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ORIGINAL RESEARCH

Evaluating the clinical significance of nontuberculous mycobacteria isolated from respiratory samples in Iran: an often overlooked disease

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Purpose: Nontuberculous mycobacteria (NTM) infection is an increasing problem worldwide whose clinical significance is still largely unknown. The aim of this study was to investigate the epidemiology of NTM infection from respiratory samples and to determine their clinical significance.

Patients and methods: This cross-sectional study was performed on 7,825 clinical samples from December 2015 to December 2017. Detection was conducted using phenotypic and genotypic (*hsp65* PCR-RFLP, *rpoB*, and *16S rRNA* genes sequencing) methods. All clinical information including symptoms and radiological findings was extracted from patients' records.

Results: A total of 478 were confirmed to have respiratory samples which were culture positive for mycobacteria, with the prevalence of NTM infection obtained as 53 (11.1%). Overall, *Mycobacterium (M.) fortuitum* was the most frequent NTM isolate, followed by *M. simiae, M. kansasii, M. gordonae*, and *M. conceptionense*. There was a relationship between NTM isolates and gender (P=0.039), symptoms (P=0.048), and radiographic findings (P=0.013). Bronchiectasis, infiltration, and cavitary lesion were the most frequent radiological findings in *M. fortuitum, M. simiae*, and *M. kansasii*, respectively, with cough being the most frequent symptom.

Conclusion: We reported five different NTM isolates in respiratory samples with a high frequency of *M. fortuitum*. NTM infections may play an important role in causing pulmonary disease and in tuberculosis management in endemic settings. Nevertheless, more studies are required to further examine the clinical significance of NTM isolates.

Keywords: nontuberculous mycobacteria, respiratory samples, clinical significance, *Mycobacterium fortuitum*

Introduction

Nontuberculous mycobacteria (NTM) which can cause pulmonary and extrapulmonary infections in susceptible persons constitute ubiquitous environmental bacteria often found in water and soil. Nowadays, NTM infections are increasingly identified as important causes of morbidity and mortality in patients owing to misdiagnosis and unsuitable treatment. In contrast with tuberculosis (TB) infection, the knowledge about NTM infections is still limited. While >190 NTM species have been recognized so far, but there are significant epidemiologic variations by species such as differences in risk factors and bacterial geographic distribution.[1–4]

The incidence of NTM infections is growing worldwide including in the Middle East countries.[5] Determining the epidemiology of NTM infection has been more challenging than for TB. Reporting NTM infection to public health authorities is

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not compulsory in most parts of the world in comparison with TB reports. Therefore, there is no accurate epidemiological information and monitoring,[6–8] thus reducing our knowledge and understanding of the effect of NTM infections on community health.[5] This eventually culminates in mycobacterial infections and their spread across healthcare units.[9] Understanding the trends and determining the real prevalence of NTM infection is an important priority for optimizing infection control programs and resources.[10] Several studies have indicated isolation of NTM infection from both TB-suspected patients and the general public in some parts of the country.[7,11] However, the prevalence of NTM infection has still remained largely unknown. Therefore, the aim of the current study was to evaluate the prevalence of NTM infection among respiratory samples and to determine their clinical importance.

Materials and methods

Sample collection and preparation

This was a cross-sectional study conducted from December 2015 to December 2017 on a total of 7,825 TB-suspected respiratory clinical samples referring to Pasteur Institute of Iran. NTM isolates were identified using the guidelines by the American Thoracic Society and Infectious Diseases Society of America (ATS/IDSA).[12] We included patients with a minimum of two positive cultures from sputum samples and/or at least one positive culture from bronchoalveolar lavage (BAL), samples were considered as clinically relevant to define NTM pulmonary disease (Figure 1). The exclusion criteria were non-respiratory samples such as skin, urine, pus, joints, lymph node and soft tissues and *Mycobacterium tuberculosis* complex (MTC) positive samples.

