Archives of Razi
Institute Journal

January & February 2026, Volume 81, Issue 1

Archives of Razi Institute Journal
"Arch. Razi Inst."

Journal Homepage: https://archrazi.areeo.ac.ir/

Research Paper

Identification of *Mycobacterium* spp. Isolates From Suspected Tuberculosis Patients Using Molecular Methods in Zahedan, Iran

Fatemeh Alimardani[1], Nader Mosavari[1*], Soheila Moradi Bidhendi[1], Ali Reza Salimi Khorashad[2], Lida Abdolmohammadi Khiav[3]

1. Department of Microbiology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
2. Department of Parasitology and Mycology, Infectious Diseases and Tropical Medicine Research Center, School of Medicine, Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, Iran.
3. Department of Anaerobic Bacterial Vaccine Production and Research, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Use your device to scan
and read the article online

How to cite this article Alimardani F, Mosavari N, Moradi Bidhendi S, Salimi Khorashad AR, Abdolmohammadi Khiav LA. Identification of *Mycobacterium* spp. Isolates From Suspected Tuberculosis Patients Using Molecular Methods in Zahedan, Iran. Archives of Razi Institute Journal. 2026; 81(1):173-182. https://doi.org/10.32598/ARI.81.1.3715

https://doi.org/10.32598/ARI.81.1.3715

Article info:
Received: 15 Sep 2025
Accepted: 10 Nov 2025
Published: 01 Jan 2026

ABSTRACT

Introduction: Tuberculosis is one of the oldest zoonotic diseases, with a high prevalence in many low-income countries. Specific and sensitive tuberculosis diagnostic methods in the early stages play a significant role in saving the lives of patients. There is little data available in Zahedan on the prevalence of the *Mycobacterium* species, so this study aimed to identify the Mycobacterium species in patients with pulmonary tuberculosis in Zahedan.

Materials & Methods: This study included 500 samples collected from sputum in Zahedan. The samples were cultured on LJ and simultaneously stained with cold Ziehl-Neelsen (ZN). After growth, DNA was extracted and used for molecular identification of the *Mycobacterium* species from the samples. RD typing was used to differentiate members of the *Mycobacterium tuberculosis* complex. Finally, the PCR-RFLP method was used as a comparison method.

Results: The typical 543 bp band was observed in all isolates via amplicon PCR-16S rRNA, emphasizing that all isolates belong to the genus *Mycobacterium*. Sixty isolates were identified as belonging to the MTBC and were classified as *M. tuberculosis* species. The PCR-RFLP analysis using Alu I on the *oxyR* gene confirmed that all 60 isolates were *M. tuberculosis*. Three samples (4.7%) were also positive for Nontubercolous mycobacteria (NTM). One isolate was categorized in the *M. terrae* complex group (MTC), and two isolates belonged to the *M. simiae* group.

Conclusion: Our results indicated that *M. tuberculosis* has a high prevalence in the human population of this city. Therefore, screening these individuals plays a significant role in reducing the prevalence of the disease in Zahedan. It is suggested that further studies be conducted on the human population to find *Mycobacterium* strains in the future.

Keywords:

Mycobacterium tuberculosis, NTM, PCR, PCR-RFLP, Zahedan

*** Corresponding Author:**
Nader Mosavari
Address: *Department of Microbiology, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.*
E-mail: *nmosavari@gmail.com*

1. Introduction

Tuberculosis (TB) is a major health problem worldwide, affecting more than one million people every year. *Mycobacterium tuberculosis* is the causative agent of TB that is transmitted through airborne particles and develops as a latent infection in most cases. In some cases, the patients experience reactivated disease and dissemination to other organs [1]. Antibiotic resistance and co-infection with COVID-19 or HIV in immunocompromised patients are also considered among the most challenging health issues [1, 2]. According to the World Health Organization's (WHO) 2023 global TB report, there was a very small increase compared with that in 2022. Most of the cases were observed in 30 countries, which accounted for 87% of the cases. Five countries accounted for 56% of the cases, including India (26%), Indonesia (10%), China (6.8%), the Philippines (6.8%), and Pakistan (6.3%) [3]. Sensitive, specific, and time-saving diagnostic tools are essential for controlling TB. However, the conventional tools for detecting TB are often inaccurate or time-consuming, particularly when distinguishing between active and latent TB infections. Polymerase chain reaction (PCR)-based methods allow for the accurate and rapid identification of mycobacterium species [4]. Iran is located in a critical region in the world due to its vicinity to Afghanistan, Pakistan, Iraq, and countries in the north of Iran. The prevalence of TB is not identical throughout the country. In 2019, the highest prevalence of TB was reported in the provinces of Sistan and Baluchestan and Golestan [5]. Limited data are available on the prevalence of members of the *M. tuberculosis* complex in the human population of Zahedan. Shakiba et al. [6] reported Mycobacterium bois from suspected patients with TB in Zahedan (unpublished data). The lack of industrial and semi-industrial livestock increases the possibility of bovine TB spreading in this city. Therefore, there is a possibility of bovine TB transmission to humans. Since *Mycobacterium bovis* is inherently resistant to pyrazinamide (the first-line drug for TB treatment), if *M. bovis* is not correctly identified, the patient may not receive appropriate and effective treatment. According to recent studies, infections caused by Nontubercolous mycobacteria (NTM) are increasing worldwide, especially in poor and developing countries. The clinical symptoms caused by this group of mycobacteria are indistinguishable from those caused by *Mycobacterium tuberculosis*. NTM are also resistant to anti-TB drugs, and have a different treatment protocol compared to MTBC. Therefore, the rapid and correct diagnosis of this group of mycobacteria is crucial. Thus, this study aimed to identify the species of *Mycobacterium* among patients with pulmonary TB in Zahedan. This will provide a clearer understanding of the epidemiological situation regarding the causative agents of this disease.

