Molecular diversity of *Mycobacterium tuberculosis* strains indifferent provinces of Iran

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ABSTRACT

Background and Objectives: Molecular epidemiology tools are widely used in determining epidemiology of tuberculosis. Spoligotyping is a molecular epidemiology method that is used for characterization and typing of *Mycobacterium tuberculosis* complex strains. The method is based on polymorphism of the chromosomal DR locus consisting of identical 36-bp DRs alternating with 35-41 unique spacers. The objective of this study was to investigate the prevalence of *M. tuberculosis* spoligotypes in different provinces of Iran.

Materials and Methods: 1242 *M. tuberculosis* strains were isolated from TB patients of Mycobacteriology Research center (MRC). DNA was extracted from patient's clinical samples. PCR was performed by using of specific primers for DR region. The amplified DNA was hybridized to the spoligotyping Membrane. Hybridized DNA was detected with ECL detection kit and by exposing ECL Hyperfilm to the membrane. The obtained result was entered to a binary format and was analyzed using SpolDB4 database.

Results: Spoligotyping resulted in 136 different patterns. Out of 1242 *M. tuberculosis* strains, 1165 strains (93.8%) were classified into 59 clusters and the remaining strains (6.2 %) were singleton.

Conclusions: The results of present study showed that strains of CAS family were more prevalent than other strains in Iran. Other prevalent families were Haarlem, T and Beijing, respectively.

Keywords: Molecular epidemiology, Tuberculosis, Spoligotyping

INTRODUCTION

Tuberculosis (TB) is one of the most urgent health problems in the Middle Eastern countries. Iran, with around 70 million inhabitants, shares geographical borders with four countries with high TB incidence rate, i.e. Pakistan, Afghanistan, Turkmenistan and Iraq. According to the World Health Organization (WHO), the estimated incidence rate of tuberculosis

within the country is 21 per 100,000 populations (1). Therefore control and prevention of TB in Iran is the main health concern of national TB program.

Genotyping of *M. tuberculosis* strains is important for TB control program, because it allows the detection of outbreaks, the tracing of transmission, to monitor species diversity and to identify secondary infections (2, 3).

Large scale genotyping of *M. tuberculosis* using *IS*6110 restriction fragment length polymorphism is labor-intensive, time consuming and the results are sometimes difficult to compare among laboratories (2, 4). Based on this knowledge we used an easier and more rapid method in order to differentiate *M. tuberculosis* strains. Spoligotyping is a PCR based method that permits genotyping of *M. tuberculosis*

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complex in a rapid, reliable and cost effective way. The method is based on hybridization of amplified DNA with spacer oligonucleotides. The DR region contains multiple short 36-bp direct repeats (DRs) interspersed with unique spacers, which are 35-41 bp in length (5). The DRs are extremely well conserved among *M. tuberculosis* complex strains, making spoligotyping a specific method for the genotyping of *M. tuberculosis* complex members (5). A total of 9 potential super families or clades of *M. tuberculosis* complex have been identified by spoligotyping method (*M. africanum*, Beijing, *M. bovis*, EAI, CAS, T group of families, Haarlem, X and LAM family) (6). In this study, we performed spoligotyping on *M. tuberculosis* complex strains collected from all over the country, which were isolated between 2010 and 2011, in order to provide preliminary insight into the population structure of *M. tuberculosis* circulating in the country as well as the distribution of MTB family strains in provinces of Iran.

MATERIALS AND METHODS

***M. tuberculosis* strains and DNA isolation.** A total of 1242 *M. tuberculosis* strains collected from 24 different provinces of Iran (2010-2011). Clinical specimens were transferred to Mycobacteriology Research Center (MRC). Mycobacterial genomic DNA was extracted from patient's samples using QIAamp® DNA mini kit (QIAGEN).

