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# Prevalence of *Mycobacterium abscessus* among the Patients with Nontuberculous Mycobacteria

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**Abstract**

**Background:** Considering the importance of the increasing incidence of non-tuberculous mycobacteria, especially *Mycobacterium abscessus* worldwide, we conducted a study to evaluate the incidence of these diseases in our area. The aim of this study was to evaluate the prevalence of *M. abscessus* in patients with non-tuberculous mycobacteria.

**Methods:** This descriptive study was performed on 18,083 samples isolated from patients with non-tuberculous mycobacteria during 2011-2017 at the Mycobacteriology Research Center (MRC), Tehran, Iran. To identify the Mycobacterium species, a 439 bp fragment of the IS6110 gene was first amplified using primers TB1 and TB2. Samples with a negative polymerase chain reaction (PCR) result were analyzed to investigate non-tuberculosis mycobacteria (NTM), especially *M. abscessus* using the RFLP method.

**Results:** Of the 18,083 samples, 5513 (30.49%, 95% CI, 12.95) strains of Complex Tuberculosis and 236 (1.31%, 95% CI, 1.84) strains of NTM were identified. The mean age of the patients with NTM was 18 years, and most of them were male. The most commonly identified species in this study were *M. abscessus* type I 32 (13.56%, 95% CI, 18.36) and *M. abscessus* type II 13 (5.51%, 95%CI, 20.04).

**Conclusion:** In this study, we observed a high prevalence of *Mycobacterium abscessus* type 1 in patients. As the treatment protocol for non-TB mycobacteria is different from *M. abscessus* complex, the diagnosis of these species as soon as possible will be significant for physicians.

**Keywords:** *Mycobacterium abscessus*, Nontuberculous mycobacteria, Polymerase chain reaction, Prevalence

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## Introduction

The mycobacterium family has more than 160 species.[1] One of their most famous species is *Mycobacterium tuberculosis* the causative agent of tuberculosis.[2] The mycobacteria other than *M. tuberculosis* are usually classified as non-tuberculosis mycobacteria (NTM).[3,4] This group of bacteria is present in all environmental sources such as water and soil, as well as dust particles in the air, and cause diseases in humans and animals as opportunistic bacteria.[5-7] The transmission of these mycobacteria from humans to humans rarely happens. However, if this transmission occurs, these organisms can lead to serious complications.[8,9] Atypical mycobacteria were classified by Runyon in 1950 based on their growth rate and pigment production.[10,11] Accordingly, NTM are classified into 4 groups: groups 1 to 3 as fast-growing, and group 4 as slow-growing.[12-14] These organisms are responsible for four distinct clinical conditions, including progressive pulmonary disease, superficial lymphadenitis, disseminated diseases, as well as skin and soft tissue infections.[14-16] Lung diseases and other diseases caused by these mycobacteria are now known in many parts of the world. Approximately 80% of patients with NTM infections are middle-aged or older women.[17,18] NTM have been dramatically associated with pulmonary diseases in humans over the past 30 years.[18-20] *M. abscessus*, as one of the NTM, is a fast-growing bacterium which can increase respiratory diseases.[21-23] Evidence suggests the association between *M. abscessus* and respiratory infections in humans.[24-26] Concerns about this issue are rising,[27] as in recent years, the role of *M. abscessus* has been identified in the development of respiratory infections, especially in patients with NTM.[25,27,28] This topic is classified as an emerging disease, which is especially serious in immunodeficient patients.[25] For example, in patients with cystic fibrosis, infection with *M. abscessus* results in a high rate of mortality.[13,29,30] Developing countries have long been researching the issue's sensitivity in terms of public health in this regard.[31,32] Increased infection with NYM and the inability of health systems in developing countries to identify them have caused non-differentiation of *M. abscessus* in patients with NTM.[8,14,33] The issue of *M. abscessus* in clinical specimens is a serious concern in advanced countries.[31,34] However, less attention is paid

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to developing countries due to technical and practical limitations.[35-38] *M. abscessus* is one of the most important known mycobacteria among non-tuberculosis. It is considered to be the most common non-tuberculosis mycobacterium in the United States, Asia, and most of Europe.[25,28,34] However, Iran as a developing country, is less concerned with this issue and limited activities are being undertaken against it. Differences in the diagnostic methods of this bacterium compared to other non-tuberculosis bacteria have made its timely diagnosis significant for both physicians and the community. The aim of this study was to determine the prevalence of *M. abscessus* in non-tuberculosis clinical specimens.

