

To the Chairman of the Scientific Jury,  
Appointed by order P-109-388/28.08.2023  
Of the Rector of MU-Varna

## **Opinion**

**by Prof. Romyana Donkova Markovska-Davidkova, MD, PhD**

**Department of Medical Microbiology,  
Medical Faculty, Medical University – Sofia**

Regarding a dissertation on the topic: "ANTIBIOTIC RESISTANCE OF THE MOST COMMON CAUSES OF BACTERIEMIA AND THE LETHALITY ASSOCIATED WITH THEM" by **Prof. Dr. Temenuga Zhekova Stoeva**, presented for the acquisition of the scientific degree "**Doctor of Science**" in the doctoral program "**Microbiology**" in the field of higher education 7.0 Health care and sports and professional direction 7.1 Medicine

The submitted documents under the procedure have been prepared correctly, according with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the rules for its implementation at the University of Varna.

### **Biography**

Prof. Stoeva's biography is impressive. She grew successively from physician and assistant to associate professor and professor at MU-Varna, and since 2016 she has been the head of the Department. In 2009, he obtained the educational and scientific "Doctor of Medicine" degree with a PhD thesis "Microbiological and molecular genetic studies on the epidemiology and resistance to antimicrobial agents in clinical isolates of *Acinetobacter baumannii*". She has acquired additional qualifications in the course of various specializations, of which the most impressive are molecular genetic methods education and working with bone marrow transplant patients. The number of Prof. Stoeva's publications is impressive - 96, of which 27 have an impact factor, impressive are as well as the implemented projects - 11 university projects and one international one, held together with Oxford University on the problems of antibiotic resistance. The number of PhD students is also impressive (six), with four having successfully defended their degrees. All these achievements were achieved against the background of the continuous educational activity of Prof. Stoeva, with lectures, exams and training of specialists. Last but not least, the extensive laboratory-diagnostic activity should be noted - introduction of modern diagnostic methods, participation in visits and consultations on antibiotic therapy of problem patients, participation in clinical trials of new antimicrobial drugs, etc. Prof. Stoeva is the head of the Microbiology Laboratory at the "St. Marina" UMBAL and a member of the Expert Council on the medical specialty "Microbiology" at the Minister of Health since April 2019.

### **Relevance and significance of the selected scientific problem**

Prof. Stoeva's dissertation is dedicated to the extremely serious and current problem of the increased antibiotic resistance of microorganisms associated with bacteremias and analysis of the etiological structure of these infections, as well as the mortality associated with them. The global increase in multidrug-resistant bacteria is a serious threat to the health of patients in hospitals and society. A group of microorganisms was designated ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*). They are a paradigm for multiple resistance and a therapeutic problem. To a large extent, they are also among the main causes of bacteremia. The problem is even more relevant for Bulgaria, which is among the first places in terms of resistance to third-generation cephalosporins in *K. pneumoniae* invasive isolates, from blood and cerebrospinal fluid, with levels reaching over 70-80%. The percentage of carbapenemase-producing isolates is increasing and pan-resistant isolates are emerging. The problem of treating infections caused by extremely resistant and pan-resistant *K. pneumoniae* and *A. baumannii* in intensive care units, in immunocompromised patients and in patients with bloodstream infections is particularly acute. The analysis of the etiological structure of blood infections, the mechanisms of antibiotic resistance of their causative agents and the risk factors associated with mortality over a long 10-year period provides important information for the creation of adequate therapy and programs for the prevention and control of infections. Even more important is the fact that the generation of comparable data on these issues allows their integration with those of European and Global Surveillance Networks. Prof. Stoeva's dissertation is dedicated to the extremely serious and current problem of the increased antibiotic resistance of microorganisms associated with bacteremia and analysis of the etiological structure of these infections, as well as the mortality associated with them. The global increase in multidrug-resistant bacteria is a serious threat to the health of patients in hospitals and society. A group of microorganisms was designated ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*). They are a paradigm for multiple resistance and a therapeutic problem. To a large extent, they are also among the main causes of bacteremia. The problem is even more relevant for Bulgaria, which is among the first places in terms of resistance to third-generation cephalosporins in *K. pneumoniae* invasive isolates, from blood and cerebrospinal fluid, with levels reaching over 70-80%. The percentage of carbapenemase-producing isolates is increasing and pan-resistant isolates are emerging. The problem of treating infections caused by extremely resistant and pan-resistant *K. pneumoniae* and *A. baumannii* in intensive care units, in immunocompromised patients and in patients with blood infections is particularly acute. The analysis of the etiological structure of blood infections, the mechanisms of antibiotic resistance of their causative agents and the risk factors associated with mortality over a long 10-year period provides important information for the creation of adequate therapy and programs for the prevention and control of infections. Even more important is the fact that the generation of comparable data on these issues allows their

