

Introducing the Best Six Loci in Mycobacterial Interspersed Repetitive Unit-Variable-Number Tandem Repeat (MIRU-VNTR) Typing for *Mycobacterium Tuberculosis* Genotyping

Mahdis Ghavidel^{1, 2}, Keyvan Tadayon³, Nader Mosavari⁴, Kimiya Nourian⁵,
Hamid Reza Bahrami Taghanaki⁶, Gholam Reza Mohammadi⁷,
Mohammad Rashtibaf⁸, Kiarash Ghazvini^{*1, 2}

Abstract

Background: Tuberculosis (TB) still remains endemic worldwide making epidemiological studies essential to mitigating efforts implicated in identifying its source, controlling, and preventing the spread of dangerous strains amongst humans such as *Mycobacterium tuberculosis* (*Mtb*).

Methods: In this study, we sought to determine the 6 Mycobacterial Interspersed Repetitive Unit-Variable-Number Tandem Repeat (MIRU-VNTR) loci with high discriminatory powers for *Mtb* genotyping as well as the loci with the highest and the lowest discriminatory powers for MIRU-VNTR. To conduct our search, we used several databases such as science direct, Embase (Elsevier), Web of Science, Scopus and Medline via PubMed. Searches were performed using key words including: *Mycobacterium tuberculosis*, MIRU-VNTR, Allele diversity, Genetic diversity and human patient. Finally, 56 articles were selected after filtering out titles, abstracts and full texts.

Results: Loci with high discriminatory powers included MIRU10 and MIRU26, while MIRU2, MIRU20, MIRU24 and ETRD had poor discriminatory powers. According to previous data in the literature, the loci MIRU10, MIRU26, MIRU40, QUB 26, QUB 11b and Mtub21 have high discriminatory powers.

Conclusions: Therefore, these loci recommended for genotyping *Mtb* to save time and cost and to ensure the production of reliable results.

Keywords: Discriminatory power, Genotyping, MIRU-VNTR, *Mycobacterium tuberculosis*.

Introduction

In spite of recent efforts to control and eliminate TB, this highly infectious disease still remains the second leading cause of death worldwide (1, 2). In 2018, WHO predicted that 10 million patients (ranging

from 9 to 11.1 million) were stricken by TB. *Mtb* is a member of the TB complex which causes TB and can be transmitted via aerosolization of bodily fluids from coughing, sneezing or speaking (2).

1: Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

2: Department of Microbiology and Virology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

3: Department of Microbiology, Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

4: PPD Tuberculin Department, Razi Vaccine and Serum Research Institute, (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

5: Doctor of Veterinary Medicine, Graduate Student of School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

6: School of Persian Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

7: Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad – Iran.

8: Deputy of Veterinary administration of Khorasan Razavi Province, Mashhad, Iran.

*Corresponding author: Ghazvini Kiarash; Tel: +98 51 38012589, Fax: +98 51 38409612; E-mail: Ghazvinik@mums.ac.ir.

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To effectively control TB, preventive policies based on transmission routes are required. Molecular epidemiology of *Mtb* involves monitoring special strains like multi-drug-resistant (MDR) *Mtb* during periods of increasing prevalence, inspecting regions of latest and potential outbreaks, locating the source and route of transmission and transmission gene sequence, discovering hidden strains and tracking circulating immigrant strains (3).

Various molecular methods are used in epidemiological studies about *Mtb* strains. Each method has a particular specificity and sensitivity. Some of these approaches include IS6110, IS6110-restriction fragment length polymorphism (RFLP), MIRU-VNTR, Spoligotyping, whole genome sequencing (WGS), Random Amplification of Polymorphic DNA PCR (RAPD_PCR), Repetitive element sequence-based PCR (rep-PCR), Pulsed-field gel electrophoresis (PFGE), Next Generation Sequencing (NGS) and finally, a combination of two or more techniques listed above will be applied. Among current typing methods, a test has to be chosen according to its feasibility, cost-benefit and discriminatory powers (4-7)

In a survey comparing PFGE, 24 locus MIRU-VNTR and IS6110-RFLP, it was revealed that the 24 locus MIRU-VNTR method was the preferred method due to a high power of discrimination and time management during epidemiological investigations (7).