This study was in accordance with the Declaration of Helsinki (1975) and local regulations. It was also approved by institutional review boards at Pasteur Institute of Iran (IR.PII.REC.1394.54). Written informed consents were received from all individual. All samples were decontaminated using N-acetyl-L-cysteine-sodium hydroxide method and were cultured on three slopes of Löwenstein–Jensen (LJ) medium.[13]

Identification of NTM strains using phenotypic and genotypic tests

The phenotypic tests for isolation of NTM strains included macroscopic and microscopic morphological characteristics, growth rate on LJ medium, growth at temperature of 25°C, 32°C, 37°C, and 42°C, Tween-80 hydrolysis, arylsulfatase, urease production, tellurite reduction, nitrate reduction, semi-quantitative catalase production, and salt tolerance, according to the Centers for Disease Control (CDC) procedures.[13]

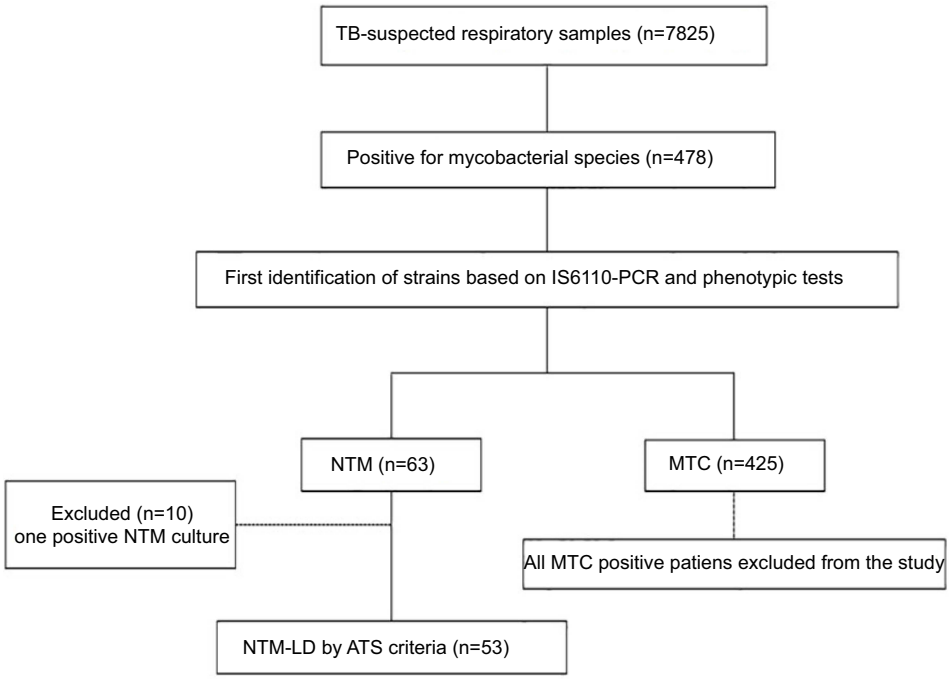

Figure 1 Population study flowchart.
Abbreviations: TB, tuberculosis; MTC, *Mycobacterium tuberculosis* complex; NTM-LD, nontuberculous mycobacteria lung disease; ATS, American Thoracic Society.

Bacterial DNA was isolated using Proba-NK DNA extraction kit (DNA-Technology Company, Moscow, Russia), according to the manufacturer's instructions. The insertion sequence 6110 (*IS6110*)-PCR (123 bp) method was applied for differentiation of MTC and NTM species in positive cultures.[14]

All NTM strains were primarily detected by using 441 bp fragment of heat shock protein 65 (*hsp65*), followed by restriction fragment length polymorphism (RFLP) via *BstEII* and *HaeIII* restriction enzymes.[15] Also, NTM isolates detected only up to *Mycobacterial* species level were investigated for sequencing two highly conserved genes, *16S rRNA* (nearly 1,500 bp) and *rpoB* (750 bp) genes,[16,17] using an ABI Automated Sequencer (Applied Biosystems, Foster City, CA, USA).

