

Opinion

from

Prof. Ilina Dimitrova Micheva, MD, PhD

Head of Clinic of Clinical Hematology,

UMBAL "St. Marina" EAD, Varna,

Head of the Department of Hematology at the Second Department of Internal Medicine,

Medical University Varna

Internal member of the Scientific Jury for the award of the scientific degree "Doctor of Sciences"

Subject: dissertation work for the award of the scientific degree "Doctor of Sciences"

Field of higher education: 7. "Health and sports"

Professional direction: 7.1. "Medicine"

Major: "Microbiology"

To: Prof. Temenuga Zhekova Stoeva, MD, PhD

Department: "Microbiology and Virology"

Topic: Antibiotic resistance of the most common causative agents of bacteremia and associated mortality

By order No. R-109-388/28.08.2023, of the Rector of the MU, Varna, based on the decision of the Faculty Council of the Faculty of Medicine under protocol No. 8/21.08.2023, I have been elected as a member of the Scientific Jury for the presentation of the dissertation work of Prof.. Temenuga Zhekova Stoeva, MD, PhD. On the basis of protocol No. 1/104.09.2023, I have been appointed to prepare an opinion on the procedure for acquiring the scientific degree "Doctor of Sciences".

1. Biographical data and professional development

Prof. Temenuga Zhekova Stoeva, MD, PhD, was born in 1969 and graduated from the Language High School with English language teaching in the city of Dobrich in 1988, and higher education "medicine" in MU Varna in 1994 with excellent results from semester and state exams. In 2006, she majored in microbiology. In 2009, she defended a thesis for the scientific degree "doctor" on the topic "Microbiological and molecular genetic studies on the epidemiology and resistance to antimicrobial agents in clinical isolates of *Acinetobacter baumannii*". From 2007 to 2016, she conducted 4 specializations - Molecular - genetic research methods. Germany, Cologne, Institute of Medical Microbiology, Immunology and Hygiene, Carbapenemase-Producing Gram-Negative Microorganisms: Detection, Epidemiology and Therapeutic Challenges. Athens, Greece, "Stem Cell Transplantation" in MU-Varna, "Immunological problems in patients with bone marrow transplantation". MU-Varna. Since 2018, she has a master's degree in health management.

Prof. Stoeva started her professional career as a doctor as a pediatrician in the city of Ruse in 1995. Since 1998, she has been a pediatrician in the pediatric nursing department of the 2nd City Hospital in the city of Varna, and in the period 1999-2003 works as a microbiologist at HEI Varna. Since 2004, she has been working as a microbiologist at UMHAT "St. Marina". Varna. Prof. Stoeva began her academic development as an assistant in the Department of Microbiology and Virology of the University of Varna in 2003, gradually moving through the positions of senior assistant and chief assistant. In 2012, she was elected as an associate professor, and in 2018, as a professor of microbiology at the Department of Microbiology and Virology at the Ministry of Education, University of Varna. Since 2014, Prof. Stoeva is the Head of the Laboratory of Microbiology at the UMHAT "St. Marina".-Varna and the head of the "Medical - Diagnostic Laboratories" department. Since 2016 she is the head of the department of Microbiology and Virology.

Prof. Stoeva is a member of SUB, BAM and ECSMID, the expert council for the medical specialty "Microbiology" at the Minister of Health since April 2019.

2. Relevance of the topic

In recent years, there has been a clear trend worldwide for increased incidence of invasive, life-threatening infections caused by multidrug-resistant pathogens. Blood infections are among the most significant public health problems and a leading cause of morbidity and mortality.

A number of studies have demonstrated, in addition to an increase in the burden of bacterial blood infections worldwide, a steady trend towards a rapid increase in the frequency of infections caused by pathogens of the so-called "ESKAPE" group (*Clostridioides difficile*, *Enterobacteriaceae*, referring to all Gram-negative intestinal bacteria, including *E. coli*, *Klebsiella pneumoniae*, *Proteus spp.* and *Enterobacter spp.*), representing between 50 and 70% of their etiological spectrum. Often, these infections are associated with prolonged hospital stays, increased economic costs, and worse outcomes due to antibiotic resistance. Because of their extremely problematic resistance, some of these pathogens (vancomycin-resistant *Enterococcus faecium*, carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae*) are also included in the World Health Organization (WHO) list of microorganisms with priority "critical" for research and development of new, effective antibiotics (WHO, 2017).