This study was performed to review published applications of the Mycobacterial Interspersed Repetitive Unit-Variable-Number Tandem Repeat (MIRU-VNTR) method in *Mtb* genotyping and to introduce the best 6 loci for MIRU-VNTR in typing *Mtb* isolated from human patients along with determining the loci with highest and lowest discriminatory powers for MIRU- VNTR.

Materials and methods

To identify the best loci of the 6 loci in MIRU-VNTR method for *Mtb* genotyping, the literature search was performed using several databases including science direct, Embase (Elsevier), Web of Science, Scopus, ISC and Medline via PubMed. Chosen keywords were: *Mtb*, MIRU-VNTR, Allele diversity, genetic diversity and human patient. Inclusion and exclusion criteria were determined by the following:

1. Isolation of *Mtb* from human patients.
2. Investigating the genetic diversity among *Mtb* just based on MIRU-VNTR.
3. Excluding the studies where lineage determination was based on MIRU-VNTR where Allele diversity “*h*” was not measured for each locus.
- 4- Excluding the studies in which MIRU-VNTR ability in cluster analysis and Hunter-Gaston discriminatory index (HGDI) for this method was investigated and *h* was not estimated for each of their locus.

Several articles were excluded from our study since allele diversity (*h*) was not mentioned for each locus. Screening the articles was done in 3 steps: 1. Title screening, 2. Abstract evaluation, 3. Full text evaluation based on these criteria.

Results

A total number of 228 articles were found collectively amongst the databases. As the title screening was performed, 90 articles were removed. Abstract screening resulted in 82 more studies to be omitted during the search. Finally, after full text screening, 56 articles remained. In the remaining articles, genotyping for *Mtb* using MIRU-VNTR was investigated from 2002 to 2019. Allele diversity (*h*) was evaluated amongst the 56 articles for each locus separately (Table 1).

Mycobacterium tuberculosis genotyping was performed on 56 studies using the MIRU-VNTR technique; 39 of which were conducted in Asia, seven in America, six in Africa, three in Europe and one in a different country. The location and number of studies are shown in Figure 1. Each individual study employed a different number of loci. The results revealed that MIRU10 and MIRU26 had the highest discriminatory powers while MIRU2, MIRU20, MIRU24 and ETRD had the lowest discriminatory powers, respectively.

Table 2 shows both the number of studies in which MIRU10, MIRU26, QUB26, MIRU40, QUB11b and Mtub21 was reported to be the loci with the highest discriminatory powers ($h > 0.6$), including the range of *h* for the remaining loci.

MIRU2 and MIRU20 (each in 21 studies), MIRU24 (17 studies) and ETRD (13 studies) were suggested as the loci with the lowest discriminatory powers ($h < 0.3$).

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Table 1. Fifty-six studies after full-text evaluation that MIRU-VNTR were used.