Drug susceptibility testing (DST)

The minimum inhibitory concentrations (MICs) of each drug were performed by the broth microdilution method and interpreted in accordance with the guidelines from the Clinical and Laboratory Standards Institute (CLSI).[18,19] The drugs tested included isoniazid, rifampicin, ethambutol, streptomycin, ethionamide, amikacin, ofloxacin, ciprofloxacin, and capreomycin. Serial double dilutions of drugs were prepared ranging from 0.06 to 512 mg/L, added to cation-adjusted Mueller–Hinton broth with the addition of 5% oleic acid-albumin-dextrose-catalase. After inoculating, all cultures were incubated aerobically at 37°C. Growth was evaluated on days 3 and 7 for rapidly growingmycobacteria (RGM) and weekly up to 4 weeks for slowly growingmycobacteria (SGM). The MIC was defined as the lowest concentration of the antimicrobial agents that inhibited visible growth. Susceptible, moderately susceptible and resistant breakpoints were assigned according to the CLSI guidelines.[18]

Statistical analysis

All clinical data and demographic characteristics were evaluated by using SPSS version 24.0 (2016; IBM Corp., Armonk, NY, USA) software package. Two-tailed *P*-value <0.05 was considered statistically significant. Data normality of continuous variables was initially verified using the Shapiro–Wilk test. Fisher's exact test/χ^2 and Mann–Whitney *U*-test were used to determine significant association between qualitative and continuous variables, respectively. Continuously distributed variables were described by reporting their mean.

Results

Patient's characteristics

A total of 7,825 TB-suspected respiratory samples were included in this study. According to the culture method, 478 (6.1%) samples yielded acid-fast bacilli on culture. Employing phenotypic and molecular methods, 425 (88.9%) and 53 (11.1%) subjects were MTC and NTM strains, respectively.

The baseline demographic characteristics of the NTM patients are summarized in Table 1. Briefly, all samples were from pulmonary site including 45 (84.9%) sputum and eight (15.1%) BAL. Cough, sputum, fever, weight loss, and night perspiration were the most frequent symptoms. The NTM patient's average age was 43.4±15.7 years, and 28 (52.8%) and 25 (47.2%) patients were male and female, respectively. In addition, 8 (15.1%), 6 (11.3%), and 5 (9.4%) cases had HIV, cystic fibrosis (CF) and, diabetes mellitus, respectively.

Phenotypic tests for detection of NTM isolates

Out of 53 isolates, 33 (62.3%) and 20 (37.7%) NTM isolates were RGM and SGM, respectively. According to the phenotypic tests, *M. fortuitum* (29 isolates) and *M. simiae* (9 isolates) were the most frequent strains, followed by *M. kansasii* (4 isolates) and *M. gordonae* (2 isolates). Among 53 isolates, only 45 (84.9%) strains were identified by phenotypic tests, while the rest of isolates were unidentifiable.

Molecular tests for detection of NTM isolates

According to molecular tests (*hsp65*-RFLP, *rpoB*, and *16S rRNA*), NTM isolates included *M. fortuitum* (31 isolates), *M. simiae* (12 isolates), *M. kansasii* (6 isolates), *M. gordonae* (2 isolates), and *M. conceptionense* (2 isolates) (Table 1).

Clinical importance of NTM isolates

The patients' characteristics according to NTM isolates are reported in Table 2. Briefly, there were no significant associations between NTM isolates and the mean age, history of smoking, presence of underlying disease, sample location, and smear microscopy results. However, there was a relationship between NTM isolates with gender, symptoms and radiographic findings. In the *M. fortuitum* positive group, AFB smears were positive for 25.8% of cases. Also, bronchiectasis (54.8%) was the most frequent chest radiography results. Here, 6 (19.4%), 4 (12.9%), and 2 (6.5%)

