To the Chairman of the Scientific Jury,
Appointed by order P-109-473/20.11.2025
Of the Rector of MU-Varna

OPINION

by Prof. Rumyana Donkova Markovska-Davidkova, MD, PhD

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Regarding a PhD thesis on the topic: „Microbiological studies on the mechanisms of resistance to beta-lactams and the nosocomial spread of clinical isolates ***Serratia marcescens***" **by Dr Stefani Dimitrova Radeva**, submitted for the award of the educational and scientific degree "Doctor", **in the doctoral program "Microbiology"** in the professional field 4.3 Biological Sciences

The submitted documents under the procedure have been prepared correctly, according with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the rules for its implementation at the University of Varna.

Biography

Dr. Radeva obtained a Master's degree in Medicine in 2018. She has a specialty in "clinical microbiology" since 2025. From 2020 to 09.2025 she is a doctoral student at the Department of Microbiology and Virology, MU-Varna. From 2023 to the present she works as a clinical microbiologist at the Heart and Brain Hospital in Burgas.

Relevance and significance of the selected scientific problem

Dr. Radeva's dissertation is dedicated to a current problem for medical practice – Serratia marcescens, an opportunistic pathogenic microorganism from the group of Gram-negative bacteria. S marcescens can be found in the external environment and causes infections associated with medical care, mainly in hospitalized patients, with prolonged antibiotic therapy and a long hospital stay, in immunocompromised patients and those with invasive diagnostic and/or

therapeutic interventions. This microorganism is extremely adaptable and is characterized by significant innate resistance, causing blood infections, urinary tract infections, respiratory infections, etc. The increasing resistance of isolates of this species indicates the need for further studies.

Structure of the PhD thesis

The dissertation submitted to me for an opinion is written on 165 pages, and is structured according to the generally accepted scheme: Abbreviations used - 2 pages; Introduction - 2 pages, Literature review - 38 pages, Aim and objectives - 1 page, Materials and methods - 16 pages, Results and discussion - 59 pages, Conclusions and contributions - 4 pages, The dissertation is illustrated with 10 tables, 32 figures. 364 sources were used, of which 3 in Cyrillic and 361 in Latin. It makes a very good impression, as 45% of the sources are from the last 5 years.

Evaluation of the qualities of the literature review

The literature review is written correctly. The taxonomy of the genus Serratia and the species characteristics are given in detail. The main emphasis is on the mechanisms of resistance, directed at the representatives of the genus *Serratia*. The mechanisms of resistance are included in detail not only to beta-lactam antibiotics, but also to aminoglycosides and quinolones. The literature review also examines the epidemiology of infections caused by *Serratia marcescens* - ways of spread, frequency and types of infections, covering the main types of infections caused by this species. The methods for epidemiological typing are considered. It would be nice to include the distribution of *S marcescens* as the main MLST types in the review. The literature review ends with a conclusion, where it logically justifies the need to develop a dissertation on such a topic.

Aim and tasks

The goal is clearly formulated, and the tasks (5 in total) correspond to the main goal and lead to its implementation. Why is a relationship sought between the frequency of isolation and the consumption of colistin only, why is this not included for carbapenems?

Section "Materials and methods"

The section includes the microbiological and molecular genetic methods used. The study design and inclusion criteria are presented in detail. The microbiological methods for identification and susceptibility determination are presented, which are fully suitable for fulfilling the aim of the dissertation work. The methods for determining beta-lactamases are both

phenotypic and molecular genetic. It would be good if the proven genes were sequenced. The evaluated methods for epidemiological typing are described in detail. The statistical methods used are appropriate.

Section "Results and Discussion"

The "Results and Discussion" section is structured in four subsections that cover the five tasks. The first subsection presents the epidemiology of *S. marcescens* – associated infections. The study is retrospective, the analysis of epidemiological data includes 488 patients with microbiologically confirmed infection with *S marcescens*. Dr. Radeva found that *S. marcescens* is among the 10 most common causes of bacteremia, with the dynamics of the frequency of infections reflecting the period of the COVID-19 pandemic, with a peak in 2018. The analysis of colistin consumption shows a lack of correlation between it and the frequency of *S. marcescens* infections. The doctoral student found that they are mainly urinary tract, cardiovascular and respiratory tract infections. She found the highest mortality in patients with neurological diseases and those with oncological diagnoses. As risk factors for the development of infection, Dr. Radeva identifies hospitalization, age between 70-79 years and cardiovascular, urological concomitant diseases, and for an unfavorable outcome, stay in an intensive care unit.

