

# EVALUATION REPORT

**from Prof. Dr. Emma Edmond Keuleyan-Hristova, PhD**

Department of Clinical Microbiology,

Medical Institute - Ministry of Interior,

Microbiology specialist, Professional field 7.1. Medicine,

Appointed as academic jury member with Order No I-841/01.07.2020 of the

Director of the Institute of Microbiology “St. Angeloff” - BAS

Regarding competition for conferral of the academic rank “Associated Professor”

Professional field 4.3. Biological sciences, Scientific specialty

“Microbiology”, announced in State Gazette 47/22 May 2020

For the needs of the Laboratory ”Molecular biology of Mycobacteria”,

Department of Infectious Microbiology, the “Stephan Angeloff” Institute of Microbiology–  
Bulgarian Academy of Sciences, according to the and

According to decisions from the first meeting of the academic jury on 24 July 2020

## GENERAL DESCRIPTION OF THE DOCUMENTS PRESENTED FOR THE COMPETITION

One candidate has submitted application and was found eligible for participation in the competition – Chief Assist. - Prof. Violeta Valcheva Russeva, PhD, member of the same department and laboratory.

The documents are in compliance with the requirements of the Law for Development of the Academic Staff and the related Statute of The “Stephan Angeloff” Institute of Microbiology–Bulgarian Academy of Sciences (BAS), and includes: Application, Copy of the announcement, copies from diplomas/certificates for: -higher education, PhD educational and scientific degree, incl. the author’s summary of PhD thesis. The applicant has prepared: Professional CV on the European sample, Abstracts of research works after the PhD thesis, List of research works with full texts, List of citations, List of scientific projects, List of participations in scientific congresses with presented copies of abstracts, Fact sheet about contributions, Fact sheets regarding: - fulfilment of minimum national requirements and requirements of The “Stephan Angeloff” Institute of Microbiology – BAS, - impact factor and Journal Rank. The presented information is precisely organised.

## PROFESSIONAL AND ACADEMIC CAREER

Chief Assist.-Prof. Violeta Valcheva was born in 1978. In 2002 r she completed bachelor degree in the “Kliment Ohridski” University of Sofia – specialty “Biotechnologies” and in 2005 graduated as magister with excellent marks (5.50) – specialty „Microbiology and microbiological control“. Her labor path starts from the end 2004 in The “Stephan Angeloff” Institute of Microbiology–BAS as a specialist-biologist, where from 2005 to 2008 is a doctoral student and after defense of dissertation “Molecular-genetic characteristics of *Mycobacterium tuberculosis* strains isolates from different regions in Bulgaria“, obtained the PhD degree. Her scientific work as Chief Assist.-Prof. expanded the areal of investigations, incl. with participation in National and International scientific projects, publications in international and Bulgarian journals, active participation in scientific forums. She increases continuously her qualification, incl. via 3-month specializations in leading International Centers: – the Institute Pasteur, St Petersburg, Russia - 2005 (fellowship of FEMS) and 2007 (fellowship from NATO project), as well as 2 weeks 2015-2019; - in the Institute Pasteur - Guadeloupe, France – 2007, 2008, 2009 (fellowships from the NATO project and Institute Pasteur - Paris); Central Children’s Hospital – Beijing, China – 2005, 2012. She completed several post-graduate-education courses – National and International, incl. in the Institute Pasteur: - Paris, St Petersburg, Athens; Institute for healthcare – Utrecht, the Netherlands; University of Gent, Belgium; Center of Diseases Control – Shanghai, China. She gradually grows up as highly competent scientist, incl. as leading author in scientific publications, participant and leader of scientific project; she is the PR of the Institute of Microbiology - BAS. She is a member of the Union of scientists in Bulgaria, as well as of the weighty International organizations: ESM (European Society of Mycobacteriology), ESCMID (European Society of Clinical Microbiology and Infectious Diseases), FEMS (Federation of the European Microbiology Societies), ISID (International Society of Infectious Diseases). She has received three scientific awards and is with the highest score from the last institutional attestation. Expression of her expert and educational activities are the scientific supervision and reviews of thesis, her work as consultant of PhD thesis. Chief Assist.-Prof. Violeta Valcheva uses English, Russian and French; she has computer skills and knowledges on bioinformatics. Her carrier curriculum characterizes her as an active and successful scientist.

## EVALUATION OF RESEARCH ACTIVITIES

### 1. Research metrics

***According to the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria:***

- Group „A“ parameters, Parameter 1: – Defense of PhD dissertation „ Molecular – genetic characteristics of *Mycobacterium tuberculosis* strains isolates from different regions in Bulgaria” – 50 points.

- Group „B“ parameters, Parameter 4 (publications indexed in Scopus, Web of science) – required – 100 points, available – 130 points.

- Group „G“ parameters: total of parameters 5 to 10 (parameters 7 and 8 (2 chapters of books)) – required 200, available 237 points - of publications after the last habitation

- Group „D“ parameters: total of points in Parameter 11: cited publications in the world databases: - 28, cited by 241 international publications – 482 points instead the required 50.