Spoligotyping. Spoligotyping was performed as previously described by Kamerbeek *et al,* (5). The DR region was amplified by PCR using primers DRa (5'-biotin -CCG AGA GGG GAC GGA AAC- 3') and DRb (5'- GGT TTT GGG TCT GAC GAC-3'), 20-50ng of DNA and 0.5 U of Taq DNA polymerase (Cinnagen, Tehran, Iran). The PCR condition was: 35 cycles of 1 min at 95°C, 1 min at 55°C and 30 sec at 72°C. The first denaturation and final extension steps were held for 10 min. The amplified DNA was hybridized to 43 immobilized oligonucleotides derived from the spacer sequences of MTB H37Rv and *M. bovis* BCG P3 by reverse line blotting. Hybridized DNA was detected by enhanced chemiluminesence (ECL, Amersham, UK) and by exposing ECL-Hyper film (Amersham) to the membrane for 10 min. Obtained results were entered in a binary format as excel spreadsheets (Microsoft) and compared with published data (7-10). The strains with spoligotype

similar to any pattern of *M. tuberculosis* strain already found in the database were automatically labeled with an already defined 'shared type'number. Any spoligotype exhibiting a profile not yet found anywhere in the SpolDB4 database was termed as orphan (not seen) strain.

RESULTS

Spoligotyping. Spoligotyping produced a total of 136 patterns for the 1242 strains. Fifty-nine patterns classified into clusters in the present study (the data are summarized in Table 1 and detailed in Table 2). Fifty nine clusters contained 1165 isolates, which amounted 93.8% of clustering rate (1165/1242).The remainers (n = 77) corresponded to singleton which were not classified into any clusters (Table 3).

Among the 1242 typed isolates, 1165 (93.8%) were classified as shared international types (SITs) according to SITVIT database. The remaining 77 isolates generated 44 new spoligotypes (orphan- not seen) that had not been previously described in the database. Among the 59 clusters, we found 46 minor spoligotypes (including 2 to 9 isolates) and 13 major spoligotypes (> 10 isolates). Isolates ST127 (20.5%; Haarlem family), ST26 (19.1%; CAS family), ST53 (11.2%; T family), ST25 (10.1%; CAS family), and ST1(8.1%; Beijing family) represent almost 70% of the total number of isolates in this study.

The spoligotyping analysis identified the strains in the familes of CAS (n = 471, 37.9%), Haarlem (n = 326, 26.2%), T (n = 195, 15.7%) and Beijing (n = 101, 8.1%). Other spoligotypes belongs to Manu (n = 25, 2%), LAM (n = 13, 1%), U (n = 17, 1.3%), EAI (n = 3, 0.2%), Bovis (n = 10 , 0.8%), X (n = 2, 0.16%) and the remaining (n = 77 , 6.1%) were orphan.

High spoligotype diversity was documented for CAS, Haarlem and T lineages. Although LAM family was not frequent in this study, a high diversity was also evidenced for this lineage (6 sublineages). Furthermore 6 *M. bovis* strains and 4 *M. bovis* BCG strains were found in this study which classified into 3 clusters.

Geographical distribution of Spoligotypes in Iran. The geographical distribution of *M. tuberculosis* spoligotypes is shown in Table 4. The most prevalent families were CAS (37.9%) followed by Haarlem (26.2%), T (15.7%), Beijing (8.1%). CAS family strains were predominant in 15 provinces (Khouzestan, Esfahan, Fars, Qazvin, Gilan, Golestan, Hamedan, Hormozgan,

Table 1. Spoligotypes of the 5 most prevalent clades with a Shared Type number in SITVIT databade.

M.tb Strain	Shared Type	No. of isolates (%)	Spoligo pattern (binary)	Spoligo pattern (octal)
H4	127	255 (20.5%)	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnonoooonnnnnn	777777777720771
CAS1_DELHI	26	238 (19.1%)	nnnooooonnnnnnnnnnnnnnnnooooooooooooonnnnnnnnn	703777740003771
T1	53	140 (11.2%)	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnooonnnnnn	777777777760771
CAS1_DELHI	25	126 (10.1%)	nnnooooonnnnnnnnnnnnnnnooooooooooooonnoonnnnn	703777740003171
Beijing	1	101 (8.1%)	oooooooooooooooooooooooooooooooooooonnnnnnnnn	000000000003771

Boushehr, Kerman, Kermanshah, Markazi, Tehran, Yazd, Lorestan); strains of Haarlem family were predominant in Qom, Semnan, Kordestan and eastern border provinces i.e. Khorasan and Sistan -Balouchestan.

