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ORIGINAL RESEARCH

Molecular Identification, and Characterization of *Mycobacterium kansasii* Strains Isolated from Four Tuberculosis Regional Reference Laboratories in Iran During 2016–2018

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Background: Non-tuberculous mycobacterial (NTM) infections are growing concern in many countries around the globe including Iran. Among them, *Mycobacterium kansasii* (*M. kansasii)* causes both pulmonary and extra-pulmonary infections. Despite the high prevalence of *M. kansasii* isolates in Iran, unfortunately little is known about the epidemiological aspects of *M. kansasii* infection. Hence, the aim of the present study was to investigate the molecular identification, determination of subtypes variation and geographic distribution of clinical isolates of *M. kansasii* isolates.

Methods: In the present study, 108 clinical pulmonary isolates suspected to NTM were collected from four Tuberculosis Regional Reference Laboratories in Iran during 2016–2018. The isolates were confirmed as NTM using conventional and molecular methods. Among them, *M. kansasii* isolates were subjected to *rpoB* gene sequencing. For determination of subtyping of *M. kansasii* isolates, polymerase chain reaction-restriction enzyme analysis (PCR-REA) based on the *hsp65* gene was performed.

Results: Based on the *rpoB* gene sequence analysis, 33 (30.5%) isolates were identified as *M. kansasii* species, compared to 31 (28.7%) isolates using phenotypic methods. The subtype I was the most frequent subtype (n=24; 72.7%), followed by subtype II (n=8; 24.2%).

Conclusion: We indicated that the rate of *M. kansasii* isolation with clinical significance appears to be increasing in Iran, especially in highly industrialized cities. The high rate of *M. kansasii* subtype I may suggest that this genotype has a particular potency for colonization, and a higher epidemiological potential for causing infection in humans. More studies are needed to provide a better understanding of the biology and pathogenicity of *M. kansasii* subtype I.

Keywords: *Mycobacterium kansasii*, subtype, PCR-restriction enzyme analysis, *rpoB*

Introduction

Non-tuberculous mycobacterial (NTM) infections are growing concern in many countries around the globe. They are considered opportunistic pathogens and are ubiquitous in the environmental sources.[1] Today, more than 200 *Mycobacterium* species were known and NTM infections have been on the rise, probably because of improved culturing techniques, the development of new molecular methods, increasing the number of susceptible hosts, and greater awareness of NTM diseases.[2,3] In recent years the attention of the Iranian health care system to NTM

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has been increased due to their clinical significance and involvement in various human diseases especially among immunocompromised individuals. Based on a meta-analysis report from Iran, 10.2% of culture positive sputum samples were belonged to NTM,[4] which this prevalence is raised to 11.1% recently,[5] and emphasizes on the important role of these bacteria in human infections.

Mycobacterium kansasii (*M. kansasii)* is a slow-growing NTM and causes both pulmonary and extra-pulmonary infections in immunocompetent and immunosuppressed individuals.[6] According to a previous global report, *M. kansasii* was the sixth most frequently isolated NTM from clinical specimens.[7] Moreover in a recent report, *M. kansasii* with a prevalence of 69.2% was the most common cause of pulmonary NTM disease in sub-Saharan Africa.[8] The frequency of this pathogen in Iran is high, exceeding 13–17% of total NTM isolations, compared to respective rates globally.[9] Treatment of *M. kansasii* infections is usually challenging, because of multi-drug therapy and long treatment periods up to 12 months.[4,10,9,11] In an economically challenged country like Iran, due to inadequate laboratory facilities, the diagnosis of tuberculosis (TB) is still only by direct smear microscopy. In many cases identification of *Mycobacterium* to the species level is not done and NTM diseases are frequently misdiagnosed as TB, particularly in the case of *M. kansasii* infections that the clinical and radiologic manifestations often indistinguishable from pulmonary TB.[12]

Species identification in *Mycobacterium* genus is challenging due to a complex and changing taxonomy, the unreliability of traditional methods and the inability of 16S rRNA to discriminate some closely related species such as *M. kansasii* and *M. gastri*.[1,3,]However, other targets such as *rpoB* gene, *dnaK* gene, *tuf* gene, *hsp65* gene, *sodA* gene and *ITS* (16S–23S internal transcribed spacer) region are usually suitable for species identification.[14]

