

To the Head of the Scientific Jury
for the Dissertation thesis for the award of
the scientific degree "Doctor of Science"
in the scientific specialty "Microbiology",
Department of Microbiology and Virology,
Medical University – Varna

Review

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Scientific specialty Microbiology

Member of the Scientific jury

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Subject: Dissertation thesis for the award of the scientific degree

"Doctor of Science":

**ANTIBIOTIC RESISTANCE OF
THE MOST COMMON CAUSATIVE AGENTS OF
BACTERAEEMIA AND THEIR ASSOCIATED LETHALITY**

Author:

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Brief biographical data about the candidate

Prof. Temenuga Zhekova Stoeva, MD, graduated from an English language school in 1988 and graduated in medicine at the Medical University, Varna in 1994. She obtained her specialty in Microbiology in 2006. Since 2004, she has been working at the University Hospital "St. Marina", progressively growing from Assistant to Associate professor in 2012 and Professor in 2018. She has specialized abroad, at the Institute of Medical Microbiology, Immunology and Hygiene in Cologne, Germany, and in Athens, Greece.

Her successfully defended PhD thesis was entitled: "Microbiological and molecular genetic studies on epidemiology and antimicrobial resistance in clinical isolates of *Acinetobacter baumannii*".

Prof. Stoeva is currently the Head of the Department of Microbiology and Virology at Medical University - Varna. She has a very large number of responsibilities, both professional and organizational, as well as educational/skill building activities.

Prof. Stoeva is also a member of the Expert Council on the Medical Specialty "Clinical microbiology" by order of the Bulgarian Ministry of Health.

Dissertation sections

The dissertation is of 296 pages, of which 234 pages are textual. The work is very well and most thoroughly illustrated with a total 36 tables, 27 figures, and an appendix of 10 tables.

Sections include: title page, table of contents and abbreviations (5 pages), introduction (3 pages), literature review (73 pages), aim and objectives (one page), materials and methods (16 pages), results and discussion (124 pages), conclusions (5 pages), contributions of the dissertation (2 pages), scientific publications and presentations related to the dissertation (4 pages), references (49 pages), appendices (13 pages) and acknowledgments (one page).

Relevance of the scientific topic

Clinically significant bacteraemia, or the presence of bacteria in the blood with the simultaneous presence of clinical symptoms that can lead to sepsis and septic shock, is an emergency and life-threatening condition for patients. The mortality rate of these infections is alarmingly high, (averaging between 14% and 37%) and is the highest among patients in intensive care units. The microorganisms causing bloodstream infections are an even more serious and growing concern due to frequent and increasing antibiotic resistance and multidrug resistance of the bacteria.

The dissertation of prof. Stoeva is dedicated to these problems of great diagnostic, clinical and therapeutic importance and one of the priorities in infection control as well. Prof. Stoeva has made an in-depth assessment of the frequency and characteristics of clinically significant bacteremias in the "St. Marina" UMBAL during the prolonged period of 10 years (from 2011 to 2020), as well as on the evolution of the frequency of the causative agents, their phenotypic and genotypic characteristics and antibiotic resistance. Research further focuses on patient groups at highest risk of mortality. She also points out the benefits and advantages of newer rapid and accurate

methods for identification and determination of antibiotic susceptibility in the diagnostic work of many more laboratories.

Literary awareness of the problem

In the literature review of 73 pages, Prof. Stoeva covers in detail all the important issues related to the topic of the study, starting from the definitions of the types of bacteremia and the criteria for laboratory-confirmed infections and going to the determination of their frequency, lethality and evolution over the years and the spectrum of causative agents. She discusses the most important risk factors for clinically significant bacteremias, the prevalence of different bacterial agents in different regions of the world and compared to our country.

The classical and most modern methods of microbiological diagnosis of blood infections are examined in detail, including automated blood culture systems, the advantages of MALDI TOF MS, MALDI Sepsityper and molecular methods such as Fluorescence in situ hybridization using peptide nucleic acid probes (PNA FISH), PCR and Multiplex PCR and DNA sequencing to accelerate identification and/or bacterial susceptibility testing directly with blood. The frequencies, mechanisms and evolution of resistance to the most common infectious agents are reviewed.

Many of the cited publications are from the last 5 years, including from 2022 and 2023, and this again emphasizes their relevance.

In short, the review is detailed and analytical, and at the same time, critically presenting the current state of the issues. It demonstrates a good understanding of the most important, including the most recent, publications on the subject of the dissertation. Therefore, I believe that it deserves to be published as a monograph.

Aim and objectives

The aim of this dissertation is the microbiological and epidemiological study of clinically significant bacteremias and their mortality rates, including in the most at-risk groups of patients, as well as the determination of the frequency and resistance of the most frequent causative agents over a 10-year period.

