



Opinion

From Assoc. Prof. Victoria Levterova, PhD
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Dissertation presented to a scientific jury formed by order
No. 581 of the Director of the National Center for Microbiology of 01. 12. 2023, for awarding
the educational and scientific degree "DOCTOR" in the scientific specialty "Microbiology" -
code 01.06.12. In the field of higher education 4. "Natural Sciences, Mathematics and
Informatics". Professional field 4.3. "Biological sciences"

Dissertation Topic: " Study on the genomic features associated with drug resistance
(resistome) and virulence (virulome) of extensively-resistant *Pseudomonas* spp."

Author of the thesis: **Ivan Ivanov Stoykov**, PhD student at the Department of Microbiology
of the National Center for Infectious and Parasitic Diseases.

Scientific supervisor: **assoc. Ivan Ivanov, PhD Scientific consultant. Prof. Stefana
Sabcheva**

I declare that I have no conflict of interest according to the Act for the Development of the
Academic Staff in the Republic of Bulgaria.

The opinion has been prepared in accordance with the requirements of the Act for the
Development of the Academic Staff in the Republic of Bulgaria and the the regulations for the
Terms and Conditions for Acquisition of Academic Degrees and Holding of Academic
Positions in the National Center for Infectious and Parasitic Diseases.

The submitted documents are in accordance with the instructions published in the
Regulations for the implementation of the Act for the Development of the Academic Staff in
the Republic of Bulgaria and the internal Regulations of the National Center for Infectious and
Parasitic Diseases.

The dissertation " Study on the genomic features associated with drug resistance (resistome)
and virulence (virulome) of extensively-resistant *Pseudomonas* spp.." by **Ivan Ivanov
Stoykov** submitted to me for official defense gives me the reason to formulate my review as
follows:

Topical relevance

As is well known, bacterial infections remain a major public health problem

worldwide. Recently, there has been a trend towards an increasing incidence and prevalence of cases with difficult-to-treat, polyresistant bacterial strains. Over the last decade, antimicrobial resistance has been a major challenge and problem worldwide.

A key strategy for limiting the development and spread of resistance to the most commonly used groups of antibiotics is the rapid identification of resistant pathogens, and the introduction of surveillance programmes to monitor changes and trends in antibiotic susceptibility among clinically important microorganisms.

In order to understand and track the evolution of this problem in medicine worldwide, modern molecular epidemiological studies are needed.

Dissertation for **Ivan Ivanov Stoykov**, includes these modern molecular epidemiological studies. The main focus of the work is on the molecular genetic characterization of the resistome and virome in *Pseudomonas* spp. using these methods such as whole genome sequencing, bioinformatic analyses and gene expression studies. This issue is of utmost importance, especially considering the increasing emergence of multi-drug-resistant (MDR), extensively drug-resistant (XDR) and pan-resistant (PDR) strains of *Pseudomonas* spp. causing difficult-to-treat infections. The problem is very topical, important and of great economic and social importance, which determines the thesis topic's relevance and offers opportunities for original and applied contributions.

Knowledge of the problem

The dissertation is constructed in a traditional form with relevant sections - introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusion, well drawn conclusions and scientific contributions, list of publications and participation in scientific events related to the dissertation and literature. It is written in 233 standard pages (including appendices) and illustrated with 31 figures (35 including sub-figures) and 20 tables (36 including sub-tables).

The bibliography includes 680 literary sources. All are in Latin, 14% of the sources are from the last 5 years and 30% of the sources are from the last 10 years, which underlines its relevance.

It includes foreign and Bulgarian authors. All of them fully correspond to the set goal and objectives.

Based on the literature sources used in the dissertation, the doctoral student has conducted a comprehensive **literature review** of 63 pages.

The material that is included in the review is proof that **Ivan Stoykov** knows the

problem well. The literature review is written informatively and presents a characterization of the river. *Pseudomonas*, and presents an overview of the methods for taxonomic identification and characterization. Mainly the literature review focuses on *P. aeruginosa* which is of greatest clinical relevance. The PhD student has presented in detail its genetic characteristics - general organization of the genome, review of genetic determinants of resistome and virology that have been found in representatives of the species. Based on this in-depth analysis, the aim of this dissertation is to investigate the genomic characteristics underlying drug resistance (resistome) and virulence (virology) in extensively drug-resistant *Pseudomonas* spp..

On the basis of the set objective, 5 tasks are formulated, which outline the specific steps to fulfill the objective of the development.

The Materials and Methods section is developed in 39 pages and illustrated with 1 figure and 31 tables. It is informative and methodologically sound.

A total of 100 MDR isolates of *Pseudomonas* were tested, of which the majority (96) were *P. Aeruginosa*, which are part of the collection of the National Reference Laboratory for "Control and Monitoring of Antibiotic Resistance" at the National Center for Infectious and Parasitic Diseases.

For the realization of the set tasks, as is evident from the section, the PhD student has mastered and applied the appropriate methods. In the development, both classical microbiological methods were used in testing the antimicrobial susceptibility of the strains under study, as well as many molecular and bioinformatic methods, which are described in great detail in the section. A good level and methodological preparedness is evident, with a wide variety of methodologies used.

Important and substantial results have been achieved. The description of the results is accurate, clear and done in good scientific language.

The results are presented in several sections, following the sequence of the set tasks. In summary, the "Results and Discussion" section is competently written in 64 pages, illustrated and documented very well with 32 figures and 7 tables. The PhD student's global knowledge of the problem is evident.

The conclusions are correctly formulated and summarize the main highlights of the work. The dissertation presents six original contributions and five applied contributions with which I fully agree.

The dissertation **results** have been published in 4 articles in refereed scientific

journals, both with Impact Factor (total impact factor: 6.17). In three of these publications, Ivan Stoykov is the first author, which shows the personal contribution and main participation in their realization.

The results of the research have been presented at five scientific forums, one of which is international, and in three of them, the PhD student is the first author.

The submitted **abstract** complies with the requirements, fully reflecting the content of the thesis. It is written on 128 pages and illustrated with 31 figures and 37 tables. It ends with a brief summary of the thesis in English.

In conclusion, the dissertation work of Ivan Stoykov reflects a high professional level of the research carried out, it is innovative and of scientific and applied importance.

The documents submitted by the candidate cover all the criteria according to the Act for the Development of the Academic Staff in the Republic of Bulgaria and the National Center for Infectious and Parasitic Diseases requirements for the acquisition of the educational and scientific degree "Doctor".

Ivan Ivanov Stoykov meets the mandatory and specific conditions and scientometric criteria for the scientific degree "Doctor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria and the National Center for Infectious and Parasitic Diseases requirements.

I confidently give my positive assessment and recommend to the respected Scientific Jury to award the educational scientific degree "Doctor" to **Ivan Ivanov Stoykov** in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", direction 4.3 "Biological Sciences", doctoral program "Microbiology".

01.02.2024г.

Assoc. Prof. Victoria Levterova, PhD