



Molecular Strain Typing of *Mycobacterium tuberculosis*: A Review of Frequently Used Methods

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Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, remains one of the most serious global health problems. Molecular typing of *M. tuberculosis* has been used for various epidemiologic purposes as well as for clinical management. Currently, many techniques are available to type *M. tuberculosis*. Choosing the most appropriate technique in accordance with the existing laboratory conditions and the specific features of the geographic region is important. Insertion sequence IS6110-based restriction fragment length polymorphism (RFLP) analysis is considered the gold standard for the molecular epidemiologic investigations of tuberculosis. However, other polymerase chain reaction-based methods such as spacer oligonucleotide typing (spoligotyping), which detects 43 spacer sequence-interspersing direct repeats (DRs) in the genomic DR region; mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR), which determines the number and size of tandem repetitive DNA sequences; repetitive-sequence-based PCR (rep-PCR), which provides high-throughput genotypic fingerprinting of multiple *Mycobacterium* species; and the recently developed genome-based whole genome sequencing methods demonstrate similar discriminatory power and greater convenience. This review focuses on techniques frequently used for the molecular typing of *M. tuberculosis* and discusses their general aspects and applications.

Keywords: Tuberculosis, *Mycobacterium tuberculosis*, Molecular Strain Typing

INTRODUCTION

Tuberculosis (TB) is one of the most common global health problems and also one of the world's deadliest communicable diseases. For example, in 2014, an estimated 9.6 million persons developed tuberculosis, and 1.5 million died. More than half (58%) of the estimated affected people were from the South-East Asia and Western Pacific Regions. Another one quarter of cases was from the African region, which also had the highest rates of cases and deaths relative to population. With the emergence of multi-drug-resistant (MDR)-TB, which is defined as *Mycobacterium tuberculosis* resistant to at least isoniazid and rifampicin, TB has become an even greater threat (1).

As recent molecular typing methods based on the direct analysis of genomic polymorphisms now are widely used and highly valuable, understanding of the evolutionary history, population dynamics, and patterns of dissemination of bacteria has been greatly improved (2). Molecular typing of *M. tuberculosis* has been used for various epidemiologic purposes such as outbreak investigations in health care settings and communities, to estimate the proportion of epidemiologic links not identified by conventional contact tracing, to identify misdiagnosis resulting from laboratory cross-contamination, to determine the distinction between re-infection and re-activation, to quantify tu-

berculosis transmission between subpopulations, and to detect the relation between drug resistance and specific genotypes (3,4).

Since the early 1990s, genotyping of *M. tuberculosis* has been used in molecular epidemiology. Recent worldwide strain distribution of *M. tuberculosis* lineages demonstrated that the Beijing strain was the most prevalent in East Asia region and found as more than 50% of all strains. Similarly, the Beijing genotypes were the most prevalent in Central Asia, Northern Asia and Southeastern Asia while only small portion appeared in Southern Asia, Western Asia, Central Asia, Austral Africa, Australasia, North America, Northern Europe and South America regions (5). Genotyping not only helps us to understand the epidemiologic aspects of the disease but also is useful for the clinical management of patients by strain classification, which has made association of *M. tuberculosis* genetic markers with important clinical consequences such as drug-resistance potential, treatment failure, or high transmissibility. Currently, molecular typing is widely used by TB control programs because genotyping results have helped to monitor epidemiologic trends, evaluate program performance, and identify instances of false-positive and cross-contamination of cultures or define the transmission dynamics of drug-resistant organisms (4,6,7).

