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**Original Article**

# Identification of Nontuberculous *Mycobacterium* Isolates in Suspected Pulmonary Tuberculosis Patients

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## ABSTRACT

*According to World Health Organization, in the global tuberculosis ranking Pakistan is in 5th position. Mycobacterium tuberculosis* bacterium is responsible for this dreadful disease, but there are other nontuberculous mycobacteria species that could also be the possible cause of this disease. Scanty data is available on the incidence and distribution of species responsible for this infection. Local studies on non-tuberculous mycobacterium species would be of great support in targeted therapy. **Methods:** This study was designed to investigate the incidence and distribution of non-tuberculous mycobacteria-associated infection in pulmonary suspected tuberculosis patients. Sputum samples were processed for microscopy and culturing on Lowenstein–Jensen regardless of age and gender suspected TB patients. Positive cultures were then processed for detection of non tuberculous mycobacteria species using commercially available Geno Type *Mycobacterium* CM hybridization strips. **Results:** A total of 1560 sputum samples were tested for *Mycobacteria* by culturing, 215 were positive, 71 contaminated and 1274 were negative. All 215 culture positive isolates were exposed to Geno Type Mycobacterium CM kit revealed, 55 cultures as Non-tuberculous mycobacteria and 160 as *Mycobacterium* complex. Adults between aged 40 to 60 years and male were predominantly (61.81%) infected than females (38.18%). **Conclusions:** Scanty data is available about the contributions of non tuberculous mycobacteria to tuberculosis-like disease, and noteworthy geographical distribution, clinical and molecular epidemiology-related knowledge gaps exist in the areas with a high burden of disease caused by *mycobacterium tuberculosis* complex. Isolation of nontuberculous mycobacteria from clinical specimens should promptly be evaluated for their clinical significance.

## INTRODUCTION

Mycobacteria are tremendously diverse with many new discovered and again classified with the advent of new molecular techniques for identification. Besides *Mycobacterium leprae* distinct heritably and phenotypically from other known mycobacterial species due to evolutionary changes and is frequently characterized in a discrete genetic clade [1]. On the basis of microbiological, inheritance, clinical and epidemiological features four major groups of human pathogens in mycobacterial genus can be described. First one is *M. tuberculosis* complex which comprises *M. tuberculosis, M. microti, M. bovis, M. canetti, M. pinnipedii, M. bovis* BCG, and *M. africanum*. Second one is *M. leprae*. Next are slowly growing Non Tuberculous Mycobacteria (NTM) (non-chromogenic, photochromogenic and scotochrmogenic respectively). Last are rapidly growing mycobacteria [2]. Convergence of

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mycobacterial species are centered on results of different biochemical tests, growth features demanding 4-6 weeks for the entire identification procedure. Later another technique was introduced in the 1990s which was more effective because it consumes less time and is more robust known as 16s DNA sequencing [3]. NTM term used to designate species of mycobacteria other than the following basic representatives of *Mycobacterium tuberculosis* such as M. *tuberculosis*, *M. caprae*, *M. africanum*, *M. microti*, *M. pinnipedii*, *M. bovis*, *M. canetti* and *M. leprae*. According to a study, more than 200 species have been documented and up to 95% contain environmental bacteria but only 25 species are highly concerned with diseases and mostly are non-infectious for animals and humans. NTM has multiple species due to evolutionary discrepancy, clinical presentation, mutable biochemical features, clinical applicability, and vulnerability to anti-mycobacterial mediators [4, 5]. Initially these microorganisms were known as Battery bacillus (*M. avium* complex) and Yellow bacillus (*M. Kansasii*) [6]. The recent observed rise in NTM cases is worrisome because of the disease's difficulty in diagnosis and treatment. Nonetheless, there is a lack of data on the geographic ranges of these species inside Pakistan. There are several potential treatments, but which one would work best will depend on a number of circumstances, such as how secluded the species is and how vulnerable it is. In-depth research requires familiarity with the similarities and differences among native species. The purpose of this research is to quantify the incidence of NTM species in patient-submitted clinical laboratory specimens at an academic medical centre in Abbottabad. The findings of this research may inform the development of new preventative measures, diagnostic tests, and therapeutic approaches for the treatment of certain species in the future.