Strains of T family were predominant in Mazandaran, East and West Azerbaijan and Ardebil provinces (North western provinces). Distribution of Beijing strains was higher in Tehran, Khorasan and Qom provinces.

DISCUSSION

This study aimed to assess the genetic diversity of *M. tuberculosis* strains collected from 24 provinces of Iran using the spoligotyping method. Although these strains were not representative of all strains presented in Iran, they provided an insight into the population structure of *M. tuberculosis* spoligotypes in the country.

Previously, the *M. tuberculosis* isolates were classified into 3 distinct genetic groups by Sreevatsan *et al* (11): Group I or ancient MTB genotype (CAS, Beijing, EAI) and Group II and III (Haarlem, T, LAM, U, X) which called Modern MTB genotypes. In a similar study, *M. tuberculosis* isolates belonged to genetic groups II and III failed to hybridize with spacers 33 to 36, suggesting that these spacers and DRs have become deleted from the genome of all these groups (12). In the present study, the prevalence of ancient TB was 47.9% and the prevalence of modern TB was 45.3%. Another study by Merza *et al*, reported that the prevalence of ancient and modern TB in Iran was almost equal, whereas in Pakistan and Afghanistan, the majority of strains belonged to ancient MTB genotype. In contrast, modern MTB strains were prevalent in Turkey. Therefore they considered Iran as the connecting geographical location between ancient and modern TB (13).

The CAS family which was the most prevalent lineage in our results is essentially localized in Central and Middle Eastern Asia (14). It belongs to ancient TB and its prevalence is steadily decreasing from south Asia to Western Asia. This genotype might be an ancestor of the Beijing lineage since it clusters close to Beijing when analyzed by a combination of MIRU-VNTR and Spoligotyping (14).

The Haarlem family was first isolated from a patient living in Haarlem, the Netherlands (7). Today its widespread distribution in different geographical regions of the world such as Asia, Europe and Africa, has been documented (15,16). A study conducted in Iran revealed that this family accounts for more than half of all clustered strains among Iranian MDR-TB (Multi-drug resistant tuberculosis) patients (16). In our study, Haarlem family was prevalent in 5 provinces (Khorasan, Kordestan, Sistan-Balouchestan, Qom and semnan) and the prevalence of T family was higher in north-western provinces (East and West Azarbaijan, Ardebil and Mazandaran).

The Beijing genotype was originally described by Van Soolingen *et al.* (21). This family has spread globally during recent years with the highest prevalence found in Asia and the territory of the former Soviet Union (17-19). The frequency of Beijing genotype was higher in Tehran, Qom and Khorasan provinces due to high migration rate from other provinces or countries with high burden of Beijing genotype to these areas. In this study the prevalence of Beijing genotype was 8.1%; in previous studies the prevalence of this genotype in Tehran, Mashhad and Shiraz was 5.5%, 7.1% and 10% respectively (6, 17, 20).

Although our results demonstrated that the most prevalent family in Iran is CAS Lineage, this family is not necessarily prevalent in each provinces of Iran.

The high frequency of Beijing strains in provinces with high migration rate should be considered, due to association of this family with drug resistance.

Table 2. Spoligotype of other Clustered strains.