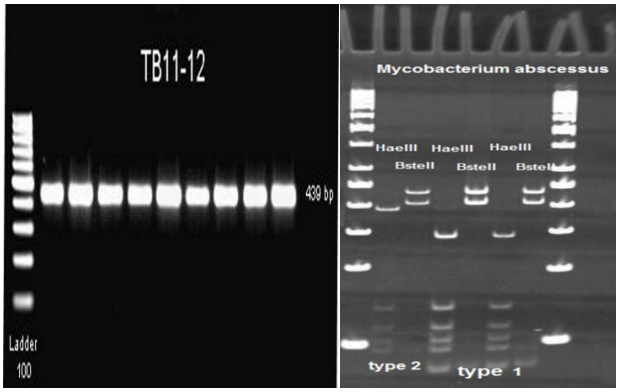


**Figure 1.** *Mycobacterium abscessus*, Hsp, 65kd, PCR Product: 439 bp, Digest with BstEII & HaeIII.

## Materials and Methods

This descriptive study was performed on 18083 samples isolated from patients during 2011-2017 at the Mycobacteriology Research Center (MRC), Tehran, Iran. Initial isolation of *Mycobacterium* strains was performed by Petrof method and Johnson culture media. In the next step, DNA of *Mycobacterium* was extracted from the sputum samples of patients using the Kiagen kit (QiAamp DNA) according to the manufacturer's instructions. To identify the *Mycobacterium* species, a 228-bp fragment from IS6110 gene was amplified using specific primers. The polymerase chain reaction (PCR) mixture with a final volume of 50 mL contained 33.5 µL distilled water, 2.5 µL X10 buffer, 4 µL primer, 1 µL dNTP mix, 2.5 µL $MgCl_2$, 2 µL DNA template, and 0.5 µL Taq DNA polymerase enzyme. The PCR reaction in the thermal cycler machine was as follows; The first cycle was 95°C for 10 minutes for initial denaturation, consisting of 35 cycles, 93°C for 20 seconds, 65°C for 1 minute, 72°C for 20 seconds continued, with the final cycle performed for 5 minutes at 72°C for final elongation. In the next step, PCR products were loaded onto 1.5% agarose gel containing iodide bromide (Figure 1, Table 1).

The samples, which were IS6110 positive, were considered as a complex of tuberculosis. The samples with a PCR result of IS6110 negative were analyzed using the PCR-RFLP method to investigate NTM using gene hsp65. Duplication of this gene was performed using Nested PCR. In the first stage, a pair of specific primers were used. Then, the PCR mix was used with a final volume of 50 µL containing 4 picomoles of special primers, 1.5 mM $MgCl_2$, 0.5 units of Taq polymerase enzyme, 1 mM dNTP, 1.5 mM X10 buffer, and 1% of DMSO. The PCR reaction occurred in the thermal cycler machine consisting of 30 cycles at an annealing temperature of 60°C. The second stage was performed using a pair of specific primers. PCR reaction was used with 50 µL final volume containing 8 picomoles of a specific primer, 1.5 mM $MgCl_2$, 1 unit of Taq polymerase enzyme, 0.2 mM dNTP, 1 mM of X10 buffer, and 2% of DMSO. The reaction temperature conditions were as follows: The first cycle was 95°C for 5 min for initial denaturation, which continued with 30 cycles of 94°C for 30 seconds, 56°C for 1 minute, and 72°C for 40 seconds, with the final cycle completed at 72°C for 10 minutes. Finally, the proliferated fragment of PCR was electrophoresed on 1.5% agarose with a band of 439 bp observed. The fragments amplified by two restriction enzymes Hael 3 and Bste 2 were digested according to the manufacturer's instructions. Digestive products were electrophoresed on 8% acrylamide gel with the digested genetic model compared with the standard strain genetic model. SPSS version 20.0 software was used to analyze the data where the frequency of percentages was calculated.

## Results

A total of 18083 patients with mycobacteria [Complex Tuberculosis strains (5513/18083; 30.49%, 95% CI, 12.95) and NTM strains (236/18083; 1.31%, 95% CI, 1.84)] were examined for the incidence of *M. abscessus* in the MRC from 2011 to 2017 (Tables 1 and 2). The mean age of patients referring to this center was 2 to 40 years. The male/female ratio for *M. abscessus* was 56% to 53% (24 males/ 21 females) (Tables 3 and 4).