## METHODS

A cross-sectional study was designed at BSL II LAB District head quarter Hospital (DHQ), Abbottabad (KPK). Samples were collected at the drug-resistant TB center Ayub teaching hospital and district Headquarters (DHQ) hospital, Abbottabad of Hazara division from September 2019 to April 2020. Pulmonary tuberculosis suspects enrolled in this study visited MDR center ATH and BSL II Lab district headquarter (DHQ) hospital Abbottabad. Patients who tested positive for Mycobacterium tuberculosis and subsequently started treatment were disqualified because of the absence of extrapulmonary TB. Decontaminant and digestant (NALC-NaOH) solutions were mixed in equal volume and then added to each sputum sample in the falcon tubes. Then phosphate buffer was added to falcon tube to make total volume of sample 50 mL and pH was maintained at 6.8. The tubes were recapped and inverted 2-3 times. After that, the samples were centrifuged in a refrigerated centrifuge for about 15 minutes at 3500 Relative Centrifugal Force (RCF). Supernatant was transferred carefully into the splash-proof container, containing the mycobactericidal disinfectant (1 % bleach). A total of 215 culture positive samples were processed for non-tuberculous mycobacteria species detection and rest 71 contaminated and 1274 negative samples were rejected. For the molecular identification, speciation and differentiation of NTM, we had used Geno Type *Mycobacterium* CM and Geno Type *Mycobacterium* CM kit (Hain Life science GmbH, Nehren, Germany). Mycobacteria were grown on Lowenstein-Jensen medium and were used for DNA extraction. We had clean the working bench to avoid any contamination. Geno Lyse® kit for extraction of DNA was used and followed the protocol provided. After the DNA extraction, samples process for amplification. Each of the two Amplification Mixes, A and B, had all of the components needed for amplification (AM-A and AM-B). The defrosted AM-A and AM-B solutions were spun separately before being carefully pipetted together. About 10 µL AM-A, 35 µL AM-B, 5 µL DNA solution with final volume of 50 µL was used. We had determined the number of samples (number of samples to be tested in addition to control samples) and prepared the tubes required. We prepared a master mix that contains AM-A and AM-B and mixed it carefully and thoroughly. All the data collected from the above mentioned assays was calculated by percentage and ratio of variables. SPSS and Endnote were also used for data analysis and references.

## RESULTS

The current research was conducted in BSL II LAB District Head quarter (DHQ) hospital, Abbottabad from September 2019 to April 2020. A total of 1560 cultures were run out of which 1274 were negative, 71 contamination and 215 were positive. Out of 215 positive cases, 160 were *Mycobacterium tuberculosis complex*, 55 were NTM species as shown in figure1.

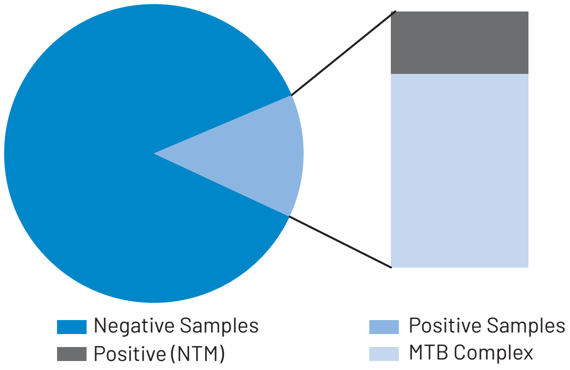

**Figure 1:** Epidemiology of overall samples in Hazara Division.

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Among the 55 NTM positive species the prevalent specie found were *Mycobacterium avium* 27(49 %), *Mycobacterium abscessus* 13 (23.6%), *Mycobacterium Kansasii* 9 (16.3%), *Mycobacterium intracellulare*6 (10.9%) respectively. Twenty-seven out of the seventeen people tested had good potential for therapy. Table 1 displays the outcomes of cases in which data on NTM isolates was collected from clinical specimens. This was feasible in certain cases when access to patient data was accessible due to the very small number of patients involved.

| Species Name | Isolates n (%) |
|---|---|
| *Mycobacterium avium* | 27 (49 %) |
| *Mycobacterium abscessus* | 13 (23.6%) |
| *Mycobacterium kansasii* | 9 (16.3%) |
| *Mycobacterium intracellulare* | 6 (10.9%) |
| *Mycobacterium fortuitum* | 0 (0%) |
| *Mycobacterium marinum* | 0(0%) |

**Table 1:** Distribution of NTM species differentiated by GenoType Mycobacteria CM/AS assay in pulmonary tuberculosis suspects (55)

The ratio between male to female according to specie were 20 (74.0%) of males and 7 (26.0%) of females diagnosed with *Mycobacterium* avium. While 7 (53.8%) male and 6 (46.2%) of female patients were diagnosed with *Mycobacterium abscessus*. Furthermore 4 (44.5%) male and 5 (55.5%) females were detected for *Mycobacterium Kansasii* along with 3 (50%) male and 3 (50%) females were positive for *Mycobacterium intracellulare* (Figure 2, 3, and 4).

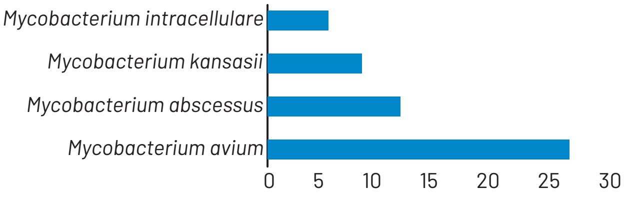

**Figure 2:** Distribution of NTM species differentiated by GenoType Mycobacteria CM/AS assay in pulmonary tuberculosis suspects (55)

Overall male to female ratio of NTM specie was 34 (61.8%) males while 21 (38.2%) females were diagnosed with NTM out of 55 patients as shown in figure 3. It was also noted that NTM lung disease affects mostly old agers.

