Polish Journal of Microbiology
2014, Vol. 63, No 2, 249–253

SHORT COMMUNICATION

Alternative Genotyping of Drug-Resistant *Mycobacterium tuberculosis* Strains

PETRO B. ANTONENKO*, VALENTIN I. KRESYUN and KATE O. ANTONENKO

Department of General and Clinical Pharmacology
Odesa National Medical University, Ukraine

Submitted 6 December 2012, revised 3 April 2014, accepted 17 April 2014

Abstract

The aim of the present research was to study the capability of a genotyping method for *M. tuberculosis* through detection of six VNTR-loci (MIRU10, MIRU26, MIRU31, MIRU39, MIRU40, ETR-A). Loci MIRU10, MIRU26, MIRU40 and ETR-A have exhibited high polymorphism in group non-Beijing, while loci MIRU26 and MIRU31 – in the Beijing family. A combined detection of all six loci for fingerprinting of the isolates both from Beijing and non-Beijing was highly effective (Hunter-Gaston index was 0.88 and 0.93 correspondently), especially in areas with limited financial resources and high prevalence of multidrug resistant *M. tuberculosis* strains.

During the last decades, a large number of DNA-fingerprinting methods for genotyping of *Mycobacterium tuberculosis* have been developed (Alonso-Rodríguez *et al.*, 2008). This issue is especially important in the study of tuberculosis, where patients with recurrent tuberculosis can be chronically infected with a given strain and relapse due to reactivation of that strain or, in contrast, can be re-infected by a different strain after cure (Anyo *et al.*, 2007). One of the most recognized typing strategies is based on the analysis of polymorphisms in the direct-repeat region. For instance, typing of *M. tuberculosis* can be based on mycobacterial interspersed repetitive units and variable numbers of tandem repeats of genetic elements (MIRU-VNTR) at 12, 15 or 24 independent minisatellite loci spreading throughout the *M. tuberculosis* genome.

Information about *M. tuberculosis* genotyping in Ukraine is almost absent. This is because of limited financing of the fundamental researches. To make a genotyping of *M. tuberculosis* more affordable, simple and cheap in a resources-limited area like Ukraine we suggest using genotyping of the six VNTR loci. According to the previous study conducted in Southwest Ukraine, the most sensitive (polymorphic) were MIRU26, MIRU31, MIRU40, ETR-A (Nikolayevskyy *et al.*, 2007). Thus, we studied the capability of a genotyping method for *M. tuberculosis* through the detection of six VNTR loci (MIRU10, MIRU26, MIRU31, MIRU39, MIRU40, ETR-A) and unravelling of the genetic profiles of drug-resistant *M. tuberculosis* strains.

Resistance of *M. tuberculosis* to first-line antituberculosis drugs (isoniazid and rifampicin) was studied according to results obtained in 2009 by the bacteriological laboratory of Odesa oblast (district) clinical tuberculosis hospital. Around 106 isolates of *M. tuberculosis* obtained from the sputum of patients treated in that hospital were studied by detection of the six VNTR loci (MIRU10, MIRU26, MIRU31, MIRU39, MIRU40, ETR-A) with use of polymerase chain reaction (PCR). For detection of each locus the appropriate pair of primers was used and the length of amplified fragments depended on the number of tandem repeats (Nikolayevskyy *et al.*, 2005). Analysis of discriminative power of VNTR-method with 6 loci determination was evaluated through calculation of Hunter-Gaston index (HGI). Index over 0.6 corresponded with high sensitivity (polymorphism), from 0.3 to 0.6 – moderate sensitivity, under 0.3 – low sensitivity. The "cross-sectional" method of culture selection was applied, and the research was approved by the bioethics commission of Odesa National Medical University.

Detection of the mutation in codon 315 *katG* gene that leads to isoniazid resistance was performed with multiplex allele-specific PCR (MAS-PCR) with modification (Antonenko *et al.*, 2010). MAS-PCR was also used in the study of the mutation at codons 516, 526, 531 in the *rpoB* gene responsible for rifampicin resistance (Mokrousov *et al.*, 2010). To recognize, whether a strain of *M. tuberculosis* belongs to the Beijing family that is characterized by high resistance or not PCR was

* Corresponding author: P. Antonenko, Department of General and Clinical Pharmacology, Odesa National Medical University, Ukraine; **e-mail – peterantonenko@yandex.ru**

