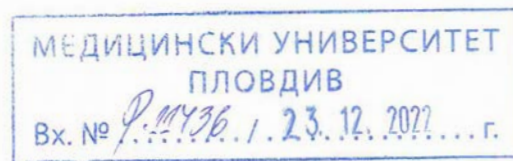


To the Chairperson of the Scientific Jury, appointed
with
Order R-2855/14.11.22 of the Rector of the MU-
Plovdiv



Review

by Prof. Todor Kantardjiev, MD, D.Sci.

of the presented dissertation of Dr. Gergana Boteva Lengerova, assistant professor at the Department of Microbiology and Immunology with the topic "Evaluation of modern microbiological techniques for rapid diagnosis of patients with bacteremia and fungemia" for the awarding of the educational and scientific degree "DOCTOR" in a doctoral program in "Microbiology", in the field of higher education 4. Natural sciences, mathematics, and informatics, professional direction 4.3. Biological sciences; Scientific supervisor Assoc. Prof. Mihail M. Petrov, MD, PhD and Statistical analysis consultant: Assoc. Prof. Ralitsa Raycheva, PhD.

I have no co-authored publications or conflict of interest of any other nature in the sense of paragraph 1, items 3 and 5 of the LDASRB with the doctoral student.

I. General presentation of the procedure

The dissertation is written on 197 pages and includes 14 tables, 68 figures, and 6 appendices. It is organized into 13 chapters. The bibliography covers 375 literary sources, the majority of which were published after 2015. The procedure is in compliance with all applicable legal requirements.

II. Relevance and importance of the research topic

The dissertation's topic is pertinent because, in the past thirty years, no comprehensive studies have been carried out in our country regarding the acceleration and optimization of microbiological diagnostics in septic conditions. In some of the previous Bulgarian dissertations, conventional and apparatus methods for accelerating laboratory results through colorimetric and turbidimetric approaches for biochemical identification and reporting of microbial growth were compared. The introduction of genetic and spectrometric techniques in the country necessitates a thorough analysis of their clinical and practical applications. The significance and utility of the dissertation are determined by its topic.

The literature review is properly structured on 31 pages and briefly traces the historical development of the problem of laboratory diagnostics in cases of bacteremia and fungemia. Particular attention is paid to the definitions, which gives depth and clarity to the subsequent exposition. Overall, the work allows the doctoral student to briefly analyze the historical evolution of the diagnosis of fungemia and bacteremia from light microscopic methods, blood cultures, and biochemical identification to genetic amplification, hybridization, and mass spectrometry analysis. This suggests that Dr. Lengerova has a solid theoretical and practical knowledge base. The review demonstrates her ability to work with literary sources, a high level of knowledge, and the capacity to make correct and well-reasoned conclusions. It is determined what inconsistencies and gaps exist in the literature data, what aspects of microbiology are unknown to us, and to which questions the dissertation will provide answers. The purpose and tasks of the dissertation are based on the summary. A complete correlation is achieved between the tasks and the work's final conclusions. The text is now more persuasive and demonstrates the doctoral candidate's ability to work with scientific literature and draw accurate scientific conclusions.

The work's materials and methods are presented consistently and thoroughly. The inclusion and exclusion criteria are discussed and justified. It is impossible to ignore the fact that the management of the department has provided cutting-edge equipment for genetic and spectrometric research, which is unmatched anywhere else in our country. In this way, it offered the doctoral student opportunities for contemporary scientific development, which are years ahead of their time. In terms of the patient selection, attention should be paid to some features of the medical facility that differentiate it from others in the country.

