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Original Research

MIRU-VNTR analysis of *Mycobacterium tuberculosis* from Tehran, Sistan-Baluchestan, Kermanshah and Hormozgan during 2014 and 2015

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Abstract: Standard 15-locus Mycobacterial Interspersed Repetitive Unit Variable Number Tandem Repeat (MIRU-VNTR) typing of *Mycobacterium tuberculosis* (MTB) is a valuable instrumentation for TB control. Our knowledge about the genetic diversity of MTB and population structure of MTB circulating in Iran is limited. During 2014–2015, 98 MTB isolates were collected from the TB centers of four provinces of Iran. Isolates were genetically characterized using 15-locus based MIRU-VNTR typing. Ninety-five distinct mycobacterial interspersed repetitive unit MIRU-VNTR patterns were found among 98 isolates. 5 (5.1%) isolates grouped into 2 clusters and 93 (94.89%) isolates had a unique pattern. The HGDI was as high as 0.99 and 10 of loci were designated as highly discriminative. Clusters belonged to Tehran only. This indicates these patterns are rotating in Tehran. Unique patterns suggest that distribution of samples in each province and population differs. HGDI is higher than previous studies for MIRU-VNTR typing in Iran. We suggest MIRU-15 because it is a valid epidemiological background for clustering defined. Limited data is available on the genetic diversity and transmission dynamics of MTB in Iran. To examine the genetic diversity and transmission dynamics of MTB strains we genotyped a collection of isolates from four different parts of Iran. The method of 15-loci MIRU-VNTR demonstrated high discriminatory power and may be applied as a first-line genotyping instrumentation in investigating the molecular epidemiology of *M. tuberculosis* in Iran.

Key words: Genotyping; *Mycobacterium tuberculosis*; MIRU-VNTR; Iran; Transmission.

Introduction

Mycobacterium tuberculosis (MTB) is an important pathogen and one of the most prevalent and deadly infectious diseases in the worldwide (1-3). Tuberculosis (TB) is cause of 8.8 million new cases and more than 1.4 million deaths each year (4, 5). The national TB control programs established and developed in Iran that resulted in reduction of TB incidence from 34 per 100 000 in 1996 to 21 per 100 000 cases in 2011 (6, 7).

Tehran is capital of Iran, with a population of around 8.4 million in the city and 14 million in the wider metropolitan area. Tehran has the largest industrial and medical centers. That is why migration to the city from other provinces is very high. Sistan-Baluchestan is a resource-limited and high TB burden area in the southeast part of Iran and has a highest estimated TB rate of 48.5 cases per 100,000 populations because it is in neighboring Afghanistan and Pakistan. The incidences of TB in Pakistan and Afghanistan are 231 and 189 per 100 000 respectively and the migration of people to Iran have changed the incidence rate of TB in Sistan-Baluchestan it has the highest incidence of TB in the country. The Hormozgan province is located in the north coast of Persian Gulf and bordered by Sistan-Baluchestan from the east part. The western part of Iran is Kermanshah that has been seriously influenced in terms of its infrastructures during the eight-year war with Iraq because incidence of TB in Iraq is 45 per 100 000 (1, 6). Therefore studies of the genetic diversity of MTB in high burden areas that are in neighborhood of Pakistan, Afghanistan and Iraq are needed in order to enable insight into dissemination dynamics and virulence pattern of the pathogen (8).

To determine epidemiological background, the MTB genotypes and their transmission patterns among Iranian patients, we accumulated a collection of isolates from different parts of Iran were genotyped (9).

Molecular genotyping of MTB isolates has become an invaluable method for epidemiology and phylogeny of TB and is a strong adjunct to TB control by allowing investigators to detect the disease transmission, outbreaks and laboratory cross-contamination, reconnais-

sance of false-positive cultures, and distinguishing between re-infections and relapses (2, 10, 11).

Nowadays, three reliable genotyping tools are the most frequently used: IS*6110* -RFLP, spoligotyping and MIRU-VNTR typing. MIRU-VNTR has been considered a fine alternative to the reference method and has demonstrated to be faster and easier to perform and presumably the most popular approach between these methods (1, 2, 7).

MIRU-VNTR genotyping is done by amplifying a panel of 12, 15 or 24 loci that genotyping 15 loci is best suited for epidemiologic studies. The utilization of the standard MIRU-15 and MIRU-24 has helped to increase insight into the transmission dynamics of MTBC and these methods have considerably increased discriminatory power over the 12 locus method (4, 11). Currently, the main method and high-resolution genotyping of *M. tuberculosis* complex isolates is MIRU-VNTR (mycobacterial interspersed repetitive unit–variable number of tandem repeats) typing and 15- locus system has been proposed as the international standard for usual epidemiological studies (12).

Several studies have been accomplished in Iran, but Limited information is existing (1, 9). So, The specific aims of this study were focused on the genetic diversity, transmission dynamics, determining pattern of MTB isolates and epidemiological background of MTB isolates from 4 important provinces of Iran including Tehran, Sistan-Baluchestan (with the most major rate of TB), Kermanshah and Hormozgan for clustering defined by using 15-locus MIRU-VNTR (1, 6, 7).