| No. | Authors reference | year | Geographical region | Continent | Locus of MIRU-VNTR | Numbers of locus | High power | Low power |
|-----|-----------------------------|------|---|-----------|--|------------------|------------------------|-----------------------|
| .1 | Cowan et al (8) | 2002 | United States (Michigan) | America | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU40 | MIRU2,20 |
| .2 | Sola et al (9) | 2003 | Different regions such as; USA, Thailand, Sicily, Guadeloupe and Russia | | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 | 15 | ETRA, MIRU40,26,10 | MIRU2,20,27 |
| .3 | Sun et al (10) | 2004 | Singapore | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU26,10, ETRE | MIRU2,20 |
| .4 | Kremer et al (11) | 2005 | China | Asia | ETR A-E MIRU10,16,26,39,40 QUB 11a,11b,26,1895 | 14 | QUB 11b,11a MIRU10 | MIRU16 ETRC |
| .5 | Kovalev et al (12) | 2005 | Russian Federation | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU26, ETRE | MIRU24,27 |
| .6 | Asgarzade et al (13) | 2007 | Iran | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU26,40 | MIRU16,39 |
| .7 | Çavuşoğlu et al (14) | 2007 | Turkey | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU16,40,26 | MIRU24,27 |
| .8 | Maes et al (15) | 2008 | Venezuela | America | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | MIRU40,26, ETRB | MIRU20,2,24 |
| .9 | Alonso-Rodríguez et al (16) | 2008 | Spain | Europe | ETR A-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,4156 | 15 | QUB,26,11b, MIRU40,10 | ETRD |
| .10 | Yun et al (17) | 2009 | Korea | Asia | ETR A-F MIRU2,10,16,20,23,24,26,27,39,40 | 6 | MIRU26, ETR E F | MIRU24,20 ETRD |
| .11 | Stavrum et al (18) | 2009 | South Africa | Africa | ETR A-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,4156 | 15 | QUB 11b | ETRD |
| .12 | Shamputa et al (19) | 2010 | South Korea | Asia | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | QUB 11b,26, Mtub4 | MIRU2,24,23 ETRB,D |
| .13 | Noguti et al (20) | 2010 | Brazil | America | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU40,23,10 | MIRU24,39 ETRD |
| .14 | Jafarian et al (21) | 2010 | Iran | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU26,10,16 | MIRU2,24 ETRD |
| .15 | Zhang et al (22) | 2011 | Cambodia | Asia | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | ETRD, Mtub 39, QUB26 | Mtub 34, MIRU2,20 |
| .16 | Asgarad et al (23) | 2011 | Iran | Asia | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 | 15 | MIRU10,26,40 | MIRU39,24 |
| .17 | Bidovec-Stojkovi et al (24) | 2011 | Slovenia | Europe | ETR A-E MIRU2,10,16,20,23,24,26,27,39,40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | QUB 26, 11b, MIRU40,10 | MIRU24,39 |
| .18 | Cerezo et al (25) | 2012 | Colombia | America | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU10,40 | MIRU24,39 |
| .19 | Chatterjee et al (26) | 2013 | India | Asia | ETR D E MIRU2,10,16,20,23,24,26,27,39,40 | 12 | MIRU26,10 | MIRU2,20 |