To this purpose, the dissertation sets five relevant objectives, related to defined criteria and numerous (27 in total) indicators. As an example, I can give the determination of the 30-day lethality of patients with bloodstream infections with six criteria, or combinations. The aim and objectives are covered in the most comprehensive and detailed manner, which is then reflected by the methods used, the results obtained, their discussion and conclusions.

Evaluation of the materials and methods used

One of the merits of the dissertation is the coverage of a prolonged period of time (10 years) and a huge number of patients (15602), which realistically characterizes the dynamics and current status of clinically significant bacteremias in our country. An impressively large number (3954) of blood cultures of patients with oncohematological diseases were studied and 457 clinically relevant isolates were detected and evaluated from the samples of 442 of the patients in this group.

Prof. Stoeva used a large number of methods to achieve the aim and objectives of the thesis. I am going to list some of them because they are crucial in determining the completeness and reliability of the results obtained:

- the automated blood culture (BACTEC) system for incubating and continuous monitoring of blood cultures,
- identification methods, including classical biochemical tests, Crystal and the Phoenix automated identification and susceptibility testing system, as well as molecular genetic tests such as PCR for *gyrB* for *A. baumannii* and *hps60* sequencing for *E. cloacae* complex,
- susceptibility testing methods, both classical such as the disk diffusion method, and state-of-the-art such as the automated Phoenix system,
- molecular methods (PCR and DNA sequencing) for detection of genes for extended-spectrum beta-lactamases (ESBL) and carbapenemases,
- epidemiological typing methods such as Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR), Randomly Amplified Polymorphic DNA (RAPD), Repetitive element sequence-based PCR (rep-PCR) and Multilocus Sequence Typing (MLST).
- The modern program IBM SPSS version 21.0 was used for statistical processing of the results.

In short, Prof. Stoeva's research work is of a very large volume and is supported by numerous methods, which show her great professional competence.

Results and Discussion

The results are presented clearly, using appropriate controls and analyzed using statistical methods. All obtained results are discussed in the great detail in comparison with the data in other countries, including also those of the European Antimicrobial Resistance Surveillance Network (EARS Net).

The incidence (9.9%) of clinically significant blood cultures was determined over the 10-year study period, strictly adhering to the criteria outlined in the Materials and Methods section.

A high frequency (66.8%) of so-called ESKAPEE*c* causative organisms (including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*) was detected.

The mortality rates in different patient groups were determined according to the type of causative agents. The highest mortality rates were found in bloodstream infections caused by *E. coli* (23.6%), *S. aureus* (20.7%) and *K. pneumoniae* (19.2%). A high (26%) 30-day mortality rate was observed in the groups of patients infected with the most pathogenic and/or antibiotic-resistant causative agents. The percentage reached the highest and alarming rate (53.6%) in bloodstream infections caused by *A. baumannii* - *calcoaceticus* complex.

The genetic mechanisms of antibiotic resistance to third-generation cephalosporins and carbapenems have been assessed in carbapenem-resistant isolates. Clonal characteristics and persistence of ST15 were identified as a contributing factor to the spread of carbapenem-resistant *K. pneumoniae*.

The results lead to the suggested recommendations for optimizing the diagnosis and treatment of bloodstream infections.

Assessment of scientific and applied contributions

I will focus on Prof. Stoeva's dissertation's contributions which have impressed me the most:

- In our country, this work represents the first long-term and large-scale study of the characteristics and frequency of the causative agents of bloodstream infections, the associated lethality as a whole and in individual risk groups of patients, as well as the bacterial resistance to antibiotics and its evolution over time.
- The 2.5-fold increase in the BCR - Blood Culture Rate - from 2011 to 2020 is alarming, corresponding to the cumulative incidence of bloodstream infections. This indicates the need for earlier and more frequent blood collection for blood culture in patients at risk of bloodstream infection, and particularly in immunosuppressed patients.
- Risk factors for 30-day lethality of patients according to age, clinic, previous hospitalization, therapy in intensive care or surgical wards and type of bacteria were studied. Patient age was found to be a significant risk factor for lethality. Other risk factors for fatal outcome according to the frequent causative agents are resistance to third-generation cephalosporins, previous hospitalization, and treatment in a surgical or intensive care unit.
- Findings regarding the most suitable antibiotics for initial empiric therapy of bloodstream infections are both scientifically and practically relevant. The study determines the antibiotics with the greatest activity
 - against *E. coli*, *Enterobacter* spp. and *K. pneumoniae* (carbapenems and amikacin),
 - against carbapenem-resistant *K. pneumoniae* (colistin and amikacin), and
 - against enterococci (glycopeptides and linezolid), despite the increasing trend of vancomycin-resistant isolates as well as
 - antibacterials against other causative agents of bacteraemia.