Of the 18083 patients referring to the MRC, 236 (236/18083; 1.31%, 95% CI, 1.84) had NTM and 5513

**Table 1.** Digest with BstEII & HaeIII

| *Mycobacterium abscessus* | | | | |
|---|---|---|---|---|
| **Rapidly Growing** | **HSP65** | | **Gene** | |
| Non-pigmented | BstEII pattern | 95% CI | HaeIII pattern | 95% CI |
| *Mycobacterium abscessus* type 1 | 235 / 210 / 0 | 0.26 | 145 / 70 / 60 / 55 | 0.61 |
| *Mycobacterium abscessus* type 2 | 235 / 210 / 0 | 0.26 | 200 / 70 / 60 / 50 | 1.02 |

(5513/18083; 30.49%, 95% CI, 12.95) had complex tuberculosis. According to our study, out of 236 people with NTM, 45 (45/236; 19.07%) were infected with *M. abscessus*. Of the 236 specimens infected with NTM of all patients (18083) referring to the MRC, only 45 (45/236; 19.07%) cases had *M. abscessus*; of this number, 5.51% (13/236; 5.51%, 95% CI, 20.04) of subjects had *M. abscessus* type 2 and 13.56% (32/236; 13.56%, 95% CI, 18.36) harbored *M. abscessus* type I (Table 3). Statistical data in this study revealed that *M. abscessus* type I (32/236; 13.56%, 95% CI, 18.36) had the highest frequency in comparison with *M. abscessus* type II (13/236; 5.51%, 95% CI, 20.04) among NTM (236/18083; 1.31%) (Table 3). According to these findings, *M. abscessus* type I could play an important role in the severity of pulmonary disease in these individuals.

Also, the study of the prevalence of *M. abscessus* in terms of gender in the patients referring to the MRC suggested that the incidence of *M. abscessus* type I was higher in both men and women as compared to *M. abscessus* type II. It was also found that the incidence of *M. abscessus* type I (14/32; 43.75%) was higher in women than men (13/32; 40.63%) referring to the MRC (Table 4). It was also observed that the incidence of *M. abscessus* type II was higher in men (10/13; 76.93%) than women (5/13; 38.47%) (Table 4).

Finally, according to the results, *M. abscessus* type I in women and *M. abscessus* type I in men were the most frequent among all samples. In general, it can be concluded that *M. abscessus* type I has a higher incidence among women and men referring to the MRC compared to *M. abscessus* type II (Table 3).

**Table 2.** Frequency of Different Species Isolated from Patients Referring to Masih Daneshvari Hospital

| Isolated Species of *Mycobacteria* | Total Number of Patients (n = 18083) | 95% CI |
|---|---|---|
| NTM | (236/18083; 1.31%) | 1.84 |
| Complex tuberculosis | (5513/18083; 30.49%) | 12.95 |
| Total number | 18083 | - |

**Table 3.** Frequency of *Mycobacterium abscessus* in Patients with Non-tuberculosis Mycobacteria

| NTM Species | Total Number of NTM (n = 236) | 95% CI |
|---|---|---|
| *Mycobacterium abscessus* 1 | (32/236; 13.56%) | 18.36 |
| *Mycobacterium abscessus* 2 | (13/236; 5.51%) | 20.04 |
| Total number of *Mycobacterium abscessus* | (45/236; 19.07%) | - |

**Table 4.** Prevalence of *Mycobacterium abscessus* in Terms of Gender

| Gender (n = 45) | *Mycobacterium abscessus* Type 1 (n = 32) | *Mycobacterium abscessus* Type 2 (n = 13) |
|---|---|---|
| Female (n = 21) | 14/32; 43.75% | 5/13; 38.47% |
| Male (n = 24) | 13/32; 40.63% | 10/13; 76.93% |

## Discussion

Non-tuberculosis *Mycobacterium* species are rapidly expanding, though the level of their infectivity is limited to humans. *M. abscessus* is a fast-growing species and the most important mycobacteria known among non-tuberculosis types.[39] It is considered to be the most common non-tuberculosis mycobacterium in the United States, Asia, and most of Europe.[40] The increasing prevalence and high rates of proliferation of this bacterium have caused a sensitivity in developed countries, while Iran as a developing country is less concerned with this issue, and limited activities have been undertaken.[29] The diagnosis principle in this bacterium in more important than other NTM for its timely diagnosis for physicians and the community.[25] The study showed that in Iran, the prevalence of non-tuberculous mycobacteria has been neglected; so, the incidence of *M. abscessus* diseases may grow in Iran. In 2010, Esther et al in the United States investigated the association between *M. abscessus* infection and lung function decline in cystic fibrosis patients.[41] They examined 1216 patients with cystic fibrosis over 8 years in the presence of mycobacteria abscesses. They found that infection with *M. abscessus* is very common in cystic fibrosis patients and reduces their lung function.[41] In another study by Van Ingen et al in the Netherlands, the clinical relevance of *M. abscessus* was studied in 95 patients. They looked at patients' clinical data from their clinical records from 1999 to 2005 where *M. abscessus* was isolated from clinical specimens using the *rpoB* sequencing. They observed that a quarter of patients with pulmonary disease were infected with *M. abscessus*.[42] Also, in another study by Benwill and Wallace in the United States, diagnosis and treatment of *M. abscessus* were evaluated in patients. They concluded that *M. abscessus* survival was improved in patients with non-tuberculous contamination.[43] In another study by Griffith in Texas, the clinical features of pulmonary patients (n = 80) caused by fast-growing mycobacterium were studied. They observed that the highest incidence rate was related to *M. abscessus* type I species.[44] All of these studies suggest that *M. abscessus* is highly prevalent in patients infected with NTM, yet many countries ignore the spread of this bacterium. The results of our studies were consistent with the findings of other studies. The results of our study revealed that *M. abscessus* (45/236; 19.07%) was the most common among the isolated NTM (236/18083; 1.31%). Other studies have shown that among *M. abscessus* isolated from NTM (236/18083; 1.31%), *M. abscessus* type I and II were isolated. Specifically, the results indicated that *M. abscessus* type I (32/236; 13.56%, 95% CI, 18.36) is more abundant than *M. abscessus* type II (13/236; 5.51%, 95% CI, 20.04). Also, by examining the prevalence of *M. abscessus* based on gender in patients referring to the MRC, *M. abscessus* type I was found to be more prevalent in men and women than *M. abscessus* type II. The results of this study are consistent with the results of the studies by

