

# **Molecular-epidemiological analysis of *Mycobacterium tuberculosis* strains isolated in the West-Bohemian Region of the Czech Republic including detailed characterisation of anti-tuberculosis drugs – resistant strains**

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## **Abstract**

Tuberculosis is contagious infectious disease that embodies significant epidemiological and clinical problem worldwide. Tuberculosis incidence differs considerably in various regions of the world but even the countries with low incidence engage strongly in epidemiology of tuberculosis. Tuberculosis belongs to one of the priorities of WHO, cooperation in this matter takes place on a global scale. Tuberculosis is a social illness; accordingly, the countries with high occurrence of tuberculosis are classified as developing countries. Mainly in Africa, there is the situation being complicated by coexistence of HIV. Generally, Europe represents a region with low incidence of tuberculosis. The Czech Republic is a country with the lowest incidence in the world with less than five new cases per 100 000 inhabitants every year. This situation is among others result of high-level tuberculosis surveillance and effective application of epidemiological arrangements based in legislation.

This dissertation thesis examines several fields of tuberculosis, mainly focused on modern opportunities of laboratory diagnosis and most importantly modern methods of molecular-epidemiological analysis. It consists of review of recent literature related to molecular-epidemiological diagnostics and application of gained knowledge on selection of the most suitable available methods for genotyping of isolates *Mycobacterium tuberculosis*, which would be fitting for putting into practice. Outcome of the thesis defines whole genome sequencing (WGS) as methods of choice for molecular epidemiological analysis of incidence and spreading of tuberculosis. Part of this thesis also represents overview of familiar genotypes *M. tuberculosis* including their geographical distribution and description of principle of designation of these genotypes and their detection. In the results section, research of strains group isolated from patients with tuberculosis isolated in the Czech Republic are described using WGS, appraisal of gained data and summary of *M. tuberculosis* genotypes that were present by infected patients. This method will be available to apply to all isolates obtained in the Czech Republic during given time period, assuming other laboratories cooperate in the future. This information can lead to discover a way of how tuberculosis spreads among people and how it can be imported from abroad, which could lead to implementing effective epidemiologic arrangements.

In this thesis, the topic of laboratory diagnostics of tuberculosis is focused on identification through mass spectrometry that belongs to the current modernist ways of identifications in microbiology and that can be applied on identification of mycobacteria. The results derive from separate research and international research, that FN Plzeň and Biomedical Center LF UK Plzeň took part in.

In recent times, the importance of latent tuberculosis diagnosis is raising. The first reason is epidemiological searching for patients with active form of tuberculosis. There are also patients with weakened cell-mediated immunity, who are mostly patients before or during immunotherapy, which uses treatment on base of TNF alfa inhibitors and so on. Last but not least, patients with primary pulmonary disease such as tumors, cystic fibrosis, interstitial pulmonary process etc. Diagnosis of this form of tuberculosis is in the paper presented by focus on IGRA methods. These methods are currently the most reliable way to detect latent tuberculosis. The thesis compares two available methods – Quantiferon TB Gold Plus and T-SPOT.TB. Results are built as comparison of both methods and there is described how, when and which one to use for routine diagnosis.

Similarly, to other fields of microbiology, diagnosis and epidemiological analysis of tuberculosis in particular develop lately very quickly. However, these methods are still only additional to the complete diagnostic spectrum they do not replace it. Standard methods in diagnosis of tuberculosis remain classic methods like microscopy and cultivation. It is appropriate to fully utilized modern methods; nonetheless, the evaluation and interpretation of result of any microbiological laboratory method are dependent on circumstances of disease of individual patient, his condition and context with other findings.