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|-----|-------------------------|------|-----------------------------|---------|---|----|--|---|
| .20 | Zamani et al (27) | 2013 | Iran | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,41,56 | 15 | MIRU16 ETRA | MIRU26, Mtub 21, 30, 39 QUB41,56 |
| .21 | Joseph et al (28) | 2013 | India | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,41,56 | 15 | ETRA,B MIRU40 | MIRU2,2 0 |
| .22 | Yasmin et al (29) | 2014 | Pakistan | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | MIRU26 QUB26 MIRU10 | MIRU2,2 0,27,24 |
| .23 | Ali et al (30) | 2014 | Pakistan | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,41,56 | 15 | QUB26 MIRU10,26 | ETRD |
| .24 | Chaoui et al (31) | 2014 | Morocco | Africa | ETRA,D,E MIRU2,10,16,20,23,24,26,27,39 40 | 12 | MIRU40,23,10 | MIRU24, 39 |
| .25 | Zheng et al (32) | 2014 | China | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB11b,26 Mtub 21 MIRU26 | MIRU24 Mtub 34 |
| .26 | Vasconcellos et al (33) | 2014 | Brazil | America | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB 41,56, 11b MIRU10 | MIRU24, 39 |
| .27 | Rindi et al (34) | 2014 | Italy | Europe | ETRA,D,E MIRU10,16,26,40 Mtub21 QUB 11b, 26 VNTR 42,43,47,52,53 | 15 | QUB 26 MIRU 40 QUB 11b | MIRU 04 ETRE |
| .28 | Boukdata et al (35) | 2015 | Morocco | Africa | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB 26, MIRU40,26 | MIRU20, 27 |
| .29 | Devi et al (36) | 2015 | India | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | Qub26, 11b MIRU10 QUB 41,56 MIRU26 Mtub 21 | MIRU2,2 0,27 |
| .30 | Zamani et al (37) | 2016 | Iran | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,41,56 | 15 | MIRU 40 | MIRU 10 QUB 41,56 |
| .31 | Hoza et al (38) | 2016 | Tanzania | Africa | ETRA,C-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,39 QUB 11b,26,41,56 | 22 | MIRU26,10,16 ETRAE QUB 26,41,56 | MIRU27, 2,20 |
| .32 | Cheng et al (39) | 2016 | China | Asia | ETRA-E MIRU10,16,20,26,27,39,40 Mtub31 QUB 11a,11b | 15 | QUB11a | ETRB,C |
| .33 | Bhembe et al (40) | 2017 | South Africa (Eastern Cape) | Africa | ETRA,D,E MIRU2,10,16,20,23,24,26,27,39 40 | 12 | ETRE, MIRU27,24 | MIRU40 |
| .34 | Zhang et al (41) | 2017 | China | Asia | ETRA-E MIRU 10,16,23,26,27,39,40 Mtub21,30,39 | 15 | Mtub 21, MIRU26,10 | ETRC,B |
| .35 | Liu et al (42) | 2017 | China | Asia | ETRA-E MIRU10,16,23,26,27,39,40 Mtub21,30,39 | 15 | MIRU26 Mtub 21 | MIRU27, 23 ETRD |
| .36 | Pasechnik et al (43) | 2017 | West Siberia | Asia | ETRA-D MIRU2,10,16,20,23,24,26,27,39 40 | 15 | MIRU26 | MIRU24 ETRB |

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|----|---------------------------|------|--|---------|---|----|--|-------------------------------|
| 37 | Pan et al (44) | 2017 | China | Asia | ETRA,DE MIRU10,16,26,39,40 Mtub4,21,24,30,39 QUB11a,11b,18,26,32,32,1895,4 156 VNTR3820,4120 | 22 | VNTR3820 QUB3232 | QUB4156 Mtub24 |
| 38 | Khosravi et al (45) | 2017 | Iran | Asia | ETR,DE MIRU2,10,16,20,23,24,26,27,39 40 | 12 | MIRU 10, 26 | MIRU2,2 0 |
| 39 | Ravansalar et al (46) | 2017 | Iran | Asia | ETRA-F MIRU10,16,26,39,40 QUB 11b | 12 | MIRU10, 26 ETRF | ETRD |
| 40 | Shah et al (47) | 2017 | Nepal | Asia | ETRA-F MIRU2,10,16,20,23,24,26,27,39 40 Mtub21,30,39 QUB 11b,11b,26,4156 | 24 | QUB 26 MIRU10 | ETRB,C |
| 41 | Rasoaha et al (48) | 2017 | Madagascar | Africa | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,4156 | 15 | MIRU26 QUB 11b Mtub21 QUB 26 | ETRD,C Mtub4 |
| 42 | Chen et al (49) | 2017 | Asian countries (Cambodia, Singapore and Taiwan) | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | Mtub21 QUB 11b | MIRU2 QUB 4156 |
| 43 | Li et al (50) | 2018 | China | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | Mtub4 MIRU40,10 | Mtub21 MIRU27 |
| 44 | Xu et al (51) | 2018 | China | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB 11b,26,4156 | 15 | QUB 11b Mtub21 MIRU26 | ETRC MIRU16 QUB 4156 |
| 45 | Esteves et al (52) | 2018 | Brazil | America | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 QUB11,26 VNTR42,1955,47,52,53,49 | 24 | QUB26 QUB11 VNTR42 | MIRU39, 24 ETRD |
| 46 | Augusto et al (6) | 2018 | Brazil | America | ETR,DE MIRU2,10,16,20,23,24,26,27,39 40 | 12 | MIRU16, 10, 26 | MIRU20, 24 |
| 47 | Riyahi Zaniani et al (53) | 2018 | Iran | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | MIRU 10 QUB 26 Mtub4,34 | ETRD, MIRU20, Mtub 29 |
| 48 | Azimi et al (54) | 2018 | Iran | Asia | ETRA,C-E MIRU10,16,26,40 Mtub4,21,30,39 QUB11b,26,4156 | 15 | MIRU26, 10 Mtub21 QUB 26 | ETR,DE |
| 49 | Lil et al (3) | 2018 | China | Asia | ETRA-E MIRU16,23,26,27,39,40 Mtub21,30,39 | 15 | ETRE, MIRU10, 26,39,40 Mtub21 | ETRB,C MIRU16, 23 |
| 50 | Mansoori et al (55) | 2018 | Iran | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,4156 | 24 | MIRU 10, 16, 26, | MIRU 2, 20 ETRD |
| 51 | Chawla et al (56) | 2018 | India | Asia | ETR,DE MIRU2,10,16,20,23,24,26,27,39 40 | 12 | MIRU 39,10,26 | MIRU 2,20 |
| 52 | Shi et al (57) | 2018 | China | Asia | ETRA-F MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,38,39 QUB 11b,26,4156 | 26 | QUB 11b Mtub21 MIRU26 | MIRU24, 2,20 |

