ORIGINAL ARTICLE

Molecular epidemiology of *Mycobacterium tuberculosis* isolates in Iran using spoligotyping

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Abstract

Spoligotyping can help assess the transmission of *Mycobacterium tuberculosis* strains. We aimed to study the genotyping of *M. tuberculosis* isolated from patients with tuberculosis from the west of Iran by spoligotyping. Forty-seven *M. tuberculosis* isolates were collected from the west of Iran. All samples were cultured on Löwenstein-Jensen medium incubated at 37°C for 8 weeks. Bacterial isolates were identified as *M. tuberculosis* using standard biochemical tests. Drug resistance patterns of *M. tuberculosis* to rifampicin and isoniazid were determined, and multidrug-resistant (MDR) strains were isolated. After DNA extraction, spoligotyping was performed. We found new spoligotypes 4162 and 4163, which correlated with atypical lineage. Atypical and unknown lineages also had correlations with the MDR tuberculosis rate (4%). The most prevalent spoligointernational types were orphan (34%), 2669 (23.4%) and 127 (14.8%) types. The most prevalent clades were Ural-2 (NEW-1) (25.53%) and atypical (23.40%) lineages. The predominant clade was Ural-2 (NEW-1) and an atypical lineage restricted to Iran. The rate of MDR was low. Knowledge of the circulating isolates in the west of Iran will help implement control programmes, so knowledge of the dynamic transmission of local isolates is crucial.

Keywords: Epidemiology, Iran, Molecular, *Mycobacterium tuberculosis*, Spoligotyping
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Introduction

Tuberculosis (TB) is a classical infectious disease that threatens the world's public health; it is caused by bacteria of the *Mycobacterium tuberculosis* complex [1,2]. The World Health Organization reports 8.6 to 9.0 million occurrences of TB, which can cause 1.3 to 1.5 million deaths annually [3,4]. Risk factors include HIV infection, drug addiction, smoking and air pollution as well as drug-resistant *M. tuberculosis* isolates that have high rates of TB incidence [5]. Combination therapy provided to treat TB includes the antibiotics rifampicin (RIF), isoniazid (INH), pyrazinamide and ethambutol or streptomycin [6]. Multidrug-resistant (MDR) TB strains are TB isolates with resistance to at least RIF and INH (the two most important anti-TB drugs available) with or without resistance to other drugs [7]. The mutations in individual drug target genes leads to the emergence of MDR-TB strains. Currently the rate of MDR-TB strains is increasing in different regions of the world. Further, MDR-TB is a major public health problem for TB control programmes in healthcare facilities [8].

In the 21st century, molecular genetics has greatly assisted the rapid diagnosis, fast initiation of therapy and evaluation of the source of TB [9]. Genotyping of TB strains includes mycobacterial interspersed repetitive unit–variable number of

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tandem repeat (MIRU-VNTR), spacer oligonucleotide typing (spoligotyping) and IS6110 restriction fragment length polymorphism (IS6110-RFLP). Such genotyping helps TB control by permitting identification of unsuspected outbreaks and laboratory cross-contamination, and it helps distinguish exogenous reinfection from endogenous reactivation, thereby permitting understanding of the genetic diversity of strains [10–13]. Spoligotyping is a major genotyping assay which is PCR based and analyses the polymorphism in the spacer sequences, which are present in the direct repeat (DR) region of strains [14,15]. Spoligotyping is an easy, rapid, economical and highly reproducible assay for typing TB strains [13].

The aim of our study was to assess the molecular epidemiology of *M. tuberculosis* genotypes by spoligotype patterns from patients with TB from the west of Iran.

Materials and methods

Collection of *M. tuberculosis* isolates

In this cross-sectional study, 47 *M. tuberculosis* isolates were collected from Ardabil, Hamadan, Qazvin, Tabriz and Kurdistan provinces (west Iran). Demographic information and clinical information regarding age, sex, TB history and residential address were recorded.

All samples were cultured on Löwenstein-Jensen medium, incubated at 37°C and kept for 8 weeks [16]. Bacterial isolates were identified as *M. tuberculosis* using catalase, production of niacin and nitrate reduction.

Drug susceptibility testing and determining MDR-TB strains

Drug resistance patterns of *M. tuberculosis* to RIF and INH, as determined by culture on Löwenstein-Jensen medium and use of the proportions method, were analysed. The concentrations and proportions were 40 mg/L and 1.0% for RIF, and 0.2 mg/L and 1.0% for INH [17,18]. *M. tuberculosis* H37Rv strain was used for quality control testing in drug susceptibility assays. TB isolates that exhibited resistance to at least RIF and INH were defined as MDR strains [6,18].

DNA extraction

DNA extraction of *M. tuberculosis* isolates grown in Löwenstein-Jensen medium was performed in accordance with the manufacturer's instructions (Qiagen, Germantown, MD, USA). All the genomic DNA preparation materials were stored at −20°C until use. The quality and quantity of the extracted DNA were assessed by a NanoDrop machine (Thermo Fisher Scientific, Waltham, MA, USA). Distilled water was used as the negative control.

Spoligotyping

Spoligotyping was performed using amplification of direct repeat regions in the genome of *M. tuberculosis* complex by DRa and DRb primers and an available spoligotyping kit according to the manufacturer's protocol. Then the amplified products were hybridized on a membrane precoated with spacer oligos that characterized the spacer region of the identified sequence. After incubation with streptavidin–peroxidase and enhanced chemiluminescence detection, the presence of spacers was imaged on X-ray films as black squares [19]. *M. tuberculosis* H37Rv was used as the positive control (spoligotyping kit for detecting tuberculosis; Mapmygenome, Hyderabad, Andhra Pradesh, India).