2. Materials and Methods

2.1. Ethics approval

All humans involved in the study were handled in accordance with the ethics guidelines and protocols approved by the "Research Ethics Committees" of Razi Vaccine and Serum Research Institute, Iran.

2.2. Patient population and sample collection

In this study, we included 500 patients suspected of having TB who were referred to the regional TB reference laboratory in Zahedan, the center of Sistan and Baluchistan Province, Iran, from 2024 to 2025. A total of 500 specimens (53.9% women and 46.1% men), between the ages of 22-99 years, were studied. The patient population exhibited symptoms of pulmonary TB, including cough, phlegm, hemoptysis, and dyspnea. They were referred to the regional TB reference laboratory in Zahedan from 2024 to 2025, where sputum samples were collected from suspected cases.

2.3. Microbiological process

The samples were cultured as follows in Zahedan. Briefly, they were decontaminated with 3.5 M NaOH for 15 min. After that, the samples were centrifuged and neutralized with HCL (0.1 N). The sediments were cultured in two slope tubes of glycerinated and pyruvate Lowenstein–Jensen (LG) medium under Biosafety Level 3 (BSL3). They were then incubated at 37 ˚C for 8 weeks. Additionally, the sediment from each sample was stained using the cold Ziehl-Neelsen (ZN) technique. The culture tubes were monitored for bacterial growth. After growth, these samples were transferred to the laboratory of the Microbiology Department at Razi Vaccine & Serum Research Institute, Karaj, Iran.

2.4. DNA extraction

DNA was extracted according to van Soolingen's method [7]. The samples were kept at -20 ˚C for further analyses.

2.5. PCR amplification

Briefly, primers were synthesized (Metabion, Germany) and used to amplify a 543 bp fragment of the *16S rRNA* gene. Then, PCR targeting IS6110 was carried out for the amplification of the TB complex. The master mix without DNA template and *M. tuberculosis* strain C were used as negative and positive controls, respectively. Subsequently, region-of-difference (RD) typing (RD1/RD4/RD9/RD12) was performed to differentiate between members of the TB complex. *M. tuberculosis* strain C, *M. bovis* AN5, and *M. bovis* BCG strain were used as positive controls. Distilled water was also used as a negative control. The oxyR PCR-RFLP method was used for final confirmation. Primers and PCR conditions are listed in Table 1.

2.6. PCR-RFLP of *oxyR* gene

PCR-RFLP of a 548-bp region of the *oxyR* gene was carried out according to Sreevatsan et al. [8]. The final volume was 12 µL, including PCR product (6 µL), AluI (2.5 µL) (Thermo Fisher Scientific, Lithuania), restriction enzyme buffer (2 µL), and distilled water (1.5 µL). The mixture was incubated at 37˚C overnight. The products were electrophoresed on an agarose gel for 100 min at 90 V. In this study, *M. tuberculosis* strain C, *M. bovis* AN5, and *M. bovis* BCG strain were used as positive controls, and water without DNA was used as the negative control.

2.7. Identification of NTM species

Targeting the *16S rRNA*, *hsp65*, and *rpoB* genes using PCR and sequence analysis was performed to identify NTM. *M. tuberculosis* strain C and master mix without DNA template were used as positive and negative controls, respectively. The outcomes of the nucleotide sequencing were analyzed using Chromas and Clustal X programs [9], and NCBI BLAST [10] was used to align the analyzed sequences.

2.8. Phylogenetic analysis of the *16S rRNA, hsp65,* and *rpoB* genes

Phylogenetic trees of NTM species were constructed using the Neighbor-Joining method using Molecular Evolutionary Genetics Analysis (MEGA) XI software.

3. Result

Sixty-three isolates grew in two LJG and LJP media after two months. However, LJG was better than LJP. Acid-fast bacilli were observed in all the samples. All purified DNAs had a concentration greater than 100 ng/µL; the 260/280 ratio of all samples was between 1.8 and 2.2. The electrophoresis results of the purified DNAs showed that all DNAs had high quality without any breaks in the electrophoresis gel. In this study, the molecular identification of 63 isolates from pulmonary TB patients was investigated using PCR-*16S rRNA*. The typical 543 bp amplicons were observed in all isolates, which showed that all isolates belonged to the genus *Mycobacterium*. In the next stage, PCR targeting *IS6110* was performed, and it was determined that 60 isolates out of 63 isolates (95.3%) were members of the MTBC. Based on the RD typing results, it was determined that all the TB complex group isolates were recognized as *M. tuberculosis* (Figure 1).

