TO
THE CHAIRMAN OF THE SCIENTIFIC JURY,
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OF THE RECTOR OF THE MEDICAL
UNIVERSITY
"PROF. DR. PARASKEV STOYANOV" - VARNA

REVIEW

By Prof. Stefana Doneva Sabtcheva
Head of Microbiology Laboratory at the
University Specialized Hospital for Active Oncology Treatment
"Prof. Ivan Chernozemsky", Sofia

Of a dissertation on the award of educational and scientific degree "Doctor"
in the field of higher education: 4. Natural sciences, mathematics and informatics;
Professional drive: 4.3. Biological Sciences;
Scientific specialty "Microbiology"

On the subject: "Microbiological Studies on the Mechanisms of Beta-Lactam Resistance and Nosocomial Spread of Clinical Isolates of *Serratia marcescens*"

By Stefani Dimitrova Radeva, MD
full-time PhD student at the Department of Microbiology and Virology, Faculty of Medicine, Medical University - Varna

Scientific supervisor:

Assoc. Prof. Milena Krasimirova Bozhkova, MD, PhD

I declare that I have no scientific works in common with the PhD student.

Relevance of the dissertation work

Serratia marcescens belongs to a specific group of *Enterobacterales*, consisting of *Enterobacter* spp., *S. marcescens*, *Citrobacter freundii*, *Providencia* spp. and *Morganella morganii*, called ESCPM. These bacteria produce species-specific, chromosomally encoded, inducible AmpC β-lactamases belonging to Ambler class C (Bush group 1). The low level of expression of these enzymes is sufficient for the hydrolysis of aminopenicillins, combinations of aminopenicillins/β-lactamase inhibitors, cephamycins, first- and second-generation cephalosporins. AmpC overproduction may occur through induction by certain β-lactams or selection of derepressed mutants during therapy with third-generation cephalosporins, leading to their hydrolysis. Furthermore, *S. marcescens* has an innate resistance to colistin, which further limits therapeutic options and contributes to their increasing prevalence. In addition to this innate set of resistance genes, *S. marcescens* can acquire, through mobile genetic elements, determinants of resistance to β-lactams, aminoglycosides, and fluoroquinolones, often associated on the same plasmid. For example, in *S. marcescens* strains isolated in Bulgaria, CTX-M-3 in association with ArmA methyltransferase, encoded on conjugative IncM plasmids, and three types of carbapenemases (OXA-48, encoded on a conjugative IncL plasmid; NDM-1, encoded on conjugative IncX3 or IncM plasmids, and chromosomally encoded VIM-4), which are often associated with CTX-M-3, ArmA, and plasmid-mediated determinants of quinolone resistance. In addition, *S. marcescens* is characterized by resistance to environmental factors and disinfectants, which is why it is widespread in hospital environments. The exceptional adaptability of *S. marcescens* to conditions in hospital ecosystems and its multiple mechanisms of antibiotic resistance place it among the ten most frequently isolated bacterial pathogens causing life-threatening healthcare-associated infections, especially among high-risk patients – immunocompromised, newborns, children, and those hospitalized in intensive care units.

All this determines the relevance of the dissertation and the need for microbiological and epidemiological studies on the mechanisms of resistance to β-lactam antibiotics and the spread of clinical isolates of *Serratia marcescens* at the University Hospital "Sveta Marina" – Varna.

Structure of the dissertation

The dissertation is 165 pages long and is structured according to the generally accepted scheme with a proportional distribution of the text in the individual sections as follows: 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, and bibliography – 40 pages. A list of publications and participation in scientific forums related to the dissertation is attached to the dissertation. The presentation is optimally illustrated with 42 precisely crafted materials (32 figures and 10 tables). The dissertation cites 364 literary sources: 3 are in Cyrillic and 361 are in Latin, most of which are from the last 10 years – proof of the relevance of the dissertation.

Evaluation of the literature review

The literature review is comprehensive, including the necessary information in terms of volume and analysis on the taxonomy of the genus *Serratia* and the species characteristics of *S. marcescens*, resistance to antibacterial agents, and infections caused by *S. marcescens*. The mechanisms of resistance to the main groups of antibiotics and infections caused by *S. marcescens* are discussed in detail. The need for rapid microbiological identification and timely application of antimicrobial therapy based on the genetic characteristics determining antibiotic resistance is justified. The conclusions from the literature review are specific and justify the aim and objectives of the scientific study.

Purpose and Goals

The author approaches the identified tasks systematically and thoroughly in accordance with the aim of the dissertation: to study the mechanisms of resistance to β-lactam antibiotics and to analyze the main aspects of the nosocomial spread of clinical isolates of *S. marcescens*.

To achieve the aim of the study, five tasks have been set, which follow the sequence of the planned research, namely: (1) To study the epidemiology of infections caused by *S. marcescens* by: determining the frequency and dynamics of isolation over time; analyzing the relationship between the frequency of isolation and colistin consumption; distributing the isolates according to the type of clinical material; characterizing the leading clinical syndromes; identifying the risk factors for the development of S. marcescens infections and assessing their impact on 30-day mortality.

(2) To analyze the dynamics of antibiotic resistance in 488 clinical isolates of *S. marcescens* and to assess the relationship between resistance and mortality, as well as the impact of therapeutic approaches on clinical outcome. (3) To determine and analyze in vitro the sensitivity of a collection of 200 *S. marcescens* isolates to a set of antimicrobial agents. (4) To determine the prevalence of genes encoding acquired β-lactamases among selected *S. marcescens* isolates. (5) To evaluate the effectiveness of RAPD and ERIC as methods for epidemiological typing of clinical *S. marcescens* isolates.