Velayati et al and Henkle et al who examined the prevalence of NTM in environmental and clinical samples. These results indicate that NTM are expanding.[25,45] Further studies have indicated that *M. abscessus* type 1 (Female, 14/32, 43.75%; male, 13/32, 40.63%) is more common in women than men. It was also found that *M. abscessus* type Π (Male,10/13, 76.93%; Female, 5/13, 38.47%) is more common in men than women. These results suggest that *M. abscessus* should be considered seriously, so as to prevent the prevalence of many pulmonary diseases. Based on different sources, the incidence of *M. abscessus* in non-tuberculosis was very low, with quantitative studies testing this matter. Through this study, we were able to take a positive step in this direction and to determine that *M. abscessus* (45/236; 19.07%) in non-tuberculosis (236/18083; 1.31%) can play a very important role in the development of various pulmonary diseases.

Our study yielded important information about the prevalence of NTM. In this study, we observed a high incidence of *M. abscessus* among patients. It can be stated that since the samples used in this study were lung and sputum specimens, if it were possible to take samples from other tissues, or soil etc., those results would be similar to other findings.[4,6,7,10,14,16,18] Another reason for the differences in our study with similar studies in the United States, Asia, and most European regions is that the patients studied here, unlike most provincial studies, were from all over Iran who were referred to the tuberculosis center of the country.[40,46,47] Therefore, the study conducted in this regard is more general and indicates the abundance of NTM throughout Iran.4,25,34 Various reports show that the prevalence of NTM, especially *M. abscessus*, is high in parts of America, Asia, and most European regions.[47-52] Considering the similarity of this mycobacterium to *M. tuberculosis*, molecular methods for differentiating *M. abscessus* from *M. tuberculosis* are more accurate, faster, and more sensitive than current tests.[53-55] As stated earlier, the results revealed that *M. abscessus* type I has a higher frequency compared to *M. abscessus* type Π. *M. abscessus*, as well as other fast-growing mycobacteria, are often found in water resources and soils.[56-58] Observation of non-tuberculosis mycobacteria has increased due to changes in behavior and for unknown reasons, with the growth and dispersal of NTM in the environment.[4,18,59,60] Boiling water is a simple method for people living in Asia which may protect them against lung colonization. Based on this protocol, NTM with *M. tuberculosis* complex (5513/18083; 30.49%, 95% CI, 12.95), which are more prevalent than NTM (236/18083; 1.31%, 95%CI, 1.84), have been different in this study. Therefore, timely diagnosis will have significant implications for physicians. The treatment of NTM varies depending on the species. However, a cure for this mycobacterium has never been easy, as it requires the use of several drugs within 24 months. In addition, they have significant side effects, some of which are often not curable. The laboratory, with timely and accurate diagnosis of NTM, can take an effective step in treatment of pulmonary diseases and guide the physician in choosing the appropriate treatment protocol.

In conclusion, the results of NTM suggested that despite the misconceptions of some physicians, NTM, such as *M. abscessus*, could be dangerous. As observed in this study, the spread of NTM, especially *M. abscessus*, was very common in Iran and was a causative agent in patients referring to the MRC. Therefore, it is crucial that they are recognized properly, which will prevent the administration of misleading and rapid acting treatments.

### Authors' Contribution

SA: wrote the paper, analyzed the data. JA: collected the data. PoF: collected the data, performed the experiments. PaF: wrote the paper, performed the experiments, conceived the experiments, collected the data, and analyzed the data. JG and AAV: collected the data, performed the experiments, conceived the experiments.

### Conflict of Interest Disclosures

None.

### Ethical Statement

The study was approved by Shahid Beheshti University of Medical Sciences, Tehran, Iran (Ethical Approval: IR.SBMU.NRITLD.REC.1396.362).

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