A First Insight into the Genetic Diversity of *Mycobacterium Tuberculosis* in Veracruz, Mexico

Raquel Almaraz-Velasco1, Daniela Munro-Rojas1,2, Javier Fuentes-Domínguez2, Raquel Muñiz-Salazar3,4, Maria Angélica Ibarra-Estela3, Alma Delia Guevara-Méndez2, Rosa Icela Chaparro-Martínez2, Monserrat Perez-Navarro2, Roberto Zeniteno-Cuevas1,4

1Ecology and Health Laboratory, Public Health Institute, University of Veracruz, Xalapa, 2Health Sciences Institute, University of Veracruz, Jalapa, 3State Program of Mycobacteriosis, Health Jurisdictions V and VII, Veracruz Health Services, Veracruz, 4Multidisciplinary Research Network on Tuberculosis, Epidemiology and Molecular Ecology Laboratory, School of Health Sciences, Autonomous University of Baja California, Ensenada, Baja California, 5Nefrology Services, General Hospital “Dr. Eduardo Liceaga”, Mexico

Abstract

**Objective/Background:** Tuberculosis (TB) remains one of the most important infectious diseases. Although Mexico is one of the Latin American countries with the largest contribution to these statistics, there are few reports that describe the genotypic characteristics of TB. The aim of this study was to use the MIRU-VNTR-24 loci to analyze the genetic diversity of *M. tuberculosis* circulating in the state of Veracruz, Mexico. **Methods:** Here, we analyze by MIRU-VNTR-24 loci 80 clinical isolates from individuals with confirmed TB from Veracruz México, also clinical and epidemiological variables were recovered and analyzed. **Results:** Of the individuals included in the analyses 65% were from men with an average age of 42 (± 17) years, 17% and 6% were drug and multi-drug resistant. 88% of the isolates were included in 20 clusters, of which 52% were classified into twelve orphan clusters and the remaining 37% were distributed among eight lineages: LAM (10%), EAI (9%), Haarlem (8%), H37Rv (4%), S (4%) and TUR (2%). **Conclusion:** An important diversity of lineages and unknown genotypes was identified; however, more studies are necessary in order to understand the characteristics of the genotypes displayed in the region. There is no doubt regarding the need for a molecular epidemiological surveillance system that can help to evaluate the dynamics of genotypes circulating in the country and support strategies for the prevention and management of populations affected by TB.

**Keywords:** Genotyping, Mexico, mycobacterial interspersed repetitive unit-variable number tandem repeat, *Mycobacterium*

**INTRODUCTION**

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* and other members of the *Mycobacterium* complex. According to the World Health Organization (WHO), about 9.5 million cases and 1.5 million deaths are caused by this disease every year.\(^1\)

According to the Pan American Health Organization, America as a continent contributes around 30% (280,000) of the annual TB cases. This represents a rate of 29/100,000 of the population. In Mexico, with a prevalence of 17.27 (19.703) cases/100,000 inhabitants, is placed among the three countries with the highest number of cases in Latin America. Within Mexico, the states of Baja California (BC), Guerrero, Tamaulipas, Veracruz, Sinaloa, and Nayarit are recognized as those with the highest incidences.\(^2\)

The strategy of the WHO “End TB” aims to end the epidemic character of this disease by 2030 and ending the death, disease, or suffering caused by TB. To reach this point is necessary to reinforce the attention centered on the patient, promote changes in the politics and health systems, and intensify basic, clinical and epidemiological research and innovation (http://www.who.int/tb/strategy/stop_tb_strategy/en/).

In recent years, the development of molecular tools for genotypic characterization such as mycobacterial interspersed repetitive unit (MIRU)-variable number tandem repeat (VNTR) and spoligotyping has enabled identification of the genetic diversity and population structure of *M. tuberculosis*

**Address for correspondence:** Dr. Roberto Zeniteno-Cuevas, Instituto de Salud Pública, Universidad Veracruzana, Av. Luis Castelazo Ayala s/n, A.P. 57, Col. Industrial Animas, Jalapa, Veracruz, CP 91190, México. E-mail: robzencue@gmail.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

**How to cite this article:** Almaraz-Velasco R, Munro-Rojas D, Fuentes-Domínguez J, Muñiz-Salazar R, Ibarra-Estela MA, Guevara-Méndez AD, et al. A first insight into the genetic diversity of *Mycobacterium Tuberculosis* in Veracruz, Mexico. Int J Mycobacteriol 2017;6:14-20.
in different geographical regions. Through this technology, it has been possible to observe how the various genotypes influence the epidemiology of the disease. Such has been the importance of these procedures that they have been used as support tools in TB care programs, and according to the genetic characteristics of *M. tuberculosis* observed in the region or country of study, it has been possible to develop specific epidemiological and health interventions of high impact in transmission prevention.

