



## Opinion

of the PhD thesis on the topic:

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

*for the award of the educational and scientific degree "Doctor of Philosophy" in scientific field 4.3. "Biological Sciences"*

**PhD Student:** *Ivan Ivanov Stoikov*, National Reference Laboratory for Control and Monitoring of Antimicrobial Resistance, National Center of Infectious and Parasitic Diseases, with **scientific supervisors Assoc. Prof. Ivan Ivanov and scientific consultant Prof. Stefana Sabcheva**

**Prepared by:** *Assoc. Svetoslav Gueorguiev Dimov*, Head of the Department "Genetics", Faculty of Biology, Sofia University "St. Kliment Ohridski"

**Scientific relevance of the PhD thesis.** The thesis work submitted for assessment is focused on a highly topical problem related to the molecular genetic characterization of resistance and virulence in *Pseudomonas* spp., carried out using whole-genome sequencing, bioinformatics analysis and gene expression studies. This problem is of extreme importance not only from a theoretical aspect but also from a purely clinical one, especially in light of the increasingly frequent appearance of this genus's MDR, XDR and PDR strains.

**General structure of the PhD thesis.** The thesis is developed within 180 pages (without the list of used literature). It is structured in a standard way, including the following main sections: literature review, materials and methods, and results and discussion (united in a common section). Introduction, aim and objectives, conclusion, conclusions and contributions are presented separately. Finally, the Ph.D. thesis finishes with an impressive bibliographic reference list covering 680 sources, most of which are from recent years.

The thesis begins with a short **abstract** of one and a half pages, in which the interest in the topic of the thesis is succinctly justified.

**"The Literature Review"** is developed within 63 pages. It is logically structured and is focused on the taxonomic characterization of the *Pseudomonas* spp. It begins with a general description of the genus and an overview of taxonomic determination and characterization methods. Following are brief presentations of the species with more limited clinical significance - *P. fluorescens* and *P. putida*. The literature review mainly focuses on the representative of the genus with the most significant clinical significance – *P. aeruginosa*. After the presentation of the morphological, biochemical, and physiological characteristics and the role of the species in nosocomial infections, its genetic characteristics are presented in detail - general organization of the genome, an overview of the genetic determinants of resistance and virulence, which were found in members of the genus. The section does not contain figures and tables. From the presentation of this part of the Ph.D. thesis work, I am convinced that the Ph.D. student has a wide scientific knowledge of the thesis topic and is well acquainted with the latest worldwide research in this field. It also says that he can skillfully interpret scientific literature from various sources and write highly scientifically. As a minimal omission, I consider that it was not omitted to present briefly the *P. kurun-egalensis*, *P. soli* and *P. protegens* found in the study, as was done for *P. fluorescens* and *P. putida*.

**"Objectives and Tasks."** One goal was formulated for the Ph.D. thesis: a genetic study of resistance and virulence in XDR strains of *Pseudomonas* spp. For its realization, five experimental tasks have been set (one of which is divided into three sub-tasks), which are entirely sufficient for its achievement and, at the same time, cover a wide range of experimental work and data interpretation, the mastery of which is necessary for the acquisition of a Ph.D. degree.

**"Materials and Methods."** The section is developed within 39 pages. Classical microbiological techniques include techniques for phenotypic identification, determination of susceptibility to various antibiotics and detection of resistance to carbapenems. Molecular genetic techniques include DNA and RNA isolation methods, different polymerase chain reaction variants, different types of electrophoresis and hybridization techniques, fragment cloning

methods, fragment sequencing techniques and whole-genome sequencing, methods for transformation, conjugative plasmid transfer techniques and a wide range of bioinformatics methods. The methods and techniques are presented in sufficient detail and a reproducible manner. The section is illustrated with 1 figure with good graphic quality and 31 tables. My sole comment on this section is that it could be structured slightly more streamlined regarding bioinformatic methods, as they are contained in other sub-sections than the 'Bioinformatics methods' sub-section.

**"The Results and Discussion"** section is developed over 65 pages. Within it, the Ph.D. student's experimental results are presented in a compact form and with the help of rich illustrative material - 7 tables and 32 figures with sufficiently good graphic quality. The section presents and analyzes the results of the phenotypic characterization and taxonomic identification of a collection of 100 *Pseudomonas* clinical isolates strains from different hospitals, mainly *P. aeruginosa*, the development, optimization and validation of a new method for RNA isolation for the needs of the study, checking for the presence of carbapenemases, studying of the expression of genes determining resistance and virulence by molecular genetic techniques, genotyping, plasmid typing, conjugation experiments and bioinformatic analyses. In general, the scientific language in which this section is written and the content of the presentation in it speak convincingly that the Ph.D. student skillfully analyzes and interprets his own experimental data, comparing them with data from other scientific groups, which are correctly cited. This skill is a testament to his maturity as an accomplished scientist, not just a skilled experimenter.

The thesis exposition ends with a short section entitled **"Summary."** It is developed over three and a half pages, concisely summarizing the work carried out and the results obtained.

**"Conclusions"**. A total of 6 conclusions were formulated, which sufficiently synthesize the performed experimental work and analyses of own experimental data. A good impression is made because no contributions were formulated as conclusions.

Within the "**Contributions**" section, six contributions of an original nature and five more of a scientifically applied nature are formulated. Again, a good impression is made because no conclusions are formulated as contributions.

**Abstract.** The presented abstract is written within 128 pages and repeats the Ph.D. thesis in an abbreviated form. It contains the main sections „Introduction, “ „Materials and Methods, “ and „Results and Discussion “. Additionally, "aim and objectives," "conclusions," and "contributions" are presented. Finally, it ends with a summary of the Ph.D. thesis work in English. It is illustrated with 31 figures and 37 tables. In my opinion, the volume of the referee exceeds the generally accepted norms.

**General remarks on the PhD thesis.** The work presented for consideration gives a good impression of a complete comprehensive scientific study on an actual problem, carried out with some of the most modern methods in molecular genetics. The overall impression I am left with is that the Ph.D. student is an accomplished and promising young scientist who is capable of carrying out a complete scientific investigation on a specific topic, as well as using a wide range of laboratory techniques and methods and adequately interpreting and justifying the results obtained by him. My only remark is that, although the Ph.D. thesis is written in a highly scientific language, it contains foreign words that have a scientific translation in Bulgarian, like "protein," "reads," and some others, as well as sometimes English sentence structure like for example "PCR screening." I would also like to draw your attention to the fact that the size of the bibliographic reference significantly exceeds the generally accepted norms for a Ph.D. thesis. However, I must point out that my remarks in no way diminish the scientific merits, and my overall impression of the Ph.D. thesis work is excellent.

**Scientific publications and contributions related to the PhD thesis.** A total of 4 scientific publications in international high-impact and refereed specialized journals are presented. In three of the publications, the Ph.D. student is the first author. The total score based on the quartiles of the journals is 59, which is twice that required 30 points in Professional Direction 4.3. "Biological Sciences" according to the Regulations for the Implementation of the

Law on the Development of the Academic Staff in the Republic of Bulgaria. A total of 6 participations in international and national scientific forums are also presented.

**CONCLUSION.** The work presented to me for consideration represents a complete and complete scientific research at a high level. Therefore, I will vote positively for awarding the educational and scientific degree "Doctor" to Ivan Ivanov Stoykov, and I will warmly recommend it to the colleagues of the Scientific Jury to do the same.

Sofia, January 28, 2024

Svetoslav  
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