

STATEMENT OF OPINION

by Prof. Tanya Vasileva Strateva, MD, PhD

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(Member of the Scientific Jury, appointed by Order No. R-109-431 / 06.10.2023 of the Rector of the Medical University – Varna)

on a Dissertation for the award of scientific degree “Doctor of Science”

Professional direction **7.1. Medicine** in the Field of higher education

7. Healthcare and sports, Scientific specialty “**Microbiology**”

Author: Prof. Temenuga Zhekova Stoeva, MD, PhD

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Topic: Antimicrobial resistance of the most common causative agents of bacteremia and the associated lethality

1. General presentation of the procedure and the author of the dissertation work

The presented set of materials on electronic media is in accordance with the requirements specified in Art. 89. (4) of Section VI. Award of a Doctor of Science scientific degree at MU – Varna from the Regulations on academic staff development at the Medical University “Prof. Dr. P. Stoyanov” – Varna / 21.11.2022, and includes all necessary documents.

Prof. Temenuga Zhekova Stoeva graduated with honors from the Medical University of Varna in 1994, where she obtained a Master degree in Medicine. In 2006, she obtained a specialty in Microbiology, and in 2018 – a master's degree in Health Management at the Medical University of Varna. In 2009 she successfully defended a PhD Thesis on the topic “Microbiological and molecular genetic studies on the epidemiology and resistance to antimicrobial agents of *Acinetobacter baumannii* clinical isolates” in the scientific specialty “Microbiology”. Since 2003, Prof. Dr. T. Stoeva has been an Assistant Professor at the Department of Microbiology and Virology of the Medical University of Varna. She qualified as an Associate Professor in Microbiology in 2012, and since 2018 she has held the academic position of “Professor” at the same department. From 2014 to the present, Prof. Stoeva is the Head of the Laboratory of Clinical Microbiology at the UMHAT “St. Marina”, and from 2016 to the present, she is also the Head of the Department of Microbiology and Virology at the Medical University of Varna.

2. Relevance of the topic

The frequency of bloodstream infections in population-based studies in Europe and North America ranges between 113 and 204 per 100,000 person-years, and the mortality rate is estimated between 15% and 30%. A number of studies have demonstrated both an increasing burden of bacterial bloodstream infections worldwide and a steady trend towards a rapidly increasing incidence

of infections caused primarily by therapeutically problematic pathogens of the ESKAPE group (50–70% of their etiological spectrum). Some of these pathogens, such as vancomycin-resistant *Enterococcus faecium*, carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae*, were included in the World Health Organization list of microorganisms that are prioritized as "critical" for research and development of new effective antibiotics.

Regarding this, bloodstream infections caused by resistant microorganisms represent a serious threat to public health. Prof. Dr. T. Stoeva has chosen an extremely relevant topic for her dissertation work, through which she makes a detailed analysis of the microbiological and epidemiological aspects of laboratory-confirmed bloodstream infections in hospitalized patients at UMHAT "St. Marina" during a 10-year period (2011-2020).

3. Structure of the dissertation

The dissertation work consists of 296 standard typewritten pages and is richly illustrated with 27 figures, 36 tables and an appendix with 10 tables. The work is well balanced between its individual parts and structured as follows: Title page – 1 page, Content – 2 pages, List of the used abbreviations – 2 pages; Introduction – 3 pages, Literature review – 73 pages, Purpose and Objectives – 1 page, Materials and Methods – 16 pages, Results and Discussion – 124 pages, Conclusions – 5 pages, Contributions – 2 pages, List of publications and scientific reports related to the topic of the dissertation – 4 pages, Bibliography – 49 pages, and Appendices – 14 pages.

4. Literary awareness and evaluation of the literature review

The bibliography includes 655 titles, of which 5 are in Cyrillic and 650 are in Latin, and 77% of the cited literary sources were published in the last 10 years and refer to the period of the study.

The literature review is very thorough and written with exceptional competence. The definitions of bloodstream infections, their classification, epidemiology and etiology, as well as the modern microbiological diagnosis of these infections are comprehensively presented. The specific aspects of bloodstream infections in oncohematologic patients, the main resistance mechanisms to antimicrobial drugs among the most common bacterial causative agents of bloodstream infections, as well as the current status of the problem are reviewed.

In Bulgaria, there is a deficiency of complex microbiological and epidemiological studies on changes in the etiological spectrum of bloodstream infections and antibiotic resistance trends of the most common bacterial causative agents, the risk factors and the lethality with which they are accompanied, which motivated Prof. Dr. T. Stoeva for the dissertation development.

5. Evaluation of the used materials and research methods

Prof. Dr. T. Stoeva presents the study design very comprehensively. The classic microbiological methods and modern molecular genetic techniques used for resistance gene detection and epidemiological typing, such as Rep-PCR, ERIC-PCR, RAPD-PCR, and MLST, are detailed in the Materials and Methods section. The applied wide range of statistical methods guarantee an adequate interpretation of the results obtained.

6. Evaluation of the dissertation and its contributions

The purpose of the dissertation is argumentative. Five objectives have been clearly and precisely formulated for its achievement.