While Mexico is one of the countries with the largest contributions to the TB problem in Latin America, there are few reports that described its genetic behavior and even less from the southeastern states of Mexico. The state of Veracruz is located in the South-East of the country and contributes 15% (2500 individuals) of the TB cases reported annually in Mexico, as well as a significant number of aggravated forms of the disease, such as poly-, multi- (combined resistance to isoniazid and rifampicin), and extreme drug-resistant TB. However, detail regarding the genotypic behavior of TB remains unknown, and therefore, the objective of this study was to analyze the genetic diversity of *M. tuberculosis* present in the state of Veracruz using the MIRU-VNTR-24 loci technique.

**METHODS**

**Study population and clinical sample isolation**

This is a cross-sectional study realized between April 2014 and May 2015, in which 113 clinical sputum samples were taken from the same number of individuals suspected of having TB. All samples were collected by the Mycobacteriology Program of the Health Department of Veracruz. Sputum samples were decontaminated using Petroff’s modified method and primary isolation was achieved with Löwenstein–Jensen medium. Susceptibility testing for the first-line drugs streptomycin (S), isoniazid (H), rifampin (R), ethambutol (E), and pyrazinamide (Z) was performed using the fluorometric method (MGIT 960 Becton-Dickinson).

Variables such as age, gender, place of residence, type of diagnosis and treatment as well as co-occurrence of diabetes, cancer, malnutrition, anemia, coinfection by Human Immunodeficiency Virus (HIV/AIDS), consumption of tobacco, alcohol, and other drugs were also recorded. Finally, the symptoms most commonly described at the moment of diagnosis and treatment as well as co-occurrence of HIV/AIDS, consumption of tobacco, alcohol, and other drugs were also recorded. Only 4% reported smoking tobacco before the diagnosis of TB. Only 50% (40/80) and 46% reported having drunk alcohol and smoked tobacco before the diagnosis of TB.

**DNA purification and 24-loci mycobacterial interspersed repetitive unit-variable number tandem repeat analysis in clinical isolates**

Extraction of DNA was conducted with a loop of cultured mycobacteria, following van Soolingen *et al.* DNA concentration was determined by spectrophotometry in a NanoDrop 1000 (Thermo Scientific).

The 24-loci MIRU-VNTR typing was conducted following the recommendations of *Supply et al.* Each locus was amplified individually, and polymerase chain reaction fragments were separated by electrophoresis using a 2% agarose gel. Fragment size was estimated by comparison with 100 bp DNA molecular weight ladders and independently verified by two separate individuals. The number of repeats at each locus was calculated by applying the corresponding conversion table.

**Lineage assignment, cluster, and clonal complex analysis**

Genotypes were expressed as numerical codes representing the number of MIRU-VNTR for each locus. Lineages were identified individually using the similarity search module of the database MIRU-VNTRplus (http://www.miru-vntrplus.org/MIRU/index.faces). The dendrogram was generated using Jaccard’s distance matrix and neighbor-joining trees. The Hunter-Gaston discriminatory index was calculated as previously reported.

Clonal complexes (CCs) were identified using the minimum spanning tree module in the database MIRU-VNTRplus, considering 12-, 15-, and 24-MIRU-VNTR loci and maximum differences within a CC of one, two, and three loci.

**Association of epidemiological variables and genotypes**

The data from the patients included in the study were analyzed using descriptive and analytical statistics. To identify associated factors, Chi-square tests were used with Yates’s correction and Fisher’s exact test. Risks for all recovered variables were evaluated using odds ratio (OR) and relative risk, considering a value of *P* < 0.05 as significant. All calculations were performed using the software SPSS (IBM, New Yor, USA) version 12 and Epidat (WHO, Ginebra Suiza) version 3.0.

**Ethical concerns**

No physical interventions occurred with the patients. All information collected were confidential, and written consent was obtained from each individual. Ethical issues derived from this study were overseen by the respective committee of the Public Health Institute of the University of Veracruz.

