

TO  
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OF THE RECTOR OF THE MEDICAL UNIVERSITY  
„PROF. DR. PARASKEV STOYANOV” - VARNA

### REVIEW

By Prof. Stefana Doneva Sabtcheva, MD, PhD,  
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Of a dissertation for the award of scientific degree „Doctor of Science”  
in the field of higher education 7. Health and Sport, Professional drive 7.1. Medicine,  
Scientific specialty „Microbiology”

**On the subject: „Antimicrobial resistance of the most common causative agents of  
bacteremia and associated lethality”**

**By Prof. Temenuga Zhekova Stoeva, MD, PhD**

I declare that I have no scientific works in common with the author of this  
dissertation.

After completing his medical education at the Medical University - Varna in 1994  
with excellent grades, prof. Temenuga Stoeva began her successful development as a  
scientist and an all-round lecturer in microbiology at the Medical University and the  
University Hospital „St. Marina”. In 2003, she joined the faculty as an assistant  
professor, in 2006 she acquired a medical specialty in microbiology, and in 2009 she  
defended her dissertation on „Microbiological and molecular studies on epidemiology  
and antimicrobial resistance in clinical isolates of *Acinetobacter baumannii*” and received  
the scientific and educational degree of „Doctor” in the scientific specialty

„Microbiology”. After winning competitions, in 2012 she was appointed to the academic position of „Associate Professor” and in 2018 – to the academic position of „Professor” in the scientific specialty „Microbiology” at MU - Varna. Since 2014 she has been the Head of the Laboratory of Microbiology of the University Hospital „St. Marina”. Since 2016 she is also the head of the Department of Microbiology and Virology of MU - Varna. The skillful combination of daily medical-diagnostic and scientific-teaching activities determines the specificity of the scientific interests of prof. Stoeva's research focuses on areas of medicine that are significant for the patients she treats – infections related to medical care, infections in immunocompromised patients and mechanisms of resistance to antimicrobial drugs in clinically important bacterial species.

#### **Relevance of the dissertation work**

The present dissertation is devoted to bloodstream infections – a leading cause of morbidity and mortality, especially in immunocompromised patients, the most common causative agents of bacteremia and their antibiotic resistance. Medical advances and treatment options for a number of diseases, accompanied by a continuous increase of immunocompromised patients, on the one hand, and the increasing antibiotic resistance of the causative agents of bacteremia against the introduction of single new antibiotics, on the other hand, determine the exceptional relevance of the dissertation submitted for review.

#### **Structure of the dissertation**

The dissertation is written in 296 pages and is structured according to the generally accepted scheme with a proportional distribution of the text in the individual sections as follows: introduction - 3 pages, literature review - 73 pages, aim and tasks - 1 page, materials and methods - 16 pages, results and discussion - 124 pages, conclusions - 5 pages, contributions - 2 pages, and references - 49 pages. A list of publications and communications related to the dissertation is also attached. The dissertation is optimally illustrated with 73 precisely crafted visual materials (27 figures and 46 tables). 655 references are cited in the dissertation: 5 are in Cyrillic and 650 in Latin, most of them from the last 10 years - proof of the relevance of the dissertation.

#### **The introduction**

The introduction justifies the relevance of research on bloodstream infections caused by resistant microorganisms. The importance of hospital-based surveillance and analysis of the etiological spectrum and antibiotic susceptibility of pathogens, their risk factors, and the lethality that accompanies them for the development of treatment algorithms, prevention and control programs at the local level is emphasized, as well as the contribution of the data obtained to the design of policies aimed at the containment and control of antibiotic resistance at the national and global levels.

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

The literature review is logically structured and addresses all aspects of bloodstream infections. The terms bacteremia and bloodstream infections are defined in detail. The current classification and epidemiology of bloodstream infections are described in detail, as well as the etiology of bacterial infections, with an emphasis on onco-hematological patients with febrile neutropenia due to their high lethality. A special place in the review is occupied by modern microbiological diagnosis of bloodstream infections. A critical analysis is made of the current status of antibiotic resistance among the major bacterial causative agents of bloodstream infections and the genetic mechanisms underlying it.

The review concludes with a discussion of the situation in Bulgaria and, since no comprehensive hospital-based studies of bloodstream infections, including microbiology, epidemiology, risk factors and lethality, have been conducted in our country, given the high mortality in transplant and onco-hematological patients, the need for a systematic study on antibiotic resistance of the most common causative agents of bacteremia and associated lethality is logically inferred.

### **Purpose and Tasks**

The author systematically and thoroughly approached the identified tasks in accordance with the aim of the thesis: to perform a microbiological and epidemiological study on antibiotic resistance of the most common causative agents of bacteremia over a 10-year period and the lethality associated with them.