For the genetic fingerprinting of *M. tuberculosis*, the gold

standard is insertion sequence IS6110-based restriction fragment length polymorphism (RFLP) analysis (8-10). However, similar discriminatory power and greater convenience has been demonstrated with other methods such as spacer oligonucleotide typing (spoligotyping), which detects 43 spacer sequences interspersed with direct repeats (DRs) in the genomic region uniquely present in members of *M. tuberculosis* complex (11); mycobacterial interspersed repetitive units-variable number tandem repeats (MIRU-VNTR) that uses polymerase chain reaction (PCR) amplification and gel electrophoresis to determine the number and size of tandem repetitive DNA sequence in 12 independent loci in the *M. tuberculosis* genome (12,13); repetitive-sequence-based PCR (rep-PCR), which is a fast and unified method for high-throughput genotypic fingerprinting of multiple *Mycobacterium* species (14); and whole genome sequencing (WGS), a recent method with high discriminatory power (15-17).

Ideally, molecular typing methods should be reproducible and stable, have high discriminatory power and epidemiologic concordance, be adaptable, be easy to perform and interpret, and be cost-effective and time-effective. We have described the currently available typing methods for *M. tuberculosis* and the current status of strain distribution in Korea (18). In this review, we described the five techniques, including whole genome sequencing (WGS), frequently used for characterizing *M. tuberculosis* isolates and their applications.

MOLECULAR TYPING

IS6110-RFLP

Mycobacterium tuberculosis insertion sequence IS6110 was first described by Thierry et al. (19), and its value as the standard approach to genotyping of *M. tuberculosis* isolates based on RFLP analysis has been proved by many studies (8,20,21). IS6110 be-

longs to the IS3 family of mobile elements and is 1,355 bp long. The IS6110 is found only within the *M. tuberculosis* complex, and, in most other members of the complex, the sequence is present in multiple copies, although *M. bovis* normally contains only one copy. In general, the copy number of IS6110 ranges from 0 to 25, depending on the frequency of transposition, which is conditioned largely by the nature of the genomic region at which transposition occurs (22). Differences in the copy number and locations within the genome are responsible for the high degree of IS6110 polymorphism and have predisposed this sequence to be useful as a specific molecular marker for genotyping *M. tuberculosis* strains (23,24).

In brief, the methodology includes genomic DNA digestion with the *PvuII* restriction enzyme that cleaves the IS6110 sequence into fragments, which are separated by gel electrophoresis (Fig. 1C). Then they are transferred to a membrane, and Southern blot hybridization is carried out with a labeled probe complementary to part of the 3' end of the IS6110 sequence. The IS6110 DNA probe is prepared by in vitro amplification of the 245-bp fragment using PCR and labeled by appropriate material. As a result, every visualized fragment represents a single copy of IS6110 surrounded by different lengths of flanking DNA sequences (8). Because the IS6110-RFLP method has been standardized and published, recommendations have been adopted by most research groups; the fingerprints generated in different laboratories can be compared and catalogued (25,26). To analyze the large number of RFLP patterns, a sophisticated pattern-matching computer program has been developed, and RFLP images on film or membrane are scanned and digitized for computer analysis. The sizes of the bands in the image are calculated by comparison with size standards run on the gel. The computer program compares the results from a new isolate with previously analyzed isolates to determine if any matches exist (26,27). A schematic diagram of the RFLP method is shown in Fig. 1.