The obtained results and their interpretation represent an essential section of the dissertation, which is illustrated with numerous figures and well-arranged tables.

The results are presented sequentially, following the previously set tasks. The trends in the development of antimicrobial drug resistance among the leading causative agents of bacteremia in UMBAL "St. Marina", Varna (*Staphylococcus aureus*, *Enterococcus faecalis*, *E. faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *A. baumannii-calcoaceticus* complex, and *P. aeruginosa*) are summarized with high competence by the author. The enzymatic mechanisms responsible for the resistance of carbapenem-resistant *K. pneumoniae* isolates to third-generation cephalosporins and carbapenems as well as hospital-circulating sequence types have been identified. Detailed studies have been carried out on the carbapenem resistance mechanisms of the invasive *A. baumannii-calcoaceticus* complex isolates, 60% of which demonstrate an XDR phenotype. There is a significant trend of increasing proportion of vancomycin-resistant *E. faecium* from blood – from 0% in the period 2011–2018 to 11.1% in 2019 and 18.2% in 2020.

A high 30-day lethality (26%) was found in the study group of 798 patients with bloodstream infections caused by *S. aureus*, *Streptococcus pneumoniae*, *E. coli*, *K. pneumoniae*, *Enterobacter cloacae* complex, *A. baumannii-calcoaceticus* complex, and *P. aeruginosa*, and this rate was higher in cases of nosocomial infections compared with those acquired in the community (31.3% vs. 20.8%). Age was the only demographic parameter identified as a significant risk factor for mortality in the study group of patients. The highest 30-day lethality was found in the Intensive care units of the hospital (35.8%), followed by the Hematology clinics (27.3%).

The discussion is written in a highly scientific style and is based on the studies published so far by Bulgarian and foreign authors.

In summary of the performed experimental and expert work, 16 conclusions have been drawn, which are logical, follow the results obtained and correspond to the set purpose and objectives.

Based on the results and conclusions drawn from the conducted studies, 7 contributions were formulated, which are divided into three categories: original contributions, contributions of a confirmatory nature and contributions of a scientific-applied nature.

I acknowledge all contributions to the dissertation work. I would like to emphasize the following as being the most significant:

- ✓ A detailed analysis of the etiological spectrum of bacteraemias and antimicrobial resistance of ESKAPEEc pathogens in an unselected group of hospitalised patients with laboratory-confirmed bloodstream infections over a decade was performed, and trends over time were assessed and compared with European and global trends.
- ✓ A detailed analysis of the etiologic spectrum of bacteremias and antimicrobial resistance of ESKAPEEc pathogens in a group of oncohematological patients with laboratory-confirmed bloodstream infections over an 11-year period was performed, and trends over time were assessed. The results provide a basis to define the recommendations for empiric antimicrobial treatment in cases of febrile neutropenia or suspected infectious complications in this group of severely immunocompromised patients.
- ✓ The 30-day lethality (general and specific) and the risk factors for fatal outcome in bloodstream infections caused by 7 bacterial species (*S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *E. cloacae* complex, *A. baumannii-calcoaceticus* complex, and *P. aeruginosa*) among 798 hospitalized patients over a 5-year period were studied, as well as the heavy burden with which these diseases are associated has been demonstrated.
- ✓ The study expands the available data and scientific information on antimicrobial resistance among the leading bacterial causes of bloodstream infections and the burden of these infections

in Bulgaria and can serve as a basis for policy making aimed at limiting and controlling the problem at local and national level.

- ✓ The study has led to the generation of high quality and comparable data and their integration into a very large international database aimed at assessing the burden of antimicrobial resistance in various infections, including bloodstream infections.

7. Evaluation of scientometric indicators

Prof. Stoeva has submitted a list of 18 publications on the topic of the dissertation work, of which 9 in authoritative scientific journals with an impact factor, 3 – with an impact rank, and 6 in peer-reviewed scientific journals. The total impact factor of the publications is 287.926. Also, Prof. Stoeva has presented part of the results obtained in the work at 6 scientific forums.

8. Author's summary

The author's summary was prepared in accordance with generally accepted standards and reflects the main results achieved in the dissertation.

CONCLUSION

The dissertation work of Prof. Temenuga Zhekova Stoeva, MD, PhD, titled "Antimicrobial resistance of the most common causative agents of bacteremia and the associated lethality" focuses on a current problem in modern medicine and makes original and scientifically-applied contributions to the fields of clinical microbiology and hospital epidemiology.

In my opinion, the dissertation corresponds to the requirements of the Law on Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for its implementation, and the Regulations of the Medical University of Varna. The attached academic report on the scientometric indicators for obtaining the scientific degree "Doctor of Sciences" shows that Prof. Dr. T. Stoeva meets and significantly exceeds the minimum requirements for this degree.

Having considered all of the foregoing, I confidently give my *positive assessment* of the conducted scientific research presented in the above-reviewed dissertation *and propose to the esteemed scientific jury to award the scientific degree "Doctor of Science"* to Prof. Temenuga Zhekova Stoeva, MD, PhD in the scientific specialty "Microbiology".

16.10.2023

Statement by: 

/Prof. Tanya Strateva, MD, PhD/