MTB Strains	Shared Type	No. of isolates	Spoligo pattern (binary)	Spoligo pattern (octal)
CAS	22	24	nnnoooonnnnnnnnnnnnoooooooooooooooooonnnnnnnnn	703777400001771
CAS	357	20	nnnoooonnnnnnnnnnnnnnooooooooooooooonnnnnnnnn	703777740000771
MANU 2	54	19	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnoonnnnnnnnnn	777777777763771
H4	656	17	nonnnnnnnnnnnnnnonnnnnnnnnnooonoooonnnnnnnn	577777377420771
H3	294	15	nonnnnnnnnnnnnnnnnnnnnnnnnonoooonnnnnnnnn	577777777720771
CAS1_KILI	21	14	nnnoooonnonnnnnnnnoooooooooooooooonnnnnnnnn	703377400001771
H4	262	12	nnnnnnnoonnnnnnnnnnnnnnnnnnoonnooonnnnnnnnn	774777777420771
Not seen		10	noonnnnnnnnnnnnnnnnnnnnnnnoonnooonnnnnnnnn	477777777420771
H4	777	8	nnnnnnnnnnnnnnnnnnnnnnnnnnnoonooonnnnnnnnn	777777777420771
Not seen		8	nnnoooonnnnnoooooooooonnnnnnnnnoonnnnnnnnn	703740007760771
H3	1908	7	nnnnnnnnnnnnnnnnnnnnnnnnnoooonoooonnnnnnnnn	777777776020771
T1	284	7	oooonnnnnnnoonnnnnnnnnnnnnnnnnoooonnnnnnnnn	37637777760771
CAS	142	6	nnnoooonnnnnnnnnnnnnoooooooooooooonnnnnnnnn	703777700003771
CAS	485	6	nnnoooonnnnnnnnnnnoooooooooooooonnnnnnnnn	703777400003771
CAS1_DELHI	381	6	nnnoooonnnnnnnnnnnnnoooooooooooonnooonnnnn	703777740003071
LAM9	42	6	nnnnnnnnnnnnnnnnnnnoooonnnnnnnnoooonnnnnnnn	777777607760771
T1	272	6	oooonnnnnnnnnnnnnnnnnnnnnnnnnnoooonnnnnnnnn	37777777760771
T1	628	6	nnnnnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnnnoo	777777777760760
U	602	6	nnnnnnnnnnnnnnnnnnnoooooooooooonnnnnnnnn	777777770000771
BOVIS	595	5	nnonnnnnonnnnnnnnnnnnnnnnnnnnnnnnnnnooooo	676777777777600
LAM3 and S/ convergent	4	5	oooooooooooooooooooooonnnnnnnnoooonnnnnnnn	000000007760771
U	1188	5	nnnnnnnnnnnnnnnnnnnnnnnoooooonnnnnnnnn	777777777003771
Not seen		5	nnnnnonnnnnnnnnnnnnnnnnnnnnnoonnnnnnnnn	757777777763771
Not seen		5	nnnnnnnnnnnnnnnnnnnnnnnnoooooooonnnnnnnnn	777777777000771
BOVIS	482	4	nnonnnnnonnnnnonnnnnnnnnnnnnnnnnnnooooo	676773777777600
CAS	486	4	nnnoooonnnnnnnnnnoooooooooooooonnnnnnnn	703777740000371
T1	1166	4	nnnnnnnnonnnnnnnnnnnnnnnnnnnoooonnnnnnnnn	777377777760771
Not seen		4	nonoonnnnnnnnnnnnnnnnnnoonnooonnnnnnnnn	517777777420771
CAS	864	3	nnnoooonnnnoooooooooooooooooooonnnnnnoo	703740000000760
CAS	1089	3	noooooonnnnnnnnnnnnoooooooooooonnnnnnnnn	403777740003771
CAS	1093	3	nnnoooonnnnnnnnnnoooooooooooooonnnnnnnnn	703777600003771
CAS	1264	3	nnnoooonnnnnnnnnnnoooooooooooooooooooooo	703777740000000
MANU 2	1634	3	nnnnnnnnnnnnnnnnnnnnnnnnnnonoonnnnnnnnnn	777777777723771
T1	520	3	nnnnnnnnnnnnnnnnnnnnnnnnnnnnoooonononnnnn	777777777760571
T1	1144	3	nnnnnnnnnnnnnnoooooonnnnnoooonnnnnnnnn	777777600760771
U(CAS_ANCESTOR?)	27	3	nnnoooonnnnnnnnnnnnnnnnnnnnoooonnnnnnnnn	703777747770371
Not seen		3	nnnnnnnnnnooooonoooooooooooooooooooonnooonn	777604000000611
CAS	599	2	nnnoooonnnnnnnnnooooooooooooooonnnnnnnnn	703777400000771
CAS1_DELHI	427	2	nnnoooonnnnooonnnnnoooooooooonnnnnnnnn	703707740003771
CAS1_DELHI	428	2	nnnoooonnnnnnnnnnnoooooooooooonnonnnnnn	703777740003371
CAS1_DELHI	1092	2	nnnoooonoonnnnnnnnooooooooooonnnnnnnnn	702777740003771
CAS1_DELHI	1314	2	nnnoooonnonnnnnnnnnoooooooonnoonnnnn	703377740003171
H4	361	2	nonnnnnnnnnnonnnnnnnnnnnnnnnooonoooonnnnnnnn	577737777420771