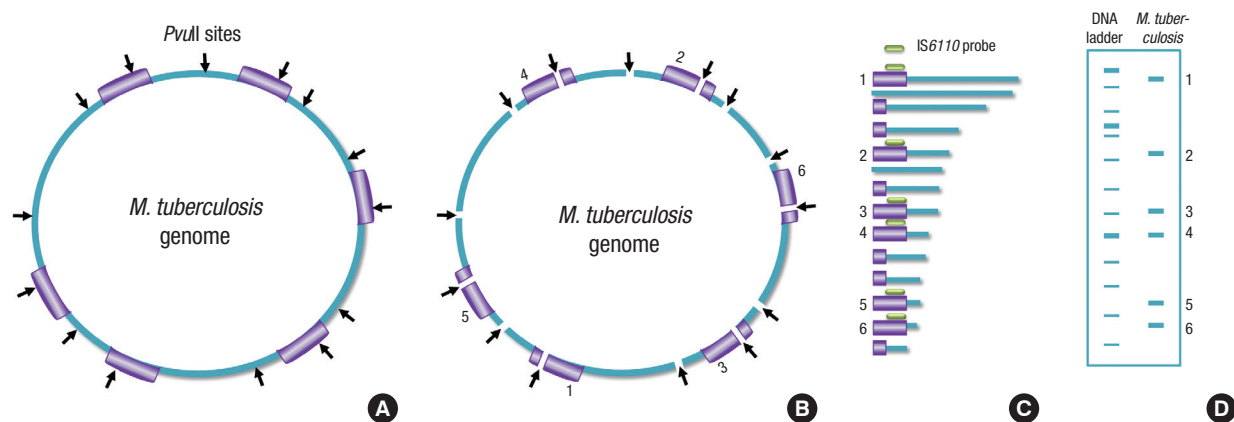


Fig. 1. Principles of RFLP. (A) *M. tuberculosis* genome with insertion segment IS6110 showing the *PvuII* cleavage sites. (B) Digestion of the whole genome with *PvuII*. (C) DNA segments of different sizes after run in the gel are transferred onto a membrane followed by hybridization. (D) Visualized fragments, which represent a single copy of IS6110 surrounded by flanking DNA of different lengths.

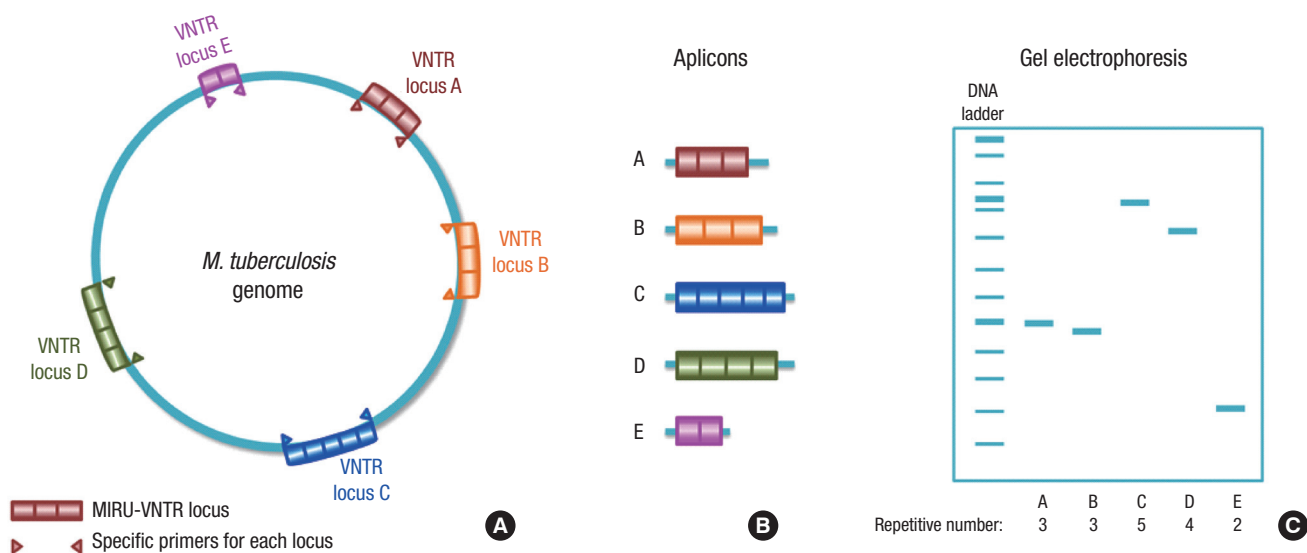


Fig. 3. Principle of MIRU-VNTR genotyping. (A) MIRU-VNTR loci of different repetitive numbers scattered in *M. tuberculosis* genome are amplified by specific primers for each locus. (B) Different sizes of amplicons after PCR. (C) Amplicons can be seen after gel electrophoresis with different sizes that reflect the repetitive number of each VNTR locus.