Materials and Methods

Studying population and samples

The study was conducted over a one year of period (from April 2014 to June 2015) in the TB centers of four provinces of Iran including Tehran, Sistan-Baluchestan (southeast province of Iran with the most major rate of TB), Kermanshah (western part of Iran, bordering with Iraq) and Hormozgan (1, 4, 6, 9).

A total of 195 clinical sputum specimens were collected from TB suspected patients attending 4 different TB center including Tehran (n=83), Sistan-Baluchestan (n=30), Kermanshah (n=47) and Hormozgan (n=35) that these patients had clinical signs and symptoms of TB and undergoing examination for possible active TB.

Identification of mycobacteria

For microscopy examination, smears were prepared with wooden swab and stained using ZiehlNeelsen method and then checked with light Microscope.

Samples from all of patients were decontaminated by Petroff's method and were inoculated into Lowenstein-Jensen (LJ) medium (Merck). The slant cultures were incubated at 37 ℃ and investigated for growth once weekly up to six weeks. All isolates were checked for colony morphology, pigmentation and growth rate.

The suspected colonies were tested for cultures production of niacin and catalase, reduction of nitrate, and inhibition by thiophene-2- carboxylic acid hydrazide.

Therefore, in this study isolates from the smear positive patients with pulmonary TB from Tehran (n=49), Sistan-Baluchestan (n=15), Kermanshah (n=18) and Hormozgan (n=16) were included (6, 7).

Genomic DNA preparation

In this study DNA was extracted from colonies grown on Lowenstein-Jensen (LJ) media. In brief, A loopful (ca. 10 µl) of colonies was suspended in TE buffer and boiled and DNA was extracted as described by by Li *et al* (13).

The pellet was suspended in 400 µL TE buffer [10 mmol/L Tris-HCl (pH 8.0), one mmol/L EDTA] (14), and in short the mixture was mixed on a vortex mixer. The suspension was placed in a boiling water bath for five min, subjected to three freeze-thaw cycles alternating between -70 ° C for 5 min and 100 ° C for two min, and then centrifuged at 10 000 *g* for 5 min. A 200 µL aliquot of the supernatant was transferred to a sterile microtube and stored at -20 ° C until PCR testing.

MIRU -VNTR typing

Standard 15-locus VNTR typing was applied to genotype the MTB clinical isolates as explained by Supply et al (4, 15, 16). Polymerase chain reactions (PCR) was performed using primers described in the MIRU-VNTR standard protocol for each isolate PCR were performed in 25 µl of reaction mixture containing 2.5 µl PCR buffer, 1 mM $MgCl_2$, 0.01 pM of each primer, 200 µM of each dNTP, 1 unit of Taq DNA polymerase and 2 µl of DNA template (1, 4, 15, 16).

Locus amplification was performed under the following conditions: initial denaturation at 95 ° C for 5 min, and then 30 cycles of 95° C for 30s, 60 ° C for 30s and 72 ° C for 45s, followed by a final extension at 72 ° C for 5 min. Negative control (sterile water) was added to each PCR reaction to accredit the assay (4, 16).

Gel electrophoresis was done in 1.5% agarose for 1 hour at 90 constant Voltage. A 100 bp marker was used to measure the size of amplicons and the obtained size compared with allelic table as disseminated by Supply et al (1, 4, 15, 16).

VNTR allelic diversity and genetic relationships Analysis

The number of repeats for each locus was defined based on the allelic table by Supply et al.(15). The clustering analysis was then performed and genetic relationships among the isolates were estimated using MIRU-VNTR plus software, and dendrogram was built based on UPGMA strategy (7). Isolates sharing identical MIRU-VNTR patterns were considered as members of a cluster. The clustering rate was specified as (nc - c)/n, where nc is the total nu

mber of clustered cases, c is the number of clusters, and n is the total number of cases in the specimen (4, 15, 16).

The allelic diversity of each VNTR locus and the discriminatory power of each locus was assessed and calculated by using Hunter – Gaston discriminatory index (HGDI) as described previously (17).

The 15-locus MIRU patterns were compared with the patterns from the MIRU-VNTR plus database to specify MTB strain lineages and relatedness.

Table 1. MIRU-VNTR fingerprinting results for 98 MTB isolates.