Seven subtypes (genotypes, I–VII) for *M. kansasii* have been described. Of these, I and II are the most prevalent types and have been associated with human disease, whereas the other five (III–VII) are predominantly of environmental origin.[15,16]

Therefore, subtyping of *M. kansasii* isolates from human samples may serve as a prediction for clinical diagnosis. There are three major methodologies used for the identification of *M. kansasii* subtypes I–VII. One is sequence analysis of either *hsp65* or *rpoB* genes, the other is 16S-23S rDNA ITS, and the third is *tuf* typing.[17–19] In a most recent work conducted by Jagielski et al, the former *M. kansasii* subtypes have been denominated as *M. kansasii* (former type I), *M. persicum* (II), *M. pseudokansasii* (III), *M. innocens* (V), and *M. attenuatum* (VI), based on the alignment fraction-average nucleotide identity, genome-to-genome distance, and core-genome phylogeny.[20]

Despite the high prevalence of *M. kansasii* isolates among NTM positive cultures in Iran, unfortunately little is known about the epidemiological aspects of *M. kansasii* infection. Hence, the aim of the present study was to investigate the molecular identification, determination of subtypes variation and geographic distribution of clinical isolates of *M. kansasii* from patients in four Tuberculosis Regional Reference Laboratories in Iran during years 2016–2018.

Materials and Methods

Data Source and Ethic Statement

In the present study, 108 clinical isolates of pulmonary origin (sputum and broncho-alveolar lavage [BAL]) suspected to NTM were collected over a 3-year period from January 2016 to May 2018. The isolates were obtained from patients referred to four regional tuberculosis reference laboratories of Iran including Khuzestan (38 isolates/35.2%), Tehran (34 isolates/31.5%), Kermanshah (19 isolates/17.6%) and Fars (17 isolates/15.7%). Demographic data including patients' gender, age, and the region of residence, type of specimen, date of collection, and source laboratory were recorded. None of the individual patients were commenced on therapy for NTM disease. The isolates not matching with the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) criteria for the definition of NTM disease[21] were excluded from the study. The study was approved by Institutional Ethics and Review Board (Code: (IR.AJUMS.REC.1397.234) after submission of the preliminary proposal, and necessary permission for sample collection was granted.

Phenotypic Identification

All 108 clinical NTM isolates were subjected to phenotypic identification. Each isolate was subcultured on Lowenstein-Jensen (LJ) medium and grown colonies were stained by the Ziehl-Neelsen method for the presence of acid-fast bacilli (AFB). For initial identification, conventional phenotypic and biochemical tests including colony morphology, growth at 25, 37, and 42°C, pigment production, semi-quantitative catalase test, Tween 80 hydrolysis, arylsulfatase test, heat-stable catalase (pH 7, 68°C), urease, and nitrate reduction test

were performed.[22] *M. kansasii* ATCC 12478 was used as reference strain.

Molecular Identification

DNA Extraction

Chromosomal DNA was extracted from mycobacterial colonies grown on LJ medium using an extraction and purification QIAamp DNA Mini Kit (Qiagen NV, Venlo, the Netherlands), according to the manufacturer's instructions. DNA concentrations and purity were determined using a Nano Drop One instrument (Thermo Fisher Scientific, Waltham, MA, USA) at 260 nm and used as template in PCR amplification.

Molecular Assignment to Species Level

A 750-bp fragment of the *rpoB* gene was amplified using primers MycoF (5′ GGCAAGGTCACCCCGAAGGG-3′) and MycoR (5′-AGCGGCTGCTGGGTGATCATC-3′) as described earlier.[23] The amplified PCR products for each isolate were purified with the Gene JETTM Gel Extraction Kit (Fermentas, Lithuania), as per manufacturer's instructions. The sequences of the products were determined using an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, United States). The sequences of *rpoB* gene for each isolate were confirmed by BLAST separately, and multiple sequence alignment (MSA) was done for our sequences and the existing relevant sequences belong to *M. kansasii* recovered from GenBank database, using MEGA7 program.[24]

Methods of Subtyping

PCR-Restriction Enzyme Analysis (REA)