In order to achieve the aim of the study, 5 tasks were set, which define the sequence of the planned research, namely: (1) to analyze the etiologic spectrum of

laboratory-confirmed bacterial bloodstream infections proven in hospitalized patients at St. Marina Hospital over a 10-year period (2011-2020), as well as the antibiotic resistance of the most common causative agents of bacteremia; (2) to investigate the genetic mechanisms of resistance to third-generation cephalosporins and carbapenems in representative carbapenem-resistant *Klebsiella pneumoniae*, *Enterobacter cloacae* complex and *Acinetobacter baumannii* blood isolates and to perform epidemiological typing; (3) to determine and analyze the etiologic spectrum of laboratory-confirmed bacterial bloodstream infections demonstrated in hospitalized patients with malignancies from 2010-2020; (4) for all hospital-treated patients with bloodstream infections caused by seven bacterial species between 2016 and 2020 (*S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *E. cloacae* complex, *A. baumannii* - *calcoaceticus* complex, *P. aeruginosa*), to extract, summarize and associate information related to 27 variables; (5) to calculate the 30-day lethality indicator (general and specific - by age groups, sex, type of clinic, site of acquisition, microbial species, „microorganism/antibiotic” combination) in the group of all hospitalised patients with laboratory-confirmed bloodstream infections caused by the 7 bacterial species in the period 2016-2020, and to identify risk factors that influence 30-day lethality.

#### **Evaluation of the Materials and Methods section**

A wide range of classical microbiological and modern molecular methods were used to perform phenotypic and genetic characterization of non-repetitive microbial isolates derived from blood in septic episodes. The methods used are highly informative and suitable for solving the set tasks.

#### **Evaluation of the Results and Discussion section**

A considerable amount and variety of research work has been carried out. In accordance with the design of the retrospective clinical-microbiological and epidemiological study, the results of the studied patients and their blood isolates over a 10-year period are presented and analysed. The results obtained and their discussion strictly follow the set objectives as follows:

1) During the period 2011-2020, the relative proportion of positive haemocultures was 16%, with clinically significant haemocultures accounting for 9.9%. The etiological spectrum of laboratory-confirmed bacterial bloodstream infections in the unselected

patient population was dominated by Gram-negative bacteria (58.9%), but demonstrated a statistically significant trend over the years of increasing proportion of Gram-positive bacteria and decreasing proportion of Gram-negative bacteria and fungi. The most frequently isolated bacterial species from haemocultures over the entire period was *S. aureus* (17.2%), followed by *E. coli* (14.6%), *K. pneumoniae* (12.0%), *E. cloacae* complex (8.0%), *A. baumannii* - *calcoaceticus* complex (6.3%) and *E. faecalis* (6.3%). The proportion of ESKAPEc pathogens in the etiological spectrum was very high, reaching 66.8%, while that of EARS-Net monitored bacterial species was 64.7%. In the course of the 10-year follow-up, a statistically significant trend of increasing relative proportion of *Staphylococcus aureus* and *Streptococcus viridans* isolates and decreasing proportion of *Klebsiella pneumoniae* was demonstrated.

The relative proportion of third generation cephalosporin and fluoroquinolone resistant *E. coli* from blood is high (more than 25%), but without a significant increasing or decreasing trend over the years. The activity of aminopenicillins against *E. coli* is highly reduced, with resistance to this antibiotic group being the highest (63.2%). Carbapenems and amikacin have preserved activity against *E. coli* (resistance <1% and <5%, respectively), making them a suitable choice for empiric therapy in cases of bloodstream infections associated with this bacterial species. A very high level of resistance to third-generation cephalosporins was demonstrated among *Klebsiella pneumoniae* isolates (74.9%), significantly exceeding the proportion resistant to the same antibiotic group *E. coli* and higher than among *Enterobacter* spp. Third-generation cephalosporins were the group with the most reduced activity against *K. pneumoniae*. Very high levels of resistance (over 50%) are also reported for fluoroquinolones and gentamicin, although the latter shows a statistically significant trend of decreasing resistance from 73% in 2011 to 40.9% in 2020. Carbapenems and amikacin are the antimicrobials with the highest activity against *K. pneumoniae* (11.8% and 13.9% resistance, respectively). Over the 10-year study period, the most dynamic and dramatic changes were evidenced in carbapenem antibiotic resistance, which went from 0% in 2011 to 32.7% in 2014 and 18.2% and 13.6% in 2019 and 2020. In the group of carbapenem-resistant *K. pneumoniae*, colistin and amikacin demonstrate the best *in vitro*

activity (6% and 17.9% resistance), making them the drugs of choice for treatment in the case of infection caused by carbapenem-resistant isolates.