Table 1 Clinical characteristic of patients infected with NTM isolates

Factors	NTM positive patients (n=53)
Mean age ± SD	52.9±13.8
Gender	
Male	28 (52.8%)
Female	25 (47.2%)
History of smoking	
Smokers	16 (30.2%)
Non-smokers	37 (69.8%)
Symptoms	
Cough	49 (92.5%)
Sputum	45 (84.9%)
Fever	45 (84.9%)
Weight loss	35 (66.1%)
Night perspiration	32 (60.4%)
Gastroesophageal	26 (49.1%)
Dyspnea	18 (33.9%)
Hemoptysis	12 (22.6%)
Underlying disease	
HIV	8 (15.1%)
Cystic fibrosis	6 (11.3%)
Diabetes mellitus	5 (9.4%)
NTM sample location	
Sputum	45 (84.9%)
Bronchoalveolar lavage	8(15.1%)
AFB smear microscopy	
Positive	12 (22.6%)
Negative	41 (77.4%)
Radiographic findings	
Bronchiectasis	26 (49.1%)
Infiltrate	19 (35.8%)
Cavitary	10 (18.9%)
Consolidation	9 (17.1%)
NTM isolates	
M. fortuitum	31 (58.5%)
M. simiae	12 (22.6%)
M. kansasii	6 (11.3%)
M. gordonae	2 (3.8%)
M. conceptionense	2 (3.8%)

Abbreviations: NTM, nontuberculous mycobacteria; AFB, acid-fast bacilli.

patients had HIV, CF, and diabetes mellitus, respectively, with 38.7% of patients being smoker. All infected patients had gastroesophageal diseases such as chronic vomiting and achalasia. The major symptoms observed in these patients included cough, sputum, fever, and weight loss (Figure 2). Most of the *M. fortuitum* strains were resistant to isoniazid, rifampicin, ethambutol, and streptomycin, and susceptible to amikacin (Table S1).

In the *M. simiae* group, 2 (16.7%), 1 (8.3%), and 1 (8.3%) patients had diabetes mellitus, HIV, and CF, respectively. Infiltrate with high frequency was seen in this group. Patients infected with *M. simiae* had an older mean age and most of them were female (83.3%). The majority of *M. simiae* strains were resistible to isoniazid, rifampicin, ethambutol, and streptomycin, while susceptible to amikacin, ofloxacin, and ciprofloxacin (Table S2).

In the *M. kansasii* group, HIV infectivity was shown in one (1.9%) patients, and one (1.9%) of the patients had diabetes mellitus. All patients had cavitary lesion in their chest radiography results. Hemoptysis was more common in this group than in other patients (Figure 2). Most of the *M. kansasii* strains were susceptible to isoniazid, rifampicin, ethambutol, and streptomycin, while resistant to amikacin, ofloxacin, and ciprofloxacin (Table S2). Infections caused by *M. kansasii, M. fortuitum*, and *M. conceptionense* were more frequent in male than in female patients.

Discussion

NTM pulmonary infections have increasingly been reported in both immunocompetent and immunocompromised individuals worldwide.[20–22] In several studies, *M. avium-intracellulare* (MAC) infection has been the most common NTM isolates, followed by *M. abscessus/chelonae, M. fortuitum*, and *M kansasii*.[22,23] The results of our study indicated that *M. fortuitum* was the most common NTM isolation species, followed by *M. simiae* and *M. kansasii* in Iranian patients.

In Iran, *M. fortuitum* is the most frequent RGM isolated from both environmental and clinical samples.[5] Predisposing factors including chronic reflux disease, HIV, malignancy, achalasia, CF, and bronchiectasis in patients infected with *M. fortuitum* usually cause pulmonary disease.[24,25] The infectious pulmonary disease pattern includes pneumonia, lung abscesses, solitary pulmonary nodules, and pleural effusions.[24] In line with other studies, most patients had underlying diseases such as HIV, CF, and diabetes mellitus. Also, bronchiectasis was the most common radiographic findings in these patients. *M. fortuitum* pulmonary infection seems to usually occur in structural lung disease.[24]

According to ATS/IDSA guideline, *M. fortuitum* was occurred in 15% of patients with pulmonary disease owing

