

OPINION

by Prof. Dr. Iliya Nikolov Iliev,

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Regarding: the dissertation titled "Drug Sensitivity and Factors of Colonization and Invasiveness in Nasopharyngeal Isolates of Staphylococcus spp." submitted by PhD student Lena Rumenova Ilieva from the Department of Biochemistry and Microbiology at Paisii Hilendarski University for the attainment of the educational and scientific degree "DOCTOR" in the professional field within the area of higher education 4. Natural Sciences, Mathematics, and Informatics; professional field 4.3 Biological Sciences; Doctoral Program: Microbiology.

Received materials: Dissertation, abstract, list of publications related to the dissertation, CD with materials for the dissertation.

By order No. R-22-1084 /29.05.2026 of the Rector of PU "Paisii Hilendarski", I have been appointed as a member of the scientific jury for the defense of a dissertation for obtaining the educational and scientific degree "Doctor" by PhD student Lena Rumenova Ilieva on the topic "Drug sensitivity and factors of colonization and invasiveness in nasopharyngeal Staphylococcus spp. isolates." The experimental work for the dissertation was conducted in the Department of Biochemistry and Microbiology at Plovdiv University "Paisii Hilendarski" and in the clinical microbiology laboratory "Sinevo". The doctoral student is supervised by Prof. Dr. Sonya Kostadinova, a specialist whose research focuses on factors of pathogenicity and antibiotic resistance in pathogenic microorganisms. The dissertation follows the standard model in Bulgaria, is written on 207 standard typed pages, and includes the following main chapters: Introduction – 1 page, Literature Review – 31 pages, Aim and Objectives – 1 page, Materials and Methods – 14 pages, Results and Discussion – 85 pages, Conclusion – 3 pages, Findings – 2 pages, References – 19 pages, Contributions – 2 pages, Appendices – 40 pages. The correct scientific style used in writing and organizing the dissertation stands out. The results are illustrated with 33 figures, 10 tables, and 8 appendices.

1. Relevance and significance of the dissertation topic

The development of microbiological science today is undergoing significant progress thanks, on one hand, to scientific advances in studying the human microbiome as a whole, and on the other hand, to the introduction of many new methods and approaches for analysis in practice, covering the concept of an interdisciplinary approach to solving important health problems. In this context, clinical microbiological practice must address issues related to the antibiotic resistance of staphylococcal bacteria, overcoming the factors of invasiveness and colonization in the human body through the lens of the human microbiome and the introduction of modern molecular-biological, immunological, and biochemical methods for conducting rapid screening and taxonomy of isolated strains. These are just some of the proofs of the relevance of the chosen dissertation topic, which covers both applied scientific and academic research.

2. Evaluation of the Structure of the Dissertation

The dissertation is structured in a traditional format with the corresponding sections: introduction, literature review, objectives and tasks, materials and methods, results and discussion, conclusion, findings, contributions, references, appendices. It is written on 207 standard computer pages. The dissertation contains 10 tables and 33 figures. The bibliography includes 377 titles.

2.1. Literature Review

The literature review presents the state of the problem and demonstrates the need to develop the dissertation thesis. A large amount of scientific information has been analyzed and summarized, showing the very strong theoretical preparation of the doctoral candidate. It can be divided into two main parts. The review covers issues related to the taxonomy of *Staphylococcus* bacteria, as well as questions related to their isolation and identification. The second part looks at some new strategies for control and new therapeutic approaches, including a completely new area of postbiotics as a potential avenue for preventing *Staphylococcus* infections.

2.2 Purpose of the Dissertation

The aim of this dissertation is to assess the role and significance of *Staphylococcus aureus* in the cause of symptomatic upper respiratory tract infections and to describe its pathobiological potential in relation to ways to limit its development.

In my opinion, the aim of the dissertation could be made more specific by focusing on a study of samples from the Bulgarian population and ecological niche. Five tasks have been formulated, which logically follow one another to prove the main hypothesis of the PhD candidate.

2.3. Materials and Methods

The 'Materials and Methods' section demonstrates an impressive set of techniques tailored to the specific requirements of the experiment. They include both routine and modern methods, covering microbiological, biochemical, molecular biology, bioinformatics analysis, and more. They are described in enough detail to be reproducible. In addition, they are clear and allow for proper execution of the experiments and obtaining reliable results.

2.4. Results and Discussion

The 'Results and Discussion' section includes experimental material, which is presented on 85 pages and demonstrates large-scale research activity. It is important to note that the study is characterized by logical consistency, with each stage being thoroughly developed and each having its significance in proving the scientific hypothesis of PhD candidate Lena Ilieva. It should also be emphasized that the dissertation is written in a very good scientific style and accurately reflects the obtained results. The supporting material is professionally presented in 10 tables and 33 figures. All these figures and tables once again demonstrate the extensive scope of the study.