University Hospital "St. George" is the country's largest medical facility with 1,490 beds, 59 of which are intensive care beds. Comparable to the majority of hospitals in the world, the number of beds allows the doctoral student to work with a large number of clinical samples from different clinics with many beds, which is impossible in the majority of our country's medical institutions with several hundred inpatients. This hospital has a long-standing tradition of making efforts to comply with an antibiotic policy. That policy has served as a model and example for other medical facilities across the country for the past three years (to the extent that it is complied with). This reflects on the etiological structure and antimicrobial drug resistance and distinguishes them from the majority of hospitals across the country. This medical institution is no exception to the chronic weaknesses of our health care system in terms of the etiological diagnosis and etiologic therapy of sepsis and the systemic inflammatory response: the small number of the taken blood cultures, lack of complete clinical microbiological monitoring of risk patients to signal the etiology of emerging sepsis, insufficient efforts to clarify the etiological value of coagulase-negative staphylococcal isolates, and many more. The change in etiological structure from coagulase-negative to coagulase-positive microorganisms indicates an improvement in the hospital's hygienic condition. However, coagulase-negative staphylococci, which are discussed as a potential contaminant in their vast majority, must be ruled out. Our system is characterized by omissions in the pre-analytical phase of research, as well as the funding authority's total disregard for the quality of medical care, particularly in the field of infectious diseases. De-escalating antimicrobial drug therapy is rarely used and usually not with cheap narrow-spectrum antibiotics to seek economic benefit in rapid etiologic diagnosis. In most cases, a rapid diagnosis using modern techniques is sought in particularly serious clinical cases, and in cases of severe pathology, the stay is longer and more expensive. Fatal outcome reduces

the length of hospital stay, and the preventable mortality should be analyzed in terms of accelerating the etiological diagnosis and de-escalating etiologic therapy.

The possibilities of the research methods are well presented and the doctoral student demonstrates good preparation, both practical and theoretical. The laboratory-economic analysis was performed expertly, and it is a first in our country. Even the National Health Insurance Fund does not have estimates of paid costs for clinical microbiology, and this has been the case for more than 20 (twenty) years.

The results are presented in detail and demonstrate the enormous amount of work that was done (p.54 - p.125). The species structure and the antimicrobial drug resistance of the causative agents are discussed in depth. The PhD candidate's conclusions are reasonable and consistent with her accumulated knowledge and experience. The three studied laboratory diagnostic and qualitatively new methods (FISH, mPCR, MALDI-TOFMS) have advantages over the conventional microbiological methods, used for many years routinely in microbiological laboratories for the diagnosis of bloodstream infections, as they accelerate and refine the results. The sensitivity, specificity, efficiency, and high speed of the studied methods enable their routine use in the microbiology laboratories of most major hospitals. Consolidation and delegation of responsibilities to larger laboratories are something that ought to be pursued by smaller medical facilities. The introduction of new methods such as FISH and mPCR provides rapid and accurate identification of the most common causative agents of bacteremia and fungemia immediately following the positivity of blood culture from the automated system. Due to their relatively limited spectra of microorganisms, FISH and mPCR can be used in addition to routine microbiological methods to identify the etiological agent and to help doctors make an accurate decision on the patient's condition and therapy. MALDI-TOF MS is a potential routine method for the identification of microorganisms due to its broad spectrum of detectable microorganisms (>2000 species), speed, high sensitivity, and the sample's low cost (if capital costs are not taken into account). The direct identification of microorganisms from blood cultures by MALDI-TOF MS has significant advantages for the identification of Gram-negative bacteria and fungi. It could be a recommended algorithm for routine microbiological laboratory testing. For the period 2015–August 2020, Gram-positive bacteria predominate over Gram-negative bacteria in blood

culture isolates and *C. albicans* is the most prevalent isolate among fungi, but non-albicans species predominate as causes of fungemia.

The conclusions of the doctoral candidate are correct: A decrease in the incidence of MRSA was reported, but there was an increase in the rates of vancomycin-resistant enterococci, ESBL-producers and KPC strains isolated from blood cultures. *A. baumannii* and *P. aeruginosa* remain problematic nosocomial pathogens with developed resistance to all targeted antibiotics except colistin.