Subtyping of *M. kansasii* isolates was performed using PCR-REA method for the *hsp65* gene, as previously described.[17] An approximately 441 bp fragment of the *hsp65* gene was amplified by PCR using two specific primers Tb11 (5′- ACCAACGATGGTGTGTCCAT-3′) and Tb12 (5′-CTTGTC GAACCGCA- TACCCT-3′). Genomic DNA of *M. fortuitum* ATCC 49404T and double distilled water were used as positive and negative controls, respectively. PCR products of *hsp65* gene were digested by the *BstEII* and *HaeIII* restriction enzymes. The fragments were compared with those of patterns deposited in a free available database (http://app.chuv.ch/prasite, n.d.), for subtyping of *M. kansasii* isolates.

rpoB Gene Sequencing

Primer Design for Sequence-Based Subtyping

The sequences of *rpoB* gene for each subtype were recovered from GenBank database and MSA were done using MEGA7 program.[24] According to the MSA results, we found a region with a suitable variation for *M. kansasii* subtyping. Based on this region, a forward primer (5′-AAT CAA CCT GTC GCG CAA CGA-3′) and a reverse primer (5′-GTT CAT CGA AGA AGT TGA CGT-3′) were designed by using Gene runner 3.05 software.

Nucleotide Sequencing

For definitive subtyping of *M. kansasii* isolates, nearly a 250-bp fragment of the *rpoB* gene was amplified using a set of above-mentioned designed primers. The cycling condition was 95°C for 1 min, followed by 30 cycles of 95°C for 30s, 64°C for 30s, and 72°C for 30s and finalized with 72°C for 5 min. The amplified PCR products of *rpoB* gene for each *M. kansasii* isolate were purified with the Gene JETTM Gel Extraction Kit (Fermentas, Lithuania), as described in the manufacturer's instructions. The sequences of the products were determined by application of an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, United States) according to the standard protocol of the supplier. Phylogenetic trees were obtained from DNA sequences based on 250 bp fragments using the Neighbor-Joining (NJ) method and Kimura's two parameter (K2P) distance correction model with 1000 bootstrap replications supported by the MEGA7 software.

Results

All 108 NTM-suspected clinical isolates were identified as NTM using conventional culture and phenotypic identification tests (Table 1). These were obtained from patients with positive cultures met (ATS/IDSA) diagnostic criteria for NTM disease.[21] Tables 2 and 3 describe the proportions of NTM isolates within our population and those meeting ATS/IDSA guidelines. According to phenotypic tests, 31 isolates (28.7%) were assigned to *M. kansasii*. For definite identification, all 108 isolates were subjected to *rpoB* gene sequencing and PRA, which revealed that 33 isolates (30.5%) showed more than 99% homology with *M. kansasii* species and were included in our study (Table 1). The *M. kansasii* isolates were obtained from samples belonged to 25 male (75.7%) and 8 female (24.3%) patients with mean age of 56.7% years, and the

Table 1 Results of *M. kansasii* Identification by Phenotypic and Genotypic Tests

Isolates No.	Growth at 37°C	Growth on MacConkey agar	Urease production	Iron Uptake	Tween 80 Hydrolysis	Thermostable Catalase	Arylsulfatase	Niacin production	Tellurite reduction	Citrate utilization	Growth on 5% Co2	Sub-Species	hsp65-PRA BsTEII/HaeIII	Phenoty-pictests	Identification by *rpoB*
84	+	–	+	–	+	+	–	–	–	–	–	IV	235, 120, 85 /130, 115, 75, 60	*M. kansasii*	*M. kansasii*
98	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
40	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
63	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
45	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
75	+	–	– /+	–	+	+		–	–	–	–	I	235, 210/130, 105, 80	Unknown	*M. kansasii*
78	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
97	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
95	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
104	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
88	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
111	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
M95	+	–	+	–	+	+	–	–	–	–	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
M88	+	–	–/+	–	+	+	–	–	–	–	–	II	235,130, 85/ 130,105	Unknown	*M. kansasii*
67	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
36	+	–	+	–	+	+	–	–	–	–	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*

35	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
M98	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
M63	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
33	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
19	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
21	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
32	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
34	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	+235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
357	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
313	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
372	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
330	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
310	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
412	+	–	+	+	+	–	–	–	–	–	–	+	+	–	II	235,130, 85/ 130,105	*M. kansasii*	*M. kansasii*
349	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
383	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*
345	+	–	+	+	+	–	–	–	–	–	–	+	+	–	I	235, 210/130, 105, 80	*M. kansasii*	*M. kansasii*

age group >60 years (n= 15, 45.4%), was the highly infected group (Table 2).

