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
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, which this prevalence is raised to 11.1% recently, 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. According to a previous global report, *M. kansasii* was the sixth most frequently isolated NTM from clinical specimens. 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. The frequency of this pathogen in Iran is high, exceeding 13–17% of total NTM isolations, compared to respective rates globally. Treatment of *M. kansasii* infections is usually challenging, because of multi-drug therapy and long treatment periods up to 12 months. 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.

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*. 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.

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.

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. 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.

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 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.
M. kansasii isolates, nearly a 441 bp fragment of the gene for each subtype were recovered from GenBank database and MSA were done using MEGA7 program. 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 CCG 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. 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 belonging to 25 male (75.7%) and 8 female (24.3%) patients with mean age of 56.7 years, and the
| Isolates | Growth at 37°C | Growth on MacConkey agar | Urease production | Iron Uptake | Tween 80 Hydrolysis | Thiosulfate Cysteine | Arylsulfatase | Niacin production | Tellurite reduction | Citrate utilization | Growth on 5% CO₂ Sub-species |
|----------|---------------|--------------------------|-------------------|------------|---------------------|----------------------|--------------|-----------------|-------------------|-----------------|-----------------------------|
| 84       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 98       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 40       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 45       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 75       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 78       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 97       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 95       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 88       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 104      | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 88       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 111      | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 95       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 98       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 67       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |
| 36       | +             | +                        | +                 | +          | +                   | +                    |             |                 | +                 |                 | hsp65-PRA 130, 115, 75, 60 |

Table 1: Results of M. kansasii Identification by Phenotypic and Genotypic Tests
| Year | 1 235 | 2 10/30 | 3 105 | 4 80 |
|------|--------|---------|-------|-------|
| 1998 | -      | +       | -     | -     |
| 1999 | -      | +       | -     | -     |
| 2000 | -      | +       | -     | -     |
| 2001 | -      | +       | -     | -     |
| 2002 | -      | +       | -     | -     |
| 2003 | -      | +       | -     | -     |
| 2004 | -      | +       | -     | -     |
| 2005 | -      | +       | -     | -     |
| 2006 | -      | +       | -     | -     |
| 2007 | -      | +       | -     | -     |

* M. kansasii

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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 *rpoB* 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                                      | I        |
| 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                                                | I        |

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

**Figure 1** Location and geographic distribution of *M. kansasii* strains during 2016–2018.
Another study conducted in Spain revealed that in contrast to our study, Tortoli et al., clinical M. kansasii type II has been found more frequently in clinical samples. 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 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.

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) 46%, (II = 3) 9%, (IV = 1) 3% |
| Gender          |                        |                      |
| Female          | (n = 8) 24%            | (I = 7) 21%, (II = 1) 33% |
| Male            | (n = 25) 76%           | (IV = 1) 3%, (IV = 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%, (II = 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%     | (I = 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%             | (I = 1) 3% |
| Alcoholic       | (n = 1) 3%             | (I = 1) 3% |

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 |
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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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.
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