Table 2 Patients properties based on NTM isolates

Factors	*M. fortuitum* (n=31)	*M. simiae* (n=12)	*M. kansasii* (n=6)	*M. gordonae* (n=2)	*M. conceptionense* (n=2)	*P*-value
Mean age ± SD	52.9±14.2	62.5±9.8	42.5±11.8	46.0±5.7	44.0±9.9	0.345
Gender						0.039*
Male	19 (61.3%)	2 (16.7%)	4 (66.7%)	1 (50.0%)	2 (100.0%)	
Female	12 (38.7%)	10 (83.3%)	2 (33.3%)	1 (50.0%)	0 (0.0%)	
History of smoking						0.424
Smokers	12 (38.7%)	2 (16.7%)	1 (16.7%)	1 (50.0%)	0 (0.0%)	
Non-smokers	19 (61.3%)	10 (83.3%)	5 (83.3%)	1 (50.0%)	2 (100.0%)	
Underlying disease						0.888
HIV	6 (19.4%)	1 (8.3%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	
Cystic fibrosis	4 (12.9%)	1 (8.3%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	
Diabetes mellitus	2 (6.5%)	2 (16.7%)	1 (16.7%)	0 (0.0%)	1 (50.0%)	
NTM sample location						0.605
Sputum	28 (90.3%)	10 (83.3%)	5 (83.3%)	1 (50.0%)	1 (50.0%)	
Bronchoalveolar lavage	3 (9.7%)	2 (16.7%)	1 (16.7%)	1 (50.0%)	1 (50.0%)	
AFB smear microscopy						0.739
Positive	8 (25.8%)	2 (16.7%)	1 (16.7%)	1 (50.0%)	0 (0.0%)	
Negative	23 (74.2%)	10 (83.3%)	5 (83.3%)	1 (50.0%)	2 (100.0%)	
Radiographic findings						0.013*
Bronchiectasis	17 (54.8%)	5 (41.7%)	1 (16.7%)	2 (100.0%)	1 (50.0%)	
Infiltrate	6 (19.4%)	9 (75.0%)	2 (33.3%)	1 (50.0%)	1 (50.0%)	
Cavitary	2 (6.5%)	1 (8.3%)	6 (100.0%)	0 (0.0%)	1 (50.0%)	
Consolidation	3 (9.7%)	4 (33.3%)	1 (16.7%)	1 (50.0%)	0 (0.0%)	

Note: *Statistically significant (P< 0.05).

Abbreviations: NTM, nontuberculous mycobacteria; AFB, acid-fast bacilli.

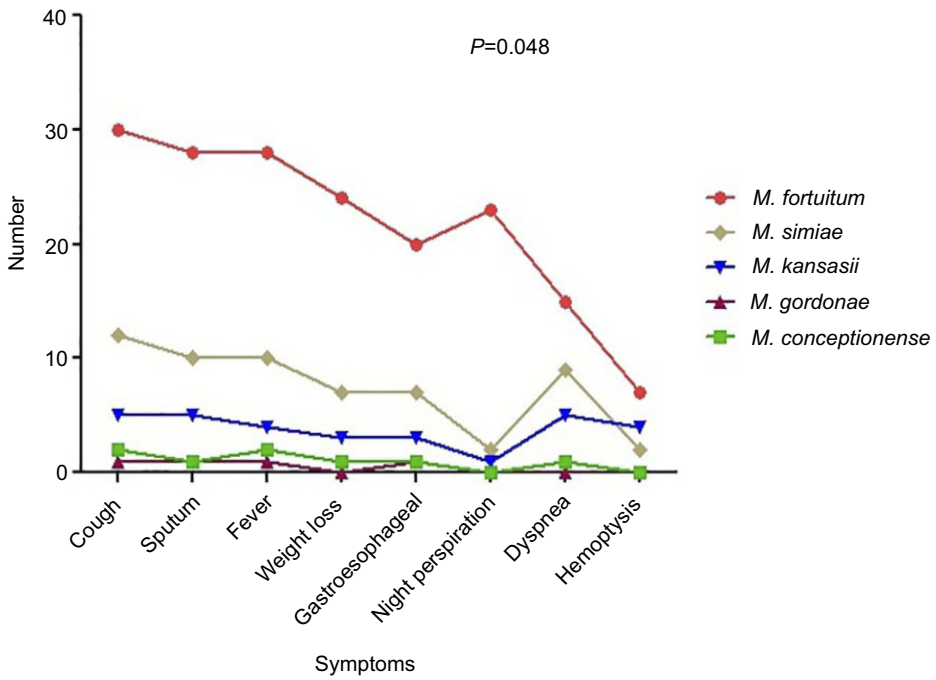

Figure 2 Symptoms of patients infected with nontuberculous mycobacteria (NTM) isolates.