2) Among the resistance mechanisms investigated, *bla*<sub>CTX-M-15</sub> has been identified as a major mechanism of resistance to third-generation cephalosporins in carbapenem-resistant *K. pneumoniae* isolates, while *bla*<sub>KPC-2</sub> and to a much lesser extent *bla*<sub>NDM-1</sub> mediate the enzymatic mechanism of carbapenem resistance in these isolates. Carbapenem-resistant *K. pneumoniae* are associated with 5 different ST types: ST15, ST76, ST151 and ST1350 for KPC-2 producers and ST11 for NDM-1 producing isolates. There is evidence of widespread intra-hospital dissemination of KPC-2 and/or CTX-M-15 producing ST15 *K. pneumoniae*. This clone persisted for several years, demonstrating high cross-transmissible, epidemic and invasive potential. In addition to ST15, resistance to third-generation cephalosporins and carbapenems has been associated with within-hospital dissemination of other ST types coexisting with the dominant ST15. In this sense, although clonal spread of ST15 contributes significantly to the dissemination of carbapenem-resistant *K. pneumoniae*, non-ST15 strains also emerge and contribute. A very high relative proportion of *Enterobacter* spp. isolates from blood resistant to third-generation cephalosporins (over 65%), as well as high levels of resistance to fluoroquinolones and gentamicin (over 45%) have been demonstrated. Compared to all other Gram-negative bacterial species, the proportion of carbapenem-resistant *A. baumannii* - *calcoaceticus* complex was the highest (68.4%), with 60% of the isolates having an XDR phenotype. Carbapenem resistance was associated with the presence of *bla*<sub>OXA-24/40-like</sub> and/or *bla*<sub>OXA-23-like</sub> genes in association with *ISAbal*. The high relative proportion of carbapenem-resistant isolates of *A. baumannii* is associated with hospital dissemination and the persistent presence of several OXA-producing MDR *A. baumannii* clones with endemic characteristics. A relatively low level of MRSA invasive isolates was found in the period 2011-2020, with a proportion lower than the European average and very close to the Bulgarian average in 2020. With the exception of macrolides, all other antibiotic groups retain good activity, with a proportion of non-susceptible isolates below 20%. There is a significant increasing trend in the proportion of vancomycin-resistant *Enterococcus faecium* from blood - from 0% in the period 2011-2018 to 11.1% in 2019 and 18.2% in 2020. *E. faecium* demonstrate very high levels of resistance to

aminopenicillins (95.3%), fluoroquinolones (84.7%) and HLARs (90.3%). In contrast to *E. faecium*, the proportion of ampicillin-resistant *E. faecalis* remains low (9.4%), making the aminopenicillin group an appropriate choice for therapy of *E. faecalis*-associated infections. The activity of fluoroquinolones over the same period among *E. faecalis* is significantly reduced (over 35%) with a high proportion of HLAR (over 45%). Despite the significant trend of increasing vancomycin resistance, glycopeptides and linezolid are still the antibiotics with the best activity against *Enterococcus* spp. isolates.

3) In the period 2010-2020, the etiological spectrum of bacteremias associated with bloodstream infections in patients with oncohematologic diseases was dominated by Gram-negative 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%). There was a statistically significant trend for a decrease in the relative proportion of *S. aureus* and an increase in that of coagulase-negative staphylococci in the etiological spectrum of bloodstream infections in this group of patients. The study performed in the group of patients with oncohematologic diseases demonstrated the emergence and persistence over the years of problematic MDR microorganisms, predominantly Gram-negative bacteria, associated with bloodstream infections in this patient population, a phenomenon that reflects increasing antibiotic resistance in the community and follows the trend in the unselected population of hospital patients with bloodstream infections. There is a persistent trend over time of high levels of resistance to third-generation cephalosporins among members of the *Enterobacteriaceae* family (49.4%) and the emergence after 2014 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 statistically significant trend of increasing resistance to third-generation cephalosporins, aminoglycosides and fluoroquinolones was demonstrated in the group of *Enterobacter cloacae* complex isolates; persistence of a high relative proportion of carbapenem-resistant *A. baumannii* - *calcoaceticus* complex against the background of a statistically significant trend for an increase in this resistance; and an increase in the proportion of MDR *Enterococcus faecium*. Similar to the general patient population, a positive finding was the low relative proportion of MRSA blood isolates. No *Staphylococcus* spp. and

*Enterococcus* spp. isolates resistant to glycopeptide antibiotics and oxazolidinones were identified during the study period, making these antibiotics the drugs of choice preferred for initial treatment in cases of suspected staphylococcal or enterococcal bloodstream infection or febrile neutropenia until an etiologic diagnosis is made.