Therefore, the minimum national requirements are not only covered (for parameter from Group A) but also substantially exceeded (for parameters from Groups B, G, D).

***According to the requirements of the Institute of Microbiology with BAS***

- the balance is analogous. According to them, the candidate for the academic rank “Assoc.-Professor” should present 20 scientific publications with IF, and to be the first author in 5 of them, while the Chief Assist.-Prof. V. Valcheva has presented 24 publications and she is the first author in 7 of them. The calculated Impact factor is 40.295 (while the required is 20); h factor (Scopus) is 13 versus the required 5. Instead of participation in three scientific projects, the applicant participates in 14 and in 7 is the head.

**2. Evaluation of the scientific publications, presented for the acquiring of the academic degree “Assoc.-Professor” – Basic scientific directions and contributions**

The applicant presents a detail description and analysis of scientific works w the basic scientific directions, formulated by her, starting with *Introduction* – a theoretical justification – a particular annotation and rationale, followed by *Main scientific contributions* and *Bibliography*. In the current evaluation the original articles are object of analysis.

***Thematic direction I: Identification and molecular epidemiology of tuberculosis in animals***

Two of the presented articles (Bibliography 1 and 2), report pioneer investigation on the spread of *Mycobacterium bovis*, the etiologic agent of tuberculosis in cattle, in Bulgaria. During the period 2015 - 2018 1193 specimens from lymph nodes and lungs of slaughtered animals or given (+) reaction with tuberculin, from 10 geographic regions in Bulgaria were investigated. From 283 isolated bacterial isolates 263 were *M. bovis*. In the performed spoligotyping (spacer oligonucleotide typing, hybridization after PCR amplification) spoligotypes were established, referred to the classical *M. bovis* (preliminary in the Nord-East Bulgaria), as well as others,

characterizing *Mycobacterium caprae*, found for the first time (preliminary in the South-West and Central Bulgaria).

***Thematic direction II: Spread of paratuberculosis in the wild animals in Bulgaria***

Investigation for the pathogens of paratuberculosis: *Mycobacterium avium* spp *paratuberculosis* (MAP) – in deers, mouphlons and roedeers from the hunting farms 2009 – 2013, was performed by examination of mesenteric lymph nodes, intestines and feces. A specific for MAP PCR confirmatory test (IS 900 PCR kit) was applied. The disease was confirmed in 8.8 % of suspected ill animals, which make accent on the epidemiological danger of infecting other wild and domestic animals – because of the emitting the pathogens in the environment (Bibliogr. 3 and 4). Bibliographic source 5 is a review, viewing MAP as a pathogen of paratuberculosis in animals and of Crohn's disease in humans. The last statement, however, is particularly disputable: the Crohn's disease is already of not fully clarified etiology; it is categorically proven as autoimmune disease, however. Participation of eventual risk factors is being discussed, incl. of some bacteria, but during the last years investigations with the most progressive technics do not find the MAP's participation.

***Thematic direction III: Molecular epidemiology, drug resistance, phylogenetics and evolution of Mycobacterium tuberculosis in Bulgaria and globally***

Phylogenetics of *Mycobacterium tuberculosis* is most related with human's migration, is the conclusion, derived from the bibliographic source 6, viewing Latin-American-Mediterranean (LAM)-cluster from different geographic regions. On the basis of investigations *de novo* of 120 LAM-family strains with 24 MIRU-VNTR method (Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats) and other methods, and by the use of 24 –MIRU-VNTR – profiles of 258 strains from all over the world, 3 sub-lines were found: RD115, RD174, SIT33: the most prevalent RD115 being the most ancient, which spread started probably from the West-Mediterranean region and is wide distributed in South Africa, Russia, Nord Eurasia and Bulgaria. RD174 probably originated from Brazil and is widely spread in S. America, Portugal, South-East Africa. SIT33 and Africa.

Distribution of the spoligotypes of *M. tuberculosis* in Bulgaria is discussed in bibliogr. sources 7, 8 and 9, 11. It was proven, that the spread in our country is heterogenic. In previously spoligotyped 133 strains (isolated 2004-2006) typing was applied by 24-MIRU-VNTR and IS6110-RFLP (a fingerprinting, determining polymorphism on the length of restricted fragments: the preliminary restricted chromosomal DNA after its electrophoretic distribution is being transferred on a membrane and hybridizing with IS6110-probe). It turns out that bearers of IS6110, the typical strains of the LAM-family are below 4 %. In Bulgaria are prevalent

different globally distributed strains from the spoligotypes ST 53, ST 47, ST 34, as well as the Balkan spoligotypes: ST 125, ST 41, ST 284. The spoligotype ST 125 is specific for Bulgaria and is not connected with drug resistance and increased transmissibility. On the basis of 21-VNTR-typing ST125 strains were distributed in 12 sub-types: T1 – T12 and conclusions were derived that they lack IS6110 and do not belong to the LAM-family, but to the S-family (they are close to ST34), first described in Sicilia and Sardinia, and are relatively ancient: > 500 years.