Digestion with Alul yielded three fragments of 79, 146, and 236 bp for DNA samples from *M. bovis* AN5 and *M. bovis* BCG strain. However, only one band at 230 bp was observed for the DNA sample from *M. tuberculosis* strain C and all of the isolates. PCR-RFLP on oxyR demonstrated that all of them were recognized as *M. tuberculosis*, which was confirmed by the result of PCR-RD typing. Three isolates were negative for PCR-IS6110. The nucleotide sequencing results based on *16S rRNA*, *hsp65*, and *rpoB* genes showed that these three samples (4.7%) were positive for NTM. It should be noted that these three isolates were isolated from three patients with the following characteristics: Patient 1 was a 70-year-old male without underlying diseases. Patient 2 was a 57-year-old male with high blood pressure and diabetes. Patient 3 was a 49-year-old male with asthma. The outcomes of the nucleotide sequencing based on *16S rRNA* showed similarity to *M. kumamotonensis* in one isolate (99.5%) and *M. simiae* in two isolates (99.6% and 99.8%, respectively). Gene sequence alignment results based on *hsp65* showed similarity to *M. senuense* in one isolate (97.8%) and *M. simiae* in two isolates (99.3% and 99.1%, respectively). Finally, the outcomes of the nucleotide sequencing based on rpoB showed similarity to *M. terrae* in one isolate (93.3%) and *M. simiae* in two isolates (99.1% and 99.4%, respectively). Therefore, the NTM isolates obtained from this study can be divided into two groups in the phylogenetic trees of the *16S rRNA*, *hsp65*, and *rpoB* genes: One isolate was categorized in the *M. terrae* complex group (MTC), and two isolates belonged to the *M. simiae* group (Figures 2, 3, and 4).

Table 1. Primers used in this study

Locus	PCR (Primer Sequence)	Size (bp)		PCR Condition	Ref.
16S rRNA	ACGGTGGGTACTAGGTGTGGGTTTC TCTGCGATTACTAGCGACTCCGACTTCA	543		Initial denaturation: 95°, 5 min; 35 cycles: 95°, 1 min, 62°, 1 min, 72°, 1 min Final extension 72°, 10 min	[11]
IS6110	CGTGAGGGCATCGAGGTGGC GCGTAGGCGTCGGTGACAAA	245		Initial denaturation: 94°, 3 min; 30 cycles: 94°, 30 sec, 65°, 33 sec, 72°, 40 sec; Final extension 72°, 10 min	[12]
RD1	AAGCGGTTGCCGCCGACCGACC CTGGCTATATTCCTGGGCCCGG GAGGCGATCTGGCGGTTTGGGG	146*	196**	Initial denaturation: 95°, 5 min; 35 cycles: 95°, 1 min, 62°, 1 min, 72°, 1 min Final extension 72°, 10 min	[13]
RD4	ATGTGCGAGCTGAGCGATG TGTACTATGCTGACCCATGCG AAAGGAGCACCATCGTCCAC	172	268	Initial denaturation: 95°, 5 min; 35 cycles: 95°, 1 min, 62°, 1 min, 72°, 1 min Final extension 72°, 10 min	[13]
RD9	CAAGTTGCCGTTTCGAGCC CAATGTTTGTTGCGCTG GCTACCCTCGACCAAGTGTT	235	108	Initial denaturation: 95°, 5 min; 35 cycles: 95°, 1 min, 62°, 1 min, 72°, 1 min Final extension 72°, 10 min	[13]
RD12	GGGAGCCCAGCATTTACCTC GTGTTGCGGGAATTACTCGG AGCAGGAGCGGTTGGATATTC	369	306	Initial denaturation: 95°, 5 min; 35 cycles: 95°, 1 min, 62°, 1 min, 72°, 1 min Final extension 72°, 10 min	[13]
PCR-RFLP on *oxyR* gene	GGTGATATATCACACCAT CTATGCGATCAGGCGTACTTG	230	79, 148, and 236	Initial denaturation: 94 °C 3 min; 30 cycles: 94 °C, 30 sec, 65 °C, 30 sec, 72 °C, 40 sec Final extension: 72 °C, 10 min	[8]
16S rRNA long	TAACACATGCAAGTCGAACGG AAAGG ACTTCGTCCCAATCGCCGATCCCA CC	1436		Initial denaturation: 95 °C 5 min; 35 cycles: 95 °C, 1 min, 60 °C, 45 sec, 72 °C, 40 sec Final extension: 72 °C, 10 min	[11]
hsp65	ACCAACGATGGTGTGTCCAT CTTGTCGAACCGCATACCCT	441		Initial denaturation: 95 °C 5 min; 35 cycles: 95 °C, 1 min, 60 °C, 45 sec, 72 °C, 40 sec Final extension: 72 °C, 10 min	[11]
rpoB	TCAAGGAGAAGCGCTACGA GGATGTTGATCAGGGTCTGC	359		Initial denaturation: 95 °C 5 min; 35 cycles: 95 °C, 1 min, 60 °C, 45 sec, 72 °C, 40 sec Final extension: 72 °C, 10 min	[11]

**M. tuberculosis*, ***M. bovis* BCG.

4. Discussion

Different genotyping methods were used to differentiate members of the *M. tuberculosis* complex, which showed a wide range of discrimination power. In this study, the molecular identification of 63 isolates from pulmonary TB patients in Zahedan was investigated. For this purpose, PCR-16S rRNA was performed, which showed that all isolates belonged to the genus *Mycobacterium*. In the next stage, PCR-IS6110 was performed, and it was determined that 60 isolates out of 63 isolates (95.3%) were members of the MTBC. Based on the RD typing result, it was determined that all 60 isolates studied were *M. tuberculosis*, similar to the Torabi study [14]. Warren reported that the multiplex PCR method based on the genomic regions of difference enabled the rapid and accurate differentiation of the *M. tuberculosis* complex [13]. In this study, we used this method. Finally, the comparative genotyping technique of PCR-RFLP on the *oxyR* gene was performed. The result showed the