**RESULTS**

**Population characteristics**

Of the 113 samples retrieved and processed, only 80 (65%) showed grow of clinical isolate and were included in the analysis. The characteristics of this population at the moment of diagnostic were: 65% (52/80) corresponded to men and the average age of the population was 42 ± 17 years. A total of 46% (36/80) stated that they were single; while 55% (44/80) presented an elementary level of education or less, all individuals claimed to be from Veracruz, with 78% (62/80) from urban areas and 31% (24/80) living in overcrowded conditions. A total of 25% were unemployed (20/80), and 50% (40/80) and 46% reported having drunk alcohol and smoked tobacco before the diagnosis of TB. Only 4% reported that they drank alcohol at the moment of diagnosis.

No individuals were found with cancer and HIV/AIDS infection. The most frequent comorbidity was type 2 diabetes mellitus, which was present in 30% (24/80) of the population,
followed by 4% (3/80) with malnutrition and anemia and 3% (2/80) with gastritis. The most frequent symptoms described were fatigue 76% (60/80), weakness 75% (60/80), cough with mucus 70% (56/80), fever 68% (54/80), night sweats 56% (44/80), and loss of appetite 51% (40/80).

All individuals were classified as a new case of TB, and 84% (67/80) were diagnosed by acid-fast bacillus testing. A total of 26% (20/80) claimed to have been in the previous contact with someone with TB before acquiring the infection, and 17% (13/80) showed resistance to at least one first-line drug while 6% (5/80) were identified as multidrug resistant (MDR).

**Molecular typing, lineage assignments, and cluster identification**

Of the 80 clinical isolates analyzed 11% (9/80) were singletons from which 9% (6/80) had not a lineage and 3% (3/80) were classified with lineages Ghana (1/80) and LAM-5 (2/80). Finally, one isolate presented the pattern of the species *Mycobacterium canetti* [Figure 1].

A total of 71 (88%) isolates were placed into two groups [Table 1]; the first consisted of 36% (29/80) of the isolates, which were included in seven lineages [Table 1 and Figure 1]. The most abundant was LAM with 11% (9/80), forming four subgroups with two isolates each, LAM-1 (strains: 121 and 146), LAM-2 (strains: 208 and 66), LAM-3 (177 and 106), and LAM-4 (239 and 240). The EAI lineage comprised 9% (7/80) of the isolates (161, 34, 140, 106, 74, 168, and 31). Haarlem comprised 8% (6/80) of the isolates, forming the two subgroups, Haarlem-1 (209, 273, and 218) and Haarlem-2 (196, 36, and 287). Two lineages included three (4%) isolates each, H37Rv (187, 87, and 108) and S (245, 28, and 92). Finally, 2% (2/80) of the isolates (275 and 110) were identified as presenting a TUR lineage [Table 1 and Figure 1].

The second group was formed by 52% of the isolates (42/80) organized into 12 clusters without a defined lineage (orphan group [OG]): OG1 was formed by 8% (6/80) of the isolates (79, 94, 96, 169, 107, and 160). OG2 comprised 7% (5/80) (strains: 157, 158, 118, 181, 33, and VNB2). OG3 comprised 5% (4/80) of the isolates (103, 125, 278, and 122). OG4 included 5% (4/80) of the isolates (69, 120, 178, and 268). In six groups with three isolates, each was found the groups: OG5 (71, 180, and 255), OG6 (68, 151, and 139), OG7 (126, 137, and 238), OG8 (149, 230, and 135), OG9 (42, 24, and 88), and GC10 (109, 115, and 111). Finally, two groups were identified that comprised 2% (2/80) of the isolates in each group: OG11 (158 and 30) and OG12 (86 and 17) [Figure 1].

The HDGI analysis displayed a global value of 0.96, which is indicative of high allelic diversity. The most diverse loci were QUB26 (0.83), QUB11b (0.83), MIRU26 (0.77), Mtub04 (0.76), MIRU10 (0.75), and MIRU40 (0.75). With medium diversity, we found MIRU23 (0.64), ETRC (0.63), ETRA (0.62), MIRU31 (0.6), Mtub39 (0.6), and MIRU2 (0.55). With low diversity, we identified ETRB (0.43), Mtub34 (0.43), MIRU20 (0.4), and MIRU 24 (0.34).

Identification of CCs, considering a difference of three loci, with the set of 24, 15, and 12 loci MIRU-VNTR, revealed one, six, and four CC, respectively [Table 2]. Considering two loci, the number of CC with the set of 24, 15, and 12 loci was 0, 4, and 5, respectively, while considering only one locus of difference, the number of clusters decreased to 0, 1, and 3, respectively [Table 2].