The isolates were mostly obtained from sputum samples (n=21, 63%), followed by BAL (n=11, 33%). All of 33 patients had positive AFB on three or more available specimens. The analyzed *M. kansasii* isolates were originated from TB centers in Khuzestan (n= 12, 36%), Tehran (n= 11, 33%), Kermanshah (n= 6, 18%), and Fars (n= 4, 12%). As shown in Figure 1, the higher rate of *M. kansasii* infection was observed in the Khuzestan followed by Tehran provinces. According to patients' archive information, the most frequent previous medical history (PMH) was human immunodeficiency virus (HIV) infection (n=5, 15%), and treated TB (n=4, 0.12%) (Table 3).

The *M. kansasii* isolates were genotyped by *hsp65*-based PRA method and partial *rpoB* gene sequencing. Three different subtypes of I, II, and IV were identified by both methods as shown in Table 4. The subtype I was the most frequent subtype (n=24; 73%), followed by subtype II (n=8; 24%). As presented in Table 4, the results of typing using *rpoB* gene sequencing (designed in this study), were identical with PRA method. Distribution of *M. kansasii* subtypes in parts of Iran under investigation is shown in Figure 1. Based on the sequencing results of the partial *ropB* gene, a dendrogram (the maximum likelihood tree generated using the Tamura 3-parameter model in MEGA7 software) illustrating the genetic distances between different *M. kansasii* types (I–VI), was generated (Figure 2). For all strains under the study, the typing results obtained with PCR-REA of *hsp65* gene and sequencing were in 100% agreement.

Table 2 Baseline Characteristics of Patients with *M. kansasii*-Positive Culture from Respiratory Samples

	Isolates	Sample source	A/G	PMH	Main symptoms	Genotype
1	**NTM 84**	BAL	60/M	HIV	Productive cough, chest wall pain & weight loss	IV
2	**NTM 98**	Sputum	76/F	Smoker	Productive cough	I
3	**NTM 40**	Sputum	57/M	Respiratory failure	Productive cough	I
4	**NTM 63**	Sputum	68/F	Open heart surgery	Productive cough	I
5	**NTM 45**	BAL	61/M	Treated tuberculosis	Productive cough	II
6	**NTM 75**	BAL	54/M	Normal	Local pain, small pale nodule	I
7	**NTM 78**	Biopsy	70/M	Pemphigus Vulgaris	Local pain, small pale nodule	I
8	**NTM 97**	Sputum	34/F	Renal failure	Productive cough, fever, body weight loss	I
9	**NTM 95**	Sputum	28/F	Normal	Fever, cough	II
10	**NTM 104**	Sputum	61/M	Normal	Fever	II
11	**NTM 88**	Sputum	60/M	Normal	Fever, inflammation and tenderness in joint	I
12	**NTM 111**	Sputum	70/M	COPD	Fever	I
13	**MOT 95**	Sputum	48/M	Normal	Productive cough, fever, weight loss	II
14	**MOT 88**	Sputum	81/M	Immunocompromised	Fever	II
15	**NTM 67**	Sputum	31/M	HIV	Fever	I
16	**NTM 36**	Sputum	69/M	HCV	Local abscess and discharge	I
17	**NTM 35**	BAL	58/M	Sarcoma	Fever, cough	II
18	**MOT 98**	BAL	31/M	Treated tuberculosis	Fever, cough	II
19	**MOT 63**	Sputum	48/M	Normal	Fever, cough	II
20	**NTM 33**	Sputum	71/M	Normal	Fever	I
21	**NTM 19**	Sputum	41/F	Normal	Local pain, small pale nodule	I
22	**NTM 21**	Sputum	50/M	Diabetic	Fever, cough	I
23	**NTM 32**	Sputum	63/F	Normal	Fever	I
24	**NTM 34**	Sputum	52/M	Normal	Fever	I
25	**NTM 349**	BAL	59/M	HIV	Fever	I
26	**NTM 383**	Sputum	55/M	Open heart surgery	Fever	I
27	**NTM 345**	Sputum	42/M	HIV	Fever, cough	I
28	**NTM 357**	BAL	60/M	Alcoholic	Fever	I
29	**NTM 313**	BAL	69/F	Treated tuberculosis	Fever	I
30	**NTM 372**	BAL	48/M	Open heart surgery	Fever	I
31	**NTM 330**	BAL	70/M	Treated tuberculosis	Fever, cough	I
32	**NTM 412**	BAL	89/F	Normal	Fever, cough	I
33	**NTM 310**	Sputum	39/M	HIV	Fever, cough	I

Abbreviations: A, age; G, gender; PMH, previous medical history; BAL, bronchoalveolar lavage; HIV, human immunodeficiency virus; HCV, hepatitis C virus.