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|----|------------------------|------|-----------|------|---|----|-----------------------------|---------------------------|
| 53 | Ei et al (58) | 2018 | Myanmar | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB 26 QUB 11b MIRU26 | Mtub34 MIRU20 |
| 54 | Weerasekera et al (59) | 2019 | Sri Lanka | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | Mtub 21,39 QUB 11b | MIRU20, 2,16 |
| 55 | Zarzour et al (60) | 2019 | Syria | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB 26 MIRU10,26 | MIRU24, 20, Mtub 29 |
| 56 | Li et al (61) | 2019 | China | Asia | ETRA-E MIRU2,10,16,20,23,24,26,27,39 40 Mtub4,21,29,30,34,39 QUB 11b,26,41,56 | 24 | QUB11b MIRU 26 Mtub21 | MIRU2, 20,24 |

Table 2. Six MIRU-VNTR loci for *Mycobacterium tuberculosis* based on the number of studies and the reported allele diversity (h) range for each locus.

| locus | Number of studies | h range |
|--------|-------------------|---------------|
| MIRU10 | 28 | 0.61 to 0.82 |
| MIRU26 | 32 | 0.61 to 0.81 |
| QUB26 | 18 | 0.604 to 0.89 |
| MIRU40 | 17 | 0.604 to 0.76 |
| QUB11b | 17 | 0.72 to 0.84 |
| Mtub21 | 12 | 0.64 to 0.83 |



Fig. 1. Location and numbers of studies was shown in the world from 2002 to 2019. Two studies were performed in different regions: Studies No. 2 and 42.

Discussion

An alarming 62% of emerging TB cases occurred in the South-East Asia and Western Pacific regions, followed by Africa, which accounts for 25% of new cases. Countries such as India, China, Indonesia, the Philippines, Pakistan, Bangladesh, Nigeria and South Africa hold a prevalence rate greater than 60% for TB. It is believed that the surge in TB research in Asia could be that TB is highly prevalent in this continent based on 2018 WHO report (2). Of the 56 studies extracted, 39 studies were conducted within Asia. More specifically, 11 were performed in China, ten in Iran, four in India, two in Pakistan, two in Korea and only one in Nepal, Singapore, Myanmar, Turkey, Cambodia, Sri Lanka, Syria, Russia, Siberia, Taiwan, and Cambodia. Among the 39 studies conducted in Asia, MIRU26 and MIRU10 was reported having high discriminatory power loci. Among the 7 studies conducted in the Western Hemisphere, four studies were carried out in Brazil, and one study was performed in Michigan, Venezuela and Colombia. Both MIRU40 and MIRU10 had high power discriminatory power loci in these continents.