integration with those of European and Global Surveillance Networks.

### **Structure of the dissertation**

The dissertation work is written on 296 pages, and is structured according to the generally accepted scheme: Abbreviations used - 2 pages; Introduction - 3 pages, Literature review - 74 pages, Aim and tasks - 1 page, Materials and methods - 16 pages, Results and discussion - 124 pages, Conclusions and contributions - 7 pages. The dissertation work is illustrated with 36 tables and 27 figures. 655 sources were used, of which over 80% from the last 10 years.

### **Evaluation of the qualities of the literature review**

The literature review is written extremely competently and at a high scientific level, using a large number of sources /over 600/, most of them from the last 5-10 years, which is proof of the relevance of the scientific information. The classification, epidemiology of blood infections (frequency, sources, risk factors) and modern methods of microbiological diagnostics are discussed in detail. The etiologic spectrum of worldwide bloodstream infections has been reviewed in great detail, as well as for individual countries and continents, covering a large number of population-based and multicenter hospital-based studies. Prof. Stoeva paid special attention to blood infections in patients with oncohematological diseases. The main mechanisms of resistance and their distribution in the leading causative agents of bacteremias were discussed. A great impression is made by Bulgaria's first place in mortality from blood infections in 2019 - both overall 70.3 per 100,000 populations, and separately for the most common causative agents - *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*. It is possible that this is related to the fact that Bulgaria is in first place in terms of resistance to third-generation cephalosporins in invasive *K pneumoniae* isolates with over 80% for 2021, and among the leading places in resistant to third-generation cephalosporins invasive *E coli* isolates, as well as with a high frequency of carbapenem-resistant invasive *K pneumoniae* - 46.3%. Levels of carbapenem-resistant invasive *A baumannii-calcoaceticus* were also increased. There are similar alarming data regarding fluoroquinolones and aminoglycosides. These data support the topicality of the problem in Bulgaria. I recommend that the literature review be published as a separate monographic work.

### **Aim and tasks**

The set goal is clearly formulated, and the tasks (5 in total) correspond to the main goal and lead to its fulfillment.

### **Section "Materials and methods"**

Prof. Stoeva's dissertation covers a long period, 2011 - 2020, including all cases of clinically significant bacteremia in patients from "St. Marina" Hospital - Varna. In addition, cases of bacteremia in oncohematological patients are included. The criteria for inclusion in the study are very well shown. A variety of microbiological and molecular genetic methods have been used. Of interest is the fact that species identification is based on proven identification systems such as Crystal and Phoenix, but molecular genetic identification has also been used for some species. Molecular genetic methods such as sequencing have also been used to identify

resistance genes, so that the exact genes encoding the beta-lactamases have been identified. Based on modern methods, the clonal relationships of the studied isolates have been proven. The results are supported by the necessary statistical methods. The detailed description of the methods used enables them to be reproduced. The studies carried out were approved by the Research Ethics Committee.

