

Opinion

By: Prof. Iskra Vitanova Ivanova, DBN

Regarding: evaluation of dissertation work for the acquisition of the educational and scientific degree "Doctor" in the field of higher education "Doctor", Field of Higher Education: 4. Natural Sciences, Mathematics and Informatics Professional field: 4.3. Biological Sciences; Doctoral program: Microbiology

Author of the dissertation work: Lena Rumenova Ilieva

Title of the dissertation work: Drug sensitivity and factors of colonization and invasiveness in nasopharyngeal isolates *Staphylococcus* spp

Scientific supervisor: Prof. Dr. Sonya Kostadinova Trifonova

By order No. PD-22-1084 of 26.05.2026 of the Rector of Plovdiv University "Paisiy Hilendarski" (PU) I am appointed as a member of the scientific jury.

1. RELEVANCE AND SIGNIFICANCE OF THE PROBLEM

Staphylococcus aureus causes infections in almost all tissues of the host. Several virulence characteristics contribute to its survival in different locations, including the ability to produce a myriad of toxins, adhesins, and immunomodulatory enzymes. *S. aureus* also possesses remarkable metabolic plasticity, which allows it to overcome host-imposed constraints. The transition to infection depends on the combined action of bacterial and host factors, with adhesins, immune evasion factors, cytolytic toxins, exoenzymes, and the ability to form a biofilm being key on the part of the microorganism. Upper respiratory tract infections continue to occupy a significant place in the structure of infectious morbidity due to their widespread distribution, significant clinical burden, tendency to relapse and the need for precise interpretation of microbiological results. In this context, *S. aureus* occupies a special place, since its presence in the upper respiratory tract requires a distinction between carriage, colonization and actual participation in the infectious process. All this gives me reason to assess the presented scientific work as up-to-date, with the potential for scientific achievements that can have a quick practical implementation.

The materials presented by the doctoral student fully meet the requirements for the defense of the doctoral thesis and present the specific evidentiary part regarding the required criteria, as well as present the overall production of the candidate. The dissertation is presented on 207 standard pages of text. The generally accepted scheme and the recommended ratios between the individual parts of the work have been observed, as follows: Introduction – 1 page; Literature review – 31 pages; Aim and objectives – 1 page; Materials and methods – 13 pages; Results and discussion – 90 pages; Conclusions 2 pages. The results obtained are illustrated with 33 figures, 10 tables, 8 appendices, 377 literary sources have been used.

2. LITERARY AWARENESS AND STATEMENT OF THE GOAL AND OBJECTIVES

The doctoral student has made a comprehensive review of the achievements of other researchers, which she was able to convey and analyse on 36 pages in the literature review. The review presents the state of the problem in detail and proves the need for development. The literature review is specific, structured correctly, and follows the logical connection of the information. The data from the review served to clearly and correctly define not only the goal, but also the formulation of the tasks. The literature (both in the overview and in the entire work) is closely related to the topic of the dissertation. The literature list includes an impressive number of 377 titles in Latin. They are mainly from recent years. This speaks of the excellent theoretical awareness of the doctoral student and the aim of finding a new scientific challenge.

3. EVALUATION OF THE METHODS AND MATERIALS USED

The aim of this dissertation is to assess the role and significance of *Staphylococcus aureus* in the etiological structure of symptomatic upper respiratory tract infections and to characterize its pathobiological potential in relation to the possibilities for limiting its development. 5 well-founded experimental tasks have been set for solution. The "Materials and Methods" section demonstrates an impressive set of methods, tailored to the specific requirements of the experiments. They are modern and adequate for the implementation of the dissertation.

They are described accurately and in detail, fully covering the multifaceted areas of work: from classical to modern research. Microbiological and biochemical methods were used. Complete genome sequencing and in silico analysis, PCR analysis of virulence-associated genes, genomic and in vitro characterization of the selected MCDs, gas chromatography analysis, confocal laser scanning microscopy and scanning electron microscopy were performed.

All this allows me to give high marks to the scientific level and the excellent preparation, which manages to correctly combine a variety of classical and modern methods for the purposes of the dissertation, successfully solving the experimental tasks set.

The doctoral student applies a combined phenotypic, biochemical and molecular approach to confirm the affiliation of the analysed collection to *S. aureus* and which allows it to be used as a model for the basis of modern diagnostic approaches. *Staphylococcus aureus* occupies a stable and significant place in the etiological structure of symptomatic upper respiratory tract infections. Determination of the species affiliation of the isolated strains has created a solid basis for the interpretation of antibiotic resistance, virulence potential and biofilm formation.