Among the six studies performed in Africa, four studies were conducted in South Africa and Morocco, respectively. One study, performed in Madagascar and Tanzania, reported that QUB26 and MIRU26 had high discriminatory power loci in this continent. Several investigations in this study used PCR-based techniques such as spacer oligonucleotide typing (spoligotyping) and mycobacterial interspersed repetitive units–variable-number of tandem repeats (MIRU-VNTR) analyses. Although spoligotyping benefits from genetic diversity, it can minimize *Mtb* clonal diversity. This method uses one direct repeat (DR) which incorporates identical alternate and variable spacers and the results are represented as a single digit pattern. The basis of MIRU-VNTR depends on the (variable) number of tandem repeat elements called mycobacterial interspersed repetitive units (MIRU). The results are represented as a code, and offers a low discriminatory power when used alone. The MIRU-VNTR method analyzes DNA segments

which involves tandem repeat sequences and the copy number which differs amongst various strains. This method depends on PCR efficiency, specifically the quantity of repeats which is based on the size of the amplified product. The results are illustrated as characters that range from 15 –24 characters, in which each character represents the number of repeats at a single locus (3, 62).

These results are evaluated in comparison to a strain database on the web-based tool MIRU-VNTR plus. Therefore, MIRU-VNTR typing is considered the gold-standard for genotypic analysis of *Mtb* (7, 9). The discriminatory power of these methods was found to be different which was determined by the Hunter and Gaston Index factor. Several studies reported different Hunter-Gaston Index for MIRU-VNTR ranging from 0.951 to 0.999 (10, 13, 45, 47, 48, 53-56).

The allele diversity index (*h*) is used to describe the discriminatory power of MIRU-VNTR loci. If the index is greater than 0.6 ($h > 0.6$), the discriminatory power of the locus is high. If the index lies between 0.3 and 0.6 ($0.3 < h < 0.6$), the locus has medium discriminatory power, however, if the index is less than 0.3 ($0.3 > h$), the discriminatory power is considered weak. Our results suggest that among the 24 defined loci for MIRU-VNTR introduced by the MIRU-VNTR plus database, the MIRU26, MIRU10, MIRU40, QUB26, QUB11b and Mtub21 loci had the highest discriminatory powers, in contrast to the MIRU2, MIRU20, MIRU24 and ETRD loci which yielded low discriminatory powers.

Mycobacterium bovis (*M. bovis*) is the causative agent of TB in humans. When comparing the discriminatory powers of loci between *Mtb* and *M. bovis*, the loci QUB 11b and QUB 3232 have the highest discriminatory powers (for *M. bovis*), whereas ETRD (for both strains) and MIRU10 (for *M. bovis*) had the lowest discriminatory powers. Therefore, it can be concluded from our study that QUB 11b in both *Mtb* and *M. bovis* has a high discriminatory power while ETRD for both strains is considered a low power locus. However, a difference exists between the two

strains in the MIRU10 locus which has a high discriminatory power in *Mtb* but low in *M. bovis* (63).

While the 24 loci MIRU-VNTR has been introduced as the best typing method for *Mtb* based on multiple comparisons between various molecular techniques, it is, therefore, highly recommended to include the following loci, QUB26, QUB11b, MIRU10, MIRU26, MIRU40 and Mtub21, provided that MIRU-VNTR is done with less than 24 loci, in order to obtain the best results when genotyping *Mtb* isolates. In this regard, data extraction becomes inexpensive and time efficient.

Among typing methods, MIRU-VNTR is considered to be one of the best. Further, the 6

loci including MIRU10, MIRU26, MIRU40, QUB 26, QUB 11b and Mtub21, all of which have high discriminatory powers, are recommended in *Mtb* genotyping to save time and cost. Indeed, epidemiological studies are critical for disease surveillance of TB as they enable us to track circulating strains on a global scale, as well as for identifying important risk factors necessary for implementing control measures in vulnerable populations worldwide.

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