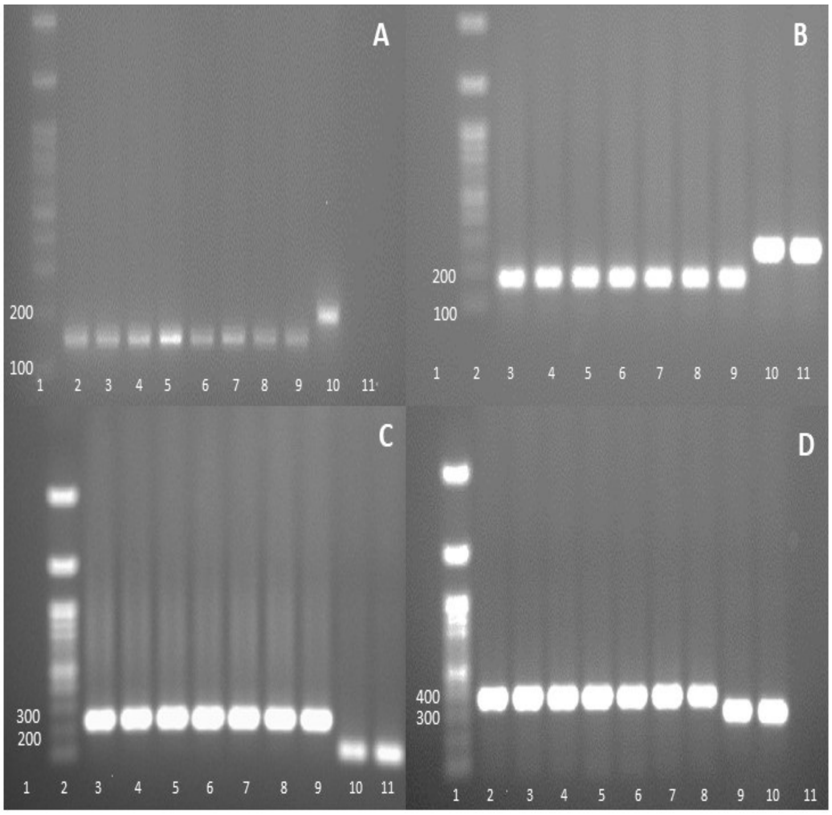

Figure 1. RD typing

A) Samples were analyzed by primers specific for RD1: Lane 1: Marker DNA (100-bp ladder); Lane 2: Positive control of *M. tuberculosis* strain C; Lanes 3–9: suspected samples, lane 10: *M. bovis* BCG strain; lane 11: Negative control (no DNA added); B) Samples were analyzed by primers specific for RD4: Lane 1: Negative control (no DNA added); Lane 2: Marker DNA (100-bp ladder); Lane 3: Positive control of *M. tuberculosis* C strain; Lanes 4–9: suspected samples; Lane 10: *M. bovis* AN5 strain; Lane 11: *M. bovis* BCG strain; C) Samples were analyzed by primers specific for RD9. Lane 1: Negative control (no DNA added); Lane 2: Marker DNA (100-bp ladder); Lane 3: Positive control of *M. tuberculosis* strain C; Lanes 4–9: suspected samples; Lane 10: M. bovis AN5 strain; Lane 11: *M. bovis* BCG strain. D) Samples were analyzed by primers specific for RD12. Lane 1: Marker DNA (100-bp ladder); Lane 2: Positive control of *M. tuberculosis* strain C; Lanes 3–8: suspected samples; Lane 9: *M. bovis* AN5 strain; Lane 10: *M. bovis* BCG strain; Lane 11: Negative control (no DNA added).

belonging of 60 isolates to the *M. tuberculosis* species, and none of them were infected with *M. bovis*; this confirmed the result of RD typing. Sreevatsan reported that *oxyR* PCR-RFLP is useful for differentiating *M. bovis* and *M. bovis* BCG from other members of the MTBC [8]. The result of this study showed the good ability of this method for confirmation of RD typing, similar to the previous study [14]. In this city, 60 isolates were *M. tuberculosis*, and none of the 60 isolates were infected with *M. bovis*, similar to the Torabi study [12]. However, in the Dehghanpour study, *M. bovis* was identified in approximately 7.4% of cases in Zanjan Province [15]. Shakiba Mehr also conducted a study on pulmonary TB and reported infection with M. bovis in Zahedan [6] (unpublished data), contrary to this study. It is perhaps due to the improvement of the health level and the use of pasteurized milk and dairy products in this city. On the other hand, due to drought and the subsequent loss of traditional livestock farming, especially in rural areas, and the development of industrial livestock farming, the consumption of local milk and dairy products has decreased. This is very important because M. bovis is considered a health indicator of the community. Based on our results, the other three isolates (4.7%) were recognized as NTM species. Nowadays, non-tuberculous mycobacteria are increasing worldwide, especially in developing countries. Karami-Zarandi et al. (2019) reported 12% of positive cases as NTM [16]. Differentiation of non-tuberculous mycobacteria from the TB group is not possible by conventional methods; therefore, molecular-based methods play a very important role in the differentiation of non-tuberculous mycobacteria. For example, Leidsema reported that the sequencing of *16S rRNA*, *hsp65*, and *rpoB* genes is a reliable method for identifying these bac-

