

Bulgarian Academy of Sciences  
The Stephan Angeloff Institute of Microbiology,

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**DOCTORAL THESIS**

**“Molecular genetic characterization of Mycobacterium tuberculosis strains,  
isolated from different regions of Bulgaria”**

Scientific Specialty 01.06.12 “Microbiology”

Directed by

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## **SUMMARY**

Tuberculosis is an infectious disease that has existed since ancient times. Over the years, its frequency has changed with varying intensity. Nowadays, there is a global trend of the increasing spread of tuberculosis. The incidence of the disease is increasing not only in the third world, but also in the economically developed countries of Europe and other continents. There is a negative trend for the manifestation of atypical and severe forms of tuberculosis, as well as an increase in cases of multidrug resistance. All this leads not only to extremely adverse effects on the individual, family and society, but also to huge damage and significant treatment costs. The current epidemiological situation requires the mobilization of all efforts of society and the state, including both national medical structures and the scientific potential and elite around the world and in our country to control this socially significant disease .

The lack of information about the population structure of *Mycobacterium tuberculosis* in Bulgaria, the frequency and spectrum of mutations, the distribution of different genotypes and the lack of data on their epidemiological and pathogenetic significance determine the relevance of systematic research on genetic variations in tuberculosis. The development of this scientific field in Bulgaria will deepen the knowledge about the features and early detection of drug-resistant strains of *Mycobacterium tuberculosis*, their importance for modern infectious pathology, prevention and treatment, and will add new information to the global database of tuberculosis in the world.

## **AIM AND TASKS**

The aim of this PhD thesis is to perform molecular genetic research and analysis of the population structure of *Mycobacterium tuberculosis* strains in terms of their genetic variability and their distribution in different regions of Bulgaria, as well as detection of specific mutations associated with molecular mechanisms of resistance to the main anti-tuberculosis drugs.

### **TASKS:**

1. Collection of clinical strains of *Mycobacterium tuberculosis* isolated in different regions of Bulgaria and study of their genomic polymorphism by molecular genetic methods - IS6110-RFLP, spoligotyping and MIRU-VNTR.

2. Determining the degree of genetic similarity and differentiation of strains by comparative genetic analysis and use of a combination of molecular-epidemiological markers (IS6110, DR, MIRU-VNTR). Identification of the predominant genotype variants in the *M. tuberculosis* population circulating in Bulgaria, and its comparison with the genotypes widespread in the neighboring countries (Balkan region) and around the world.
3. Determination of the frequency of drug resistance to the main anti-tuberculosis drugs - rifampicin, isoniazid, ethambutol and streptomycin (RIF, INH, EMB, STR) by detecting mutations in the genes *rpoB*507-533, *katG*315, *inhA* promoter region (up to positions - - 25), *embB*306 and *rpsL*43.
4. Selection of a combination of appropriate molecular-epidemiological markers in order to create an optimized scheme for epidemiological typing of the genotype variants of *M. tuberculosis* in Bulgaria.

## **CONCLUSIONS:**

1. For the first time a molecular genetic study of the *M. tuberculosis* strains circulating in Bulgaria was performed. It was proved that the studied population is quite heterogeneous and it is dominated by several globally distributed international (ST53, ST47, ST34), Balkan (ST41, ST284) and specific for Bulgaria (ST125, ST2905) spoligotypes.
2. Transmission of multidrug-resistant tuberculosis strains of the Beijing genotype was not detected in Bulgaria, which obviously shows that this genotype has not yet reached our country.
3. Typing of *M. tuberculosis* strains with the MIRU-VNTR method showed the highest discrimination ability compared to the spoligotyping and IS6110-RFLP method. The combination of different methods and MIRU-VNTR schemes allows more detailed analysis and strain differentiation.
4. Our proposed optimized 5-MIRU-VNTR locus scheme gives good discrimination for the studied strains and can be used for primary genotyping of *M. tuberculosis* strains circulating in Bulgaria.
5. The prevalence of gene mutations linked to resistance to major anti-TB drugs (rifampicin, isoniazid, ethambutol and streptomycin) was determined. Mutations in *rpoB* (507-533), *katG*315, *embB*306, *inhA* and *rpsL*43 can be used for rapid genetic detection of resistant strains of *M. tuberculosis* in Bulgaria.

6. The combination of spoligotyping and MIRU-VNTR methods showed that the emergence and spread of drug-resistant and MDR-TB in Bulgaria is not associated with any specific spoligotype or MIRU-VNTR cluster.

7. A local circulation of the particular area-specific clones appears to be an important factor to take into consideration in the molecular epidemiological studies of tuberculosis in Bulgaria.

## **CONTRIBUTIONS**

1. The first in-depth molecular genetic study of the *M.tuberculosis* strains circulating in Bulgaria for the purpose of epidemiological research was carried out.

2. The discrimination abilities of three main molecular genetic methods - IS6110-RFLP, spoligotyping and MIRU-VNTR allow more detailed analysis and differentiation of *M. tuberculosis* strains in our country.