Profile (ETR A, ETR C, MIRU 04, ETR E, MIRU 10, MIRU 16, MIRU 26, MIRU 40, QUB11b, QUB 26, MTUB 30, MTUB 39, MTUB 04, MTUB 21, QUB4156)	Frequency- Provinces	Profile (ETR A, ETR C, MIRU 04, ETR E, MIRU 10, MIRU 16, MIRU 26, MIRU 40, QUB11b, QUB 26, MTUB 30, MTUB 39, MTUB 04, MTUB 21, QUB4156)	Frequency- Provinces
4, 4, 3, 7, 2, 4, 1, 3, 2, 3, 3, 3, 3, 3, 3	3 - Tehran	3, 4, 2, 2, 3, 3, 6, 4, 2, 9, 2, 3, 3, 5, 4	1 - Tehran
3, 4, 3, 4, 1, 4, 6, 4, 2, 8, 3, 4, 3, 5, 4	2 - Tehran	4, 6, 2, 3, 4, 3, 4, 1, 2, 6, 2, 3, 3, 4, 4	1 - Tehran
1, 4, 2, 3, 4, 3, 6, 4, 2, 9, 1, 2, 4, 2, 1	1 -Hormozgan	3, 4, 2, 4, 3, 3, 8, 4, 2, 9, 3, 4, 4, 5, 4	1 - Tehran
1, 4, 2, 3, 4, 5, 6, 4, 2, 4, 1, 2, 4, 3, 2	1 -Hormozgan	6, 4, 6, 5, 4, 3, 3, 4, 3, 8, 1, 4, 3, 14, 3	1 - Tehran
3, 4, 3, 4, 1, 5, 7, 2, 1, 7, 3, 4, 2, 6, 2	1 -Hormozgan	3, 4, 2, 4, 3, 4, 6, 4, 3, 7, 3, 4, 3, 4, 4	1 -Kermanshah
3, 4, 3, 4, 4, 3, 1, 1, 2, 11, 4, 4, 3, 3, 1	1 -Hormozgan	3, 4, 2, 4, 2, 4, 6, 1, 2, 5, 2, 4, 3, 5, 4	1 - Sistan-Bluchestan
2, 4, 3, 4, 4, 3, 6, 1, 2, 5, 1, 2, 4, 3, 2	1 -Hormozgan	3, 4, 2, 4, 3, 4, 7, 4, 2, 9, 2, 4, 3, 5, 4	1 -Hormozgan
3, 4, 3, 4, 4, 3, 4, 1, 5, 6, 3, 6, 3, 2, 2	1 - Tehran	3, 4, 2, 4, 6, 4, 6, 7, 2, 8, 1, 3, 4, 3, 4	1 -Hormozgan
3, 4, 3, 4, 1, 4, 6, 4, 2, 9, 3, 4, 4, 5, 2	1 - Tehran	3, 4, 2, 5, 3, 4, 7, 3, 2, 8, 2, 4, 3, 5, 3	1 -Kermanshah
2, 4, 3, 4, 1, 4, 7, 3, 2, 8, 3, 4, 3, 5, 3	1 - Tehran	4, 2, 2, 7, 8, 5, 8, 3, 2, 3, 2, 4, 4, 4, 7	1 -Hormozgan
3, 4, 2, 4, 2, 4, 6, 4, 2, 8, 2, 4, 3, 5, 4	1 - Tehran	3, 4, 2, 6, 3, 4, 7, 4, 2, 8, 2, 4, 3, 4, 4	1 -Kermanshah
5, 5, 3, 7, 4, 4, 6, 4, 2, 9, 4, 4, 4, 6, 4	1 - Tehran	4, 2, 2, 8, 8, 5, 9, 4, 2, 8, 3, 4, 4, 4, 4	1 - Tehran
3, 5, 3, 4, 2, 4, 6, 4, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	4, 6, 2, 8, 6, 4, 6, 4, 5, 6, 3, 4, 3, 3, 4	1 - Tehran
2, 5, 3, 4, 1, 4, 6, 5, 2, 8, 3, 4, 3, 6, 4	1 - Tehran	2, 3, 2, 4, 4, 4, 6, 2, 3, 6, 3, 4, 3, 2, 4	1 - Tehran
3, 5, 3, 4, 1, 5, 6, 5, 2, 8, 3, 4, 4, 5, 4	1 - Tehran	2, 3, 2, 4, 2, 4, 6, 4, 2, 6, 2, 3, 2, 4, 4	1 -Hormozgan
5, 2, 3, 7, 7, 6, 10, 5, 2, 9, 3, 4, 4, 4, 6	1 - Tehran	1, 4, 2, 3, 4, 4, 6, 4, 2, 8, 1, 2, 4, 4, 4	1 -Hormozgan
4, 5, 3, 4, 7, 2, 2, 4, 2, 9, 4, 4, 3, 4, 4	1 - Tehran	3, 4, 2, 4, 3, 4, 6, 4, 2, 8, 2, 3, 2, 5, 4	1 - Sistan-Bluchestan
3, 5, 3, 4, 1, 3, 9, 6, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	4, 2, 2, 5, 8, 5, 1, 2, 2, 6, 2, 2, 4, 4, 6	1 - Sistan-Bluchestan
4, 5, 3, 4, 1, 2, 6, 6, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	3, 3, 2, 4, 3, 4, 6, 4, 2, 9, 2, 3, 4, 8, 4	1 - Sistan-Bluchestan
2, 3, 3, 4, 1, 4, 6, 3, 2, 8, 3, 4, 2, 5, 4	1 - Tehran	3, 4, 2, 4, 3, 4, 5, 4, 2, 9, 2, 3, 3, 4, 4	1 -Kermanshah
4, 2, 3, 4, 7, 7, 6, 4, 2, 10, 4, 4, 6, 4, 4	1 - Tehran	3, 4, 2, 4, 3, 5, 6, 4, 2, 6, 2, 4, 2, 5, 4	1 - Sistan-Bluchestan
4, 4, 3, 4, 1, 4, 6, 2, 2, 8, 3, 4, 3, 4, 4	1 - Tehran	4, 3, 2, 4, 5, 4, 6, 3, 2, 3, 2, 4, 2, 4, 4	1 -Kermanshah
3, 4, 3, 4, 1, 4, 6, 4, 2, 8, 3, 3, 3, 5, 4	1 - Tehran	3, 4, 2, 4, 3, 4, 6, 4, 2, 8, 2, 4, 3, 4, 4	1 -Hormozgan