Each of the spacers produces either a dark band (indicating the presence of the spacer) or no band (indicating the spacer's absence). For each *M. tuberculosis* isolate, the spoligotyping assay produces a series of bands. The pattern is converted to a 43-digit binary code system that has 1 second and 0 seconds (1 means that the band is present and 0 means it is absent). Then the 43-digit binary code is converted to a 15-digit octal numbers. Each octal designation is unique and represents one specific banding pattern. From an octal designation, the binary code of the spoligotyping pattern can be re-created. A simple spreadsheet application can be used to convert binary to octal and octal to binary (4,11,37). Fig. 2 shows the principle of the spoligotyping method.

The sensitivity of spoligotyping is estimated to be 10 fg of chromosomal DNA, considerably less than what is needed for RFLP (38). The results can easily be interpreted and compared by different laboratories. An international spoligotyping database, which can be accessed online (<http://www.pasteur-guadeloupe.fr:8081>), describes 1,939 shared types (STs; i.e., spoligotype patterns shared by two or more isolates) and 3,370 orphan types (i.e., spoligotype patterns reported for only single isolates) from a total of 39,295 *M. tuberculosis* complex isolates from 122 countries, classified temporarily into 62 clades/lineages. The SITVIT database was incorporated, and it describes a total of 7,105 spoligotype patterns, corresponding to 58,180 clinical isolates, grouped into 2,740 STs representing 53,816 clinical isolates and 4,364 orphan patterns (39,40).

Many studies reported spoligotyping as a simple, cost-effective, and high-throughput method, although it has a lower discriminatory power than IS6110-RFLP typing. However, spoligotyping can differentiate *M. tuberculosis* strains with low IS6110

copy numbers (≤ 5 bands in RFLP patterns). Spoligotyping could be performed as a first-line screening test, to be followed by another typing method(s) of greater discriminatory power if necessary (30,41).

MIRU-VNTR typing

Almost all higher eukaryote genomes possess tandemly repeated sequences that are dispersed by thousands of copies (42). Their repeat numbers are highly variable in many loci and therefore are called "variable number tandem repeat" (VNTR) loci (43,44). Small repetitive DNA sequences with different unique characters were found in *M. tuberculosis* and other mycobacterial genomes by different scientists (45-50). In 1997, Supply et al. (13) identified a novel minisatellite-like structure in the *M. tuberculosis* genome composed of 40- to 100-bp repetitive sequences and named them "mycobacterial interspersed repetitive units" (MIRU). These are scattered in 41 locations throughout the genome of *M. tuberculosis* H37Rv. Among those 41 locations, 12 show polymorphisms in copy number of non-related *M. tuberculosis* isolates (51). These MIRUs are located mainly in intergenic regions and are dispersed throughout the mycobacterial chromosome. They have different characteristics from other repetitive sequences. For example, there are no obvious palindromic sequences; rather, they are direct tandem repeats. Orientation occurs in one direction relative to transcription of the adjacent gene, and they contain small open reading frames (ORFs) (11). In 2001, Supply and colleagues proved the usefulness of MIRUs in mycobacterial strain identification for epidemiologic study by developing an automated PCR method with computerized automation of the genotyping. The principle of the typing system is PCR analysis of 12 variable tandem repeat loci

with specific primers complementary to the flanking regions followed by gel electrophoresis. The size (bp) of the amplicon reflects the tandem repeat unit and is converted into numerical code to get digital format results in which each digit represents the number of copies at a particular locus (52).