The second main subsection in the sections "Results" and "Discussion" includes determining the resistance of 488 isolates to the main groups of antimicrobial agents for the period 2013-2017. The relationship between the antibiotic used and mortality is also shown. A very interesting result is that the use of aminoglycosides and levofloxacin is an independent predictive factor for reducing 30-day mortality. Dr. Radeva found that resistance to amikacin is associated with a statistically significant increased risk of fatal outcome.

The third section returns to resistance, presenting the levels of resistance of *S. marcescens* isolates to the main groups of antimicrobial agents. In materials and methods, it is described that the 200 isolates were selected according to criteria to be hospital-acquired. This would be good to be clarify in the discussion too. It is noteworthy that the frequency of resistance to ceftriaxone is 55% in the 200 isolates, while in the 488 it is 35.9%, in total these 200 isolates have increased levels of resistance. The difference is significant and is probably related to the fact that they are hospital-acquired. Dr. Radeva also studied the susceptibility of 108 isolates resistant to third-generation cephalosporins. She found high levels of resistance to gentamycin - over 85%, to ciprofloxacin and levofloxacin - about 77% - 79%, a positive result is

the relatively low percentage of resistance to amikacin - 21.5%. The doctoral student found the presence of CTX-M beta-lactamases in 77 of the 108 isolates, mostly alone or in combination with TEM enzymes. The positive result for TEM and SHV enzymes should be interpreted with caution due to the presence of SHV-1 TEM-1,2 enzymes. Dr. Radeva establishes the presence of two isolates producing KPC and VIM enzymes, sequencing of the genes encoding the identified carbapenemases, as well as SHV, TEM and CTX-M is recommended. The fourth section provides an answer to the last task – to evaluate ERIC and RAPD PCR as a method for epidemiological typing in *S. marcescens*. The title of this subsection should be corrected. The doctoral candidate establishes that ERIC and RAPD-4,-5,-6 primers are not applicable for typing. With the introduction of MLST typing and whole-genome sequencing, the applicability of these methods for assessing clonal spread decreases. As a result of the presented work, Dr. Radeva formulates 11 main conclusions that meet the set goals and objectives. I have only one comment, from conclusion 10 the reader would understand that the study proves 52% TEM ESBL and 0.9% SHV ESBL, based only on PCR without sequencing this is impossible and this conclusion should be corrected.

Evaluation of the contributions of the PhD thesis

From the results obtained and conclusions drawn, Dr. Radeva formulates 10 contributions, which I fully accept. Three of them are original.

Scientific indicators

Dr. Radeva presents 3 full-text publications, related to the PhD thesis, published in refereed journals and one in non-refereed. Two of the publications are with Q3 and one with Q4 and so the total number of points for the publications is 42, which exceeds the national requirements and those of MU-Varna. The fact that in three of them she is the first author gave an excellent impression.

Abstract

The attached abstract presents the dissertation work and its results, contributions and conclusions well. The requirements of the regulations have been met.

In conclusion, the presented PhD thesis by Dr. Radeva in terms of structure, content and volume meets the criteria in the ZRASRB and the Regulations of MU-Varna for acquiring the educational and scientific degree "doctor". The topic is relevant, the selected methods are

appropriate, Dr. Radeva shows thoroughness and precision in the presentation, statistical processing and analysis of the results. I give my positive assessment and recommend to the members of the Scientific Jury to vote for awarding the educational and scientific degree "doctor" in the scientific specialty "Microbiology" in the professional field 4.3 Biological Sciences to **Dr. Stefani Dimitrova Radeva**.

12.01.2026г. **Prepared the review:**

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

/ Prof. Rumyana Donkova Markovska-Davidkova, MD, PhD /