REVIEW

on the procedure for acquiring the educational and scientific degree of "Doctor"
in the scientific field of "Microbiology"
by Dr. Stefani Dimitrova Radeva
with a dissertation on the topic:
"Microbiological studies on the mechanisms of resistance to beta-lactams and the nosocomial spread of clinical isolates of *Serratia marcescens*",

with scientific supervisor
Assoc. Prof. Milena Krasimirova Bozhkova, MD, phD

by
Prof. Temenuga Zhekova Stoeva, MD, Dsc
Medical University, Varna
Chair of the Scientific Jury, appointed
by Order NoP-109-473/20.11.2025

This review has been prepared on the basis of Order NoP-109-473/20.11.2025 of the Rector of MU-Varna, determining the composition of the scientific jury. The materials submitted for the procedure meet the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for the Development of Academic Staff at Medical University - Varna for the award of the academic degree of "Doctor".

Biographical data and career development:

Dr. Stefani Dimitrova Radeva was born on January 31, 1993. In 2018, she completed her higher education with a master's degree in medicine at the Medical University of Sofia. Since 2019, she began working as a doctor in the Microbiology Laboratory at St. Anna Hospital, Varna, and in 2020 she was appointed as an assistant at the Department of Microbiology and Virology at MU-Varna, where she worked until September 1, 2025. Since 2020, she has been a doctoral student at the same department, and in 2025 she obtained a specialization in Clinical Microbiology. Currently, Dr. Radeva works as a clinical microbiologist at the Heart and Brain Hospital in Burgas. Dr. Radeva has excellent written and spoken English skills and is computer literate.

Relevance of the dissertation:

In 2019, the WHO declared "antibiotic resistance" as one of the 10 threats to public health, and in 2023, the UN set a target of a 10% reduction in mortality associated with this phenomenon by 2030. A large-scale study on global mortality associated with bacterial antibiotic resistance in 2019 reported approximately 5 million deaths

worldwide, with over 3.5 million of them related to several pathogens - representatives of the order *Enterobacterales* (*E. coli, Klebsiella pneumoniae*), *Staphylococcus aureus, Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*.

An European study on nosocomial infections conducted in 2022-2023 by ECDC also reports *Enterobacterales* among the most common causes of infections associated with medical care. In 2017 and 2024, the WHO placed third-generation cephalosporin- and carbapenem-resistant *Enterobacterales* on the list of microorganisms that are of "critical priority" for research and the development of new, effective antibiotics.

In light of these facts, the topic of this dissertation is highly relevant. It is related to one of the most medically important representatives of the *Enterobacterales* order - *Serratia marcescens*, and more specifically - with the species' resistance to antibiotics (including those from the "Watch" and "Reserve" groups), the hospital epidemiology of infections associated with it, as well as with the possibilities for etiological therapy.

Structure of the dissertation:

The dissertation submitted for review is 165 pages long and is structured according to the generally accepted scheme: Abbreviations used - 2 pages; Introduction - 2 pages; Literature review - 37 pages; Materials and methods - 16 pages; Results and Discussion - 59 pages; Conclusions - 2 pages. The dissertation is illustrated with 32 figures and 10 tables. A total of 364 literature sources are used, of which 3 are in Cyrillic and 361 are in Latin, with 45% of them published in the last 5 years.

Assessment of the quality of the literature review:

The literature review is well structured and competently written, using over 300 titles, which demonstrates the doctoral student's extensive knowledge of the literature. The review begins with data on the current taxonomic status of *S. marcescens* and continues with a detailed description of the species characteristics of this microorganism and the methods for its identification. An important part of the review is devoted to the mechanisms of resistance and their genetic determinants in *S. marcescens* to the main groups of antimicrobial drugs (beta-lactams, aminoglycosides, quinolones, tetracyclines, sulfonamides, and polymyxins). Of particular interest in the review is the section devoted to the medical significance of *S. marcescens* as an important nosocomial pathogen and the epidemiology of infections associated with this microbial species, with a detailed review of the spread of *S. marcescens* in the hospital environment, the frequency and type of infections/epidemic outbreaks, the main risk factors, and the methods used for epidemiological typing.