In this context, blood infections, caused by resistant microorganisms, represent a very current medical problem.

Adequate surveillance at the local level contributes significantly to the expansion of data and scientific information on antibiotic resistance at the national level and to the analysis of the generated information in order to assess the burden of antibiotic resistance in various infections, in particular those of the blood. That is why Prof. Stoeva's interest is focused on conducting a microbiological and epidemiological study on the antibiotic resistance of the most common causes of bacteremia and the mortality associated with them over a 10-year period at the UMHAT "St. Marina".

3. Structure and content of the dissertation work

The dissertation is written in a volume of 296 pages, - 234 pages of text, 27 figures, 36 tables and an appendix with 10 tables.

The literature review is presented on 72 pages. It presents an in-depth analysis of the research problem in the light of state-of-the-art research. The content is fully focused on the main aim and objectives of the dissertation.

The purpose of the study is clearly and precisely formulated, namely, to perform a microbiological and epidemiological study on the antibiotic resistance of the most common causative agents of bacteremia over a 10-year period and the mortality associated with them.

The 5 main tasks set are clearly formulated and follow the logically set goal.

The Materials and Methods section is presented in 15 standard pages. The study has a retrospective descriptive design, including all clinically significant episodes of bacteremia (related to infection),

proven in patients treated at the UMHAT "St. Marina", Varna in the period 2011-2020, regardless of their age, gender, diagnosis and clinic. All clinically significant episodes of bacteremia in hospitalized patients with oncohematological diseases over an 11-year period (2010-2020) were studied separately, regardless of their gender, age and diagnosis. A total of 798 patients were included, stratified on the basis of the clinical outcome of the hospitalization into two groups: the first group that survived within at least 30 days and the second group that died within 30 days from the time of the positive blood culture.

Criteria and concepts, as well as indications for patient inclusion, are clearly defined. Microbiological methods, methods for testing susceptibility to antimicrobial drugs, molecular genetic methods for proving genes encoding ESBLs and carbapenemases, and epidemiological typing are described.

Reliable and adequate statistical methods were used for data analysis, which guarantee the reliability of the results obtained and the conclusions drawn.

The section "Results and Discussion" is 123 standard pages long. The results of the set 6 tasks are analyzed, systematized, presented concisely and consistently and illustrated with the help of tables, figures, graphs and box plots.

The etiological spectrum of bacterial infections of the blood in hospitalized patients for a 10-year period (2011-2020), the total number of examined patients, the proportion of positive blood cultures, the spectrum of causative agents of laboratory-confirmed blood infections, detailed etiological structure and the division are presented by year of the 10 most frequently isolated microbial species (*S. aureus*, *E. faecalis*, *E. faecium*, *E. coli*, *K. pneumoniae*, *Acinetobacter* spp. and *P. aeruginosa*).

A high proportion of true positive (clinically significant) blood cultures was established - 9.9% and a 2.5-fold increase in the "BCR" indicator. Gram-negative bacteria dominate (58.9%), the share of ESKAPEE pathogens in the etiological spectrum reaches 66.8%, and that of the bacterial species monitored by EARS Net - 64.7%. The relative proportion of *E. coli* resistant to third-generation cephalosporins and fluoroquinolones from blood is high (over 25%).

Carbapenems and amikacin have conserved activity against *E. coli* (resistance <1% and <5%, respectively), making them appropriate choices for empiric therapy.