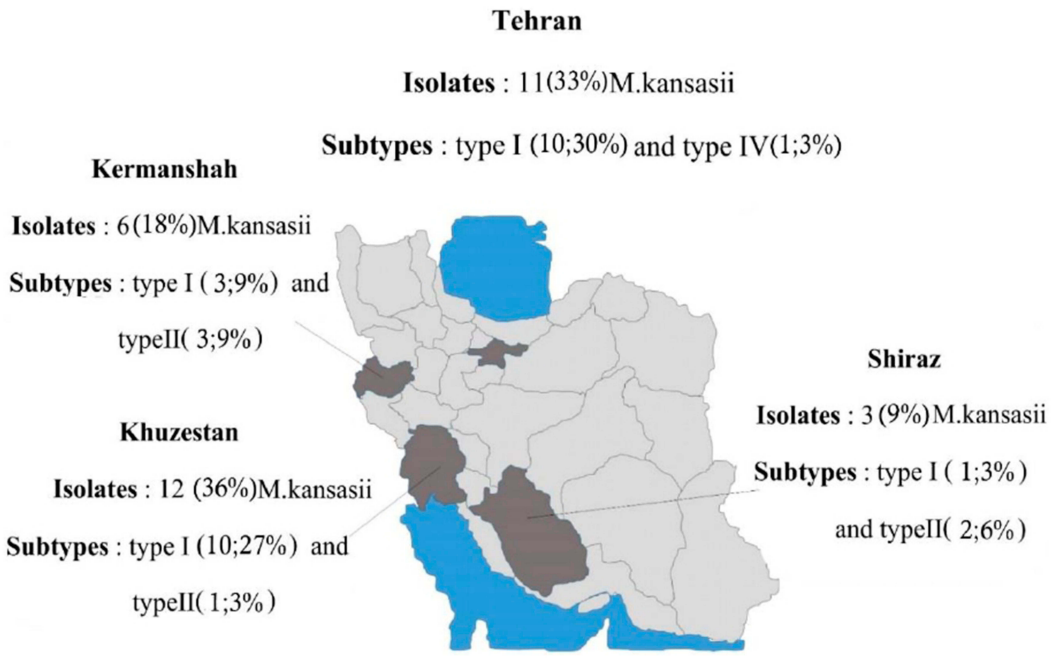

Figure 1 Location and geographic distribution of *M. kansasii* strains during 2016–2018.

Discussion

Currently, the incidence and prevalence of NTM diseases are increasing, and their impact on human health has been a topic of interest.[25,26] Iran is an endemic country where TB remains a major public health problem. Although the epidemiology of TB is well described, the prevalence of NTM disease in Iran remains largely undetermined. *M. kansasii* is one of the most frequently encountered NTM species among culture positive sputum samples in Iran.[4] Unfortunately, there are no reliable data on the epidemiology of *M. kansasii* infections in Iranian patients. Hence, to our best knowledge, this is one of the limited studies on the frequency, epidemiology and distribution of *M. kansasii* species from different regions in Iran, as the first report belonged to Velayati et al from Iran.[27] In the present study, we applied a combination of phenotypic and molecular methods for the species identification of NTM isolates. Based on the *rpoB* gene sequencing and PRA method, 33 (30.5%) isolates were identified correctly as *M. kansasii* species, compared to 31 (28.7%) isolates using phenotypic methods, so it was confirmed that sequence-based identification offers much more resolution than the phenotypic methods, as shown in several studies.[28–30]