5, 2, 3, 7, 8, 4, 6, 4, 2, 10, 3, 4, 4, 6, 6	1 - Tehran	2, 3, 2, 4, 3, 4, 8, 4, 2, 7, 2, 4, 3, 5, 4	1 - Tehran
3, 4, 3, 4, 1, 7, 8, 4, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	3, 4, 2, 6, 3, 4, 6, 4, 2, 8, 2, 4, 3, 6, 3	1 - Tehran
3, 4, 3, 4, 1, 4, 6, 1, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	4, 2, 2, 7, 8, 5, 9, 4, 2, 9, 2, 4, 4, 4, 3	1 - Tehran
3, 5, 3, 3, 1, 4, 6, 5, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	3, 4, 2, 4, 3, 3, 5, 4, 2, 7, 2, 4, 2, 4, 2	1 - Sistan-Bluchestan
4, 5, 3, 4, 1, 4, 7, 5, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	3, 4, 2, 4, 2, 3, 5, 4, 2, 8, 2, 4, 2, 5, 2	1 - Sistan-Bluchestan
4, 5, 3, 4, 1, 4, 8, 4, 2, 8, 3, 4, 3, 5, 4	1 - Tehran	2, 4, 2, 3, 5, 3, 5, 4, 2, 3, 1, 3, 3, 3, 2	1 - Hormozgan
4, 2, 2, 8, 7, 8, 7, 4, 1, 9, 3, 2, 7, 4, 2	1 -Kermanshah	4, 2, 2, 5, 8, 3, 5, 4, 2, 9, 2, 4, 1, 3, 4	1 - Tehran
2, 3, 2, 6, 1, 3, 7, 4, 3, 7, 2, 4, 4, 2, 4	1 -Kermanshah	3, 4, 2, 4, 3, 3, 6, 3, 2, 8, 3, 4, 2, 4, 2	1 - Tehran
3, 3, 2, 6, 1, 4, 7, 4, 1, 9, 2, 4, 4, 5, 4	1 -Kermanshah	2, 4, 2, 4, 3, 3, 5, 4, 2, 5, 2, 4, 2, 5, 2	1 - Tehran
3, 3, 2, 6, 1, 3, 7, 4, 1, 10, 2, 2, 4, 5, 4	1 -Kermanshah	5, 3, 2, 6, 5, 5, 8, 4, 2, 7, 2, 4, 4, 4, 6	1 - Tehran
3, 3, 2, 6, 1, 4, 7, 4, 1, 9, 2, 4, 2, 5, 4	1 - Sistan-Bluchestan	2, 4, 2, 3, 4, 2, 6, 6, 2, 5, 1, 3, 4, 4, 4	1 -Hormozgan
3, 3, 2, 4, 1, 4, 7, 4, 2, 8, 2, 4, 3, 4, 4	1 -Kermanshah	3, 6, 2, 3, 2, 4, 6, 4, 2, 3, 2, 4, 1, 4, 4	1 - Tehran
5, 2, 2, 5, 7, 8, 6, 4, 2, 8, 4, 4, 6, 4, 6	1 -Kermanshah	4, 4, 2, 6, 3, 4, 6, 4, 6, 9, 3, 4, 4, 6, 4	1 - Tehran
2, 3, 2, 5, 4, 2, 4, 4, 3, 7, 2, 4, 4, 2, 4	1 -Kermanshah	3, 2, 2, 5, 8, 5, 6, 4, 2, 7, 2, 4, 4, 6, 6	1 - Tehran
3, 3, 2, 5, 2, 4, 8, 4, 2, 8, 2, 4, 3, 4, 4	1 -Kermanshah	3, 4, 2, 3, 2, 5, 5, 4, 2, 8, 2, 4, 2, 5, 4	1 - Sistan-Bluchestan
3, 2, 2, 7, 8, 5, 9, 3, 2, 7, 2, 4, 9, 4, 6	1 - Sistan-Bluchestan	3, 4, 2, 5, 2, 4, 6, 2, 2, 8, 3, 4, 2, 5, 4	1 - Tehran
3, 3, 2, 6, 7, 5, 7, 3, 3, 6, 4, 4, 3, 4, 4	1 -Kermanshah	2, 4, 2, 3, 5, 4, 6, 5, 2, 8, 1, 3, 4, 4, 4	1 -Hormozgan
4, 2, 2, 7, 4, 7, 12, 4, 2, 8, 3, 4, 4, 4, 6	1 - Sistan-Bluchestan	3, 2, 2, 7, 8, 7, 6, 4, 2, 8, 3, 3, 4, 3, 6	1 -Kermanshah
4, 2, 2, 7, 8, 7, 4, 4, 2, 8, 2, 4, 4, 4, 6	1 - Sistan-Bluchestan	3, 3, 2, 5, 2, 5, 6, 4, 2, 8, 3, 4, 2, 4, 2	1 - Sistan-Bluchestan
3, 4, 2, 5, 2, 4, 6, 4, 2, 8, 2, 4, 2, 6, 4	1 -Kermanshah	4, 3, 1, 4, 3, 4, 5, 4, 2, 3, 2, 4, 1, 4, 2	1 -Kermanshah
3, 4, 2, 5, 2, 4, 6, 4, 2, 8, 2, 2, 2, 5, 4	1 - Tehran	2, 3, 2, 4, 4, 4, 7, 4, 4, 7, 2, 3, 2, 4, 2	1 -Hormozgan
4, 2, 2, 5, 7, 5, 6, 4, 2, 9, 4, 4, 4, 3, 4	1 - Tehran	4, 2, 2, 8, 8, 9, 12, 2, 2, 10, 3, 4, 4, 5, 6	1 - Sistan-Bluchestan
4, 3, 2, 5, 4, 3, 6, 2, 5, 8, 2, 4, 1, 4, 3	1 -Kermanshah	2, 4, 2, 3, 5, 3, 5, 4, 2, 3, 2, 4, 3, 3, 2	1 -Hormozgan
4, 2, 2, 8, 8, 4, 6, 4, 2, 8, 2, 4, 3, 6, 4	1 - Sistan-Bluchestan	6, 4, 2, 5, 4, 3, 6, 4, 3, 8, 3, 4, 4, 5, 3	1 - Tehran
4, 4, 2, 6, 13, 3, 1, 2, 2, 8, 4, 4, 4, 3, 4	1 - Tehran		