Typing by comparison of these numeric codes makes the method easier to handle a large number of strains. Furthermore, the MIRU-VNTR technique is a reliable genotyping method, as it is 100% reproducible (51-54), sensitive, and specific for *M. tuberculosis* complex isolates. At the same time, a website was set up for the analysis of *M. tuberculosis* MIRU-VNTR genotypes via the Internet (54). Development of this method has been used for the real-time tracing of transmission, comparison of inter-laboratory data, and global database construction (52,55). The way for worldwide epidemiologic surveillance of tuberculosis therefore has been opened.

The MIRU-VNTRs are remarkably stable, and their evolution rate is slightly slower than that of IS6110-RFLP, so that the method is appropriate for long-term epidemiologic analyses (56). The discriminatory power of MIRU-VNTR typing based on 12 loci is slightly less than that of IS6110-RFLP analysis when *M. tuberculosis* isolates have high copy numbers of IS6110 (36,54,56) but is more discriminatory than the IS6110-RFLP if isolates have low copy numbers of IS6110 (57). Another drawback of 12 loci MIRU-VNTR is its limited power for discriminating the Beijing family strains of *M. tuberculosis* (58,59). In 2006, Supply et al. (60) selected 15 loci (including 6 previously investigated) as the new standard for epidemiologic discrimination of *M. tuberculosis* as well as 24 loci (including 12 previously investigated) as a high-resolution tool for phylogenetic studies. This newly proposed set of 15 loci of MIRU-VNTR for strain typing has been accepted as highly discriminatory in a population dominated by Beijing family strains (61) and 24 loci as equal in discriminatory power to that of IS6110-RFLP (62,63). Additional use of hypervariable loci such as VNTRs 3232, 3820, and 4120 has been recommended to be added to standardized loci set for second-line typing if more detailed genotyping is necessary to differentiate Beijing strains (61).

While doing amplification of tandem repeats, PCR failure was found in some loci, which occurred repeatedly even though the tests were performed under different PCR conditions and with different primer sets. This can be assumed to be characteristic of certain strains. It may be attributable to the presence of unexpectedly large numbers of repeat units or deletion of this region (61). In some cases, double alleles are found at a single locus in MIRU-VNTR profiles, and they are considered to be a clonal variant of the same strain. But when double alleles are found at two or more loci of the same isolate, the sample should be considered a mixed infection (64,65).

Identical MIRU-VNTR patterns are considered to be in a cluster. Using the dice coefficient and the un-weighted pair group

method with arithmetic averages (UPGMA), dendrograms can be generated. The discriminatory power of strain typing methods is calculated using the method described by Hunter and Gaston (66). Free software named MIRU-VNTR_{plus} is appropriate to analyze the multi-locus variable number tandem repeat analysis (MLVA) and spoligotyping, large sequence polymorphism, and single nucleotide polymorphism data. A weighted combination of these markers has been applicable from the Web since 2010; it is now widely used. By this Web tool, strain similarity search, generating phylogenetic trees and minimum spanning trees, and geographic information mapping all can be done (67). A schematic diagram of the MIRU-VNTR genotyping method is shown in Fig. 3.

Rep-PCR

The rep-PCR is one of the PCR amplification-based methods in which spacer fragments lying between repeat motifs of the genome are amplified using two outwardly directed primers at high stringency (68). The fragments are repetitive extragenic palindromic repetitive elements 38 bp long that consist of six degenerate positions and a 5-bp variable loop between each side of the conserved palindromic stem (69). The genetic relatedness is determined by comparing the banding pattern after gel electrophoresis of the amplicons, differences in the number of repetitive elements, and their relative position within the bacterial genome.