A very high level of resistance to third-generation cephalosporins was demonstrated among *Klebsiella pneumoniae* isolates (74.9%). In the group of carbapenem-resistant *K. pneumoniae*, colistin and amikacin demonstrated the best in vitro activity (6% and 17.9% resistance), making them the drugs of choice for treatment in case of infection caused by carbapenem-resistant isolates.

blaCTX-M-15 was identified as a major mechanism of resistance to third-generation cephalosporins in carbapenem-resistant *K. pneumoniae* isolates.

A very high relative proportion of *Enterobacter* spp isolates was demonstrated. from blood resistant to third-generation cephalosporins (over 65%), as well as high levels of resistance to fluoroquinolones and gentamicin (over 45%).

Throughout the 10-year study period, *A. baumannii* - *calcoaceticus* complex blood isolates consistently demonstrated very high levels of resistance to all antimicrobials tested (over 60%), except colistin.

In the period 2011-2020 a relatively low level of MRSA invasive isolates was found. A significant trend was found to increase the proportion of vancomycin-resistant *Enterococcus faecium* from blood - from 0% in the period 2011 - 2018 up to 11.1% in 2019 and 18.2% in 2020.

In patients with oncohematological diseases, Gram-negative bacteria predominated over Gram-positive bacteria (54.3% vs. 38.0%), with the most common bacterial pathogens being *S. aureus* (17.3%), *E. coli* (16.0%), *Enterobacter* spp. (10.9%), *Klebsiella* spp. (10.3%) and *Enterococcus* spp. (8.8%). A persistent tendency over time for a high level of resistance to third generation cephalosporins among representatives of the *Enterobacteriaceae* family (49.4%) and the appearance after 2014 was established of invasive carbapenem-resistant isolates from the same family, with the most affected species in terms of both types of resistance being *K. pneumoniae* (57.8% and 6.7%, respectively).

A high 30-day mortality rate (26%) was found. The highest 30-day mortality is demonstrated in the intensive care structures of the hospital (35.8%), followed by the Hematology Clinics (27.3%). *E. coli* (23.6%), *S. aureus* (20.7%) and *K. pneumoniae* (19.2%) were associated with the highest number of deaths. When comparing the bacterial species, the highest 30-day mortality was found in infections caused by *A. baumannii* - *calcoaceticus* complex (53.6%).

The risk factors influencing prognosis and survival up to the 30th day were also determined.

The results are summarized in 15 conclusions, analyzed and discussed in the context of the data published so far from the scientific literature. Contributions of an original, confirmatory and scientific-applied nature also stand out.

The book collection contains 655 literary sources, of which 5 are in Bulgarian and 650 are in English.

4. Publications related to the dissertation work

18 full-text publications related to the dissertation are presented. Nine are with IF, including in *Lancet* (IF 202.731) and *Lancet Public Health* (IF 72.427). In seven of the presented publications, the dissertation student is the first author. All are indexed in Scopus and Web of Science.

5. **The abstract** makes a brief summary of the dissertation's work and is set out on 77 pages. The most important studies, results and discussions on the scientific problem are summarized.

6. **Conclusion**

The presented project of the dissertation work of Prof. Stoeva on the topic "Antibiotic resistance of the most common causative agents of bacteremia and the mortality associated with them" examines an actual problem of fundamental clinical importance and contains important scientific and scientific-applied results. The study covers a huge number of patients over a ten-year period, which makes it possible not only for the reliability of the statistical methods, but also for outlining trends. The methods used are modern and adequate, and the results obtained lead to important conclusions of great scientific and practical significance.

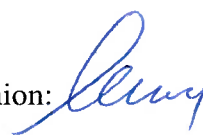
The dissertation meets all the requirements for the acquisition of the Doctor of Science degree, laid down in the Law on the Development of the Academic Staff in the Republic of Bulgaria and the ordinance for the Development of the Academic Staff of the University of Varna.

I confidently give my positive assessment and recommend to the respected members of the scientific jury to vote positively for awarding the scientific degree "Doctor of Sciences" to Prof.. Temenuga Zhekova Stoeva, MD, PhD in the scientific specialty "Microbiology".

Date: September 27, 2023

Varna

Prepared the opinion:



/ Prof.. Ilina Micheva, MD, PhD/