To the Scientific Jury

appointed by Order No. R-109-473/20.11.2025

of the Rector of the Medical University – Varna

Opinion

Regarding the procedure for obtaining the educational and scientific degree "Doctor"

In the scientific specialty "Microbiology" by Stefani Dimitrova Radeva, MD, with a dissertation entitled: "Microbiological studies on the mechanisms of resistance to beta-lactams and the nosocomial spread of clinical isolates *Serratia marcescens*."

by Assoc. Prof. Kalinka Dineva Bozhkova, MD, PhD

External Member of the Scientific Jury, appointed by Order No. R-109-473/20.11.2025 of the Rector of the Medical University – Varna

The submitted materials for the procedure comply with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the Development of the Academic Staff of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, for awarding the educational and scientific degree "Doctor."

Biographical Data and Career Development

Dr. Stefani Radeva graduated in Medicine from the Medical University – Sofia in 2018. In the period 2019–2020 she worked as a part-time lecturer in Microbiology at the Department of Microbiology and Virology at the Medical University – Varna. From 2019 to 2023 she was a resident physician in Clinical Microbiology at St. Anna University Hospital – Varna. From 2020 to 2025 Dr. Radeva served as an Assistant Professor at the Department of Microbiology and Virology at the Medical University – Varna. Since 2023 to the present, she has been working as a clinical microbiologist at Heart and Brain Hospital – Burgas.

Relevance of the Research Topic

The dissertation of Stefani Radeva, MD is dedicated to investigating the nosocomial spread of *Serratia marcescens* in a university multiprofile hospital – St. Marina University Hospital, Varna – and the mechanisms of beta-lactam resistance among the isolates. Although the relative share of

Serratia marcescens-associated hospital-acquired infections in Bulgaria is low, hospitalized patients undergoing invasive procedures and intensive care remain at risk due to the wide environmental distribution and high adaptability of this bacterial species. Furthermore, the intrinsic multiresistance of *Serratia marcescens* to the most commonly used antimicrobial agents poses a serious therapeutic challenge when infections occur. The persistent global trend of increasing antimicrobial resistance observed over recent decades has restricted adequate therapeutic options, particularly in healthcare-associated infections. In Bulgaria, studies on the hospital epidemiology of *Serratia marcescens* and the elucidation of the genetic mechanisms of resistance still represent significant challenges for clinicians and clinical microbiologists.

The present work is the first study of its kind in our country to present a contemporary approach to investigating the hospital spread and resistance mechanisms of *Serratia marcescens*, which determines its high relevance and significance for clinical microbiology and hospital epidemiology.

Structure of the Dissertation

The dissertation consists of 165 standard typed pages. The title reflects the essence of the work. The dissertation is logically structured into the following sections: Introduction – 2 pages; Literature Review – 31 pages; Aim and Objectives – 1 page; Materials and Methods – 14 pages; Results and Discussion – 58 pages; Conclusions – 2 pages; Summary of Contributions and lists of publications and participation in scientific events – 1 page; Bibliography – 32 pages.

The proportions between the sections are appropriate. The presented material is illustrated with 32 figures and 10 tables. The exposition is easy to follow, and the data is clearly presented.

Assessment of the Quality of the Literature Review

The literature review includes a total of 364 references, of which 3 are in Cyrillic and 361 in Latin script. Publications from recent years predominate. This demonstrates the excellent academic awareness of Dr. Radeva and reflects both the chronology and the current global state of the problem.

The structure of the review correlates with the defined aim and objectives of the dissertation. The literature review thoroughly examines the questions and issues related to the taxonomy and identification of the genus *Serratia*, the spectrum of infections caused by this microorganism, as well as the main mechanisms of bacterial resistance and methods for epidemiological typing. The

literature review presents the problem and the existing data in sufficient depth, while also outlining the need for further research. The review concludes with a summary that logically motivates the aim and objectives of the dissertation.

Aim and Objectives of the Dissertation

The aim of the dissertation is precisely formulated. Five objectives have been set to achieve it, each scientifically justified and reflecting the sequential execution of the major stages of the research.