Results

Demographic and clinical characteristics

Of 195 specimens, 88 (45.1%) were excluded because these were either culture negative or had culture contamination. Furthermore, another 9 specimens (4.6%) were excluded because these cultures were mycobacteria other than TB (MOTT).

Therefore, 98 culture positive were further tested in this study. These MTB cases with culture-positive TB were included 98 patients Tehran (n=49), Sistan-Baluchestan (n=15), Kermanshah (n=18) and Hormozgan (n=16) (6, 7, 16).

Typing of strains and clustering analysis by MIRU-VNTR

Five cases (5.1%) were distributed into two clusters and the remaining 93 (94.89%) isolates had a unique pattern (17).

One cluster with three members and other cluster with two members were also identified and these two clusters belonged to Tehran only. Conversely, isolates from Sistan-Baluchestan, Kermanshah and Hormozgan were not grouped together in any cluster. The discriminatory power of MIRU-VNTR typing for all isolates was high (HGDI _ 0.99) (1).

Results of allelic profile frequencies are shown in Table 1.

Allelic diversity and Discriminatory power of the MIRU-VNTR loci

To identify the allelic diversity of the MIRU-VNTR 15-loci among the strains in this study, the discriminatory power (the allelic diversity index) was calculated using HGDI, 10 MIRU loci were determined as very discriminant ($h \geq 0.6$) in our samples (MIRU10, MIRU16, MIRU26, ETRA, ETRC, ETRE, QUB26, MTUB30, MTUB04 and MTUB21), five MIRU loci were determined as moderately discriminant ($0.3 \geq h \geq 0.6$; MIRU04, MIRU40, QUB11b, MTUB39 and QUB4156), and none of MIRU loci not found to be poorly discriminant ($h \leq 0.3$). Results of the distribution of the MIRU alleles are shown in Table 2 (1, 12).

The allelic diversity and Hunter – Gaston discriminatory index (HGDI) of the MIRU-VNTR 15-loci are defined in four provinces including Tehran, Sistan-Baluchestan, Kermanshah and Hormozgan in tables 3.

Phylogenetic analysis

The dendrogram begotten using the UPGMA algorithm based on the MIRU-VNTR data explains the genetic relationships of the 98 isolates and in the Figure 2 clusters are specified (Figure 1).

Analysis the 15-loci MIRU-VNTR patterns in this study and comparison the data with those in the international MIRU-VNTR plus database (http://www.miru-vntrplus.org) revealed that none of the strains out of the 186 present in this database matched our patterns (1, 7).

Discussion

Mycobacterium tuberculosis a significant public health problem worldwide, particularly in developing countries such as Iran. Based on the recent WHO report in the year 2011, the incidence rate of TB in Iran was nearly moderate, 21 cases per 100,000 populations. However, the existence of extended borders with countries where TB is endemic Afghanistan: 189 per 100 000 and Pakistan: 231 per 100 000 and immigrations of these populations influenced TB distribution in the region and threatens the control strategies (6, 7).