These results are important to avoid delayed or inappropriate antibiotic treatment of the patients and, on the other hand, to optimize antibiotic choice pending microbiological outcome.

- Among the Gram-negative bacteria, the highest frequency (68.4%) of carbapenem resistance was found in *A. baumannii* - *calcoaceticus* complex isolates, which is explained by the prevalence and persistence of OXA-producing clones with multiple resistance.
- Another important result is the persistence of the ST15 clone of *K. pneumoniae*, commonly associated with carbapenem resistance, and the presence of *bla*_{KPC-2} in 12 hospital clinics between 2014 and 2017. The isolation of bacteria of this clone from the hands of medical staff in one of the clinics indicates the need for increased monitoring of compliance with hand hygiene practices as well as sanitary microbiological control. Here I would like to remind us that in the study

by Wetzker et al. [2016] in Germany, hand hygiene compliance was suboptimal, ranging from 67% to 84% depending on the recommendations.

- Data on the etiological spectrum and the evolution of resistance in one of the most at-risk groups, the patients with oncohematological diseases, is presented separately. The results are useful for determining guidelines for empirical therapy for these and other patients with severe immunosuppression.
- The results and conclusions of this dissertation demonstrate the need for more widespread implementation and use of MALDI TOF MS and molecular genetic methods for rapid identification and susceptibility testing of bloodstream infectious agents. In parallel, diagnostic optimization is necessary, which includes shortening blood collection and incubation time, as well as (and last but not least), a constant and good communication between clinicians and microbiologists. Optimizing the diagnostic working hours of laboratories is another factor that can be improved, e.g. by duty hours, given that the clinically significant bacteraemia is an emergency condition.

Publications related to the dissertation

Prof. Stoeva presents 18 publications in her dissertation and is the first author of 7 of them. Of all publications listed, 9 are in journals with impact factors.

Impressively, two of the 2022 publications are in the *Lancet* and *Lancet Public Health* with impact factors above 202 and, respectively, over 72. There are also publications in other prestigious international medical journals such as *Infection*, *Genetics and Evolution*, *Journal of Global Antimicrobial Resistance*, *Diagnostic Microbiology & Infectious Disease*, *APMIS* and *The Brazilian Journal of Infectious Diseases*.

The candidate has also participated in scientific forums, including abroad in Germany and Spain.

Prof. Stoeva's publications have been frequently cited in the medical literature. For instance, the *Lancet* article has been cited over 4,000 times, the publication in the *Journal of Global Antimicrobial Resistance* has been cited 29 times, and the article in *APMIS* has obtained 63 citations. These citations confirm the importance of the articles as a contribution to the overall understanding and guidelines for controlling antibiotic resistance and mortality in bacteremia, and reveal the high appreciation of the publications worldwide.

Evaluation of the thesis abstract:

The presented abstract is written in 77 pages and correctly reflects the structure, data and their analysis in the dissertation, as well as the contributions of the author.

Critical comments and recommendations

I have some minor comments that do not affect at all the value of the thesis. For instance, in Materials and Methods/Study Design, "*Propionibacterium*" could be replaced with "*Cutibacterium*", referring to *Cutibacterium acnes*. The abbreviation CoNSCH should not be in italics. On some pages, the family name of *Enterobacteriaceae* should be replaced by the name of the order *Enterobacterales*.

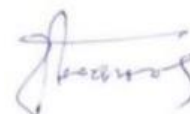
Conclusion

After reviewing Prof. Stoeva's dissertation, I believe that it meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the regulations for its application and the regulations of the Medical University - Varna for awarding an educational and scientific degree "Doctor of medical science".

This work is very up-to-date, diagnostically and clinically important, precisely planned and competently and thoroughly developed. This is the first such large-scale and long-term study of patients and the causative agents of blood infections in our country. The coverage of the 10-year research period and the corresponding number of patients (over 15,600), as well as the number and complexity of the used classical and state-of-the-art research methods, including automated and molecular genetic tests, outline the characteristics, current status, risk factors and guidelines for the control of clinically significant bacteremias in our country.

As the author of the dissertation, Prof. Stoeva appears as a motivated, diligent and highly competent scientific researcher who successfully conducts long-term and large-scale scientific studies. Her contributions, both theoretical and scientific-applied, are clearly outlined and appreciated by the international scientific community, as evidenced by their numerous citations.

Based on the above considerations, I strongly and confidently recommend the respected members of the Scientific Jury to award the educational and scientific degree "Doctor of Science" in the scientific specialty "Microbiology" to Prof. Dr. Temenuga Zhekova Stoeva, PhD, for her dissertation work.



16 Oct. 2023.

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