For convenience, a semi-automated rep-PCR commercial system (DiversiLab microbial Genotyping System; bio Merieux, Marcy-l'Etoile, France) has been developed using microfluidic chip-based DNA fragment separation that can overcome the reproducibility problem (70). Analysis can be performed with the Web-based DiversiLab software, which uses the Pearson correlation coefficient and an un-weighted pair group method with arithmetic means to perform automatic comparison of the rep-PCR-based DNA fingerprints of unknown isolates. By this system, a dendrogram, a scatter plot for sample comparison electropherogram, gel-like images, and selectable demographic fields can be generated. Like spoligotyping and MIRU-VNTR typing, rep-PCR is a fast time- and labour-saving method that can generate real-time strain typing results. Its discriminatory power is high, and it can be used for samples that are not typable by RFLP because of low copy number of the target insertion elements (14). Above all, this method has proved to discriminate strains of Beijing family members well, whereas discrimination of members of this family is unsatisfactory using the gold standard RFLP method (71). Therefore, by combination with another amplification method such as MIRU-VNTR, the rep-PCR method can be considered to replace the currently accepted gold standard method, especially in countries where Beijing family strains are prevalent. As its significant advantage is its broad applicability, it is especially useful to laboratories

that also work on non-tuberculous mycobacteria (NTM).

WGS

In recent years, WGS has been used for genotyping of *M. tuberculosis* and is especially useful to examine outbreaks by identifying transmission events where strains are genetically indistinguishable by current methods (15,72-74). WGS-based genotyping also offers an optimal resolution of *M. tuberculosis* complex isolates in molecular epidemiologic studies and can provide additional information (e.g., on drug resistance) (73,75). Bryant et al. (76) performed a randomized controlled trial of tuberculosis treatment using WGS and MIRU typing as molecular tools and reported that WGS enables the differentiation of relapse and re-infection with better resolution than MIRU-VNTR. A recent population-based study using a long-term large-scale WGS approach in a high-prevalence area provided strong evidence for differences in transmission patterns and virulence in various *M. tuberculosis* lineages (77).

One major obstacle to WGS-based genotyping is the difficulty of data standardization and integration into a readily accessible and expandable database. A number of studies were carried out to improve the standardization of WGS genotyping and the creation of Web-assessable databases for global TB surveillance (78). Bio-informative programs such as CLC Genomics Workbench (www.clcbio.com/products/clc-main-workbench/), and UniproUGENE (<http://ugene.unipro.ru/>) can be used for WGS data analysis (75). With the decreasing price of WGS, which has a higher discriminatory power and a single rapid analysis for identification, drug resistance prediction, and epidemiologic typing, the method is expected to become a new standard for routine typing of *M. tuberculosis* in the near future.

Other molecular typing methods

Apart from WGS, the methods described above belong to methods based on repetitive sequences. There are many other methods based on repetitive sequences that are less frequently used for *M. tuberculosis* typing. One of them is the polymorphic GC-rich repetitive PGRS-RFLP method which has the same principle as IS6110-RFLP with the exception of the use of a different restriction enzyme and a recombinant plasmid pTBN12 for the probe. The pTBN12 fingerprinting method has limitations similar to those of IS6110-RFLP typing, and in addition, the hybridization patterns produced by PGRS typing are more complex and difficult to interpret (79,80). Before development of WGS, the following are the methods based on nonrepetitive sequences also used for TB genotyping. During the 1990s, there were reports that randomly amplified polymorphic DNA (RAPD) analysis, which uses the PCR with arbitrarily designed primers, can provide highly pleomorphic and easily interpretable result profiles. However, its poor reproducibility limits its use for molecular typing (81,82). Pulse-field gel electrophoresis (PFGE) is of

limited use because of its requirement for a large amount of DNA and lack of a standard protocol for analysis of *M. tuberculosis* patterns (83). Amplified fragment length polymorphism (AFLP) can be used as a complement to RFLP because AFLP can differentiate between clusters identified by RFLP (84,85). The conventional radioactive AFLP method has a lower discriminatory potential than IS6110-RFLP typing, but the improved fluorescent AFLP (fAFLP) provides greater discriminatory ability, and these methods are widely used for species differentiation of NTM isolates (86,87). In 1998, Maiden et al. (88), proposed a typing scheme for pathogenic microorganisms called multilocus sequence typing (MLST) which enables electronic portability of nucleotide sequence data for the characterization of microorganisms. MLST method has proved to be valuable in investigations of MTB when seven high discrimination gene loci (*rec X*, *rps L*, *rmlC*, *rpmG1*, *mprA*, *gcvH*, *ideR*) were chosen (89) and also useful in detecting NTM in laboratory outbreak settings (90). Although single nucleotide polymorphisms (SNPs) represent the most reliable markers for lineage classification of *M. tuberculosis* complex, their use is hampered by the need to test a large set of genes to achieve satisfactory resolution (2,91).