4) The indicators „age”, „type of clinic”, „prior hospitalization”, „type of therapy administered (ICU and/or surgical treatment)”, „bacterial species” and „infectious syndrome” were identified as independent factors that significantly influenced the prognosis for survival to 30 days (predictors of death). Grouped according to bacterial species in cases of *E. coli* infections, the factors „resistance to third-generation cephalosporins”, „prior hospitalization”, and „surgical/ICU treatment” were significantly associated with the risk of dying, and in *S. aureus* infections, so were „age” and „surgical/ICU treatment”. For *P. aeruginosa* and *A. baumannii* - *calcoaceticus* complex infections, a statistically significant predictor of death was the variable „surgical treatment”, and in the case of bloodstream infections caused by *K. pneumoniae* - „surgical/ICU treatment”.

5) A high 30-day lethality (26%) was found in the study group of 798 patients with bloodstream infections caused by *S. aureus*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *E. cloacae*, *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%). With the exception of age, all other demographic characteristics did not prove to be significant risk factors for lethality in the study group of patients. The highest 30-day lethality was evidenced in the intensive care units of the hospital (35.8%), followed by 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. In a comparison between bacterial species, the highest 30-day lethality was demonstrated for infections caused by *A. baumannii* - *calcoaceticus* complex (53.6%). When 30-day lethality was evaluated against the bacterial species/antibiotic combination, aminoglycoside, quinolone and meropenem-resistant *A. baumannii* - *calcoaceticus* complex (15.9-16.3%) were associated with the highest number of deaths, followed by ampicillin-resistant *E. coli* (15.9%) and third-generation cephalosporin-resistant *K. pneumoniae* (14.9%).

#### **Evaluation of the scientific contributions**



In the thesis prof. Stoeva has achieved significant original and confirmatory contributions of a scientific and applied nature.

Among the contributions of original nature, the following stand out: (1) An analysis of the etiological spectrum of bacteremias and antibiotic resistance of ESKAPEE<sub>c</sub> associated pathogens in an unselected group of hospitalized patients with laboratory-confirmed bloodstream infections over a decade was performed, and trends over time were assessed and compared with European and global trends. (2) A detailed analysis of the aetiological spectrum of bacteraemias and antibiotic resistance of ESKAPEE<sub>c</sub> associated pathogens in a group of oncohaematology patients with laboratory-confirmed bloodstream infections over an 11-year period was performed and trends over time were assessed. The results provide a basis on which to define recommendations for empiric antibiotic treatment in cases of febrile neutropenia or suspected infectious complications in this group of immunocompromised patients. (3) The 30-day lethality (general and specific) and 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, and the heavy burden with which these diseases are associated has been demonstrated. (4) The study expands the available data and scientific information on antibiotic resistance among the leading 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.

Contributions of a confirmatory nature are distinguished by a clear concentration on problems of clinical microbiology. The genetic mechanisms of carbapenem resistance among the major pathogens (*K. pneumoniae*, *A. baumannii*) associated with clinically significant bacteremias have been investigated, and their widespread hospital dissemination and association with certain sequence types and international clones have been demonstrated.

Among the contributions of a scientific and applied nature of great clinical relevance are the findings of the detailed study of the factors „Antibiotic resistance” and „Lethality” accompanying bloodstream infections in hospitalized patients, especially in

immunocompromised patients. The results clearly demonstrate the need to introduce modern laboratory methods for rapid microbial identification and antimicrobial susceptibility testing in life-threatening bloodstream infections.

#### **Publications related to the dissertation**

The results of the extensive research work of prof. Temenuga Stoeva is reflected in 18 publications, in 11 of which she is the first or second author - evidence of a leading contribution to the development of the publication. The dissertant has also 6 congress participations, 2 of which were abroad. It should be noted that the results of Prof. Stoeva have been published in prestigious journals with a total Impact Factor of 287.926. Overall, these scientific metrics present the dissertant as an established and respected scientist with a significant contribution to the development of clinical microbiology and knowledge in the field of healthcare-associated infections, infections in immunocompromised patients and mechanisms of antimicrobial drug resistance in clinically relevant bacterial species.

#### **Conclusion**

The presented dissertation is on an extremely important problem for medical science and practice with significant scientific and applied contributions related to all clinical and microbiological aspects of bacteremias in hospitalized patients. 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 scientific degree „Doctor of Sciences” in the scientific specialty 01.06.12 - Microbiology to Prof. Temenuga Zhekova Stoeva, MD, PhD.

Reviewer:



/ Prof. Stefana Sabcheva, MD, PhD /

16.10.2023