Statement

By Assoc. Prof. Victoria Stefanova Levterova, PhD,

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On a dissertation presented to a scientific jury formed by order 439/20.12.2024 of the Director of NCIPD, for awarding of the educational and scientific degree "DOCTOR" in the Professional field: 4.3. "Biological sciences", Doctoral Programme in "Microbiology"

Dissertation topic: 'Metagenomic studies on determinants of antibiotic resistance in model environmental samples'

Dissertation author: Deyan Valentinov Donchev, National Reference Laboratory Control and Monitoring of Antimicrobial Resistance" (NRL CMAR), Department of Microbiology of the NCIPD.

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

I declare that I have no conflict of interest within the meaning of Article 4, paragraph 5 of the Law on the Development of the Academic Staff of the Republic of Bulgaria (LPASB). I have no joint publications with Deyan Donchev.

The documents submitted under the procedure fully comply with the requirements of the LPASB and the Regulations for the Implementation of the Law on the Development of Academic Staff of the Republic of Bulgaria, for the award of the degree of Doctor of Education and Science of the National Centre for Academic and Scientific Research, Sofia.

The dissertation thesis 'Metagenomic studies on determinants of antibiotic resistance in model environmental samples' by Deyan Valentinov Donchev, submitted to me for official statement, gives me the grounds to formulate my opinion as follows:

Relevance of the topic

The topic of Deyan Donchev's dissertation is extremely urgent due to the growing threat of antimicrobial resistance worldwide. The World Health Organization identifies the increasing antimicrobial resistance as one of the most serious challenges for society. This necessitates strong measures to monitor and control the use of antibiotics worldwide.

Deyan Donchev's dissertation has presented an extremely important problem worldwide, namely the study of the presence of antimicrobial resistance determinants in the environment. Recently, the environment has been recognized as a reservoir and mediator in the spread of antibiotic resistance genes. In Bulgaria, studies on the spread of antimicrobial resistance in the environment have been lacking. This pilot comprehensive study makes the dissertation work of Deyan Donchev relevant, innovative and significant.

The dissertation work of Deyan Valentinov Donchev includes modern molecular studies, namely next-generation sequencing, which allows to overcome the shortcomings of classical microbiological methods. The main focus of the work is the use of metagenomic studies for the analysis of microbial communities and antibiotic resistance genes, which provides a modern approach and allows detailed characterization of bacterial composition and resistance mechanisms.

The problem is relevant, significant and of great economical and social importance, which determines the dissertation's suitability and offers opportunities for original and applied contributions.

Evaluation of the structure and content of the dissertation

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 references. It is written in 202 standard pages (including appendices) and is illustrated extremely well with 30 figures and 20 tables.

The bibliography includes 540 references. All are in Latin, and most of the sources presented are from the last 5 years, underlining its current relevance. All of them fully correspond to the stated aim and objectives.

Based on the literature sources used in the dissertation, the PhD student has conducted a comprehensive literature review of 66 pages.

The material that is included in the review is proof that Deyan Donchev, has a good knowledge of the problem. The literature review is written informatively and presents the essence of metagenomics, antimicrobial resistance and bioinformatic analysis methods.

The PhD student discusses in detail the applications of metagenomic studies as well as the two main approaches, amplicon-based metagenomics and whole metagenomic sequencing, and he points out their advantages and disadvantages. The bioinformatic processing of metagenomic sequencing data is also thoroughly discussed.

The PhD student's good knowledge and skills in the field of metagenomic research are impressive.

Based on this in-depth analysis, the aim of this thesis is to investigate the prevalence and type of determinants of antimicrobial resistance, mobile genetic elements and bacterial biodiversity in environmental samples and the potential risk to human health.

On the basis of the stated aim, 8 objectives were formulated, which outline the specific steps to be taken to fulfil the aim of the study.

The Materials and Methods section is developed in 19 pages and illustrated with two figures and nine tables. It is informative and methodologically sound. The methods used are described in detail.

The student has mastered and applied the appropriate methods to realize the set tasks. In the development, both collection and processing of samples, isolation of total DNA from different water sources, sequencing and a range of bioinformatic methods were used. A good level and methodological sophistication is evident, using a wide variety of methodologies.

The results are presented in several sections based on the different study sites (wastewater, rivers and groundwater), following the sequence of the set tasks. In summary, the "Results and Discussion" section is competently written in 62 pages, illustrated and documented very well with 5 tables and 24 figures. Important and significant results have been achieved. The description of the results is accurate, clear and done in good scientific language.

The PhD student's global knowledge of the problem is evident and he has the necessary knowledge and skills to implement and interpret the results obtained, analyzing and comparing them with other scientific studies.

There are 7 conclusions, which are correctly formulated and summarize the main highlights of the work.

The dissertation presents eight contributions. One of a fundamental nature, 5 scientifically applied and 2 as methodological with which I fully agree.

The dissertation results have been published in 2 articles in refereed scientific journals, and they have a total impact factor of 9, one scientific publication with quartile Q1, and the other with quartile Q2. In both publications, the PhD student is the first author, which shows the personal contribution and main involvement in their realization.

The results of the research have been presented at two scientific forums, one of which is international.

The submitted abstract of the dissertation complies with the requirements, fully reflecting the content of the thesis. It is written on 78 pages and illustrated with 22 figures and 13 tables. It concludes with a brief summary of the thesis in English.

Conclusion

In conclusion, Deyan Donchev's dissertation reflects a high professional level of research, it is innovative and of scientific and applied importance.

In view of the above, I believe that the dissertation fully meets the requirements of the LPASB of Bulgaria and the Regulations for its implementation and the local Regulations of the National Center of Infectious and Parasitic Diseases for granting of the educational and scientific degree "Doctor". It also collects and exceeds the points required under the Regulations for the Implementation of the LPASB.

I give my positive assessment and support the award of the degree of Doctor of Education and Science in the scientific specialty of Microbiology to **Deyan Valentinov Donchev**.

Assoc. Prof. Victoria Levterova, PhD