MTB Strains	Shared Type	No. of isolates	Spoligo pattern (binary)	Spoligo pattern (octal)
H4	597	2	nonnnonnnnnnnnnnnnnnnnnnnnooonooooonnnnnn	567777777420771
H4	764	2	nnnnnnnnnnnonnnnnnnnnnnnnnnonooooonnnnnn	777757777720771
H4	1568	2	nnnnnnnoonnnnnnnnnonnnnnnnooonooooonnnnnn	774777377420771
MANU 2	1690	2	nnnnnnnnnnnnnnnnnnnnnnnnnnnnnoononnnnnnn	777777777762771
T1	1626	2	nnnnnnnnnnnnnnnnnnnnnnonnnnnoooonnnnnnn	777777776760771
T3	37	2	nnnnnnnnnnonnnnnnnnnnnnnnnnnnoooonnnnnn	777737777760771
U	374	2	nnnnnnnnnnnnnnnnnnnnononooooooooooooooooo	777777752000000
Not seen		2	nnnnnnnnnnnonnnnnnnnnnnnnoooooooooonnnnnn	777604000000611
Not seen		2	nonnooooonnnnnnnnnnnnnnnnnooonooooonnnnno	517777777420771
Not seen		2	nonnooooonnnnnnnnnnnnnnnnnnooonooooonnnnnoo	517777777420771
Not seen		2	nnnnnnnnnnonnnnnnnnnnnnnnooooooooonnnnnn	777737777000771

Table 3. Spoligotype of non-clustered strains.

MTB Strains	Shared Type	No. of isolates	Spoligo pattern (binary)	Spoligo pattern (octal)
BOVIS	694	1	nnonnnnnonnnnooonnnnnnnnnnnnnnnnnnnnnoooooo	676743777777600
CAS	1422	1	nnnoooonnnnnnnnnnooooooooooooooooooonnnnnn	703777000000771
CAS	1616	1	ooooooonnnnnnnnnnnooooooooooooonnnnnnnn	003777740003771
CAS1_DELHI	289	1	nnnoooonnnnnnnnnnnooooooooooooonnnonnnnnn	703777740003571
CAS1_DELHI	754	1	nononnoonnnnnnnnnnooooooooooooonnnnnnnn	503777740003771
CAS2	288	1	nnnooooooonnnnnnnnnnooooooooooooonnnnnnnn	700377740003771
EAI5	236	1	nnnnnnnnnnnnnnnnnnnnnnnnooooonononnnnnnn	777777777413771
EAI5orEAI3	8	1	noooooooooooonnnnnnnnnnnnooooonononnnnnnn	400037777413771
EAI8_MDG	109	1	noonnnnnnnnnnnnnnonnnnnnnnnooooonononnnnnnn	477777377413771
H3	50	1	nnnnnnnnnnnnnnnnnnnnnnnnnonooooonnnnnnn	777777777720771
H3	511	1	nnnnnnnnnnnnnnnnnooooooooooonooooonnnnnn	777777700020771
H3-T3	36	1	nnnnnnnnnnonnnnnnnnnnnnnnnonooooonnnnnnn	777737777720771
H4	35	1	nnnnnnnnnnonnnnnnnnnnnnnnnooonooooonnnnnn	777737777420771
LAM11-ZWE	59	1	nnnnnnnnnnnnnnnooooonnoooonnoooonnnnnnn	777777606060771
LAM6	64	1	nnnnnnnnnnnnnnooooonnnnnonnoooonnnnnnn	777777607560771
LAM9	492	1	nnnnnonnnnnnnnnnoooonnnnnnnooooonnnnnnn	773777607760771
LAM9	770	1	nnnnnnnnnnonnnnnnnoooonnnnnnnooooonnnnnnn	777677607760771
MANU 2	1192	1	nnnnnnnnnnnnnnnnnnonnnnnnnnnnoonnnnnnnn	777777677763771
T1	7	1	onnnnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	377777777760771
T1	65	1	nnnnnnnnnnnnnnnnnnnnnnnnnnooooonoonnnn	777777777760471
T1	102	1	nnnnnnnnnnnooooonnnnnnnnnnnnooooonnnnnnn	777703777760771
T1	131	1	nnnnnnnnnnoonnnnnnnnnnnnnnnooooonnnnnnn	777717777760771
T1	154	1	nnnnonnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	757777777760771
T1	205	1	nnnonnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	737777777760771
T1	243	1	nnnonnnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	737777777760771
T1	291	1	nnnnnnnnnnnnnnnnnonnnnnnnnnooooonnnnnnn	777777677760771
T1	535	1	nnnnnnnnnnnnnnnnooonnnnnnooooonnnnnnn	777777707760771
T1	635	1	ooooooooooooooooooooonnnnonnnooooonnnnnnn	000000007560771
T1	801	1	nnnnnnnoonnnnnnnnnnnnnnnnnnooooonnnnnnn	774777777760771
T1	1073	1	nnnnnnnnnnoonnnnnnnnnnnnnnnnooooonnnnnnn	777637777760771
T1	245	1	nnnnnnnnnnnnnnnnnnnnnnnnnnooooonnooooo	777777777760600
T1	411	1	noooooonnnnnnnnnnnnnnnnnnnnnnooooonnnnnnn	407777777760771

