To the Chairperson
of the Scientific Jury

OPINION

by Assoc. Prof. Denis Sunay Niyazi, MD, PhD
Laboratory of Clinical Microbiology, St. Marina University Hospital – Varna
Department of Microbiology and Virology, Medical University – Varna

regarding a dissertation for awarding the educational and scientific degree "Doctor"

Professional field: 4. Natural sciences, mathematics and informatics, 4.3. Biological sciences
Scientific specialty: "Microbiology"
Author: Stefanie Dimitrova Radeva, MD
Form of doctoral training: full-time
Scientific institution: Medical University – Varna
Dissertation topic: "Microbiological studies on mechanisms of resistance to beta-lactams and the nosocomial spread of clinical isolates of *Serratia marcescens*"
Supervisor: Assoc. Prof. Milena Krasimirova Bozhkova, MD, PhD

By order of the Rector of the Medical University – Varna (No. P-109-473/20.11.2025), I was appointed as an internal member of the Scientific Jury and, pursuant to Protocol No. 1/03.12.2025, I was designated to prepare this opinion.

Biographical data and career development of the candidate:

Dr. Stefanie Dimitrova Radeva graduated from a high school for foreign languages in her hometown (Stara Zagora) in 2012, after which she was immediately admitted to study Medicine at the Medical University – Sofia, graduating in 2018 with excellence. In 2023, she obtained a second Master's degree in Public Administration with a specialization in Health Management at the University of National and World Economy. After graduating as a medical doctor, Dr. Radeva began her specialization in Clinical Microbiology at St. Anna Hospital – Varna (2019–2023). During the same period, she was appointed as honorary assistant professor at the Department of Microbiology and Virology, Medical University – Varna (2019–2020). In 2020, following a successful competition, she was enrolled as a full-time PhD student at the same department. From 2020 to September 2025, Dr. Radeva held the position of Assistant Professor at the Medical University – Varna. In December 2024, she acquired the medical specialty Clinical Microbiology. She currently works as a specialist physician at the Microbiology Laboratory of Heart and Brain Hospital, Burgas. She is fluent in English.

Relevance of the problem:

The etiological spectrum of nosocomial infections worldwide is changing, with an increasing incidence of infections caused by opportunistic Gram-negative bacteria, among which *Serratia marcescens* occupies an important place. Although it rarely causes community-acquired infections, it is a significant nosocomial pathogen, widely distributed and highly adaptable to the hospital environment, often associated with epidemic outbreaks. The most common infections include urinary tract, respiratory, and wound infections, which may progress to sepsis, especially in high-risk patient groups—immunocompromised individuals, those with prolonged hospital stay, antibiotic therapy, or invasive procedures. *Serratia* spp. consistently rank among the ten most frequent causes of severe healthcare-associated infections.

The growing antibiotic resistance represents a serious global public health threat, with Bulgaria being among the countries with high antibiotic consumption, especially β-lactams. The intrinsic and acquired resistance of *S. marcescens* significantly limits

therapeutic options and increases clinical risk. Therefore, this species is of major importance for clinical practice and necessitates rapid microbiological diagnostics, appropriate antimicrobial therapy tailored to local resistance levels, as well as systematic investigation of resistance mechanisms and hospital epidemiology.

Structure and layout of the dissertation:

The dissertation comprises a total of 165 pages and meets all specific requirements. It includes a title page, table of contents, list of abbreviations (4 pages), introduction (2 pages), literature review (38 pages), aim and objectives (1 page), materials and methods (16 pages), results and discussion (59 pages), conclusions (2 pages), contributions (1 page), publications related to the dissertation (1 page), and references (40 pages). The bibliography includes 364 sources, 45.1% of which are from the last five years, demonstrating the relevance of the review and presented data.

Literature review (23%): The PhD candidate provides a detailed presentation of taxonomy, species characteristics, and infectious complications associated with *S. marcescens*, thoroughly examines antimicrobial resistance, and discusses epidemiological typing methods. The review demonstrates excellent handling of scientific literature.