The exiguous molecular epidemiology data in Iran such as Tehran, Sistan-Baluchestan, Kermanshah and Hormozgan provinces could provide information on the TB transmission status in these regions (18). Thus, this study done to illustrate the genetic diversity of the *M. tuberculosis* isolates circulating in TB-prevalent provinces (19). Reconnaissance of epidemiologically linked *M. tuberculosis* strains helps to disclose the provenance of infection, to rejection the transmission ways of different strains, and to characterize the risk factors for

Table 2. Allelic diversity for each locus.

Allele	ETR A	ETR C	MIRU 04	ETR E	MIRU 10	MIRU 16	MIRU 26	MIRU 40	QUB 11b	QUB 26	MTUB 30	MTUB 39	MTUB 04	MTUB 21	QUB 4156
1	3	-	1	-	22	-	6	6	5	-	9	-	4	-	2
2	15	18	67	1	15	4	1	8	81	-	43	8	17	5	15
3	46	19	29	11	18	21	1	11	7	9	38	16	41	14	10
4	27	48	-	44	16	49	4	63	1	1	8	73	32	34	59
5	5	10	-	15	5	15	9	6	3	4	-	-	-	34	-
6	2	3	1	10	2	1	49	3	1	8	-	1	2	9	11
7	-	-	-	12	7	5	14	1	-	10	-	-	1	-	1
8	-	-	-	5	12	2	7	-	-	44	-	-	-	1	-
9	-	-	-	-	-	1	4	-	-	17	-	-	1	-	-
10	-	-	-	-	-	-	1	-	-	4	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
12	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
13	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Total	6	5	4	7	9	8	11	7	6	9	4	4	7	7	6
HGDI	0.68	0.68	0.44	0.74	0.85	0.70	0.71	0.56	0.31	0.74	0.64	0.41	0.69	0.73	0.59

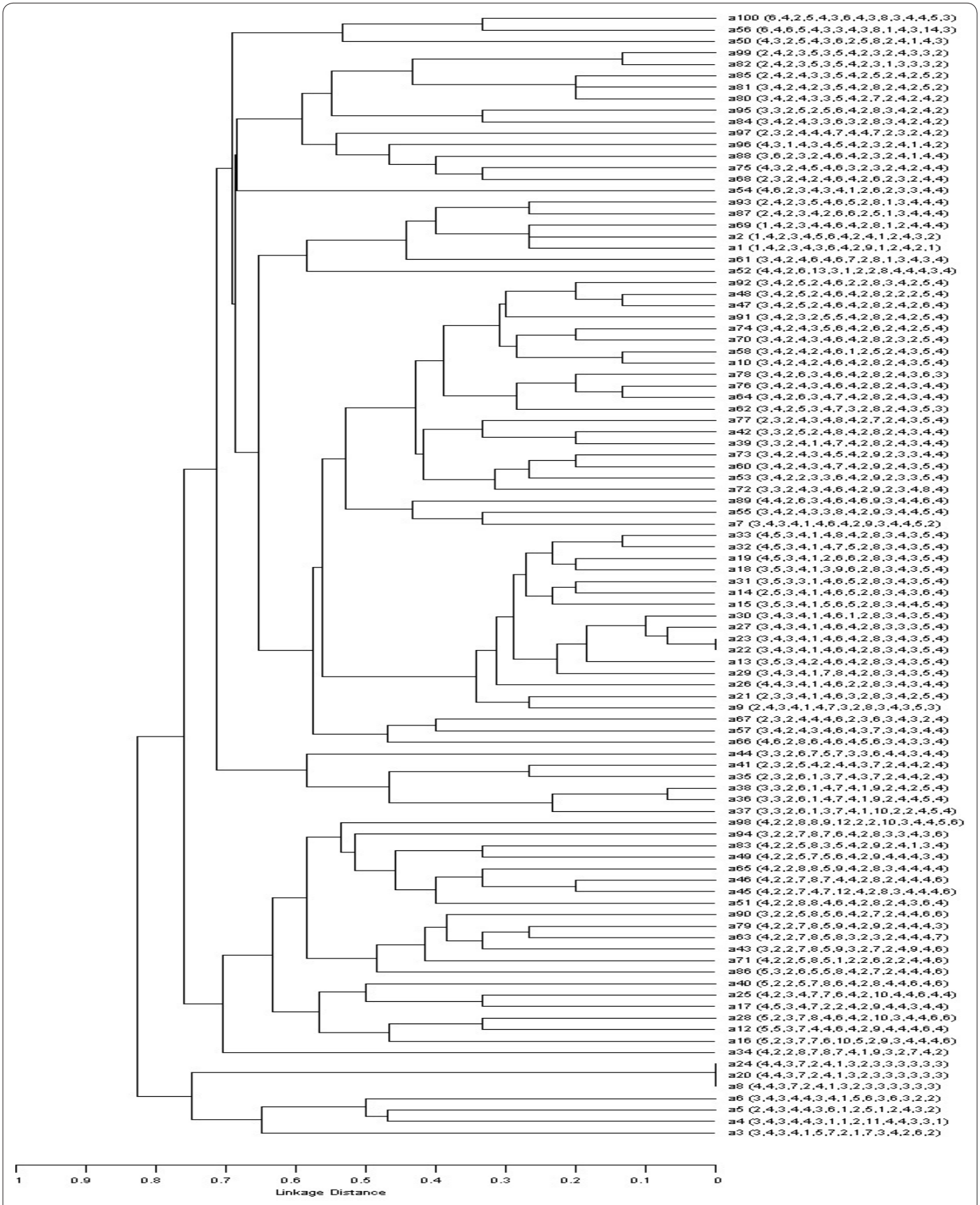

Figure 1. Genetic relationships dendrogram showing clustering among the genotyped MTB strains. five (5.1%) of 98 patients fell into 2 clusters that belonged to patients from Tehran province only.