MTB Strains	Shared Type	No. of isolates	Spoligo pattern (binary)	Spoligo pattern (octal)
T1	926	1	nnnnnnonnnnnnnnnnnnnnnnnnnnnoooonnnnnn	773777777760771
T1	1566	1	nnnnoonnnnnnnnnnnnnnnnnnnnnnoooonnnnnn	747777777760771
T1	1735	1	nnnnnnnnnnnnnnnnnnnnooonnonoooonnnnnn	777777774320771
T1_rus2	280	1	nnnnnnooooooooooooonnnnnnnnnnnnoooonnnnnn	770000777760771
T1_RUS2	899	1	nnonnnoooooooooooonnnnnnnnnnnnoooonnnnnn	670000777760771
T1_RUS2	1173	1	nnnnnnooooooooooooonnnnnnnnnnnoooonnnonnn	770000777760731
T2	52	1	nnnnnnnnnnnnnnnnnnnnnnnnnnnooonnnonnn	777777777760731
T3	1655	1	nnnnnnnnnnnonoonnnnnnnnnnnnnooonnnnnn	777723777760771
U	172	1	nnnnnnnnnnnnnnnnnnnnnnnnnoooonnnnnn	777777777740771
X1	708	1	nnnonnnnnnnnnnnnonnnnnnnnnnnooonnnnnn	737776777760771
X2	302	1	nnnnnnnnnnnonnnonnnnnnnnnnooonnnnnn	777756777760771
Not seen		1	nononnnnnoooooonnnnnnnnnnnnnnooonnnnnn	537017777760771
Not seen		1	nonnnnnooooonnnnnnnnnnnnnnooonooonnnnnn	576037777420771
Not seen		1	nnnooooonnnnnnnnnnnnnnnnnonnnnoonnnnnn	703777777363771
Not seen		1	nnnnnnnnnnnnnnnnnnnnnnnnooooooooooooonnn	777777777400031
Not seen		1	oooooooooooonnnnnnnnnnnnnnooonooonnnnnn	000177777420771
Not seen		1	nnnnnnnnnnnnnnnnnnnnnnnnnnoonnnnnnn	777377777763771
Not seen		1	nnnoooonnnnnnnnnnnnnnnoooononoonnnnnn	703777776123771
Not seen		1	nnnnnnnnononnnnnnnnnnnnnnooonooonnnnnn	777257777620771
Not seen		1	nnnnnoonnoonnnnoooooonnnnnnnnooonnnnnn	763170077760371
Not seen		1	nnnooonnnnnnnnnooooooooooooooooonnnnnn	703777000000771
Not seen		1	nnnooonnonnnooooooooooooooooooonnnnnn	703360000000371
Not seen		1	nonnnnnnonnnnnnnnnnnnooonoooononnnnn	577357777420571
Not seen		1	nnnnnnnnnnnnoonnnnnnnnnoonnnnnnnnnnnonon	777747774777661
Not seen		1	nnnnnnnnoooonnnnnnnnnnooooonnonnnnnnnn	777027776027771
Not seen		1	nnnnnnnnooonnnnnnooooonnnnnnooonnnnnn	777637607760771
Not seen		1	nnnnnnnnnnnnnnnonnnnnnnnnooooonnnnnn	777777377701771
Not seen		1	nnnoooonnnnnnnnnnooooooooooooonnnnnn	703777700001771
Not seen		1	nnnoooonnonnnnnnnnnooooooooooooonnnnnn	703377700000771
Not seen		1	nnnoooonnnnnoooooooooooooooooonnnnnn	703740000000771
Not seen		1	nnnnnonnnonnnnnnnnnnnnooonooonnnnnn	757337777420771
Not seen		1	nnnoooonnnnnnnnooonnoooooooooooonnnnnn	703776140003771
Not seen		1	nnnoooonnnnnnoonnnnnoooooooooooonnnnon	703743740000761
Not seen		1	nnnnnonnoonnnnnnnnnnnnnooonooonnnnnn	756337777420771
Not seen		1	nnnnnnnnnonnonnnonnnonnnnnnnnoooonoonnnn	777356737760471
Not seen		1	nnnnnnnnnnnnnnnnnnnnnnooonoonnnnnn	777777777423771
Not seen		1	nnnoooooonooooonnonnnnnnnnnooonnnnnn	701207377760771
Not seen		1	nnnnnnonnonnoonnnnnnnnnonnnnooononnnnnn	773347776761271
Not seen		1	nnnnnnnnnnnooooooooooonnnnnnnooonnnnnn	777740003760771
Not seen		1	nonnnnnooonnnnnnnnnnoonnnoonooonnnnnn	574377763420771
Not seen		1	oooonnnnnnoonnnnnnnnnnnnnnnooonnnonnn	037637777760731
Not seen		1	oonoooonnnnnnnnnnnooooooooooonnnnnnnn	103777740003771
Not seen		1	noonnnnnnnnnnnnnnnnoooonnoooooonnnn	477777777010071
Not seen		1	nonnnnnnnnnnnnnnnnnnnnooonoooonnnnoo	577777777420760
Not seen		1	nnnnnnnoonnooonnnnnnoooooooooooonnoonnnnn	776307740003171