The clinical significance of *S. marcescens* and the potential of this bacterial species for intra-hospital epidemic spread highlights the relevance of the chosen topic and logically leads to the need for in-depth studies on the chronological trends in the development of resistance, on the mechanisms of

resistance to strategic antimicrobial agents in clinical isolates of *S. marcescens*, the epidemiology of related infections, which will serve as a basis for optimizing the therapeutic approach and improving strategies for the prevention and control of nosocomial infections and outbreaks.

Objective and tasks

The objective is clearly formulated and the tasks (5 in total) correspond to the main objective.

Evaluation of the "Materials and Methods" section:

The "Materials and Methods" section describes the study design in detail. It describes in detail the various classical methods used for biochemical identification and modern methods such as MALDI-TOF MS. For the detection and analysis of beta-lactamase production and identification of the mechanisms of resistance to beta-lactam antibiotics, both phenotypic and molecular genetic methods (PCR) were used simultaneously. Molecular genetic methods (RAPD and ERIC PCR) were also used for the epidemiological typing of *S. marcescens* isolates in order to assess their applicability in studies of the bacterial species.

Evaluation of the section "Results and Discussion":

The results obtained are presented in four subsections and illustrated with a total of 10 tables and 32 figures. One of the strengths of the dissertation is the results related to the study of the epidemiology of infections associated with *S. marcescens*, providing a clinical perspective on the problem. Among them, I would highlight the following most important ones:
1. The frequency of *S. marcescens* isolation varies significantly during the study period, with the highest rate recorded in 2018, followed by a marked decrease in 2021, coinciding with the period of restricted planned hospitalisation during the COVID-19 pandemic.
2. The study did not demonstrate a statistically significant difference in gender distribution between the individual years of the observation period, nor a relationship between gender and the risk of developing *S. marcescens*-associated infection or fatal outcome. The study identified age as an important risk factor for acquiring infection. The 70-79 age group has the highest incidence, and the 20-29 age group has the lowest. A high proportion of cases is also found in the 0-3 month subgroup, with the difference between the different subgroups being statistically significant.
3. Over 95% of the *S. marcescens*-associated infections studied were identified as healthcare-associated infections, clearly demonstrating the clinical significance of this bacterial species as an important nosocomial pathogen and defining hospitalization as a leading risk factor for the development of a related infection.
4. The average length of hospitalization was found to be 11.9 days, with the type

of infection proven to be an independent prognostic factor for the length of hospital stay. The longest hospitalization was found in cases of blood stream and respiratory infections, and the shortest in cases of urinary tract infections.
5. The highest relative proportion of patients with *S. marcescens*-associated infection was found in clinics with a therapeutic profile, followed by those in surgical and intensive care clinics, with hospitalization in a therapeutic clinic being identified as a significant risk factor for acquiring an infection.
6. The presence of foreign bodies (urethral catheters and CVCs), followed by invasive procedures, have been identified as leading risk factors for acquiring *S. marcescens*-associated infections.
7. The study demonstrates that cardiovascular (65.8%), endocrinological (31.8%), and urological diseases (13.9%) are statistically significant risk factors for acquiring infection.
8. The study reports a 10.5% overall 30-day mortality rate among all 488 patients with *S. marcescens*-associated infection. The highest 30-day mortality was observed in patients with concomitant neurological (28.6%) and malignant diseases (17.1%), with the difference between the groups and the outcome of the disease being statistically significant.
9. The highest mortality rate was found in the 60-79 age group (12.6%), and the lowest in the 20-39 age group (4.8%).
10. Hospitalization in an intensive care unit was identified as an important predictor not only for the occurrence of infection, but also for unfavorable outcomes.
11. The study did not find a statistically significant association between infection caused by ESBLs-producing *S. marcesens* strains and fatal outcomes. However, the therapeutic use of aminoglycosides (amikacin, gentamicin) and levofloxacin was identified as an independent protective factor for reducing 30-day lethality, while infection caused by an amikacin-resistant isolate was associated with an increased risk of adverse outcome.