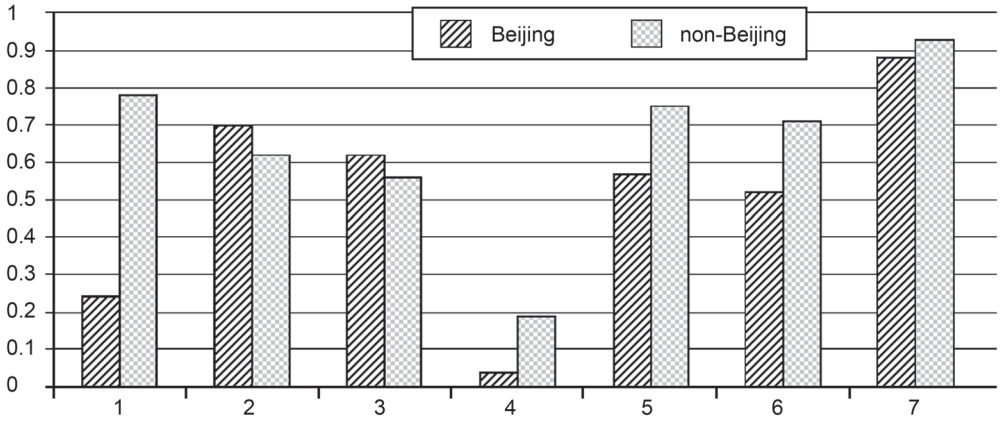

Fig. 1. Discriminative power of VNTR-typing for *M. tuberculosis* from Beijing or non-Beijing group Footnote: at abscissa – meaning of Hunter-Gaston index; at ordinate axis – studiet VNTR-loci – 1-MIRU-10, 2- MIRU-26, MIRU-31, MIRU-39, MIRU-40, 6-ETR-A, 7-all six loci together.

used as well (Banu *et al.*, 2004). The method is based on demonstrating insertion fragment IS6110 in intergenes locus *dnaA-dnaN* by two primers. Statistical analysis of results was done with Microsoft Excel involving χ-square criteria.

Out of 106 DNA-isolates, 46 samples belonged to the Beijing family (43.4%), while 60 (56.6%) samples belonged to non-Beijing strains. Data for numbers of tandem repeat regions in the 6 VNTR loci were obtained. According to the numbers of tandem repeat regions Beijing family strains were particularly distinguished from the non-Beijing group by loci MIRU31 and MIRU39 (Fig. 1).

To detect the capability of presented VNTR-genotyping an analysis of polymorphism and discriminative power of studied VNTR loci was conducted. It is interesting that in isolates of the Beijing family low sensitivity was noticed in MIRU10 and MIRU39 loci; moderate polymorphism – in MIRU40 and ETR-A; high polymorphism – in MIRU26 and MIRU31. However, the proposed method showed higher sensitivity in the strains from the non-Beijing group. For instance, low polymorphism was in MIRU39; moderate polymorphism – in MIRU31 and high polymorphism – in MIRU10, MIRU26, MIRU40 and ETR-A. A combined detection of all six loci for fingerprinting of the isolates both from Beijing and non-Beijing was highly effective (0.88 and 0.93 correspondently).

Out of 46 DNA-isolates that represent the Beijing family, 27 (58.7%) were multidrug resistant (simultaneous resistant to isoniazid and rifampicin). It was discovered that all isolates with profiles 355335, 375344 and 385334 were multidrug resistant (Tab. 1). Out of 60 DNA-isolates from the non-Beijing group, 24 (40.0%) were multidrug resistant. The majority of the isolates from cluster 452242 (83.3%) were multidrug resistant as well as all isolates from clusters 562242 and 712234.

Half of the isolates from Beijing family carried a mutation at codon 315 *katG* gene simultaneously with a mutation at codon 516/526/531 *rpoB* gene (Tab. I). In addition, all isolates from the clusters 375344 and 385334 have had both mutations mentioned above. Out of 60 DNA-isolates from non-Beijing strains only 15 (25.0%) carried both studied mutations in the *katG* and *rpoB* genes. The majority isolates (83.3%) with 452242 profile have had both studied mutations.

The isolates from Beijing family with profiles 365334 and 375334 in 75.0% cases and with profile 375344 in 100% cases were received from HIV-infected patients (average meaning in the Beijing group was 23.9%). In the patients who carried *M. tuberculosis* with profiles 375334 and 375344 lethal outcome during one year (2009) was observed in 50% cases (average 8.7%). All strains of *M. tuberculosis* from the clusters 356335, 375334, 375344, and 385334 were obtained from patients, who had contact with patients with TB before the disease started (average 65,2%). All non-Beijing strains with profile 553213 were obtained from the patients, who had a prison history and contact with patients with TB before the disease started (average meaning in the non-Beijing group was 26.7% and 55.0% correspondently).

In general, the obtained data relatively high and moderate polymorphism of MIRU10, MIRU26, MIRU31, MIRU40 and ETR-A of the non-Beijing isolates are matching with previous research in the Southwest Ukraine (Nikolayevskyy, 2005).

We have revealed moderate polymorphism of loci MIRU40 and ETR-A accompanied by high polymorphism of loci MIRU26 and MIRU31 of the isolates from the Beijing family.