Evaluation of the "Materials and Methods" Section

The scientific work includes 488 clinical isolates of *Serratia marcescens*, collected between 2016 and 2023. Both standard microbiological methods for identification and antimicrobial susceptibility testing and a large number of modern molecular-genetic methods were used—for identification, for determining exact mechanisms of beta-lactam resistance, and for epidemiological typing. The detailed description of the methods allows for their reproducibility. All experimental procedures were carried out in accordance with the requirements for scientific research.

Evaluation of the "Results and Discussion" Section

Extensive and diverse experimental work was conducted. The obtained results and their discussion strictly follow the defined objectives.

It was demonstrated that:

(1) The highest relative share of *Serratia marcescens* isolates was found in urine, wound, and respiratory samples, while isolation from blood was significantly less common. Most patients with *S. marcescens*-associated infections were hospitalized due to diseases of the urinary, cardiovascular, and respiratory systems. The dissertation discusses the leading clinical syndromes at admission, distribution by sex, prior hospitalization, place of acquisition of the infection, comorbidities, distribution of patients across hospital wards, ICU stay/surgical treatment, risk factors associated with medical interventions, and average hospital stay as interrelated predisposing factors for the occurrence and development of *Serratia marcescens*-associated infections. (2) A high percentage of *Serratia marcescens* isolates resistant to third- and fourth-generation

cephalosporins was established. A statistically significant association was demonstrated between resistance to third-generation cephalosporins and resistance to other antimicrobial groups. (3) Regardless of the anatomical site of isolation, imipenem, meropenem, and amikacin demonstrated the highest activity against *Serratia marcescens*. The two carbapenem-resistant isolates showed susceptibility only to amikacin. (4) The double-disk synergy test used to detect ESBL beta-lactamases in *Serratia marcescens* isolates demonstrated lower sensitivity, whereas the modified Hodge test for carbapenemase detection showed excellent sensitivity. The rapid immunochromatographic assay yielded a positive result in only one of the two carbapenem-resistant isolates. (5) The presence of bla_{KPC} was detected in one of the carbapenem-resistant isolates, representing the first reported case in Bulgaria. (6) For epidemiological typing of *Serratia marcescens* in routine practice, the application of highly discriminatory methods such as PFGE or WGS is necessary.

The results presented in the dissertation are scientifically grounded, thorough, applicable in practice, and suitable for future scientific research. Dr. Radeva provides a competent and well-reasoned interpretation of the findings.

The discussion of the results is in-depth and comprehensive. All findings are analyzed and compared with published data, demonstrating excellent knowledge of the topic.

Conclusions, Contributions, and Publications on the Topic

Dr. Radeva has formulated 11 conclusions in a consistent, clear, and structured manner, all of which fully correspond to the defined objectives and the obtained scientific results. Each conclusion represents original contributions both for the microbiological community in Bulgaria and for a wide range of clinical specialties.

Based on the conclusions, ten contributions have been formulated- three original, three confirmatory, and four of applied scientific significance. The contributions of original character have considerable scientific and practical importance, particularly for national science and practice. This work, concerning *Serratia marcescens*-associated infections, is the first of its kind in Bulgaria. The confirmatory and scientific–applied contributions have substantial practical relevance for any microbiology laboratory, establishing the dissertation as not solely theoretical.

I accept the stated contributions without remarks.

The results of Dr. Radeva's research are presented in four publications. The doctoral candidate also presents two conference communications. In five of the six total publications and communications, Dr. Radeva is the leading author, which is indisputable evidence that the dissertation is her own independent work.

The dissertation is written in clear, precise, and well-structured scientific language, with logical consistency, making the material easy to read and understand.

Conclusion

The dissertation submitted by Stefani Radeva, MD addresses a topic of high relevance for medical microbiology, with significant contributions to clinical microbiology, antibacterial therapy, and hospital epidemiology. I consider that the dissertation meets the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria, the relevant regulations, and the Rules of the Medical University – Varna, with respect to relevance, scope, and content. The submitted materials and dissertation results fully comply with the scientific standards defined in these regulations.

The dissertation demonstrates that Dr. Radeva possesses profound theoretical knowledge and professional skills, scientific reasoning on the topic, and the qualities necessary for conducting independent scientific research.

These considerations provide sufficient grounds for me to express a positive opinion and to recommend to the esteemed Scientific Jury to award Stefani Dimitrova Radeva, MD the educational and scientific degree "Doctor."

Prepared by:

Assoc. Prof. Kalinka Bozhkova, MD, PhD

07.01.2026