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Original Article

# Genetic diversity of multidrug-resistant *Mycobacterium tuberculosis* strains isolated from tuberculosis patients in Iran using MIRU-VNTR technique

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**Abstract** Tuberculosis (TB) is considered as one of the most important infectious diseases in the world, and recent rise and spread of multidrug-resistant (MDR) *Mycobacterium tuberculosis* (MTB) strains, have made the matter worsened. Due to the importance of TB prevalence in Iran, this study was designed to investigate the genetic diversity among MDR strains of MTB by MIRU-VNTR typing scheme. A total of 88 drug resistant *M. tuberculosis* isolates belong to pulmonary TB cases were collected from several TB reference centers of Iran. Drug susceptibility testing for Isoniazid and Rifampin was performed using the agar proportion method and MDR isolates were underwent genotyping by using 12-locus- based MIRU-VNTR typing. On performing proportion method, 22 isolates were identified as MDR. By typing of MDR isolates using 12-loci MIRU-VNTR technique, high diversity were demonstrated in MDR strains and these were classified into 20 distinct MIRU-VNTR genotypes. MIRU loci 10 and 26 were the most discriminatory loci with 8 and 7 alleles respectively; while MIRU loci 2, 20, 24 and 39 were found to be the least discriminatory with 1–2 alleles each. We noticed a mixed infection in isolate

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53, as this isolate comprised simultaneous two alleles in MIRU loci 40, 10, 16 and 39. In conclusion, this result represents MIRU-VNTR typing as a useful tool for studying genetic diversity of MDR-MTB in regional settings, and will help the health sectors to construct a preventive program for MDR-TB. Additionally, it can detect mixed infection which can facilitate management of treatment.



## Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is the major cause of morbidity and mortality worldwide especially in developing countries [1]. Recently, the World Health Organization (WHO) announced, on the Global Tuberculosis Report [2], an estimated 9 million new active cases and 1.5 million TB deaths annually, and has raised concern about the emergence of different forms of drug resistance and the ever-increasing global drug resistant TB epidemic. In fact, despite the existence of an efficient anti-TB treatment, the emergence of multidrug-resistant TB (resistant to at least isoniazid and rifampicin) [MDR-TB] and extensively drug-resistant TB (XDR-TB) with additional resistance to a fluoroquinolone and one of the injectable antibiotics (kanamycin, amikacin or capreomycin), has become a great threat to TB control [3,4]. According to WHO report [5], more than 80 countries have reported cases of XDR-TB.

In Iran, TB has become a public health problem due to comprising vast borders with neighboring countries with high burden of tuberculosis such as Azerbaijan, Armenia, Afghanistan, and Pakistan. According to the national survey, the TB incidence in Iran is significantly lower from neighboring countries (22 cases per 100,000 populations). The incidence of MDR TB in recent years as a consequence of illegal migration from these countries has made the matter worsen. This incidence is 0.8% for TB new cases and 12% for re-treatment cases [5,6]. These alarming figures emphasize the need for improved epidemiological understanding, including better descriptions of the molecular epidemiology and transmission dynamics of MDR-TB as a health priority in Iran.

Molecular typing of MTBC is a powerful adjunct to TB control to monitor the disease transmission, and to detect or confirm outbreaks [7]. In last decades, several genotyping methods have been used for studying of molecular epidemiology of MTB. Among them, *IS6110*-restriction fragment length polymorphism (RFLP) DNA fingerprinting has been the genotyping technique used most widely initially considered a gold-standard. However, despite providing high discriminatory power, this method is limited by its requirement for large quantities of high-quality DNA and complicated procedures [8], Recently, several PCR-based genotyping methods have been developed to compensate for the limitations of RFLP, including spoligotyping and mycobacterial interspersed repetitive units (MIRU), which occur in variable number tandem repeats (VNTR) consisting of multiple loci scattered throughout the genome [9]. MIRU-VNTR has been considered as a good alternative method to RFLP [10]. The discriminatory power of this technique is similar to *IS6110*-RFLP for high copy-number strains, and also is more discriminatory for low *IS6110*-copy number isolates [8]. Although MIRU-VNTR was founded by Supply et al. [11], based on the variability found in 12 specific loci interspersed throughout the mycobacterial genome, However, currently this typing method approaches employing 15 or 24 loci [12,13]. Studies in western European countries show the high resolution power of this technique for the study of TB transmission [14]. The technique is also useful for studying the diversity and clonal expansion of particular strain or lineages [15].

We have previously employed MIRU-VNTR for investigation of genotypic diversity among MTB isolates in the region of present study successfully [16]. However, since we are witnessing the current emergence of drug resistance in our country, the present study was aimed to perform a detailed molecular epidemiological assessment of MDR strains of MTB to evaluate the genotypic variation among the resistant isolates by using MIRU-VNTR.

## Methods

### Sample collection and processing

This study included a total of 88 *M. tuberculosis* drug resistant isolates corresponding to 88 patients with pulmonary tuberculosis. The isolates were collected from several regional TB reference centers in Iran, over a period of one year started from October 2013. The preliminary proposal of the work was approved by the Research Ethics Committee of the Ahvaz Jundishapur University of Medical Sciences as sponsor of the study. The strains were identified as MTB complex by routine mycobacterial processing procedure including acid fast stain microscopy, culture and identification biochemical tests (Niacin, Nitrate reductase, and catalase at 37 °C and 68 °C). Initial drug susceptibility testing for the collected isolates was performed in regional laboratories.

### Drug susceptibility testing (DST)

Since multidrug-resistant (MDR)-MTB was defined as combined resistance to isoniazid (INH), and rifampin (RIF) [17], to identify the MDR among collected drug resistant isolates, DST was performed using the 1% proportion method, at the concentrations of 0.2 mg/ml for INH, and 40 mg/ml for RIF,