Thus, the genotyping of *M. tuberculosis* via combined detection of the six VNTR loci can be considered as a quite informative and sensitive method, especially in areas with limited financial resources, where

Table I
Spreading of drug-resistance and certain epidemiologic factors among different clusters of *M. tuberculosis* (%)

VNTR-clusters (number of isolates)	Mutationin gene		Multidrug resistance	HIV-infection	Prison history	TB-contact
	rpoB	*katG*				
Beijing family						
355335(5)	80.0	60.0	100	20.0	40.0	80.0
355344(2)	0*	0	0	0	0	50.0
355345(2)	100	50.0	50.0	0	50.0	50.0
356335(2)	50.0	50.0	50.0	0	50.0	100
356344(2)	0*	50.0	50.0	0	50.0	50.0
375334(2)	100	50.0	0	50.0	50.0	100
375344(2)	100	100	100	100*	50.0	100
385344(2)	100	100	100	0	100	100
385345(3)	100	66.7	100	33.3	66.7	66.7
average (46)	69.6	58.7	58.7	23.9	34.8	65.2
Non-Beijing group						
353233(2)	0	0	0	0	0	0
363233(2)	50.0	0	0	0	50.0	100
452242(6)	83.3	83.3*	83.3*	0	0	66.7
452252(2)	50.0	100	0	50.0	0	50.0
553213(2)	100	50.0	50.0	100*	100*	100
562242(3)	66.7	33.3	100*	66.7	33.3	33.3
712234(3)	100	100*	100*	33.3	0	33.3
average (60)	45.0	33.3	40.0	26.7	26.7	55.0

* – $P < 0,05$ (correspondently to average spreading in group)

the detection of all 12, 15 or 24 loci is complicated. In fact, in the scientific literature one can find information about the genotyping of *M. tuberculosis* through detection of certain six VNTR-loci, but they were different from the VNTR-loci presented in the current research (Allix-Béguec *et al.*, 2008).

Taking into account an international database of VNTR available on http://www.MIRU-VNTRplus.org and considering loci MIRU10-MIRU26-MIRU31-MIRU39-MIRU40-ETRA one can conclude that clusters 353233 and 363233 belong to the Cameroon group (Euro-American group); the most spread clusters 452242 and 452252 – to LAM group (Euro-American group); cluster 553213 is close to the Haarlem group (Euro-American group); cluster 712234 – to the URAL group (Euro-American group). According to our results and the international database, the isolates from Haarlem family had extremely high level of the multidrug resistance. In fact, both isolates that are close to Haarlem family were obtained from HIV-infected patients with a prison history.

It is known that in the central regions of Russian Federation the majority of *M. tuberculosis* isolates represent LAM and Beijing families (Ivanov *et al.*, 2004). These strains are characterized by significant spreading worldwide and they play an important role in tuberculosis infection epidemics.

It is known that HIV-infection, a prison history are risk factors for the development of TB, for the appearance of drug resistance in *M. tuberculosis* infection, and for the malignant course of tuberculosis process. That is why genotyping of the strains in HIV-infection with tuberculosis is extremely important for the monitoring of tuberculosis epidemics and successful treatment of tuberculosis. According to previous research, Beijing strains with profile 375334 were very common in patients with a prison history in the Southwest of Ukraine (Nikolayevskyy *et al.*, 2007) and this has been proved by our work.

Acknowledgments

This work was supported by the special scientific research program of the Healthcare Ministry of Ukraine (No 0113U001634 and 0110U006662).

Literature

Allix-Béguec C., D. Harmsen and T. Weniger. 2008. 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.* 46: 2692.

Alonso-Rodríguez N., M. Martínez-Lirola and M. Herránz. 2008. Evaluation of the new advanced 15-loci MIRU-VNTR genotyping tool in *Mycobacterium tuberculosis* molecular epidemiology studies, *BMC Microbiol.* available: http://www.biomedcentral.com/1471-2180/8/34

Antonenko K.O., V.I. Kresyun and P.B. Antonenko. 2010. Mutations leading to drug-resistant Mycobacterium tuberculosis infection in Ukraine, *Central European Journal of Medicine* 5: 30.

Anyo G., J. Allen and C. Reddy. 2007. Re-infection vs reactivation as causes of tuberculosis recurrence in a high-burden country, *The International journal of tuberculosis and lung diseases*, 11, suppl. 1: 162.

Banu S., V.G. Stephen and P. Si P. 2004. Genotypic Analysis of *Mycobacterium tuberculosis* in Bangladesh and Prevalence of the Beijing Strain, *J. Clin. Microbiol*. 42: 674.

Ivanov I. Iu, V.N. Stepanshina and M. Iu. Lipin. 2004. Spoligotypes of clinical Mycobacterium tuberculosis strains isolated in patients with tuberculosis in the Central Region of Russia, *Probl. Tuberk. Bolezn. Legk.* 5: 23.

Mokrousov I., T. Otten and B. Vyshnevskiy. 2003. Allele-Specific *rpoB* PCR Assays for Detection of Rifampin-Resistant *Mycobacterium tuberculosis* in Sputum Smears, *Antimicrobial agents and chemotherapy* 47: 2231.

Nikolayevskyy V.V., T.J. Brown and Y.I. Bazhora. 2007. Molecular epidemiology and prevalence of mutations conferring rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* strains from the southern Ukraine. *Clin. Microbiol. Infect.* 13: 129.

Nikolayevskyy V.V. 2005. Optimization of genotyping strategy of Mycobacterium tuberculosis in Odessa district of Ukraine: comparing of spolygotyping method and VNTR, *The Odessa Medical Journal* (in Ukrainian) 3: 32.