**Association of epidemiological factors and genotypic characteristics**

The search for association between sociodemographic and epidemiological factors with the clusters showed OR with a significant relationship. A significant difference was only found with two variables and four clusters. Being a male and belonging within the clusters, OG1 (P = 0.01), OG2 (P = 0.03), and OG9 (P = 0.03) and having type 2 diabetes mellitus and belonging to the OG10 (P = 0.02) cluster.

**Discussion**

The MIRU-VNTR 24 loci analysis shows that 88% (71/80) of the isolates were located within 20 clusters, displaying an important level of genotypic diversity circulating in the population.

---

**Table 1: Distribution of lineages of mycobacteria isolated from Veracruz, Mexico, based on classification by SITVITWEB of the 24 loci mycobacterial interspersed repetitive units-variable number tandem repeat set**

| Species         | Lineage | Frequency (n) | Prevalence in % (n=80) | Number of clusters (number isolates included) | Singletons |
|-----------------|---------|---------------|------------------------|-----------------------------------------------|------------|
| *M. tuberculosis* | LAM     | 9             | 11                     | 1 (8)                                         | 1          |
| *M. tuberculosis* | EAI     | 7             | 9                      | 1 (7)                                         | -          |
| *M. tuberculosis* | Haarlem | 6             | 8                      | 1 (6)                                         | -          |
| *M. tuberculosis* | H37     | 3             | 4                      | 1 (3)                                         | -          |
| *M. tuberculosis* | S       | 3             | 4                      | 1 (3)                                         | -          |
| *M. tuberculosis* | TUR     | 2             | 2                      | 1 (2)                                         | -          |
| *M. tuberculosis* | Ghana   | 1             | 1                      | -                                              | 1          |
| *M. canetti*     | Canetti | 1             | 1                      | -                                              | 1          |
| **Group 2**     |         |               |                        |                                               |            |
| *M. tuberculosis* | Orphans | 48            | 60                     | 12 (42)                                       | 6          |

*M. tuberculosis: Mycobacterium tuberculosis, M. canetti: Mycobacterium canetti, TUR: Turkey*
The LAM lineage was the most abundant (11%); this coincides with national and Latin American reports where it ranks as one of the most frequent.\textsuperscript{[11,14,22,23]} No association of this lineage with epidemiological or clinical features was found. Finally, four LAM subgroups were identified, which could highlight several points of dispersion of this lineage within the population.

**Figure 1:** Neighbor-joining tree showing the genetic diversity of 80 tuberculosis isolates from Veracruz, based on 24 mycobacterial interspersed repetitive unit-variable number tandem repeat loci.
EAI lineage was found in 9% of individuals. This lineage has been described in the center and southwest of the country. All isolates in this lineage formed a single major group, demonstrating the expansion of this group in the population. Despite the fact that no drug resistance was identified in these isolates, it is considered necessary to implement close monitoring of individuals carrying this strain, given its virulent character, and tendency to develop resistance.

Regarding the Haarlem lineage, this was observed at a frequency of 8% and has been described frequently in countries from Northern Europe and Central America. In Mexico, it has been reported with a frequency ranking from 2% to 15%.

The remaining lineages found (H37, S, Tur, and Ghana) have been previously reported in Mexico; however, it is important to note that the X, T, and Beijing lineages were absent in the samples analyzed, despite the fact that they are frequently referred to in other states.

Isolate number 25 showed the characteristic pattern of the species _M. canetti_ and was also identified as MDR. The individual bearing this strain was a man 56 years of age, who lives in an urban area and did not report having had previous contact with a TB-infected person. The occurrence of this species is notable since it usually does not affect humans and is considered as a zoonosis that is predominantly described in North African countries (Miltgen et al. 2002). However, it was not possible to identify the source of infection in this specific case. Further studies will be required to identify the origin of this isolate, considering its MDR character, and the fact that it is one of the first descriptions of this species in Mexico.

A total of 60% (48/80) of the isolates were without a specific lineage, possibly due to the high discriminative power of the technique of genotyping MIRU-VNTR 24 loci. The OGs were the only ones who showed a significant association with certain sociodemographic variables, and these included four of the five isolates identified as MDR-TB. There is no doubt that the application of other genotyping techniques will help improve the assignation of these isolates to specific lineages or confirm their orphan status.