to RGM usually observed as a pathogen in patients with gastroesophageal disease such as chronic vomiting, achalasia, and exogenous lipoid pneumonia.[12] Studying patients with NTM pulmonary disease as well as esophageal disorders, Hadjiliadis et al revealed that most of them had achalasia and lung infection by *M. fortuitum*.[26] In the current study, most of *M. fortuitum* infected patients complained about gastroesophageal disease and achalasia, suggests them being an important contributor to *M. fortuitum* pulmonary disease.[25]

All *M. fortuitum* positive patients had two or more positive culture results. Several studies observing *M. fortuitum* isolation in two of the three respiratory sample cultures proposed that although *M. fortuitum* lung disease is rare, this could potentially be the cause of disease.[12] However, the results of our study have suggested a high pathogenicity of *M. fortuitum* in respiratory samples.

DST results against *M. fortuitum* isolates indicated that this isolate was susceptible to amikacin and intermediate to ofloxacin, ciprofloxacin, and capreomycin in our study. The *M. fortuitum* group is generally more susceptible to drugs than other RGM species. They are usually susceptible or intermediate to doxycycline, fluoroquinolones, sulfonamides, and macrolides.[27] However, Park et al suggested that long-term treatment with antibiotics may not be necessary for most *M. fortuitum* positive patients from respiratory samples. For this group of patients, infection with *M. fortuitum* can be seen as colonization or a transient infection, and thus less invasive treatment strategies may be indicated.[24]

The second frequent NTM in our study was *M. simiae*. The presence of *M. simiae* in respiratory samples could suggest a real infection or colonization.[11] The current study indicated that most patients infected with *M. simiae* were female and older than those with other pulmonary NTM isolates. Several studies have revealed that men outnumber women in pulmonary disease caused by all NTM species, except for *M. abscessus, M. chelonae*, and *M. simiae*, which is in line with our results.[28,29]

The *M. simiae* positive cases in our study had underlying diseases such as HIV, CF and diabetes mellitus. According to early surveillance reports, 21% of *M. simiae* patient isolates had underlying disease, although other reports have shown a far lower incidence of clinical disease.[12] Nevertheless, earlier reports revealed that NTM disease mainly occurs in patients with diabetes, CF, silicosis, and pneumoconiosis.[30] Although the HIV infection is very important for increased NTM infection, Maoz et al reported that *M. simiae* positive patients were HIV negative. These findings may demonstrate that a clinical suspicion for *M. simiae* infection should also be considered more frequently in non-HIV subjects.[28]

M. simiae may have similar clinical and radiologic manifestations with *M. tuberculosis*.[11] In the current study, *M. simiae* was isolated from patients previously diagnosed as multidrug-resistant TB, who had received anti-TB drugs. As a result, for every *M. simiae* isolate, DST should be performed.[31] In our isolated strain, due to financial reasons and lack of access to all drugs, DST was not conducted with all drugs. However, *M. simiae* isolates from the present study were mainly susceptible to amikacin, ofloxacin, and ciprofloxacin, unlike the findings by Van Ingen et al, which indicated susceptibility rates of 14–40% for amikacin and 33–62% for ciprofloxacin.[31] These results confirm the variable susceptibly profile of *M. simiae* in various geographic regions, emphasizing the need to do the susceptibility testing before starting treatment. However, there is little information linking in vitro susceptibility to treatment responses.[30]

M. kansasii was the third most frequent pulmonary NTM in the current study. It has clinical and antigenic properties similar to *M. tuberculosis* whose high prevalence has been shown in polluted cities.[5,32]

In this study, radiographic findings for *M. kansasii* pulmonary disease were diverse. Cavitary lesion was the most frequent radiographic features. Several studies have demonstrated cavitation in 75–96% of patients with *M. kansasii* pulmonary disease.[33,34] The radiographic characteristics of *M. kansasii* pulmonary disease are commonly known as being indiscernible from those of *M. tuberculosis*.