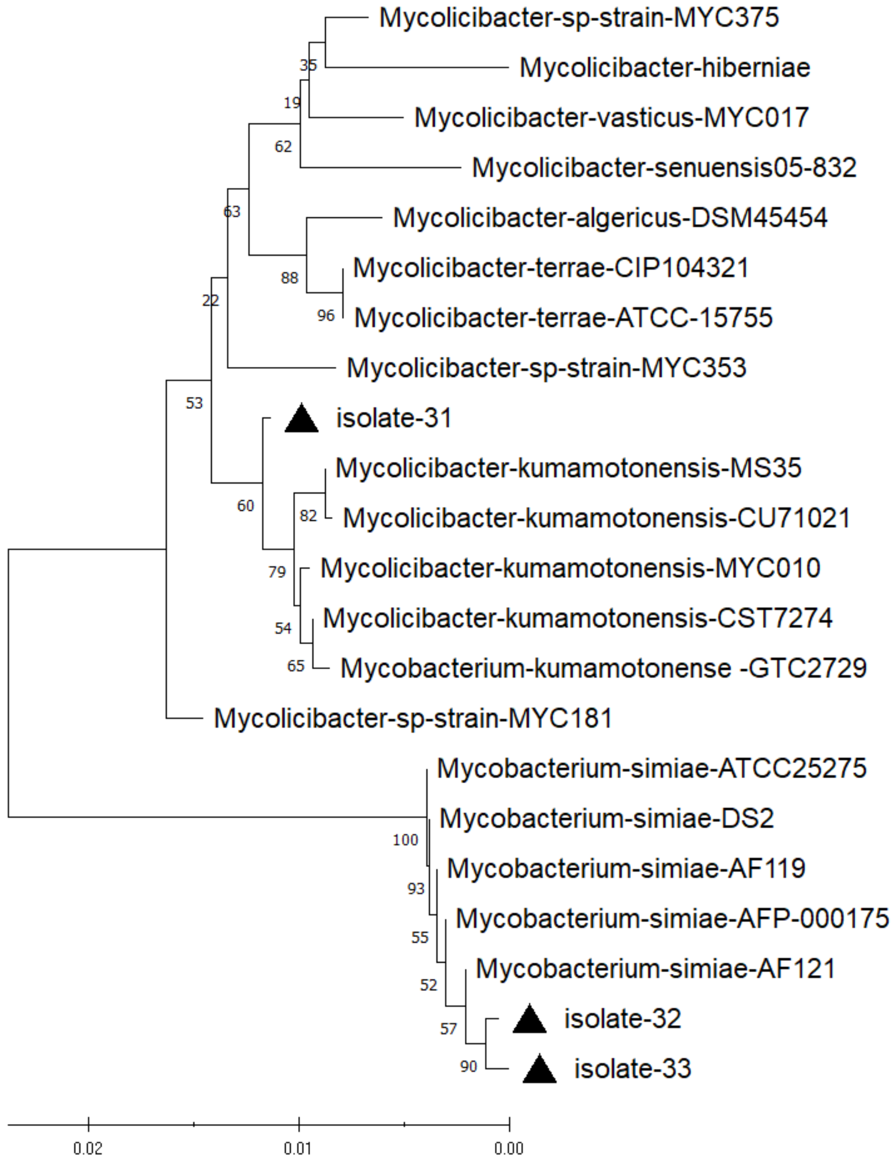

Figure 2. Phylogenetic tree of different strains and isolates of *Mycobacterium* based on *16S rRNA*

teria [17]. In this study, we used the sequencing of *16S rRNA*, *hsp65*, and *rpoB* genes for identifying non-tuberculous mycobacteria and phylogenetic analysis. Previous studies reported that the geographical distribution of the NTM group varies worldwide [18]. In this study, *M. simiae* was the most predominant among the NTM group, similar to the Shafipour study [19]. Another study showed that the *M. avium* complex was the most predominant among NTM species in Asia including, South Korea, Japan, and Taiwan [20]. Firoozeh et al. reported that the prevalence of non-tuberculous mycobacteria has increased in Iran. *M. simiae* was the most common slow-growing NTM group, similar to our study, while *M. fortuitum*, *M. terrae*, and *M. gastri* were the most common fast-growing NTM species, respectively [21]. In this study, one NTM isolate showed heterogeneity resulting from the sequencing of all three genes. Based on our result, sequencing using the *16S rRNA* gene showed higher discriminatory power and percentage similarities (99.5%) than the *hsp65* gene (97.8%) and the *rpoB* gene (93.3%). *16S rRNA* gene produces a 1436 bp fragment, which is the largest amplified fragment of the *16S rRNA* gene; therefore, the results of the *16S rRNA* gene are more valid than those of the two other genes, similar to the Hassansoltan Solaghani study [22]. Lee et al. also reported that the sequencing of *16S rRNA* and *hsp65* genes is useful for the identification of the MTC, but the discriminatory power of the *hsp65* gene is lower compared to *16S rRNA*, similar to our study [23]. In this study, one NTM isolate was identified as *M. kumamotonensis*.