DISCUSSION

Molecular strain-typing methods have been rapidly advancing toward computer-assisted design and increasing automation, resolution, throughput, and reproducibility. Tools of bioinformatics, such as computer-based programs for genotyping markers, genotyping design and comparison of genotyping data, development of software analysis, and phylogenetic analysis lead to convenience in retrospective studies, inter-laboratory comparison, and long-term epidemiologic surveillance. In this review, we have provided an overview of five molecular typing techniques that are used mostly for the differentiation of *M. tuberculosis* isolates. We compare the advantages and disadvantages of each method in Table 1. Although we have said that IS6110-RFLP typing is universally accepted as the gold standard for tuberculosis genotyping, one of its drawbacks is insufficient discrimination for those strains whose copy number of IS6110 is 5 or less. For those strains, additional typing method(s) such as spoligotyping or MIRU-VNTR usually is needed to complement the genotype pattern. Moreover, some NTM have multiple copies of sequences homologous to IS6110 and may hybridize with the IS6110 probe (92). Thus, PCR-based typing is more reliable than other genotyping methods.

Spoligotyping, for which the discriminatory power is less than that of IS6110-RFLP and MIRU-VNTR (93,94), nevertheless has advantages over IS6110-based genotyping, as it requires only small amounts of DNA so that it can be performed on clinical samples or liquid culture shortly after inoculation. The method is simple, the results can be obtained from a *M. tuberculosis*

Table 1. Comparison of frequently used molecular typing methods for *M. tuberculosis*

| Method | Advantages | Disadvantages |
|---------------|--|--|
| RFLP | High discriminatory power Reproducible Stable | Lower discriminatory power in isolates with ≤ 5 copies Need large amount of high-quality DNA (2-3 µg) Laborious and time consuming Requires sophisticated and expensive computer software and experienced personnel with considerable technical expertise |
| Spoligotyping | Simple Need only small amount of chromosomal DNA Cost effective and high throughput Results are easy to interpret Useful for the differentiation of low IS6110 copy-number strains Can be compared between laboratories using online database | Lower discriminatory power than RFLP Single pattern in Beijing family strains |
| MIRU-VNTR | Simple to perform, fast and labor saving Stable, and evolution rate is slightly slower than that of RFLP Need only small amount of DNA Easier to handle large number of strains by comparison of numeric codes Cost effective and 100% reproducible Real-time tracing of spreading strains A free web tool is available to compare the strains internationally | 12 loci and 15 loci MIRU VNTR has lower discriminatory power than IS6110 RFLP |
| Rep-PCR | Simple to perform, fast and labor saving Generate real-time strain-typing results Analysis can be performed with Web-based software Highly discriminatory for Beijing strains Broadly applicable to different strains of mycobacteria | Relatively less reproducible |
| WGS | High discriminatory power Precise genetic information can be obtained | Expensive Difficulty of data standardization Do not have readily accessible and expandable database |

RFLP = restriction fragment length polymorphism, MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number of tandem repeat, Rep-PCR = repetitive element palindromic PCR, WGS = whole genome sequencing.

culture within one day, and they are expressed in digital format. Because of its rapidity, this method is useful in the clinical setting to detect causative bacteria and to provide epidemiologic information on strain identities (95). In recent years, some researchers have proved that VNTR typing has a discriminatory power equal to that of IS6110-RFLP (96), and the combination of MIRU-VNTR typing and spoligotyping is accepted as an appropriate way to obtain a rapid and reliable genotyping result (97). Automated MIRU typing is now available, which is based on multiplex PCR in which one of each primer set is labeled with a different fluorescent dye. To estimate the PCR size, an automated sequencer is used to electrophorese the fluorescently labeled amplicons (54). This method is highly reproducible and fast, as the results are obtained through a computerized analysis of the signals, although it has the disadvantage of a need for a particular sequencer and a specialized software package (60).