The high allelic diversity observed in 25% of the loci QUB26, QUB11b, MIRU26, Mtub04, MIRU10, and MIRU40 coincides with a previous report from Mexico. This diversity could help explain the low presence of CCs, even considering 1, 2, or 3 loci differences in the three sets of MIRU-VNTR loci analyzed. While Mexico is one of the largest contributors of TB in Latin America, there are few studies exploring the genotypic characteristics of the isolates circulating in the country; this is even truer for reports related to description of the set of MIRU-VNTR-24 loci.

Cluster analysis of the genotypes described here versus 140 isolates characterized by MIRU-VNTR-24 loci from BC, Mexico, shows the occurrence of 22 isolates, 13 from Veracruz and 9 from BC, forming five groups with similarities in their genotypes [Figure 2]. The first group included one isolate with LAM-4 lineage from Veracruz and two isolates from BC. The second comprised two isolates with a LAM-3 lineage from Veracruz and three from BC. The third group included seven isolates with EAI lineage from Veracruz and two from BC. The fourth group comprised one isolate with S lineage from Veracruz and one from BC. Finally, two isolates with TUR lineage from Veracruz showed similarity to one isolate of BC [Figure 2]. This evidences the possible existence of genotypes circulating in both states, perhaps with a wide distribution across the country. However, the absence of patterns of MIRU-VNTR-24 loci from other states precludes a broader analysis to confirm the occurrence of these genotypes. This reinforces the need to implement a system of molecular epidemiological surveillance of TB in Mexico, where the information generated will help assess at greater depth and detail the behavior of the different TB genotypes circulating within the country.

The main limitation of our study was related to the small number of isolates analyzed and the inability to perform additional
genotyping techniques, such as spoligotyping. Undoubtedly, an increased number of isolates and incorporation of genotypes would generate a better description of the genotypes and lineages present as well as identifying factors that might be associated with some lineages, clusters, or CCs.