TB transmission in a community (11).

A strong method for perusing the molecular epidemiology of tuberculosis (TB) is DNA fingerprinting of *M. tuberculosis* (20). Molecular genotyping methods should be inexpensive, highly discriminative, deliver rapid results, be direct forward to done, and generate easily expository (21). MIRU-VNTR method by using well-selected loci can be beneficial in discriminating the clinical *M. tuberculosis* isolates in different provinces where the MTB is predominant (22).

Sufficient treatment, fast diagnosis and contact detection to prevent further transmission are important factors in the control of tuberculosis. Molecular genotyping can be pragmatic at the population level, and the

Table 3. Allelic diversity and frequencies in 4 provinces for each locus (T= Tehran, S=Sistan-Bluchestan, K=Kermanshah, H=Hormozgan).

Allele	*MTUB30*				*MTUB39*				*MTUB04*				*MTUB21*				*QUB4156*				*MIRU04*				*ETR E*			
	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H
1	1	-	-	8	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-
2	12	12	13	6	1	1	2	4	5	7	2	3	2	-	2	1	4	3	2	6	22	15	17	13	1	-	-	-
3	31	3	3	1	6	2	2	6	27	2	7	5	7	-	1	6	8	-	2	-	26	-	-	3	3	1	-	7
4	5	-	2	1	41	12	14	6	14	5	5	8	10	6	11	7	33	7	12	7	-	-	-	-	25	6	5	8
5	-	-	-	-	-	-	-	-	-	-	-	-	23	7	3	1	-	-	-	-	-	-	-	-	7	2	6	-
6	-	-	-	-	1	-	-	-	1	-	1	-	6	1	1	1	4	5	2	-	1	-	-	-	4	1	5	-
7	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	7	3	1	1
8	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	2	1	-
9	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	**4**	**2**	**3**	**4**	**4**	**3**	**3**	**3**	**5**	**4**	**6**	**3**	**6**	**4**	**5**	**5**	**4**	**3**	**4**	**4**	**3**	**1**	**2**	**2**	**7**	**6**	**5**	**3**
HGDI	**0.54**	**0.34**	**0.46**	**0.64**	**0.29**	**0.36**	**0.39**	**0.7**	**0.61**	**0.69**	**0.78**	**0.65**	**0.71**	**0.65**	**0.61**	**0.7**	**0.51**	**0.67**	**0.54**	**0.69**	**0.52**	**0**	**0.11**	**0.32**	**0.7**	**0.8**	**0.77**	**0.59**

Allele	*MTUB30*				*MTUB39*				*MTUB04*				*MTUB21*				*QUB4156*				*MIRU04*				*ETR E*			
	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H	T	S	K	H
1	1	-	-	8	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-	-
2	12	12	13	6	1	1	2	4	5	7	2	3	2	-	2	1	4	3	2	6	22	15	17	13	1	-	-	-
3	31	3	3	1	6	2	2	6	27	2	7	5	7	-	1	6	8	-	2	-	26	-	-	3	3	1	-	7
4	5	-	2	1	41	12	14	6	14	5	5	8	10	6	11	7	33	7	12	7	-	-	-	-	25	6	5	8
5	-	-	-	-	-	-	-	-	-	-	-	-	23	7	3	1	-	-	-	-	-	-	-	-	7	2	6	-
6	-	-	-	-	1	-	-	-	1	-	1	-	6	1	1	1	4	5	2	-	1	-	-	-	4	1	5	-
7	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	7	3	1	1
8	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	2	2	1	-
9	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	**4**	**2**	**3**	**4**	**4**	**3**	**3**	**3**	**5**	**4**	**6**	**3**	**6**	**4**	**5**	**5**	**4**	**3**	**4**	**4**	**3**	**1**	**2**	**2**	**7**	**6**	**5**	**3**
HGDI	**0.54**	**0.34**	**0.46**	**0.64**	**0.29**	**0.36**	**0.39**	**0.7**	**0.61**	**0.69**	**0.78**	**0.65**	**0.71**	**0.65**	**0.61**	**0.7**	**0.51**	**0.67**	**0.54**	**0.69**	**0.52**	**0**	**0.11**	**0.32**	**0.7**	**0.8**	**0.77**	**0.59**

acquired clustering of isolates enables clues about the patterns and dynamics of transmission in the population (19). Therefore, tracking the molecular characteristics of MTB is beneficial for controlling and realization the spread of strains in the population with a high incidence of tuberculosis (23).