Appropriate treatment regimen for *M. kansasii* pulmonary disease is a three-drug combination including isoniazid, rifampin, and ethambutol for at least 12 months after negative sputum conversion. In our study, DST for *M. kansasii* pulmonary disease, in accordance with other studies, had the same results with the treatment regimen. All *M. kansasii* isolate were resistant to isoniazid, rifampin, and the highest resistance to ciprofloxacin.[35] In line with our study, Shitrit et al showed that *M. kansasii* isolates had highest sensitivity to rifampin, ethambutol, and the highest resistance to ciprofloxacin.[19]

Hemoptysis occurred in 4 (66.7%) of the *M. kansasii* cases in our group patients, which was higher than the rates of other studies.[12,33] Hemoptysis in lung infections can be related to the incidence of endobronchial disease and lead to disintegration of bronchial vessels by cavitation. Although there is no practical information on the relative incidence of endobronchial disease, the incidence of cavitation in the *M. kansasii* pulmonary infection is reported to be 57%, which is slightly close to our study. Note that cavitations were far less common in patients with other NTM infections.[34–36]

We found higher patients with hemoptysis, dyspnea, and cough and fewer patients with fever and night perspiration. This result was inconsistent with the report of Shitrit et al[19]. These discrepancies may have been owing to the short interval from symptom onset to diagnosis.[19]

In the current study, *M. conceptionense* was rarely identified in respiratory samples. It was reported that this isolate caused skin and subcutaneous fat infections under surgery in immunocompromised patients.[37] Furthermore, few studies confirmed that this isolate may be a pulmonary pathogen.[38,39] In Iran, only one study had revealed *M. conceptionense* in two patients with pulmonary disease.[40]

For the second time in Iran, we identified *M. conceptionense* in 37-year-old male with HIV infection and 51-year-old male with diabetes mellitus. Interestingly, both patients had underlying disease and DST showed susceptibility to ofloxacin, ciprofloxacin, and amikacin as well as intermediate to isoniazid, rifampicin, and ethionamide. These findings were in agreement with the Kim et al's study[39] suggesting that *M. conceptionense* can cause pulmonary disease, though it is a rare NTM in Iran.

The main limitations of our study were the lack of access to follow-up information. Also, the radiological evaluation focused mainly on chest radiography, not on CT scan.

Conclusion

We detected five NTM species in respiratory samples. *M. fortuitum* was the most frequent NTM species followed by *M. simiae* and *M. kansasii* with different radiographic findings, clinical symptoms, and drug resistance. *M. conceptionense* as a rare NTM isolate was reported for the second time in Iran. However, future studies are required to assess the epidemiology of respiratory NTM in Iran to increase the knowledge of Iranian physicians for the diagnosis and treatment of NTM isolates.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Antimycobacterial susceptibility testing results for clinical isolates of RGM

