



*To the members of the scientific jury,
determined by order no. 439 of 20.12.2025 of
the Director of the National Center for
Infectious and Parasitic Diseases*

OPINION

FROM Prof. Dr. Svetla Trifonova Danova, DSc.

Institute of Microbiology, "Stefan Angelov" – Bulgarian Academy of Science

ABOUT dissertation work, titled: "**Metagenomic studies on the determinants of antibiotic resistance in model environmental samples**" submitted for the educational and scientific degree "Doctor" in the field of higher education: 4. *Natural Sciences, Mathematics and Informatics*, in the professional field 4.3 *Biological Sciences*

Author of the dissertation work: **Deyan Valentinov Donchev**

Scientific supervisor: Assoc. **Prof. Ivan Ivanov, PhD**

1. According to the procedure:

On the basis of Art. 31 of the Regulations for the implementation of the ZRASRB of the National Center for Communicable and Parasitic Diseases in connection with Art. 9 of the ZRASRB and the decision of the National Council of the NCIPD, Protocol No. 04/17.12.2024 and Order No. 439 of 20.12.2024 of the Director, I am elected as a member of the scientific jury in the procedure for awarding the ONS Doctor, in professional field 4.3 Biological Sciences to full-time doctoral student Deyan Donchev.

I declare that I have no common publications or any conflict of interest of any other nature with the candidate within the meaning of para. 1, items 3 and 5 of the ZRASRB.

2. Relevance and significance of the dissertation topic:

The dissertation submitted for review is a modern and innovative scientific study of the distribution and type of antimicrobial resistance (AMR) determinants in the environment in order to assess the potential risk to human health. A study provoked by the growing antibiotic resistance and the major public health crisis as a result of the battle with SARS-CoV-2, which led to 6.6 million deaths due to coronavirus disease 2019 (COVID-19). As a result of one of the most severe pandemics in human history, an indisputable fact is the increased use of antibiotics and increasing resistance. The search for approaches to its assessment and prevention of its spread are problems of not only scientific but also social importance. In Bulgaria, there is no active monitoring of antimicrobial resistance (AMR) in the environment and in the future it is necessary to adopt such measures. All this gives me reason to assess the scientific work submitted for evaluation as extremely relevant.

3. Assessment of the structure and content of the dissertation work

The dissertation is presented on 200 standard pages of text. The generally accepted scheme and the recommended ratios between the individual parts of the work are observed, as follows: *Introduction* – 3 pages, *Literature review* – 63 pages, *Aim and objectives* – 1 page,

Materials and methods – 18 pages, *Results and discussion* – 56 pages, *Implications*-1 page, *Contributions* - 2 pages; *Conclusion* - 4 pages. *Appendix* – 4 pages. *Cited and used sources* – 32 pages. The bibliography includes an impressive 540 titles, in Latin, even for a large doctoral thesis. All articles are from the last 10 years, which shows excellent theoretical awareness of the problem developed by the doctoral student.

The dissertation is technically very well-designed and richly illustrated with 30 figures and 20 tables. The correct expression, the grammatically sound and concise scientific style used in the writing and the overall layout of the work make an excellent impression.

3.1. Literature review

The literature review is specific, very-well structured, following the logical connection of the information. Genomics, Metagenomics with their basics and Metagenomic studies, and bioinformatics analysis have been consistently reviewed, as well as all modern aspects of the problem of Antimicrobial Resistance (Antibiotics, antimicrobial resistance, basic mechanisms of AMR - basic concepts, the status of AMR, reaching methods for research and monitoring of AMR in the environment). Thus, the clearly formulated and logically derived goal has been reached. The modern molecular genetic and metagenomic approaches for monitoring microbial communities and environmental samples have been reviewed in a very systematic and erudite manner.

The review shows the excellent theoretical preparation of the doctoral student and knowledge of the problems he is working on. The knowledge of the current European Directives for monitoring AMR and the justified need for such development in our country are impressive.

3.2. Aim and objectives of the dissertation

The aim is specific and logically follows from the data presented in the review: "To study the distribution and type of antimicrobial resistance determinants, mobile genetic elements and bacterial biodiversity in environmental samples and the potential risk to human health". To achieve this aim, 8 experimental tasks have been defined, which predetermine a voluminous research work.