Table 4. geographical distribution of *Mycobacterium tuberculosis* spoligotypes in Iran.

	Khouzestan	East and West Azarbaijian/Ardebil	Esfahan	Fars	Qazvin	Gilan	Golestan	Hamedan	Hormozgan /Boushehr	Kerman	Kermanshah	Khorasan	Kordestan	Markazi	Mazandaran	Qom	Semnan	Tehran	Yazd	Sistan- Balouchestan	Lorestan	Total	
BEIJING	5	7	6	5			6	1	5		1	12		3	1	10	1	35		3		101	8.1%
LAM	7		2	1		1			1			1						2				15	1.2%
BOVIS	1	2		1												2		4				10	0.8%
CAS	67	16	17	16	6	18	23	7	19	10	9	34	4	9	4	18	7	107	6	59	15	471	37.9%
EAI	2																	1				3	0.2%
HAARLEM	7	10	2	14	2	13	8	5	8	8	2	42	9	3	6	20	8	85	2	65	7	326	26.2%
MANU	2	1		1			1	1				3						14		2		25	2%
T	13	29	12	1		2	8	3	4	2	4	19	3		13	8	5	47	4	16	2	195	15.7%
U	6								1			2				6				2		17	1.3%
X															1					1		2	0.16%
UNKNOWN	9		3	3		5	2	4	3			4				1	3	23		17		77	6.1%
TOTAL	119	65	42	40	10	39	47	21	38	24	16	117	16	15	26	61	21	324	12	165	24	1242	

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