Results

Spoligotype patterns of *M. tuberculosis* isolates are shown in Table 1. Orphans indicate spoligotyping patterns that are unique to the analysis and are not found in the SITVIT database (http://www.pasteur-guadeloupe.fr:8081/SITVIT2/). The frequency of the new spoligointernational types (SITs) 4162 and 4163 were 8.51% and 4.26% respectively. Orphan spoligotype patterns (34%) by definition were found only once and have not yet been described.

Table 2 lists the prevalence of lineages in this study. Ural-2 (NEW-1) had the highest rate (25.53%) of the lineage and of other clades, including atypical, unknown and Ural-2. The S lineage had the lowest rate.

Differences in the prevalence of drug resistance to RIF and INH are shown in Table 3. Atypical lineages had the highest proportion of members resistant to RIF and INH. Atypical and unknown lineages also had the highest correlation with the MDR-TB rate (4%).

Discussion

Molecular epidemiology may be applied for several aims, including comparing isolates worldwide [20]. TB control programmes depend on information about molecular epidemiology to obtain insight into TB's distribution and diversity in a specific area. In considering the SITVIT database, our study is the first to analyse spoligotyping in the west of Iran. Our study found the new spoligotypes 4162 and 4163 in this region. Orphan spoligotypes represent patterns that have been found only once and that have not been found in the past. They may indicate recent and/or sporadic TB infection in the studied area. For a profile to be considered a SIT, this profile must have been found at least twice in the present study. New SITs are created after a match

TABLE 1. Spoligotype patterns of *Mycobacterium tuberculosis* isolates

SIT	*M. tuberculosis* (%)	Total (%)
26	2.13	2.13
127	14.89	14.89
252	2.13	2.13
284	2.13	2.13
602	6.38	6.38
1965	2.13	2.13
2669	23.40	23.40
4162	8.51	8.51
4163	4.26	4.26
Orphan	34.04	34.04
Total	100	100

SIT, spoligointernational type.

with another orphan in the database, or if two or more strains belonging to the new pattern within this study were found. These SITs are from the Ural-2 (NEW-1) lineage. On the basis of the SITVIT database, this lineage is more prevalent in Iran. The most prevalent SITs were the orphan (34%), 2669 (23.4%) and 127 (14.8%) types. The orphan types mostly belong to the Ural-2 (NEW-1) lineage reported from Iran. The 2669 spoligotype belongs to atypical lineages mostly isolated from Saudi Arabia. The 127 spoligotype belongs to the Ural-2 lineage, which has mostly been isolated from Iran, Iraq and Saudi Arabia. Finding the most potent circulating clades is important in controlling TB and comparing them to other isolates.

Iran has a variety of geographical conditions; therefore, distribution of *M. tuberculosis* strains differs from state to state. Prevalence of lineages Ural-2 (NEW-1), atypical, Ural-2 and unknown were 25.53%, 23.4%, 14.89% and 14.89% respectively. As indicated earlier, the predominant lineage is reported from Iran. Therefore, most isolates are unique to our region. We are located on the border with Iraq, and because of ethnic group relationships, there is close communication between peoples; it was therefore obvious that the second prevalent lineage would be Ural-2.

The resistance rates to RIF and INH were as follows: atypical lineage, 8%; unknown lineage, 4%; and Ural-2, 4%. The rate of frequency of MDR strains was 4%, with strains belonging to atypical, Ural-2 and unknown lineages. The reported prevalence of drug resistance in Colombia is 2.38% [21]. The MDR-TB rate

TABLE 2. Prevalence of lineage in *Mycobacterium tuberculosis* isolates

Lineage	*M. tuberculosis* (%)	Total (%)
AFRI	6.38	6.38
Atypical	23.40	23.40
CAS1-Delhi	4.26	4.26
LAM9	2.13	2.13
S	2.13	2.13
T1	6.38	6.38
Unknown	14.89	14.89
Ural-2	14.89	14.89
Ural-2 (NEW-1)	25.53	25.53
Total	100	100

TABLE 3. Prevalence of lineage and multidrug resistance

	Rifampin (%)		Isoniazid (%)		MDR (%)	
Lineage	R	S	R	S	No	Yes
Atypical	8	36	8	36	40	4
CAS1-Delhi	0	8	0	8	8	0
LAM9	0	4	0	4	4	0
T1	0	4	0	4	4	0
Unknown	4	8	4	8	8	4
Ural-2	4	24	4	24	24	4
Total	16	84	16	84	88	12

MDR, multidrug resistant; R, resistant; S, susceptible.

is 11% in India [22]. In a previous study, the MDR rate was higher than in our study [23,24]. Therefore, TB control was successful in the west of Iran. Most resistant isolates are from the Beijing family. Interestingly, the Beijing genotype was not found in this study; most Beijing strains are prominent in populations of Afghani origin [24–27]. Because we do not have any Beijing types in this region, the rates of resistance are lower than in other parts of the world.

Conclusions

To our knowledge, ours is the first study to introduce the new spoligotypes 4162 and 4163 from the west of Iran. The predominant lineages were restricted to Iran. Also, the rate of MDR was low. A good control programme needs to understand the dynamic transmission of local isolates; therefore, we performed this study to identify circulating isolates in the west of Iran.

Conflicts of interest

None declared.

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