There are several reports on the frequency of *M. kansasii* from Iran and around the world. In studies from Iran, the clinical *M. kansasii* respiratory isolates has been shown to vary from 11% to 26%.[4,5,30] Additionally, in South American and some European countries, *M. kansasii* was reported as the second most common cause of NTM diseases.[31] Also, high rates of *M. kansasii* incidence have been consistently reported in Japan and South Africa.[32,33] This wide range of differences might be explained by different sample sizes, difference in diagnostic criteria and changing in NTM epidemiology and distribution. The results of this study showed that the majority of Iranian patients with *M. kansasii* lung infection were from highly industrialized provinces. Among the 33 patients positive for *M. kansasii*, 23 (69.7%) lived in highly industrialized provinces of Tehran and Khuzestan, which this finding is in line with other studies.[28,34]

The hsp65-PRA assay is one of the most common techniques currently used for *M. kansasii* subtypes identification.[29] Here, we applied both PRA and partial *rpoB* gene sequencing, designed in this study. Of the seven currently recognized *M. kansasii* subtypes (genotypes, I–VII), genotype I has predominated among clinical samples, making up 42–100% of *M. kansasii* clinical isolates.[16] This was confirmed by this study too, and we found that the subtype I is the predominant genotype of *M. kansasii* (72%), as reported worldwide.[7,35,36] In comparison, the frequency of this genotype was found to be lower in Switzerland (68%),[37] and much higher in Brazil (98%).[38] However, only 24% and 3% of *M. kansasii* isolates in this study were classified as subtype II and subtype

Table 3 Frequency of *M. kansasii* Isolates and Subtypes by Characteristic, Symptoms at Presentation and Previous Medical History

Characteristics	*M. kansasii* N = 33 (%)	*M. kansasii* Subtypes
Year		
2016	(n = 7) 21%	(I= 5) 9%,(II = 2) 6%
2017	(n = 10) 30%	(I= 7) 21%,(II = 3) 9%
2018	(n = 16) 48%	(I= 12) 36%,(II = 3) 9%, (IV = 1) 3%
Age		
0–19	–	–
20–39	(n = 5) 15%	(I= 3) 9%,(II = 2) 6%
40–59	(n = 11) 33%	(I= 9)27%,(II = 3) 9%
≥60	(n = 17) 51%	(I= 12)66%, (II = 3) 9%, (IV = 1) 3%
Gender		
Female	(n = 8) 24%	(I= 7)21%, (II = 1)3%
Male	(n = 25) 76%	(IV = 1) 3%,(I= 19) 57%, (II = 5) 18%
Symptoms at presentation		
Fever, cough	(n = 9) 27%	(I= 5) 15 %,(II = 4) 12%
Fever	(n = 12) 36%	(I= 9) 27 %,(II = 3) 9%
Productive cough	(n = 4) 12%	(I= 3) 9 %,(II = 1) 3%
Local pain, small pale nodule	(n = 3) 9%	(I= 3) 9%
Fever, inflammation and tenderness in joint	(n = 1) 3%	(I= 1) 3%
Local abscess and discharge	(n = 1) 3%	(I= 1) 3%
Productive cough, thoracic pain & weight loss	(n =1) 3%	(IV= 1)3%
Productive cough, fever, weight loss	(n = 2) 6%	(I= 1) 3%
Underlying condition		
Treated tuberculosis	(n = 4) 12%	(I= 2) 6%, (II= 2) 6%
HIV infection	(n = 5) 15%	(I= 4) 12 %, (IV= 1)3 %
Open heart surgery	(n = 3) 9%	(I= 3) 9%
COPD	(n = 1) 3%	(I= 1) 3%
HCV	(n = 1) 3%	(I= 1) 3%
Immunocompromised	(n = 1) 3%	(II=1) 3%
Renal failure	(n = 1) 3%	(I= 1) 3%
Diabetic	(n = 1) 3%	(I= 1) 3%
Pemphigus Vulgaris	(n = 1) 3%	(I= 1) 3%
Respiratory failure	(n = 1) 3%	(I= 1) 3%
Smoking	(n = 1) 3%	(I= 2) 6%
Sarcoma	(n = 1) 3%	(II= 1) 3%
Alcoholic	(n = 1) 3%	(I= 1) 3 %

IV, respectively, which is lower from other similar previous studies.[7] Another study conducted in Spain revealed the absence of genotype II among *M. kansasii* clinical isolates, with 91 (97.8%) of the 93 isolates tested representing genotype I.[39] In contrast to our study, Tortoli et al, reported that *M. kansasii* type II has been found more