Aim and tasks (0.6%): Based on the literature review, the aim is clearly defined—to investigate mechanisms of resistance to beta-lactam antibiotics and analyze key aspects of the nosocomial spread of clinical isolates of *S. marcescens*. Five tasks are logically formulated.

Materials and methods (9.7%): The PhD candidate provides a comprehensive description of the study design and presents in detail the applied research methods (microbiological, molecular-genetic, and statistical), which are appropriately selected in accordance with the stated aim and tasks. The dissertation of Dr. Stefanie Radeva is based on a retrospective study covering the period 2016–2023. It can be conventionally divided into two parts:

(1) an epidemiological and statistical study aimed at investigating the epidemiology of *S. marcescens*–associated infections, the correlation between colistin use and the incidence of *S. marcescens* infections, as well as the risk factors associated with the occurrence of these infections and the related mortality. This part includes data from

488 patients hospitalized at St. Marina University Hospital – Varna during the study period;

(2) a microbiological and molecular-genetic study of a collection of 200 non-duplicate clinical isolates of *S. marcescens*, which were subjected to tests for the detection and confirmation of determinants of antimicrobial resistance to beta-lactam antibiotics (double-disk synergy test, modified Hodge test, immunochromatographic test, and polymerase chain reaction, PCR), as well as analysis of the genetic relatedness among the isolates (using ERIC-PCR and RAPD-PCR).

Results and discussion (35.8%): In her dissertation, Dr. Radeva presents an impressive number of scientific results, which are precisely illustrated with figures (32) and tables (10). The results are described in detail and arranged in a logical sequence in accordance with the stated tasks. Some of the more important findings include:

- *Serratia marcescens* stands out as a frequent causative agent of respiratory and urinary tract infections and, to a much lesser extent, bloodstream infections.
- Extended-spectrum beta-lactamase (ESBL)-producing *S. marcescens* strains are also associated with resistance to other antibiotic groups, such as aminoglycosides and quinolones.
- Isolates from urine and blood exhibit the highest levels of resistance to various groups of antimicrobial agents.
- Carriage of $bla_{\text{CTX-M}}$ has been identified as the most common mechanism of resistance to beta-lactam antibiotics.
- Two carbapenemase-producing strains, KPC and VIM, have been confirmed.
- The results obtained by ERIC-PCR and RAPD-PCR demonstrate their limited discriminatory power for *S. marcescens* isolates.

In addition, Dr. Radeva skillfully discusses the obtained results by comparing them with data from other studies and drawing logical conclusions.

Conclusions: Based on the obtained results, the PhD candidate formulates 11 conclusions that are reliable and logically structured. They correspond fully to the stated aim and tasks.

Contributions: Dr. Radeva formulates 10 contributions, which are grouped into three categories: original contributions, confirmatory contributions, and contributions of scientific and applied significance.

I accept the formulated conclusions and contributions without reservations.

Publication activity: In connection with the dissertation, Dr. Radeva has presented four scientific publications, in three of which (75%) she is the first author and in one the second author, confirming her leading role in the preparation of the dissertation. Three of the publications are indexed in international databases (Scopus/Clarivate), and two of them have an impact factor. In addition, Dr. Radeva has presented her results at two scientific forums.

The **thesis summary** comprises 88 pages and has been prepared in accordance with the requirements, fully presenting the essence of the dissertation.

Other: Dr. Radeva's study was approved by the Ethics Committee of the Medical University – Varna (No. 115/31.03.2022) and was funded by Project No. 21010 of the Science Fund (MU – Varna).

Dr. Radeva's dissertation is topical and represents a work of high scientific value. It presents Dr. Radeva as a thorough and capable researcher in the field of microbiology and molecular-genetic methods.

In view of the above, I give my **positive** evaluation of the submitted dissertation and propose that the Scientific Jury **award** the educational and scientific degree "**Doctor**" in Microbiology to Stefanie Dimitrova Radeva, MD.

Varna
05.01.2026

Assoc. Prof. I