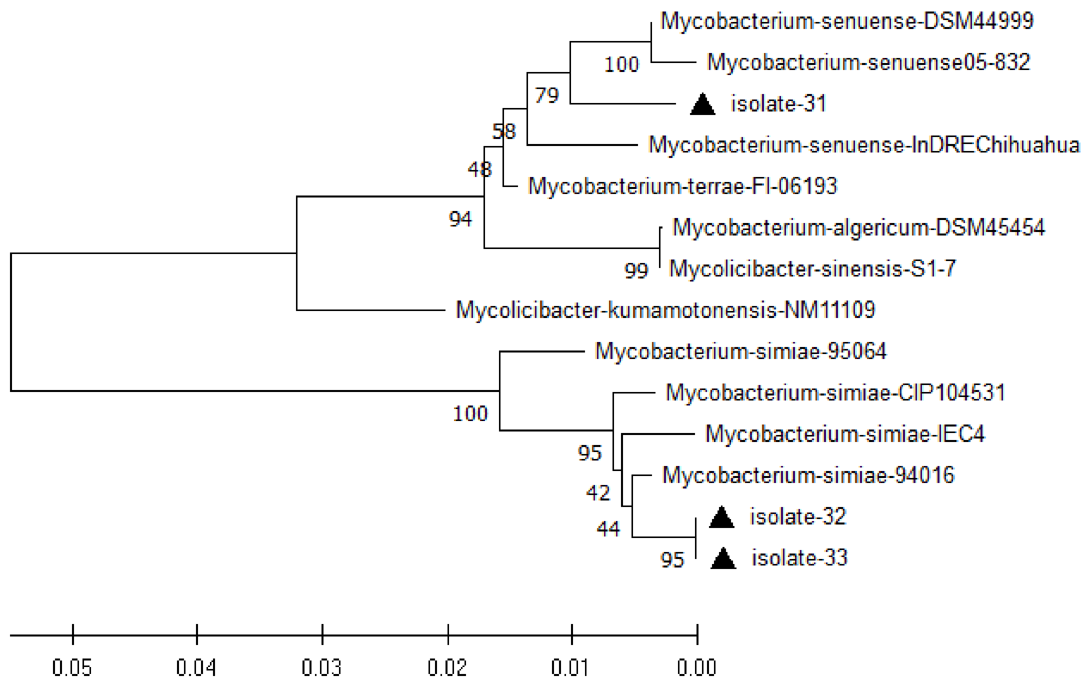

Figure 3. Phylogenetic tree of different strains and isolates of *Mycobacterium* based on hsp65

Also, the similarity of this isolate to *M. senuense* and *M. terrae* was identified through the *hsp65* and *rpoB* genes, respectively. According to studies, *M. kumamotonensis* and *M. senuense* are new species of the MTC [24]. *M. kumamotonensis* has a great similarity to members of the MTC based on the sequencing of *16S rRNA*, *hsp65*, and *rpoB* genes [25]. *M. senuense* is also classified in the MTC based on phenotypic characterization [26]. Therefore, one of the three NTM isolates belonged to the MTC, and two of the three NTM isolates were identified as *M. simiae* by sequencing of *16S rRNA*, *hsp65*, and *rpoB* genes. Two out of three NTM isolates were isolated from sputum samples of patients with underlying diseases. The risk of infection with NTM in underlying diseases, such as diabetes and immunodeficiency diseases, is greater than in patients without underlying disease [27], similar to our study. Wang reported a relationship between patients with diabetes mellitus and NTM, although there were no significant differences reported among age and gender groups [27]. According to the previous study and our study, *M. simiae* is the most common slow-growing nontuberculous mycobacterium among NTM species in Iran. Because *M. simiae* is the only niacin-positive NTM, similar to *M. tuberculosis*, and it can cause pulmonary infections in both immunocompromised and immunocompetent individuals [28], its differentiation from *M. tuberculosis* is very vital and will help in the rapid recovery of patients. Another NTM species, *M. kumamotonensis* was isolated from the sputum sample of a 70-year-old male patient without underlying disease. As mentioned, this bacterium is a new species of the MTC. So far, no report has been announced of its isolation in Iran. Based on our results, *M. tuberculosis* has a high prevalence in this city, similar to the previous study, due to its proximity to the borders of Afghanistan and Pakistan. Khammarnia reported a significant relationship between non-Iranian nationality and TB in Sistan and Balochistan Province [29]. In this study, 5% of disease cases belonged to the non-Iranian population; therefore, the possibility of disease transmission and circulating strains within non-Iranian populations should be considered one of the important contributing factors in this city. Other factors, such as malnutrition, smoking, tropical climate, population density, and poverty, should also be taken into account. Thus, the high prevalence of circulating *M. tuberculosis* in the human population of Zahedan highlights the need for screening programs to reduce the number of disease cases. Therefore, further studies on the human population to identify *Mycobacterium* strains are required in the future.

Acknowledgements

The authors are very grateful to the staff of Microbiology Department.