4. EVALUATION OF THE OBTAINED RESULTS

The section "Results and Discussion" is well structured, supported by tabular and graphical material, with an appropriate interpretation of the results obtained by foreign scientific teams. The author consistently presents evidence for her scientific thesis, thus logically finalizing experimental work.

A large-scale and diverse experimental work has been carried out within the framework of a complex study. The doctoral student applies a combined phenotypic, biochemical and molecular approach to confirm the belonging of the analysed collection to *S. aureus* and which allows it to be used as a model for the basis of modern diagnostic approaches. *Staphylococcus aureus* occupies a stable and significant place in the etiological structure of symptomatic upper respiratory tract infections. Determination of the species belonging of the isolated strains has created a solid basis for the interpretation of antibiotic resistance, virulence potential and biofilm formation. The *Staphylococcus aureus* strains isolated within the study form a population characterized by dominant resistance to β -lactam antibiotics, increased methicillin resistance and practically significant levels of resistance to macrolides, lincosamides. Studies have shown that clinical isolates of *Staphylococcus aureus* possess a stable set of genes associated with adhesion, persistence and interaction with the host immune system and demonstrate the ability to form a biofilm. The screening of lactic acid bacteria showed that there is a clear strain specificity of antagonistic activity against *Staphylococcus aureus*, with a wide range of degrees of inhibition of growth and biofilm formation between individual isolates. In the work carried out with fractionation by FPLC, it was shown that the main antistaphylococcal effects of cell-free lactic acid bacteria supernatants are associated with low-

molecular-weight protein bacteriocin-like fractions, while the lipid component does not exhibit independent inhibitory activity against the growth and biofilm formation of *Staphylococcus aureus*. The genomic analysis of the selected lactic acid bacteria confirmed the presence of gene clusters associated with the potential for synthesis of antimicrobial peptides. Lyophilized cell-free supernatants from *Levilactobacillus brevis* DPL5, *Lactiplantibacillus paraplantarum* IZITR_13 and *Lactiplantibacillus plantarum* IZITR_24 exhibited pronounced dose-dependent antistaphylococcal activity. Of interest are the data from the scanning electron microscopy, proving that the cell-free supernatants of the three strains modify the biofilm-associated behavior of *Staphylococcus aureus*.

The results obtained, presented in “Results and Discussion”, logically follow the course of solving the tasks set. They are summarized and discussed in the light of published data from recent years.

Both the idea and the volume of research conducted on the implementation of this task and throughout the work deserve high praise. The discussion of each experiment, the comparison of the results and the comparison with the literature data, once again emphasize the qualities of the doctoral student in mastering experimental theory. With this, she proves that she has fully mastered the third stage of her training and is a complete experimenter.

Is it possible that there is regulatory control over metabolic plasticity in *S. aureus* with important implications for pathogenesis, and whether *S. aureus* coordinates metabolic flux through transcriptional and posttranscriptional mechanisms to promote adaptation and survival during infection?

5. CONTRIBUTIONS AND SIGNIFICANCE OF THE DEVELOPMENT FOR SCIENCE AND PRACTICE, REMARKS AND QUESTIONS

I accept the made contributions. Ilieva is an author in 2 scientific publications, in one of which she is a leading researcher, which shows her creative and research activity in their development and shaping.

I have the following question for the doctoral candidate:

Is it possible that there is regulatory control over metabolic plasticity in *S. aureus* with important implications for pathogenesis, and whether *S. aureus* coordinates metabolic flux

through transcriptional and posttranscriptional mechanisms to promote adaptation and survival during infection?

The dissertation contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the relevant Regulations of the University "Paisiy Hilendarski" and I give my high assessment and recommend to the members of the scientific jury to award the dissertation candidate Lena Rumenova Ilieva the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences, Specialty Microbiology.

CONCLUSION

The topic is relevant, the doctoral student has mastered modern methods, the experiments are methodically set up correctly, the results obtained are reliable and are a solid basis for further scientific and applied developments.

Based on the above, I can confidently state that the peer-reviewed dissertation is an original scientific work, with theoretical and applied significance. The proposed dissertation is evidence that Lena Rumenova Ilieva has developed competencies necessary for the award of the degree of doctor, including theoretical preparation, methodological knowledge, independence and experience in planning experiments and the ability to analyze the results. The dissertation contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ADSRB), the Regulations for the Implementation of the ADSRB and the relevant Regulations of the University "Paisiy Hilendarski" and I give my high assessment and recommend to the members of the scientific jury to award the dissertation candidate Lena Rumenova Ilieva the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences, Specialty Microbiology.

07.08.2026

Signature:

Prof. Iskra Ivanova