3.3. Materials and methods

The doctoral student has selected for study samples from habitats with different levels of microbial contamination. Sampling is according to the standards of accredited control, which predetermines an objective assessment of the microbiota. The coordinates are precisely marked, according to the requirements of the Nagoya Protocol. A wide range of modern molecular genetic methods, correctly selected and adequate to achieve the goal of the dissertation work, has been used. They are described precisely, fully covering the various aspects of the study, guaranteeing reliable and significant scientific results. The wonderfully designed experimental schemes and the systematized presentation of the molecular approaches are impressive. I appreciate the author's approach for comparative parallel analysis of the specific sample, in the stages at which methodological approaches are developed/adapted. E.g. when solving task 3: "Testing the method *Flocculation with skimmed milk for concentrating bacterial biomass from water samples by comparative analysis versus vacuum filtration*".

3.4. Results and discussion

The dissertation is based on scientific evidence that the study of AMR is possible only through a comprehensive metagenomic study. A significant volume and diverse experimental work was carried out within the framework of a complex genetic and bioinformatics study, based on a set of 4 integral samples taken from habitats with different intensities of microbial contamination - Iskar River, groundwater around the city of Stara Zagora, WWTP, Stara Zagora and WWTP, poultry slaughterhouse, subdivided into their individual elements. Deyan Donchev correctly assessed the need to obtain sufficient and high-quality isolated DNA and therefore the initial stage of the work is the optimization and adaptation of an in-house protocol for DNA isolation. The level of preparedness of the Deyan Donchev is also shown by the fact that when optimizing the protocols, attention was paid even to the residual DNA in the DNA polymerase enzyme in commercial amplification kits. I.e. In this and the following stages of genetic analyses, all elements that may affect the objective results have been taken into account. Deyan follows a well-structured and methodically secured experimental scheme, as the results obtained in the previous task determine the setting of the subsequent experiments and bioinformatics analyses. I highly appreciate the solutions and implementation of each of the tasks. Both model bacteria with difficult-to-lyse cell walls and the experimental protocols used have been correctly selected. The results are presented correctly and analytically and are discussed in the light of data from recent years. The results obtained from the comparative study of mixed methanogenic consortia are original. The results of the evaluation of the results of the detection of antibiotic resistance genes in water from Treatment Plants have been discussed very critically. The doctoral student correctly assesses the bioinformatics algorithms used and the need for a standardized methodology for objective assessment of health risk. This proves that the he is a well-rounded molecular biologist with excellent knowledge in bioinformatics and genetic analyses, seeking original solutions to complex experimental problems.

3.5. Conclusions and contributions

The dissertation represents a large-scale and targeted modern molecular-genetic and bioinformatics study from which original results of scientific and applied significance were obtained. They are summarized in 7 conclusions and 5 contributions, which reflect the fulfillment of all the tasks set. The doctoral student has correctly divided them into contributions of a fundamental nature and of a scientifically applied nature - a total of 6 and 2 of a methodological nature. I accept the original contributions and would like to emphasize that this is the first metagenomic study that provides an assessment of the effect of the treatment plant on the resistome, mobilome and microbiome in the Iskar River, a major freshwater source of drinking water for the Sofia region of great economic importance.

3.6. Recommendations, notes and questions:

In the dissertation work of Deyan Donchev, important scientific results have been obtained and have contributed to subsequent metagenomic studies. The results that have been reported for the first time, which are an indisputable merit of the work, can be more boldly summarized. In this regard, I would like to ask the doctoral student:

1. Why do you label your work as a metagenomic study in model environmental samples, when real habitats have been systematically and in an original scheme studied, with key importance for the ecology of the regions?

2. How can these important results be used to assess health risks and to which institutions should they be addressed, in addition to the scientific community?

4. Publications related to the dissertation work

The results have been included in 2 publications, in international refereed scientific journals, with a high impact factor and have been reported at two scientific forums, in Bulgaria and in Germany respectively. Deyan Donchev is the first author in both publications, which gives me reason to conclude that the results are the personal work of the doctoral student and the scientific community is familiar with them.

5. Conclusion:

In conclusion, I would like to emphasize that the material is dissertable. The topic is extremely relevant, the doctoral student has mastered a wide range of modern molecular genetic and bioinformatics methods, the experiments are methodically correct, the results obtained are reliable and are the basis for further scientific and applied developments. Original scientific and applied contributions stand out.

Based on the above, assessing the merits of the development and full compliance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of the NCIPD for the Development of the Academic Staff, I give my excellent assessment, on the basis of which I would like to propose to the esteemed scientific jury to award the educational and scientific degree "Doctor" to the doctoral student **Deyan Vladimirov Donchev**.

Reviewer:.....
(Prof. Dr. Svetla Danova, PhD)