Among the results of the study on antimicrobial susceptibility of this large collection of clinical isolates of *S. marcescens* (n=488), I would note the following important ones:

1. A high relative proportion of isolates (over 25%) resistant to third- and fourth-generation cephalosporins (ceftriaxone 55.0%; ceftazidime 53.5%; cefepime 52.0%); gentamicin, 48.5%; quinolones 44.5-46.0%; trimethoprim/sulfamethoxazole, 44.5%; piperacillin/tazobactam 38.5%; amikacin, 28.0% is found. Resistance to carbapenems is below 1%. In the group of carbapenem-resistant *S. marcescens*, only amikacin demonstrated good in vitro activity.
2. Isolates resistant to third- and fourth-generation cephalosporins (n=108) demonstrated very high resistance to other antibiotic groups [gentamicin (85.3%), ciprofloxacin (79.8%), levofloxacin (77.0%), piperacillin/tazobactam (72.5%), trimethoprim/sulfamethoxazole (69.7%)], but to a much lesser extent to

amikacin (21.5%) and carbapenems (1.8%).

3. Invasive isolates and those from urine were identified as significantly more resistant to all tested antibiotics compared to isolates from other clinical samples, with the highest resistance to third-generation cephalosporins. Regardless of the anatomical site, carbapenem antibiotics (imipenem, meropenem) and amikacin demonstrated the highest activity.
4. Resistance to third-generation cephalosporins in the studied collection of *S. marcescens* isolates is mainly mediated by ESBL production, predominantly by CTX-M type enzymes (71%) and, to a lesser extent, TEM beta-lactamases. *Bla*$_{\text{KPC}}$ and *bla*$_{\text{VIM-like}}$ are the genetic determinants responsible for carbapenem resistance.

The discussion of the results is an important part of the dissertation. All results obtained were analyzed in depth and compared with similar studies from the scientific literature. This allows Dr. Radeva to formulate 10 main conclusions from his work, which I fully accept.

Assessment of the contributions of the dissertation:

Based on the results obtained and conclusions drawn, a total of 10 contributions have been formulated, which I accept. The most significant contributions of an original nature are as follows:
1. An in-depth analysis of the epidemiology of *S. marcescens*-associated infections among hospitalized patients over an 8-year period has been performed. The main risk factors for acquiring these infections and the overall 30-day mortality rate in the study group have been established.
2. A detailed study of the mechanisms of resistance to beta-lactam antibiotics (including carbapenems) in a large collection of clinical isolates of *S. marcescens* has been performed.

Of the scientific and applied contributions, I would like to highlight the following:
1. The sensitivity of phenotypic tests for the detection of ESBLs in *S. marcescens* isolates resistant to third-generation cephalosporins has been evaluated.
2. Rapid molecular genetic methods for detecting genes encoding beta-lactamases and rapid immunochromatographic tests for detecting carbapenemases in clinical isolates of *S. marcescens* have been evaluated.

Scientometric indicators:

There are four publications related to the dissertation, two of which are in journals with IF. In three of the articles, the doctoral student is the first author. There are two participations in scientific forums.

Critical comments:

Dr. Radeva has taken into account my comments as an internal reviewer.

Conclusions:

The dissertation, written by Dr. Stefani Dimitrova Radeva, submitted to me for review is an in-depth study of a current clinical and microbiological problem, namely infections associated with *S. marcescens* and the antibiotic resistance of this important nosocomial pathogen. The study uses both classical and molecular genetic and statistical methods. In terms of structure, content, and volume, the dissertation meets the requirements of the LDASRB and the Regulations for the Development of Academic Staff at MU-Varna. The work contains numerous results in accordance with the set goals and objectives. These results are discussed in depth and basic conclusions are formulated, both original and confirmatory, as well as contributions of an applied nature.
Based on the above, I give a positive assessment of the dissertation and propose that the members of the scientific jury vote in favor of awarding the educational and scientific degree of "Doctor" in the scientific specialty of "Microbiology" to Dr. Stephani Dimitrova Radeva.

31.12.2025 **prof. T. Stoeva, MD, Dsc**