Epidemiological studies with strains, isolated in some other countries, were reported in separate publications: from 56 DNA from sputum of patients from prisons in Kirgizstan, *M. tuberculosis*, similar to those, widely spread in Russia were found, but also of the Fam. Beijing, in addition a resistance to Rifampicin (RIF) 28 % and to Isoniazid (INH) 5.5 % was discovered (bibliogr. 12). As expected, in the region of Beijing, China (bibliogr. 14), the strains Beijing are predominant: established in 113 of 123 investigated strains and typically, MDR.

Establishment of drug resistance in *M. tuberculosis* as a reason of failure of therapy or an epidemiological factor in the spread of disease becomes more actual. Studies by V. Valcheva and co-authors (8, 9, 13, 14) are among the first, demonstrating the diagnostic significance of the molecular-genetic technics for the rapid prove of resistance to anti-tuberculosis agents and necessity of change of therapy. Basically in bibliogr. source 10, as well as in 9 (respectively a review and a chapter from monography), research based on the prove of mutational resistance to rifampicin, pyrazinamide (PZA), ethambutol (EMB), isoniazid, streptomycin (STR) via PCR with specific primers is discussed. Three type of mutations in gene *rpoB* (RIF) – were reported in Bulgarian strains - in 20 from 27 resistant strains, *rpoBS5312* being the most distributed; 11 from 23 INH resistant isolates were with mutational resistance *katG* S315T (48 %); *inhA*-15C>T –mutation was found in both resistant and susceptible strains; in 7 of 11 EMB resistant strains mutationa *embB306* was discovered; in resistant to STR *M. tuberculosis* the presence of mutation in *rpsL43* was not proven. It has been established that the drug resistance is not related to the population structure of strains; looking for mutations in *rpoB* and *embB306* is a reliable rapid method for proving resistance to RIF and EMB.

#### ***Thematic direction IV: L- forms in Mycobacteria and staphylococci***

L- forms of *M. tuberculosis* (bibliogr. source 15) are characterized by quicker growth on liquid nutritive media and it has been proven that they are important mean for survival of tuberculosis bacteria, incl. in latent tuberculosis. Experiments *in vivo* were performed, via infecting of rats, followed by the cultivating in culture media and their proving by electronic microscopy and spoligotyping.

### ***Thematic direction V: Development of new compounds with antituberculosis activity***

The long course of combined application of antituberculosis drugs for therapy, and especially the developed and spread during the last years resistant and multiple-drug resistant (MDR) strains are the ground for studying new drugs – a priority according the WHO. The Chief Assist.-Prof. V. Valcheva participates actively in equips studying new antituberculosis agents (bibliography 17-24). Using the method of Canetti for determination of Minimal Inhibitory Concentrations (MIC), she performed a screening of the newly synthesized compound, obligatory against the etalon strain H<sub>37</sub>Rv, as well as against the MDR strain 43 (RIF, INH). The obtained results show a significant *in vitro* activity of some of studied compounds: pyrazol and coumarin-derivatives, contained hidrazide-hidrazones (demonstrate *in vitro* activity, comparative to INH (MIC 0.28-1.69 IM); acyl-hydrazones: 2H-chromene or coumarin-liked, which representatives 7m, 7o и 7к are with MIC respectively 0.13, 0.15 and 0.17 μM, compared to INH and EMB; chiral amino-alcohols with a camphane scaffold: the 4 most active of which are with MIC 0.27-0.72 mM, i.e., higher than EMB; 22 derivatives of carbamid, thioureas, and acyl-thioureas with R-amino-butanol motive – derivatives of acyl-thioureas are with MIC of 0.36 – 7.46 mM; amidoalcohols and amidodiols with a camphor skaffold, some of them 25 time more active than EMB; diamide derivatives of mandelic acid with similar activity to EMB; new camphane-derivatives of isoindolin with similar activity to EMB; 47 derivatives of 2-amino-butanol: 5 compounds of R form are 11 time more active than EMB.

### **CONCLUSION**

I believe that Chief Assist.- Prof. Violeta Valcheva is a recognised researcher, with substantial contributions to experimental and clinical microbiology, and especially in Mycobacteriology: - for the dynamics and evolution of the strains *Mycobacterium tuberculosis*; for the phylogeography of pathogens in humans and animals and the epidemiology of infections, - for the diagnosis of tuberculosis with rapid molecular-genetic methods: for the quick detection of resistance to antituberculosis drugs, - for the development of new antituberculosis agents. She is one of the scientists contributing to the presence of Bulgarian science on the global scene. She is an author of numerous research publications, published in weighty International editions, with IF over 40; she has a substantial number of citations - 241, with enviable participation in research projects and programmes, as well as a head. Her research metrics scores exceed the minimum national requirements of the Law for Development of the Academic Staff/requirements of the Institute of Microbiology - BAS. Based on these facts, I am convinced in voting for conferral of the academic rank “Associate Professor” to the Department of

Infectious Microbiology, Laboratory "Molecular biology of Mycobacteria", Institute of Microbiology - BAS to Chief Assist.- Prof. Violeta Valcheva Russeva.

30 August 2020

Prof. Dr Emma Keuleyan, PhD

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