Bacteria (no.) and antimicrobial agent		MIC (μg/mL)		No. (%) of isolates		
	Range	50%	90%	Susceptible	Intermediate	Resistant
***M. fortuitum* (31)**						
Isoniazid	2–>256	>256	>256	0 (0.0%)	0 (0.0%)	31 (100.0%)
Rifampicin	1–>256	128	>256	0 (0.0%)	0 (0.0%)	31 (100.0%)
Ethambutol	32–>256	256	>256	0 (0.0%)	0 (0.0%)	31 (100.0%)
Streptomycin	8–>256	32	128	0 (0.0%)	0 (0.0%)	31 (100.0%)
Amikacin	0.06–16	1	8	28 (90.3%)	2 (6.5%)	1 (3.2%)
Ofloxacin	0.06–32	2	8	15 (48.4%)	4 (12.9%)	12 (38.7%)
Ciprofloxacin	0.06–8	0.25	2	15 (48.4%)	4 (12.9%)	12 (38.7%)
Capreomycin	0.03–2	0.5	2	14 (45.2%)	7 (22.6%)	10 (32.2%)
***M. conceptionense* (2)**						
Isoniazid	16–>256	>256	>256	0 (0.0%)	1 (50.0%)	1 (50.0%)
Rifampicin	2–8	1	8	0 (0.0%)	1 (50.0%)	1 (50.0%)
Ethambutol	128–>256	>256	>256	0 (0.0%)	0 (0.0%)	2 (100.0%)
Streptomycin	4–64	32	64	0 (0.0%)	0 (0.0%)	2 (100.0%)
Amikacin	0.25–>32	16	64	2 (100.0%)	0 (0.0%)	0 (0.0%)
Ofloxacin	0.03–8	0.5	8	2 (100.0%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin	0.13–8	2	4	2 (100.0%)	0 (0.0%)	0 (0.0%)
Capreomycin	0.03–32	2	16	1 (50.0%)	1 (50.0%)	0 (0.0%)

Abbreviations: MIC, minimum inhibitory concentrations; RGM, rapid-growing mycobacteria.

Table S2 Antimycobacterial susceptibility testing results for clinical isolates of SGM

Bacteria (no.) and antimicrobial agent		MIC (μg/mL)		No. (%) of isolates		
	Range	50%	90%	Susceptible	Intermediate	Resistant
***M. simiae* (12)**						
Isoniazid	16–128	32	64	0 (0.0%)	0 (0.0%)	12 (100.0%)
Rifampicin	0.5–128	2	8	1 (8.3%)	0 (0.0%)	11(91.7%)
Ethambutol	4–64	16	64	3 (25.0%)	0 (0.0%)	9 (75.0%)
Streptomycin	2–64	16	32	2 (16.7%)	0 (0.0%)	10 (83.3%)
Amikacin	0.06–64	2	16	10 (83.3%)	0 (0.0%)	2 (16.7%)
Ofloxacin	2–64	8	32	1 (16.7%)	0 (0.0%)	10 (83.3%)
Ciprofloxacin	0.25–64	8	32	3 (25.0%)	0 (0.0%)	9 (75.0%)
***M. kansasii* (6)**						
Isoniazid	0.25–16	2	4	6 (100.0%)	0 (0.0%)	0 (0.0%)
Rifampicin	0.125–16	2	8	6 (100.0%)	0 (0.0%)	0 (0.0%)
Ethambutol	0.25–64	2	4	5 (83.3%)	0 (0.0%)	1 (16.7%)
Streptomycin	0.5–32	8	32	4 (66.7%)	0 (0.0%)	2 (33.3%)
Amikacin	0.125–32	2	16	1 (16.7%)	0 (0.0%)	5 (83.3%)
Ofloxacin	0.25–4	1	2	2 (33.3%)	0 (0.0%)	4 (66.7%)
Ciprofloxacin	0.25–16	2	4	2 (33.3%)	0 (0.0%)	4 (66.7%)
***M. gordonae* (2)**						
Isoniazid	0.5–>256	2	>256	1 (50.0%)	0 (0.0%)	1 (50.0%)
Rifampicin	0.125–16	0.25	8	0 (0.0%)	0 (0.0%)	2 (100.0%)
Ethambutol	0.5–64	1	16	1 (50.0%)	0 (0.0%)	1 (50.0%)
Streptomycin	0.25–32	1	8	1 (50.0%)	0 (0.0%)	1 (50.0%)
Amikacin	0.5–128	2	32	0 (0.0%)	0 (0.0%)	2 (100.0%)
Ofloxacin	1–32	2	16	1 (50.0%)	0 (0.0%)	1 (50.0%)
Ciprofloxacin	0.125–16	0.5	8	0 (0.0%)	0 (0.0%)	2 (100.0%)

Abbreviations: MIC, minimum inhibitory concentrations; SGM, slow-growing mycobacteria.