**CONCLUSION**

Notwithstanding the preliminary nature of this study, it was possible to appreciate an important diversity of genotypes circulating within the study region. Further studies related to the epidemiological and molecular characterization of TB in Mexico are required to obtain a more precise description of the behavior of this disease within the population of the country.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. WHO, Global Tuberculosis Control; 2015. Available from: http://apps.who.int/iris/bitstream/10665/191102/1/9789241565059_eng.pdf. [Last accessed on 2017 Jan 19].
2. Pan-American Health Organization, Tuberculosis in the Americas. Regional Report 2014; 2014. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=31284&lang=es. [Last accessed on 2017 Jan 19].
3. Castellanos-Joya M. Actual Situation of Tuberculosis in Mexico; 2014. Available from: http://www.cenaprece.salud.gob.mx/programas/interior/micobacteriosis/descargas/pdf/SituacionActualTbMexico.pdf. [Last accessed on 2017 Jan 19].
4. García de Viedma D, Mokrousov I, Rastogi N. Innovations in the molecular epidemiology of tuberculosis. Enferm Infecc Microbiol Clin 2011;29 Suppl 1:8-13.
5. Pedersen MK, Andersen AB, Andersen PH, Svensson E, Jensen SG, Lillebaek T. Occupational tuberculosis in Denmark through 21 years analysed by nationwide genotyping. PLoS One 2016;11:e0153668.
6. Case C, Kandola K, Chui L, Li V, Nix N, Johnson R. Examining DNA fingerprinting as an epidemiology tool in the tuberculosis program in the Northwest Territories, Canada. Int J Circumpolar Health 2013;72:20067-74.
7. López-Rocha E, Juárez-Álvarez J, Riego-Ruiz L, Enciso-Moreno L, Ortega-Aguilar F, Hernández-Nieto J, et al. Genetic diversity of the Mycobacterium tuberculosis complex in San Luis Potosí, México. BMC Res Notes 2013;6:172.
8. Molina-Torres CA, Moreno-Torres E, Ocampo-Candiani J, Rendon A, Blackwood K, Kremer K, et al. Mycobacterium tuberculosis spoligotypes in Monterrey, Mexico. J Clin Microbiol 2010;48:448-55.
9. Nava-Aguilera E, López-Vidal Y, Harris E, Morales-Pérez A, Mitchell S, Flores-Moreno M, et al. Clustering of Mycobacterium tuberculosis cases in Acapulco: Spoligotyping and risk factors. Clin Dev Immunol 2011;2011:408375.
10. Flores-Treviño S, Mendoza-Olazárra S, Garza-González E. Drug resistance and molecular epidemiology of Mycobacterium tuberculosis in Mexico: A systematic review. Salud Públ Méx 2014;56:63-77.
11. Flores-Treviño S, Morfin-Otero R, Rodríguez-Noriega E, González-Díaz E, Pérez-Gómez HR, Bocanegra-García V, et al. Genetic diversity of Mycobacterium tuberculosis from Guadalajara, México and identification of a rare multidrug resistant Beijing genotype. PLoS One 2015;10:e0118095.
12. Macías Parra M, Kumate Rodríguez J, Arredondo García JL, López-Vidal Y, Castaño-Areola M, Balandrano S, et al. Mycobacterium tuberculosis complex genotype diversity and drug resistance profiles in a pediatric population in Mexico. Tuberculosis 2011;2011:239042.
13. Zenteno-Cuevas R, Mendoza-Damián F, Muñoz IC, Enciso-Moreno L, Pérez-Navarro LM, Ramírez-Hernández MD, et al. Description of the population structure and genetic diversity of tuberculosis in Estado de México, a low prevalence setting from Mexico. APMS 2015;123:116-22.
14. Flores-López CA, Zenteno-Cuevas R, Laniado-Laborín R, Reynaud Y, García-Ortiz RA, González-Y-Merchán JA, et al. Molecular epidemiology of Mycobacterium tuberculosis in Baja California, México: A result of human migration? Infect Genet Evol 2016. pii: S1675-6433(16)00279-9.
15. Zenteno-Cuevas R, Cuevas-Cordoba B, Enciso A, Enciso L, Cuellar A. Assessing the utility of three TaqMan probes for the diagnosis of tuberculosis and resistance to rifampin and isoniazid in Veracruz, México. Can J Microbiol 2012;58:318-25.
16. Petroff SA. A New and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. J Exp Med 1915;21:38-42.
17. van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. Occurrence and stability of insertion sequences in Mycobacterium tuberculosis complex strains: Evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J Clin Microbiol 1991;29:2578-86.
18. Supply P, Allix C, Lesjean S, Cardoso-Oleemnn M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable number tandem repeat typing of Mycobacterium tuberculosis. J Clin Microbiol 2006;44:4498-510.
19. Willet D, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: A web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res 2010;38:W326-31.
20. Allix-Béguec C, Harmsen D, Weniger T, Supply P, Niemann S. Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of Mycobacterium tuberculosis complex isolates. J Clin Microbiol 2008;46:2692-9.
21. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: An application of Simpson’s index of diversity. J Clin Microbiol 1988;26:2465-6.
22. Cerezo I, Jiménez Y, Hernández J, Zozio T, Murcia MI, Rastogi N. A first insight on the population structure of Mycobacterium tuberculosis complex as studied by spoligotyping and MIRU-VNTRs in Bogotá, Colombia. Infect Genet Evol 2012;12:657-63.
23. Dantas NG, Suffys PN, Carvalho Wda S, Gomes HM, de Almeida IN, de Assis LJ, et al. Genetic diversity and molecular epidemiology of multidrug-resistant Mycobacterium tuberculosis in Minas Gerais State, Brazil. BMC Infect Dis 2015;15:306.
24. Zenteno-Cuevas R, Silva-Hernández FX, Mendoza-Damián F, Ramírez-Hernández MD, Vázquez-Medina K, Widrobo-García L, et al. Characterisation of pks15/1 in clinical isolates of Mycobacterium tuberculosis from Mexico. Mem Inst Oswaldo Cruz 2013;108:718-23.
25. Kamerbeek J, Schols L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol 1997;35:907-14.
26. Driscoll JR. Spoligotyping for molecular epidemiology of the Mycobacterium tuberculosis complex. Methods Mol Biol 2009;551:117-28.
27. Stucki D, Malla B, Hostettler S, Huna T, Feldmann J, Yeboah-Manu D, et al. Two new rapid SNP-typing methods for classifying Mycobacterium tuberculosis complex into the main phylogenetic lineages. PLoS One 2012;7:e41253.
28. Bolado-Martínez E, Candia-Plata Mdel C, Zenteno-Cuevas R, Mendoza Damián F, Avilés-Acosta M, Álvarez-Hernández G. Proposal of a five MIRU-VNTR panel to screen clinical isolates of Mycobacterium tuberculosis in Mexico. Enferm Infecc Microbiol Clin 2015;33:609-12.