One of the most promising methods is MIRU-VNTR genotyping, a PCR-based method that used to genotype *M. tuberculosis*. It is very faster, requires a smaller amount of DNA, and has nearly the same discriminatory power as the standard RFLP IS*6110* method (10, 24).

MIRU-VNTR genotyping method is done by amplifying a panel of 12, 15, or 24 loci (5). Standardized 15-locus MIRU-VNTR profiling of MTB isolates has been recommended for progressing discriminatory power on the basis of clonal stability and evolutionary rates. Different combinations of MIRU and other VNTR loci have been proposed to supplement the standard MIRU-15 scheme to attain higher discrimination (4).

Results collected from such studies clearly disclosed that due to the strong phylogeographic structure exhibited by MTBC, the most relevant MIRU-VNTR typing schemes will presumably differ depending on the specific geographical setting (4). For example, Shamputa et al. (25) successfully defined a reduced set of eight loci from standard MIRU-24, which could be used to discriminate, specimens from the Republic of Korea (4). Similarly, Murase et al. (26), Dong et al. (17) and Zhou et al. (27) successfully identified a minimal set of 12 loci for genotyping Beijing strains that made up more than 90% of the isolates investigated from Asia (4).

In the present study, we evaluated such an approach for genotyping MTBC strains from Iran on the basis of MIRU 15 as proposed by Supply et al. (15). As a result, during 2014–2015, we examined 98 smear-positive TB patients for genotyping, analysis of these data identified important epidemiologic aspects of TB in four provinces of Iran (16).

This typing method identified five (5.1%) of 98 patients in two clusters that these two clusters belonged to Tehran only. Cluster sizes were small, ranging from two to three isolates that the isolates with the same MIRU pattern possibly have the common epidemic origin (18). Conversely, isolates from Sistan-Baluchestan, Kermanshah and Hormozgan were not grouped together in any cluster (1). 93 (94.89%) isolates had a unique pattern, which was significantly higher than those from previous studies in Iran (17, 18). Whether the isolates belong to endogenous or exogenous infection we have to do further investigation (18).

The Hunter- Gaston discriminatory index (HGDI) was 0.99, that is higher than previous studies for MIRU-VNTR typing in Iran (9). Based on the discriminatory index, 10 MIRU loci were determined as highly discriminative ($h \geq 0.6$) in our sample (MIRU10, MIRU16, MIRU26, ETRA, ETRC, ETRE, QUB26, MTUB30, MTUB04 and MTUB21), five were determined as moderately discriminative ($0.3 \geq h \geq 0.6$; MIRU04, MIRU40, QUB11b, MTUB39 and QUB4156), and none of MIRU loci not found to be poorly discriminative ($h \leq 0.3$).

The results demonstrated that in Tehran, Sistan-Bluchestan, Kermanshah and Hormozgan MIRU10, MIRU26, MIRU10 and QUB26 had the highest discriminatory power respectively. Furthermore, in all of the samples, discriminatory index of 6 MIRU loci (MIRU10, MIRU16, MIRU26, QUB26, MTUB04 and MTUB21) were highly discriminative, these results suggest that these 5 MIRU loci had the most discriminative loci in the samples of all provinces (Table 3).

In this study MIRU loci were designated as highly discriminative in our isolates was similar with another study (1, 9) and one of pattern is equal with pattern of other study (1) and these similarities indicate that this pattern circulation in Iran.

The high genetic diversity of MTB strains and unique patterns indicate that the distribution of samples in each province and population differentiate is high which is likely due to the reactivation of latent MTB infection and the migration of people within the country as well as the migration of people from neighboring countries with high incidence of TB.

Tehran is the most population of city in Iran and has the largest industrial and medical centers in the country that is why human migration to the city from other provinces is very high until the present. Therefore, population differentiate in this province is high and these two clusters belonged to Tehran. Clusters indicated that these patterns are rotating in Tehran and may be transferred to other provinces because of immigration.

In this study MIRU-VNTR typing disclosed high genetic diversity in four provinces of Iran. The results indicated that with a satisfactory discrimination power exhibited, the 15 loci based MIRU typing could be a valuable tool for epidemiological studies in MTB isolates from Iran.

Recent developments in molecular microbiology have licensed rapid reconnaissance, the expansion of molecular methods for the genetic analysis and exploring particular strains of MTB spreading through the population, which consequently can supply better insights on the epidemiology of TB in Iran (28). MIRU typing based on a capillary electrophoresis system displays convenience, automation, a brief rotation time, great throughput and complete reproducibility, and may be the procedure of election for M. tuberculosis strain typing in this country (20).

Establishment of MIRU digital profiles at the nearest future is essential for the control of tuberculosis in Iran, especially in the regions of high occurrence of TB like Sistan-Baluchestan and Kermanshah (18).

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Interest conflict

The authors declare no conflict of interest related to this work.

Author's contribution

M.B. performed the experiments, collected of specimens, assembled and collected the data and wrote the manuscript. S.Z. helped to analyze the data and manus-

cript evaluation. H.Z., S.J. and G.R.H. authors contributed to the work presented in this paper and prepared specimens in their laboratory. M.M.F. developed the concept, designed the experiments, supervised its analysis and edited the manuscript.

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