Table 4 Restriction Patterns and *M. kansasii* Sub-Typing by PRA-Hsp65 and *rpoB* Gene Sequencing

Number of Isolates	*BstE* II Patterns	*Hae* III Patterns	Best Matches by PRA	Sequence Typing
24	235, 210	130, 105, 80	*M. kansasii* type I	*M. kansasii* type I
8	235, 130, 85	130, 105	*M. kansasii* type II	*M. kansasii* type II
1	235, 120, 85	130, 115, 75, 60	*M. kansasii* type IV	*M. kansasii* type IV

likely to be recovered from patients with HIV-positive status, whereas type I has also been associated with pulmonary infections in HIV-negative patients with pre-existing pulmonary diseases.[40]

The high prevalence of type I among the isolates of *M. kansasii* of HIV-positiveindividuals indicates that this type had a greater capacity for colonization and/or pathogenic activity for human beings, while the data available from type II suggest that this type less able to overcome natural resistance mechanisms. Many epidemiological and pathogenic aspects of *M. kansasii* are still far from being understood, and further investigation is necessary to elucidate the virulence factors of the *M. kansasii* subtypes I and II. However, the present study emphasizes on the identification of *M. kansasii* to the subtype level, may be not only an interesting epidemiological tool, but also a process relevant to clinical management, as it allows the differentiation of pathogenic from the nonpathogenic subtypes. Performance of drug susceptibility testing and comparing the results of drug resistance between *M. kansasii* subtypes, though was desired but has not been done in this study, and this was the limitation of our study due to the extreme lack of budget, however, will be our goal for future studies.

In conclusion, we showed that the rate of *M. kansasii* isolation with clinical significance appears to be increasing in Iran, especially in highly industrialized cities. Management of *M. kansasii* infections requires a multidisciplinary effort from primary care physicians, pulmonologists and infectious diseases specialists. The high detection rate of *M. kansasii* subtype I in clinical samples may suggest that this genotype has a particular propensity for colonization, and thus a higher epidemiological potential for causing infection in humans. More comprehensive studies, on large collections of *M. kansasii* isolates, are

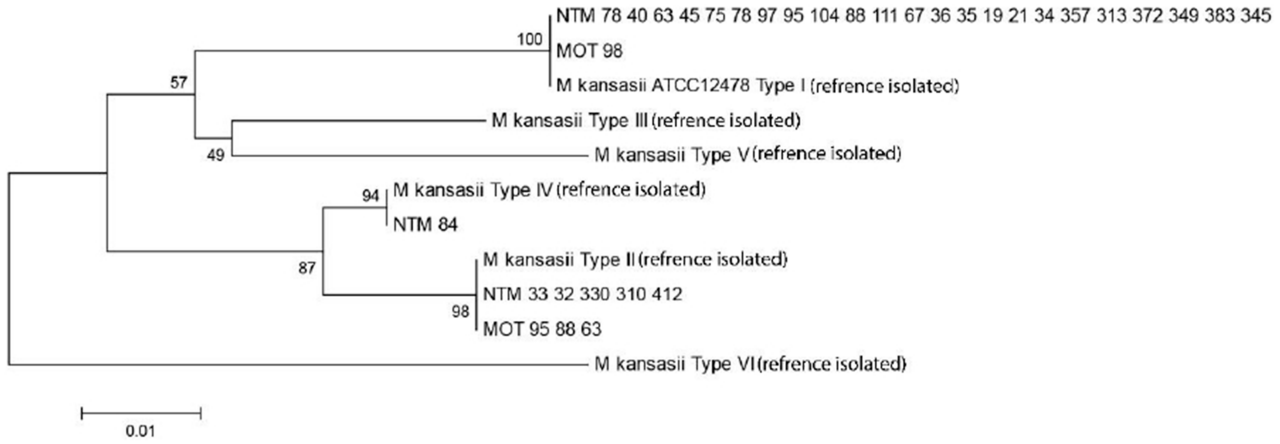

Figure 2 A dendrogram (the maximum likelihood tree generated using the Tamura 3-parameter model in MEGA6 software) illustrating the genetic distances between different *M. kansasii* types (I–VI), based on the sequencing results of the partial *rpoB* gene.

needed to provide a better understanding of the biology and pathogenicity of *M. kansasii* subtype I.

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Disclosure

The authors declare no conflicts of interest in this research work.

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