Spread of MDR- and extensively drug resistant (XDR)-TB is endangering the world; real-time tracing of tuberculosis strains therefore is of major importance for control of these super spreaders. This aspect makes PCR-based genotyping a suitable method for tuberculosis surveillance systems and tracing current spreading routes. Moreover, WGS, a recently developed high-cost technique, is expected to become a new standard method for routine typing of *M. tuberculosis* in the near future because it provides a single rapid analysis that contains identification,

drug resistance profiles, selection of locus-specific typing markers, and rational design of genotyping strategies.

In a short term outbreak of tuberculosis, the strains might be derived from one main family like Beijing or Haarlam, and discrimination and tracing of source and the transmission route may be difficult to work out. Studies working on the differentiation of clustered strains from one method with other methods revealed different results and give us a light to choose the typing methods when a short term outbreak occur (75,98). In the study of Kremer et al. (98), 51 isolates (74% of the study samples) were found to be Beijing genotype by spoligotyping with HGDI of 0.253. However, the HGDI of MIRU-VNTR and IS6110 RFLP for those isolates were 0.982 and 0.997 respectively, and discriminating powers were much better than that of spoligotyping. In another study, a large cluster of Haarlem lineage was identified by two classical strain typing methods, IS6110 DNA fingerprinting and 24-loci MIRU-VNTR typing, and clustered strains from those typing results had undergone WGS. The analysis of WGS data showed SNP and small deletions which subdivided 53% of that 86 cluster isolates (75). A study on a 3-year period outbreak in Canada also revealed that WGS could differentiate two genetically distinct lineages of *M. tuberculosis* with identical MIRU-VNTR genotypes, and suggesting that there were two concomitant outbreaks (15). Those researches not only revealed that WGS has higher discriminatory power than classical genotyping, but also showed better correlation with

the spatial distribution of the cases, contact tracing data, and involvement of multiple source cases (15,75).

CONCLUSION

Each technique has its own advantages and disadvantages, and we have to consider these facts when we select, implement, and combine those techniques according to our specific laboratory conditions and the particular features of the geographic region. Currently, molecular typing is widely used by TB control programs because it offers a powerful tool to monitor epidemiologic trends and transmission dynamics of drug-resistant strains. It has been witnessed that simple conventional technologies moved to more advanced ones, such as molecular diagnostics, and now we are facing a brand new technology of 'WGS' even though still there are many things to be solved; normalization of the data, mining meaningful data sets from enormous amounts of data, high costs, etc. WGS will soon replace most of the typing methods that are currently being used. However, it will take quite a long time to replace the whole typing settings in the world. Meanwhile, we need to, and will, use current molecular typing methods in combination with WGS. It will be important not only for the transition phase of moving to the new technology, but also important that through such a combination, we will get deeper knowledge that relies on the genetic characters and differences of each *M. tuberculosis* strain. We support the fact that TB molecular typing may play as an essential role in monitoring of the progress toward eliminating TB transmission.

DISCLOSURE

The authors have no potential conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Prepared first manuscript and drawing figures: Ei PW. Contributed some parts of manuscript writing and revised the first manuscript: Aung WW. Revised the final manuscript: Lee JS. Finalized and revised the figures: Choi GE. Supervised the review outline and proof read the final version of the manuscript: Chang CL. Approval of final manuscript: all authors.

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