### **Section "Results and Discussion"**

The presented dissertation is a well-structured retrospective study, with a large time span of 10 years and a large number of cases - 15602 hospitalized patients were covered. The results and discussion are grouped together logically in a common section. It includes five main subsections with proprietary studies. A detailed analysis of the frequency of clinically significant bacteremia and their etiological spectrum in patients treated at UMHAT "Saint Marina" is presented. The large number of studied isolates is extremely impressive - 2727 unique isolates. Prof. Stoeva found 9.9% positive clinically significant blood cultures, with the main isolates being *S. aureus* (17.2%), followed by *E. coli* (14.6%) and *K. pneumoniae*. She reports that for the studied period, Gram-negatives dominate (58.9%) as the main causes of bacteremia, but there is a tendency to increase Gram-positives, which confirms the European and world trends. The results are similar in oncohematological patients, where *Enterobacter spp* appears in third place, ahead of *K. pneumoniae*. Antibiotic susceptibility was studied in detail, and the most important conclusions can be noted: the tendency to increase the frequency of carbapenem-resistant isolates especially in *K pneumoniae*, the high frequency of third-generation cephalosporin-resistant *K pneumoniae* /74.9%/, as they are often resistant to aminoglycosides and fluoroquinolones, similar /65%/ percentage of resistant to third generation cephalosporins *Enterobacter spp*. *A. baumannii calcoaceticus complex* isolates consistently demonstrated very high levels of resistance to all antimicrobials tested (over 60%), except colistin. The share of carbapenem-resistant *A. baumannii - calcoaceticus complex* was the highest (68.4%), with 60% of the isolates having an extremely resistant phenotype. The work also found a trend for an increase in vancomycin-resistant *Enterococcus faecium* /up to 18.2% in 2020/. The level of MRSA remains relatively low. Of the broad-spectrum beta lactamases, *bla*<sub>CTX-M-15</sub> predominates, and of the carbapenemases *bla*<sub>KPC-2</sub> in *K. pneumoniae* and *bla*<sub>OXA-24/40-like</sub> or *bla*<sub>OXA-23-like</sub> in *A. baumannii - calcoaceticus complex*. In this study, the main MLST types associated with blood infections ST11, ST15 among *K pneumoniae* are studied and discussed. A major advantage of the work is the studied 30-day mortality in 798 patients and the risk factors associated with it. The doctoral student found that it was relatively high at 26% and the main risk factors were the age of the patient and the type of clinic, with intensive care and hematology clinics being associated with the greatest mortality. The fact that the bacterial type also has an impact is very interesting - the highest mortality is found in *Acinetobacter baumannii-calcoaceticus complex* isolates, and when antimicrobial sensitivity is taken into account - in ampicillin-resistant *E. coli* and in third-generation cephalosporin-resistant *K pneumoniae*, which exclude carbapenem-resistant isolates. As a result of the presented study, Prof. Stoeva formulated 16 main conclusions, which correspond to the set goals and objectives.

### **Evaluation of the contributions of the dissertation work**

From the results obtained and conclusions drawn, Prof. Stoeva formulated 7 contributions: 5 of an original nature, 1 of a confirmatory nature and 1 of a scientific-applied nature, which I fully accept.

### **Scientific indicators**

Prof. Stoeva presents 18 full-text publications in connection with the dissertation, of which 9 are in IF journals. An excellent impression is made by the fact that publications with IF are in renowned journals such as Lancet with IF above 200.

### **Abstract**

The attached abstract critically presents the dissertation work and its results, contributions and conclusions. The requirements of the regulations have been met.

**In conclusion**, the dissertation presented by Prof. Temenuga Stoeva is a thorough, very precisely executed and written work, on a relevant topic, which is also a problem for Bulgaria. The large scale of the work covering a large area of medical microbiology is very impressive. The results are interesting, with a significant applied and theoretical contribution to the field of modern microbiological practice. They were achieved as a result of the candidate's many years of highly professional work. The contributions are not only of national, but also of international importance, as evidenced by the publication of the results in international journals such as Lancet. In terms of structure, content and volume, the dissertation work fully meets the criteria in the ŽRASRB and the Regulations of MU-Varna for the acquisition of the scientific degree "Doctor of Sciences". In view of the above, I confidently give my positive assessment and recommend to the members of the Scientific Jury to vote for awarding the scientific degree "Doctor of Sciences" in the scientific specialty "Microbiology" to Prof. Dr. Temenuga Stoeva.



15.10.2021г.

**Prepared the opinion:**

**/ Prof. Romyana Donkova Markovska-Davidkova, MD, PhD /**