The author studies the structure of nasopharyngeal infections over a five-year period (2016-2020), processing more than 27,000 clinical samples at the 'Synevo' laboratory, mostly from the Plovdiv region. An analysis of the patients' age structure was also performed, showing a clear dominance of the 0-10 age group, which makes up two-thirds of all samples and nearly 60% of throat swabs. This analysis is relevant for developing methods and therapeutic approaches for this specific target group, which has its own physiological and psychological characteristics.

Additionally, the seasonal dynamics of the infections were analyzed, as an important factor in their spread. Regarding these studies, I have one specific suggestion: the focus should be on the ethics committee protocol for working with human clinical material, which is necessary in such research. I'm aware that most of the work was done in a registered clinical laboratory, but some of the research with clinical samples was conducted in microbiology laboratories in Department of Biochemistry & Microbiology.

The research work continues with the phenotypic identification of the isolates, where 776 strains were processed and it was proven that 97% of them are *S. aureus*. A correlation analysis was also carried out, but the results do not show the presence of any correlation dependencies. The species identification is shown in Fig. 5, which clearly outlines the short protocol for identifying *S. aureus* specifically. In subsequent studies, the author used two techniques for genotypic identification: 16S rRNA gene sequencing and whole genome sequencing. Here, a practical approach should be taken to assess whether, in the case of clinical practice, it is already necessary to routinely use whole genome sequencing.

An important stage in Lena Ilieva's scientific research is tracking the antibiotic resistance of the isolated staphylococcal strains. The results have both scientific and practical significance. The PhD student receives confirmations regarding antimicrobial agents. Lena Ilieva deepens her research on antimicrobial resistance by conducting an in silico analysis of the resistome of *S. aureus*, as well as an analysis of the correlation between genotype and phenotype in WGS-characterized strains. The next research task that the PhD student undertakes is the genetic characterization of the virulence potential of newly isolated *S. aureus* strains.

A new aspect of the current study is the development of a strategy for using LAB to control the spread of *S. aureus*. Strains of LAB have been sequentially isolated and identified, and they have been characterized for their probiotic potential. The study also looked at their secretion of substances with antimicrobial effects against *S. aureus*. Lena Ilieva was prompted to investigate the production of postbiotic substances that inhibit staphylococcal strains. I consider this research a novelty in the field of staphylococcus studies, with potential for future applied use.

In this aspect of the research, I would recommend comparing the data on the new MCB strains with established probiotic strains using experimental data in the specific studies of the dissertation. This is important not only to demonstrate the probiotic and postbiotic potential but also as a strategy to protect the scientific data. Additionally, in future research on postbiotics, it would be very useful to carry out a more in-depth analysis of the component composition of the postbiotic fraction to prove its nature and describe the mechanism of inhibitory action against staphylococci. This is generally an important requirement if the goal is to develop a final postbiotic product. As a result of the conducted studies, PhD candidate Lena Ilieva has formulated a conclusion summarizing the main results of her research.

2.5. Conclusions and Contributions

The conclusions are a logical result of the experiments conducted. In my opinion, they are formulated in accordance with what has been achieved. Regarding the conclusions, I would make the following comment: the first and second conclusions are quite general and descriptive and have a generally confirmatory nature, which makes them somewhat unnecessary in their current form. Considering the large-scale study of a significant number of samples, the doctoral candidate should show more boldness and demonstrate the significance of the research through its scale.

I accept the contributions made.

2.6. Publications Related to the Dissertation

The PhD student has published a significant part of her results in two articles in prestigious scientific journals, one in Q1 and the other in Q2. In this way, she significantly exceeds the required minimum number of points.

3. Conclusion

All of this gives me reason to believe that the PhD student Lena Rumenova Ilieva has acquired the competences and skills required to obtain the educational and scientific degree of "Doctor." I believe that the scope of this work is impressive, many analyses have been conducted, valuable results have been obtained, important conclusions have been drawn, and the research has scientific and practical contributions.

I have the following question for the PhD student:

1. On what basis did you select the twelve *Lactobacillus* strains to carry out your specific screening for postbiotic properties?

Conclusion:

My assessment of the doctoral thesis, the abstract, the scientific publications, and the scientific contributions of PhD student Lena Rumenova Ilieva is entirely positive. Despite the comments and recommendations made, I would like to note once again her merits – a meticulously conducted research work, beginning with a comprehensive study of staphylococcal infections in the oral cavity of children. The dissertation fully meets the requirements for the educational and scientific degree of "Doctor," containing a sufficient amount of results, correctly interpreted and properly formulated conclusions. Two original publications have been made in reputable scientific journals.

The submitted dissertation fully meets the requirements of the ZRASRB and its Implementation Rules. The results achieved give me reason to propose that Lena Rumenova Ilieva be awarded the educational and scientific degree of 'Doctor' in the professional field of higher education 4. Natural Sciences, Mathematics, and Informatics; professional field 4.3 Biological Sciences; doctoral program: Microbiology.

10.08.2026

Prepared the opinion:

Plovdiv

(Prof. Dr. Iliya Iliev)