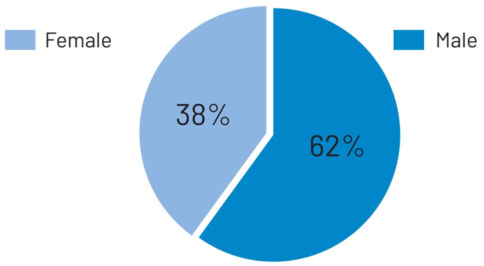

**Figure 3**: Prevalence of Non-tuberculosis mycobacteria among male and female suspects of pulmonary tuberculosi

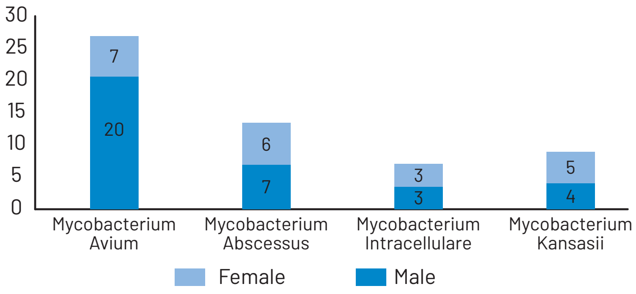

**Figure 4:** Distribution of different Non-tuberculosis mycobacteria species across gender

## DISCUSSION

The predisposing risk factors for NTM infection are advanced age, immuno-suppression (including HIV infection), and pulmonary disease (Chronic obstructive pulmonary disease, bronchiectasis, sequelae of previous TB, cystic fibrosis [7]. The current study also showed the same data that the infected patients were lying between ages 40 to 60 years mostly because they were immuno-compromised and easily got infected by NTM infection so we agree with the previous study conducted by [8]. NTM infections related to healthcare are well described in the published literature [9-11]. We were capable of evaluating three extra-pulmonary NTM infections associated to healthcare. Washing with tap water carrying NTM can easily contaminate the clinical instruments and/or without use of mycobactericidal disinfectants. Similarly, if proper sterile fluids are not used in the irrigation of surgical wounds or during surgical practices, wounds can be contaminated with NTM, as a result wounds does not heal properly. NTM has been linked not only to the spread of infections in healthcare settings but also to the contamination of samples at the time of collection (such as the introduction of NTM into sputum during washing with tape water) [12]. In laboratories reporting a high incidence of NTM, reagent contamination may have occurred during specimen processing [13]. Identification of NTM is global challenge which required rapid and sensitive testing protocol.. The diagnosis and treatment of disorders caused by MTB and NTM are significantly hindered by the fact that the clinical signs of the two infections are frequently quite similar [14]. About 40% of NTM isolates were linked to the Effective therapy requires knowledge of the kind of NTM, the location of infection, and the medications' efficacy Mycobacterium avium complex, as reported in the aforementioned Taiwanese investigation [15, 16]. According to the survey, males made up more than half of India's NTM. chelonae, with the M subtype accounting for 41% of all NTM isolates. Comparison to other animals, we're really lucky, M. was fortunate, according to researchers from 14 different nations [17]. MAC was the most abundant species in Brazil and Europe, whereas fortuitum was the most common in Iran and Turkey. In Belgium, xenopi virus isolations were highest, followed by *M. gordonae*. Following M. The writing is on the wall, literally. M. are C. are *C. gordonae* and C. m. When investigating rates of isolation, researchers from the Czech Republic found that the kansasii species was the most often seen. These findings imply shifts in the global distribution of NTM species. The ratio between male to female according to specie were 20 (74.0%) of males and 7 (26.0%) of females diagnosed with *Mycobacterium* avium. While 7(53.8%) male and 6(46.2%) of female patients were diagnosed with *Mycobacterium abscessus*. Furthermore 4 (44.5%) male and 5 (55.5%) females were detected for *Mycobacterium kansasii* along with 3 (50%) male and 3 (50%) females were positive for *Mycobacterium intracellulare*. We compared our findings across sex, age, and species with data from other places and found that 34 men and 21 females were NTM-positive overall. Infections with NTM have been demonstrated to vary in prevalence from species to species. Additionally, *M. kansasii* is frequently isolated in a number of nations or areas. Surprisingly, compared to the reported rates in Japan (3.9%) [17]. and the USA (7.7%), the isolation rate of this pathogen from NTM pulmonary illnesses in Norther Tunisia was very high, reaching 23.3% of all NTM isolations [18]. With Central Europe being one of the hotspots, *M. kansasii* related pulmonary illness tends to concentrate in particular geographic areas [19, 20]. Nevertheless, because *M. kansasii* was specifically extracted from municipal tap water.

## CONCLUSIONS

Currently available information suggests that once novel molecular diagnostic procedures are developed, widespread access to diverse NTM species will be possible. Four NTM species, which we will refer to collectively as "M," were detected as a consequence of our research. The letter "M" is the appropriate symbol for species kansasii and intracellulare. Due to the broad range of ages and sexes across which clinical disease symptoms were recorded, it is possible that many pathogenic and potentially pathogenic NTM species can be found in the isolated species.

## Conflicts of Interest

The authors declare no conflict of interest

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