Evaluation of the Materials and Methods section

A wide range of classical microbiological and modern molecular genetic methods were used to conduct the research for phenotypic and genotypic characterization of clinical strains of *S. marcescens* isolated from hospitalized patients at the University Hospital "Sveta Marina" from 2016 to 2023. The study design includes a retrospective analysis of data related to ten variables in 488 patients with clinically manifested *S. marcescens* infection. Of these, 200 strains were selected based on invasiveness and resistance to third-generation cephalosporins, which were tested for antimicrobial susceptibility to a unified set of antimicrobial agents. Additional phenotypic methods were used to detect ESBL and carbapenemase activity. The presence of genes encoding the most common ESBL (bla_{TEM}, bla_{SHV}, bla_{CTX-M}) and carbapenemases (bla_{KPC}, bla_{VIM}, bla_{NDM}, bla_{IMP}, bla_{OXA-48}) was confirmed by PCR. PCR-based techniques (RAPD and ERIC) were used for the epidemiological typing of *S. marcescens* isolates. Statistical processing was performed using highly informative methods suitable for solving the tasks at hand.

Evaluation of the Results and Discussion section

A significant amount of diverse research work has been carried out. The results obtained and their discussion strictly follow the tasks set.

1) It has been established that in 97.5% of cases, infections caused by *S. marcescens* occur during hospital treatment, which makes hospitalization the most significant risk factor for the development of these infections. In this regard, the dynamics in the frequency of isolation of *S. marcescens* reached its highest values in 2018, followed by a marked decrease in 2021, which coincides with the period of restriction of planned hospitalisation during the COVID-19 pandemic, and a moderate increase in the period 2022-2023, which

coincides with the restoration of normal hospital activity. Other risk factors have also been identified, the leading ones being the presence of a foreign body (80.0%), invasive procedures (16.0%), and intubation/mechanical ventilation (7.9%); the 70–79 age group, cardiovascular (65.8%), endocrinological (31.6%), and urological (13.9%) comorbidities have also been proven to be significant additional risk factors. It has been established that stay in the intensive care unit is a significant predictor of both infection and fatal outcome. The highest mortality rate was observed in patients with neurological (28.6%) and malignant diseases (17.1%). It has also been established that the type of *S. marcescens* infection is an independent prognostic factor for the length of hospital stay, with the longest hospitalisation observed in blood infections and the shortest in urinary tract infections.

2) No statistically significant association was found between ESBL production and mortality in infections caused by *S. marcescens*. The use of amikacin, gentamicin, and levofloxacin has been found to be an independent protective factor for reducing 30-day mortality, while resistance to amikacin is associated with a statistically significant increased risk of adverse outcomes.

3) A statistically significant correlation was found between resistance to cephalosporins and antibiotics from other groups, such as gentamicin (85.3%), ciprofloxacin (79.8%), levofloxacin (77.0%), piperacillin/tazobactam (72.5%), and trimethoprim-sulfamethoxazole (69.7%). Imipenem, meropenem, and amikacin are the drugs with the best activity, making them suitable for empirical treatment in cases of invasive infections caused by *S. marcescens*.

4) The main mechanism of resistance to third-generation cephalosporins has been identified – production of CTX-M ESBL. Carbapenem resistance is caused by bla_{VIM} and bla_{KPC}, which was identified for the first time in *S. marcescens* in Bulgaria and is probably associated with the endemic spread of KPC producers in the hospital.

5) It has been established that epidemiological typing by RAPD-PCR and ERIC-PCR has limited discriminatory power with regard to *S. marcescens*. The genetic stability and low variability of the species reduce the effectiveness of these techniques in routine practice, which necessitates the use of highly discriminatory methods such as PFGE or WGS to establish the genetic relationship between isolates.

Evaluation of the conclusions and contributions of the dissertation

The dissertation concludes with 11 conclusions and 10 contributions, which are formulated in great detail and clarity. Presented in this way, they are informative and lend weight to each result obtained. The contributions are highly significant due to the fact that this is the first clinical-microbiological and epidemiological study of *S. marcescens* infections covering consecutive clinical cases over a long period of time (2016-2023).

Publications related to the dissertation

The results of Dr. Stefani Radeva's extensive research work are reflected in four publications, three of which she is the first author and the fourth the second author. The publications are in English (two in Bulgarian journals and two in international journals), with three of the journals indexed in Scopus with an SJR ranging from 0.154 to 0.410. The doctoral candidate has presented her achievements at two scientific forums. These scientometric indicators present the doctoral student as an established researcher with a significant contribution to the development of clinical microbiology and knowledge of the diagnosis, antibiotic resistance, and epidemiology of bacterial infections in hospitalized patients.

Conclusion

The presented dissertation deals with a problem of current interest to medical science and practice, with significant scientific and applied scientific contributions related to the clinical-microbiological and epidemiological aspects of nosocomial infections caused by *S. marcescens* in Bulgaria. The dissertation repeatedly exceeds the requirements of the Academic Staff Development Act in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of MU-Varna. This gives me reason to confidently support the awarding of the educational and scientific degree "Doctor" in the scientific specialty "Microbiology" to Dr. Stefani Dimitrova Radeva.

11.12.2025

Reviewer:

/ Prof. Stefana Sabcheva /