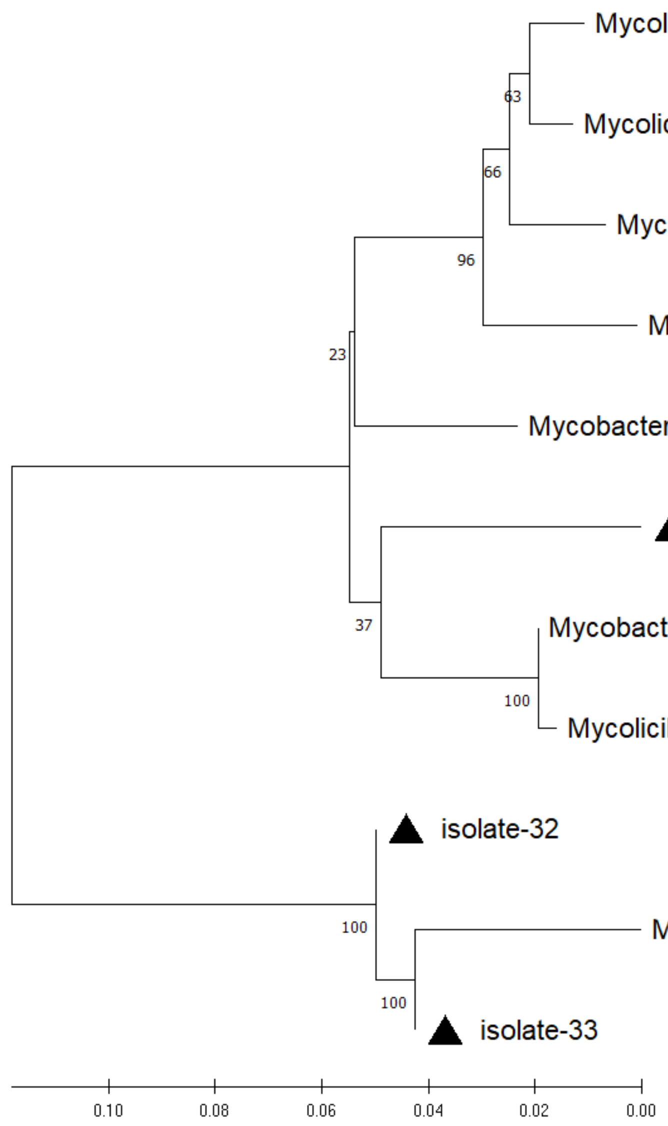

Figure 4. Phylogenetic tree of different strains and isolates of *Mycobacterium* based on rpoB

Compliance with ethical guidelines

This study was approved by the Research Ethics Committees of Razi Vaccine and Serum Research Institute, Karaj, Iran (Code: IR.RVSRI.REC.1403.006).

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Data acquisition, analysis, interpretation: Fatemeh Alimardani; Writing the original draft: All authors; Review and editing: Nader Mosavari, Soheila Moradi Bidhendi, Ali Reza Salimi Khorashad, and Lida Abdolmohammadi Khiav; Nader Mosavari, Soheila Moradi Bidhendi, Ali Reza Salimi Khorashad, and Lida Abdolmohammadi Khiav; Supervision: Nader Mosavari, Soheila Moradi Bidhendi, and Ali Reza Salimi Khorashad.

Conflict of interest

The authors declared no conflict of interest.

References

[1] Behr MA, Kaufmann E, Duffin J, Edelstein PH, Ramakrishnan L. Latent tuberculosis: Two centuries of confusion. Am J Respir Crit Care Med. 2021; 204(2):142-8. [DOI:10.1164/rccm.202011-4239PP] [PMID]

[2] Ravansalar H, Tadayon K, Ghazvini K. Molecular typing methods used in studies of Mycobacterium tuberculosis in Iran: a systematic review. Iran J Microbiol. 2016; 8(5):338-46. [PMID]

[3] World Health Organization. Global tuberculosis report. Geneva: WHO; 2023. [Link]

[4] Parsons LM, Brosch R, Cole ST, Somoskövi A, Loder A, Bretzel G, et al. Rapid and simple approach for identification of Mycobacterium tuberculosis complex isolates by PCR-based genomic deletion analysis. J Clin Microbiol. 2002; 40(7):2339-45. [DOI:10.1128/JCM.40.7.2339-2345.2002] [PMID]

[5] Fallahzadeh H, Khazaei Z, Najafi ML, Pordanjani SR, Goodarzi E. Distribution incidence, mortality of tuberculosis and human development index in Iran: Estimates from the global burden of disease study 2019. BMC Public Health. 2023; 23(1):2404. [DOI:10.1186/s12889-023-17114-4] [PMID]

[6] Shakiba Mehr. Reported Mycobacterium bois from suspected patients with TB in Zahedan. 2015. [Unpublished]

[7] Van Soolingen D, Hoogenboezem T, De Haas PE, Hermans PW, Koedam MA, Teppema KS, et al. A novel pathogenic taxon of the Mycobacterium tuberculosis complex, Canetti: characterization of an exceptional isolate from Africa. Int J Syst Bacteriol. 1997; 47(4):1236-45. [DOI:10.1099/00207713-47-4-1236] [PMID]

[8] Sreevatsan S, Escalante P, Pan X, Gillies DA 2nd, Siddiqui S, Khalaf CN, et al. Identification of a polymorphic nucleotide in oxyR specific for Mycobacterium bovis. J Clin Microbiol. 1996; 34(8):2007-10. [DOI:10.1128/jcm.34.8.2007-2010.1996] [PMID]

[9] Clustal. Chromas and Clustal X programs [Internet]. 2026 [Uupdated 7 April 2026]. Available from: [link]

[10] Blast.cgi. NCBI BLAST. 2026 [Uupdated 7 April 2026]. Available from: [Link]

[11] Huard RC, Lazzarini LC, Butler WR, van Soolingen D, Ho JL. PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. J Clin Microbiol. 2003; 41(4):1637-50. [DOI:10.1128/JCM.41.4.1637-1650.2003] [PMID]

[12] van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, et al. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: Recommendations for a standardized methodology. J Clin Microbiol. 1993; 31(2):406-9. [DOI:10.1128/jcm.31.2.406-409.1993] [PMID]

[13] Warren RM, Gey van Pittius NC, Barnard M, Hesseling A, Engelke E, de Kock M, et al. Differentiation of Mycobacterium tuberculosis complex by PCR amplification of genomic regions of difference. Int J Tuberc Lung Dis. 2006; 10(7):818-22. [PMID]

