avium</i>	<i>M. fortuitum</i>	<i>M. Avium</i> complex

V.P. Myneedu study shows the lesser percentage (0.38%) of NTM isolates as compared to our study (0.66%) but a study by A.K. Mourya shows higher percentage (27.4%) of NTM isolates as compared to our study (0.66%) [2,8]. In our study we observed that predominant age group is 40-50 years of age which is similar to study done by Ahmed *et al.*, [1].

The most common isolate observed in our study was *M. avium* complex. This finding is similar with the studies done by Ahmed *et al.*, Marios Panagiotou *et al.*, 2014, Mohammad nasiri *et al.*, 2015. In other studies, depending upon geographic areas various other species were observed. Overall in most of the studies *M. Avium* complex is the most common isolate observed.

Conclusion

- ▶ The most common isolated species of NTM is *M. avium* complex followed by *M. haemophilicum*.
- ▶ Although the prevalence of NTM is comparatively low, screening and reporting is important because of similarities in the clinical presentation with *Mycobacterium tuberculosis* and the difference in treatment of both.
- ▶ Further molecular evaluation is to be strengthened for diagnosis of NTM

Abbreviations:

NTM: Non-Tuberculous Mycobacteria

TB: Tuberculosis

ZN: Ziehl-Neelsen

CBNAAT: Cartridge based nucleic acid amplification test

BSC: Biosafety cabinet

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Author statement: All authors read, agree and approved the final manuscript

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human

participants or animals performed by any of the authors.

References

- [1] Ahmed I., Jabeen K. and Hasan R. (2013) *BMC Infect Dis.*, 13: 493. doi:10.1186/1471-2334-13-493.
- [2] Maurya AK, Nag VL, Kant S, Kushwaha RA, Kumar M, Singh AK, Dhole TN. (2015) *Biomed Res Int.*, 465403. doi: 10.1155/2015/465403
- [3] Al-Anazi, Khalid & M AL-JASSER, ASMA & Khalid Al-Anazi, Waleed (2014) *Frontiers in Oncology*, 4. 10.3389/fonc.2014.00311.
- [4] Khosravi A.D., Hashemi Shahraki A., Hashemzadeh M., Sheini Mehrabzadeh R. and Teimoori A. (2016) *Front. Cell. Infect. Microbiol.* 6:42. doi: 10.3389/fcimb.2016.00042
- [5] Koh W.J., Kwon O.J., Jeon K., Kim T.S., Lee K.S., Park Y.K., Bai G.H. (2006) *Chest*, 129(2), 341-8.
- [6] Otchere I.D., Asante-Poku A., Osei-Wusu S., Aboagye S.Y. and Yeboah-Manu D. (2017) *Int J Mycobacteriol*, 6(1): 70-75. doi: 10.4103/2212-5531.201895
- [7] Rindi L. and Garzelli C. (2016) *BMC Infect Dis.*, 1-5, doi:10.1186/s12879-016-1380-y.
- [8] Maurya A.K., Nag V.L., Kant S., Kushwaha R.A.S., Kumar M., Singh A.K. and Dhole T.N. (2015) *Biomed Res Int.*, 465403