International Journal of Mycobacteriology 4 (2015) 22–23

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Mycobacterium tuberculosis Latin–American–Mediterranean family in Bulgaria

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ARTICLE INFO

Article history:
Received 18 October 2014
Accepted 21 October 2014
Available online 23 December 2014

Keywords:
Mycobacterium tuberculosis
Latin–American–Mediterranean family
Genotyping
Spoligotyping
IS*6110*
Bulgaria

ABSTRACT

Introduction: Tuberculosis (TB) remains an important public health issue for Bulgaria. Although a number of new cases are showing a steady decline (44/100,000 in 2000, 40/100,000 in 2005), the TB incidence rate in Bulgaria is still sufficiently high (26.7/100,000 in 2013). The current population structure of *Mycobacterium tuberculosis* is clonal. Certain genetic families of this species have justly attracted more attention due to their global dissemination and/or remarkable pathogenic properties. Beijing, Haarlem, and LAM are the most known examples. The latter family LAM (Latin–American–Mediterranean) was shown in an increasing number of studies to possess increased transmissibility, hence the importance of its rapid detection and correct estimation of its prevalence in a population. Spoligotyping signature of LAM is absence of signals 21–24 and 33–36, although abridged spoligo-profiles with long blocks of deleted spacers result in an uncertain definition of such strains. The use of other molecular markers may be helpful. This study aimed to evaluate the prevalence of LAM strains among *M. tuberculosis* strains in Bulgaria based on the use of different molecular markers.

Materials and methods: *M. tuberculosis* isolates were randomly selected among *M. tuberculosis* strains isolated from newly diagnosed, adult, pulmonary TB patients in different regions of Bulgaria from December 2004 to March 2006. Spoligotyping was used to analyze a variation in the DR locus. The spoligotyping patterns were compared with the international database SITVIT_WEB (Institut Pasteur de Guadeloupe). Analysis of the IS6110 element specific for the LAM genetic family was performed as described previously (Marais et al., 2006).

Results: A study sample included 133 strains from different regions of the country and characterized in previous publications (Valcheva et al., 2008, 2010). Application of the published rules for the definition of the major spoligotype clades and comparison with SITVIT_WEB global database permitted this study to assign most of the 133 strains to the known spoligotype families. All available strains ($n = 101$) were tested for LAM-specific IS*6110* insertion. Comparison of results by different methods identified 3 groups of strains. The first group included 11 strains with 2 amplified bands which present an apparent discrepancy; further study is warranted. The second group included 86 strains with a single amplified band, specific for the absence of the IS6110 insertion in the target locus, hence indicative of the other than LAM family. The third group included 4 strains with a LAM-specific band only.

Conclusions: Application of the LAM-specific PCR revealed double-sided discrepancies when the obtained results were compared with those obtained by spoligotyping. As a whole, a

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http://dx.doi.org/10.1016/j.ijmyco.2014.10.044

phylogenetic family of 38 strains was revised or questioned: 27 strains were shown not to belong to LAM, while 11 more strains showed apparently discrepant results that question the global utility of such PCR or at least highlight an importance of using multiple markers for molecular detection of the LAM family of *M. tuberculosis*.

Acknowledgements: Dr. Nadya Markova, Prof. Olga Narvskaya, and Prof. Nalin Rastogi are gratefully acknowledged for kind support and guidance.