3. Our proposed optimized 5-MIRU-VNTR locus scheme can be successfully used for discrimination and primary genotyping of *M. tuberculosis* strains in Bulgaria.

4. Mutations in genes associated with resistance to major anti-TB drugs (rifampicin, isoniazid, ethambutol and streptomycin) have been identified. The possibilities of genotypic methods for rapid detection of resistant strains in our country have been evaluated.

5. The importance of locally-specific clones as an important factor in the molecular-epidemiological studies of tuberculosis in Bulgaria was underlined.

6. It was found that the drug resistance and the spread of resistant strains in Bulgaria are not associated with the circulation of specific genotypic variants/clones.

## **SCIENTIFIC PUBLICATIONS RELATED TO THIS PhD THESIS**

1. Valcheva V., Mokrousov I, Narvskaya O, Rastogi N, Markova N. 2008. Genetic Diversity of Mycobacterium tuberculosis Population in Bulgaria. In: Genetic Diversity. Nova Science Publishers. NY, USA.

2. Valcheva V., Mokrousov I, Narvskaya O., Rastogi N., Markova N. 2008. Molecular snapshot of drug-resistant and drug-susceptible Mycobacterium tuberculosis strains circulating in Bulgaria. Infect Genet Evol.; 8: 657-663.

3. Valcheva V., Mokrousov I., Narvskaya O., Rastogi N., Markova N. 2008. Utility of New 24-locus VNTR Typing for Discriminating *Mycobacterium tuberculosis* Clinical Isolates in Bulgaria. *J. Clin. Microbiol.*; 46: 3005-3011.
4. Valcheva V., Mokrousov I., Rastogi N., Narvskaya O., Markova N. 2008. Molecular characterization of *Mycobacterium tuberculosis* isolates from different regions of Bulgaria. *J. Clin. Microbiol.*; 46: 1014-1018.
5. Mokrousov I., Jiao W.W., Valcheva V., Vyazovaya A., Otten T., Ly HM., Lan N.N., Limeschenko E., Markova N., Vyshnevskiy B., Shen A.D., Narvskaya O. 2006. Rapid detection of the *Mycobacterium tuberculosis* Beijing genotype and its ancient and modern sublineages by IS6110-based inverse PCR. *J Clin Microbiol.*; 44: 2851-2856.

## **PARTICIPATION IN THE CONFERENCES**

1. Valcheva V., Mokrousov I., Narvskaya O., Rastogi N., Markova N. Utility of new 24-locus VNTR format for discriminating *Mycobacterium tuberculosis* isolates in Bulgaria. 29th Congress of the European Society of Mycobacteriology. Plovdiv-Bulgaria, 6-9 July, 2008
2. Valcheva V., Mokrousov I., Rastogi N., Narvskaya O., Markova N. Molecular insight into drug resistant tuberculosis in Bulgaria. 29th Congress of the European Society of Mycobacteriology, Plovdiv-Bulgaria, 6-9 July, 2008
3. Valcheva V., Mokrousov I., Narvskaya O., Rastogi N., Markova N. Use of MIRU-VNTR Typing to differentiate *Mycobacterium tuberculosis* isolates from Bulgaria and comparison with IS6110-RFLP Typing and Spoligotyping. 18th European Congress of Clinical Microbiology and Infectious Diseases. Barcelona-Spain, 19-22 April, 2008
4. Valcheva V., Mokrousov I., Narvskaya O., Rastogi N., Markova N. Molecular characterization of *Mycobacterium tuberculosis* strains from Bulgaria. 28th Congress of the European Society of Mycobacteriology. Athens-Greece, 1-4 July, 2007
5. Valcheva V., Mokrousov I., Narvskaya O., Rastogi N., Markova N. Genetic strain diversity in *Mycobacterium tuberculosis* isolates from Bulgaria. 4th Congress of the International Union Against Tuberculosis and Lung Disease, Europe Region, Riga-Latvia, 27-30 June 2007
6. Valcheva V., Mokrousov I., Narvskaya O., Markova N. 2006. Genetic biodiversity of *Mycobacterium tuberculosis* strains from patients with pulmonary tuberculosis in Bulgaria. 60 years Institute of Microbiology. Sofia-Bulgaria, 14 -16 March, 2007

7. Valcheva V., Mokrousov I., Narvskaya O., Markova N. Using of molecular markers for studying the population structure of *Mycobacterium tuberculosis* strains from Bulgaria. 2<sup>nd</sup> National Student's Conference of Technical University. Sofia-Bulgaria, 11-13 October, 2006
8. Valcheva V., Mokrousov I., Narvskaya O., Markova N. 2006. Molecular-epidemiological characterization of *Mycobacterium tuberculosis* strains originating from different region of Bulgaria. 11th Congress of the Bulgarian Society of Microbiologists. Varna-Bulgaria, 5-7 October, 2006