The introduced new methods reduce the length of stay of patients in a very serious condition with a timely change of antimicrobial treatment (25.5 days versus 34 days!), improve the outcome of the disease, significantly reduce the direct and indirect costs of treatment (approximately BGN 12,500 per patient!) and lead to a lower overall expenditure of financial resources for the medical facility.

For patients diagnosed only by a culture test, it was found that this resulted in a 34% increase in the mean hospital stay. It also increased the mean duration of empiric antibiotic therapy by 6% and added 35% to the direct and indirect costs and the clinical pathways and procedures costs. These negative trends are eliminated with the rational and optimized application of the new diagnostic methods.

The implementation of new equipment in the microbiological practice addresses the lack of medical personnel in the country by saving human resources, time, labor and consumables for determining the final diagnosis of patients with bacteremia/fungemia.

III. Characterization and evaluation of the dissertation and its contributions

Scientific and theoretical contributions

This pilot study in Bulgaria has conducted a comparative assessment of the diagnostic value of rapid methods for diagnosing positive blood cultures - FISH, multiplex PCR and MALDI-TOF MS in patients with bloodstream infections. This is a comparison of the most innovative techniques in microbiology with those that have been in use for decades.

Pioneering for our health care was the development of an algorithm that recognizes the diagnostic potential of MALDI-TOF MS for the identification of microorganisms directly from positive blood cultures, which has not been done previously.

A complete analysis (a six-year ambispective study) of the dynamics and trends of the changes in the etiology and evolution of antimicrobial drug resistance of the isolates from positive blood cultures in the largest university hospital in the country has been conducted and the leading pathogens and types of antimicrobial drug resistance have been identified.

The first major financial analysis of the direct costs of treating ICU patients with bacteremia/fungemia during intensive care stay and evaluation of the efficacy of the applied methods for the etiologic diagnosis of positive blood cultures have been conducted.

The scientific practical contributions are the following:

A form was developed and tested, through which a request for molecular genetic analysis (mPCR) is made by the clinic where the patient is hospitalized. It contains both the patient's clinical data entered by the attending physician and the established laboratory deviations (Appendix No. 1). Appendix No. 2 contains the result of the performed multiplex PCR, which is sent back to the unit that requested the test and attached to the patient's History of Present Illness. FISH, multiplex PCR, and MALDI-TOF MS were implemented for rapid microbiological diagnosis in cases of patients with symptoms of bacteremia/fungemia, and their effectiveness was assessed and compared with classical methods. A practical algorithm for diagnostic process optimization in cases of bacteremia or fungemia has been proposed and tested.

A modified algorithm for direct identification by MALDI-TOF MS of pathogens from blood cultures, immediately after their positivity, has been developed and implemented, which has not been done previously in our country.

For the first time in our country, the diagnostic significance and economic impact of the application of express methods for diagnosing pathogens from positive blood cultures are assessed.

V. Conclusion

The doctoral student has been provided with excellent opportunities such as unique equipment and the opportunity to compare innovative methods with classic ones, which is pioneering work

for our country. The opportunities for inclusion in a large number of research funding programs are excellent, which is a credit to the university management. There are very few doctorate positions in clinical microbiology in the country which are provided with such large apparatus, financial and laboratory resources. The methodical help of the supervisor, as well as the head of the Department and the help of the academic staff for providing and utilizing these opportunities are evident.

Based on the accepted scientific-theoretical and scientific-practical contributions and due to the fact that the dissertation is the personal work of Dr. Gergana Boteva Lengerova, assistant professor in the Department of Microbiology and Immunology, I believe that the dissertation meets all the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for implementation of the LDASRB and the Regulations of MU - Plovdiv regarding the awarding of the ESN "Doctor". All this gives me grounds for an extremely high evaluation of the dissertation thesis, based on which I support the award and propose to the esteemed scientific jury to award the educational and scientific degree 'Doctor' to Dr. Gergana Boteva Lengerova.

Заличено на основание
Чл.5 §1, б."В" Регламент (ЕС)2016/679
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