[14] Torabi N, Mosavari N, Nazari R. [Molecular identification of Mycobacterium tuberculosis complex by RD-Typing and PCR-RFLP Method (Persian)]. Vet Res Biol Prod. 2017; 30(3):16-25. [Link]

[15] Dehghanpour M, Baghcheh Saraee H, Mosavari N, Mazloom Zadeh S. [Isolation of Mycobacterium bovis from tuberculosis patients in Zanjan Province (2013) (Persian)]. J Adv Med Biomed Res. 2016; 24(107):71-83. [Link]

[16] Karami-Zarandi M, Bahador A, Gizaw Feysia S, Kardan-Yamchi J, Hasan-Nejad M, Vosough H, et al. Identification of non-tuberculosis mycobacteria by line probe assay and determination of drug resistance patterns of isolates in Iranian patients. Arch Razi Inst. 2019; 74(4):375-84. [Link]

[17] Ledesma Y, Echeverría G, Claro-Almea FE, Silva D, Guerrero-Freire S, Rojas Y, et al. The re-identification of previously unidentifiable clinical non-tuberculous mycobacterial isolates shows great species diversity and the presence of other acid-fast genera. Pathogens. 2022; 11(10):1159. [DOI:10.3390/pathogens11101159] [PMID]

[18] Hoefsloot W, Van Ingen J, Andrejak C, Ängeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: An NTM-NET collaborative study. Eur Respir J. 2013; 42(6):1604-13. [DOI:10.1183/09031936.00149212] [PMID]

[19] Shafipour M, Shirzad-Aski H, Ghaemi EA, Sohrabi A, Taziki M, Kochkaksaraei MB, et al. Occurrence and risk factors of nontuberculous mycobacteria in tuberculosis-suspected patients in the north of Iran. Iran J Microbiol. 2021; 13(2):190-8. [DOI:10.18502/ijm.v13i2.5980] [PMID]

[20] Ong LT. Epidemiology of nontuberculous mycobacteria infection in Asia: A Narrative Review. Indian J Tuberc. 2025; 72(2):259-65 [DOI:10.1016/j.ijtb.2024.08.006] [PMID]

[21] Firoozeh F, Firoozeh A, Salmani A. An update on the prevalence of nontuberculous in clinical samples in Iran during 2000-2022: A Retrospective Systematic Review and Meta-Analysis. Med Lab J. 2023; 17(3):22-31. [DOI:10.61186/mlj.17.3.22]

[22] Hassansoltan Solaghani T, Nazari R, Mosavari N, Tadayon K, Zolfaghari MR. Isolation and identification of nontuberculous mycobacteria from raw milk and traditional cheese based on the 16S rRNA and hsp65 genes, Tehran, Iran. Folia Microbiol (Praha). 2024; 69(1):81-9. [DOI:10.1007/s12223-023-01073-9] [PMID]

[23] Lee CK, Gi HM, Cho Y, Kim YK, Lee KN, Song KJ, et al. The genomic heterogeneity among Mycobacterium terrae complex displayed by sequencing of 16S rRNA and hsp65 genes. Microbiol Immunol. 2004; 48(2):83-90. [DOI:10.1111/j.1348-0421.2004.tb03492.x] [PMID]

[24] Abudaff NN, Beam E. Mycobacterium arupense: A review article on an emerging potential pathogen in the Mycobacterium terrae complex. J Clin Tuberc Other Mycobact Dis. 2017; 10:1-5. [DOI:10.1016/j.jctube.2017.11.001] [PMID]

[25] Masaki T, Ohkusu K, Hata H, Fujiwara N, Iihara H, Yamada-Noda M, et al. Mycobacterium kumamotonense sp. nov. recovered from clinical specimen and the first isolation report of Mycobacterium arupense in Japan: Novel slowly growing, nonchromogenic clinical isolates related to Mycobacterium terrae complex. Microbiol Immunol. 2006; 50(11):889-97. [DOI:10.1111/j.1348-0421.2006.tb03865.x] [PMID]

[26] Mun HS, Park JH, Kim H, Yu HK, Park YG, Cha CY, et al. Mycobacterium senuense sp. nov., a slowly growing, nonchromogenic species closely related to the Mycobacterium terrae complex. Int J Syst Evol Microbiol. 2008; 58(Pt 3):641-6. [DOI:10.1099/ijs.0.65374-0] [PMID]

[27] Wang JY, Lin HC, Lin HA, Chung CH, Chen LC, Huang KY, et al. Associations between diabetes mellitus and non-tuberculous mycobacterium-caused diseases in Taiwan: A nationwide cohort study. Am J Trop Med Hyg. 2021; 105(6):1672-19. [DOI:10.4269/ajtmh.20-1441] [PMID]

[28] Malekshahian D, Tabarsi P, Farnia P, Javanmard P, Seif S, Teymouri H. Mycobacterium simiae isolates subtypes and molecular drug susceptibility in Iran. Arch Clin Infect Dis. 2022; 17(3):e127866. [DOI:10.5812/archcid-127866]

[29] Khammarnia M, Daghighi M, Peyvand M. Analysis of the epidemiological trend of tuberculosis in Sistan and Baluchestan province during 2015-2019. J Shahid Sadoughi Univ Med Sci. 2024, 31(11):7194-203. [DOI:10.18502/ssu.v31i11.14784]