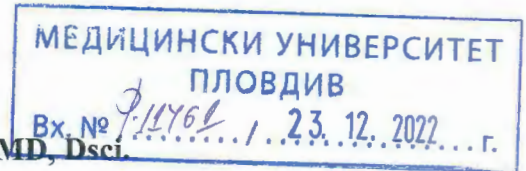


REVIEW

by Prof. Chavdar Stefanov Stefanov, MD, Dsci.



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on a dissertation for awarding the educational and scientific degree 'doctor'

professional direction 4.3. Biological sciences in the field of higher education 4. Natural sciences,
mathematics and informatics

Doctoral program in Microbiology

Author: Gergana Boteva Lengerova, MD

Form of doctoral studies: independent study

Department: Medical Microbiology and Immunology "Prof. Elissay Yanev". Medical University -
Plovdiv and the Innovative Diagnostic Methods Section of the Scientific and Research Institute of
Medical University - Plovdiv.

**Topic: Evaluation of modern microbiological techniques for rapid identification of
microorganisms in patients with bacteremia and fungemia**

**Research supervisor: Assoc. Prof. Michael Mihailov Petrov, MD, PhD; Medical University -
Plovdiv**

1. General presentation of the procedure and the doctoral student

The set of electronically presented materials is in accordance with Article 70 (1) of Section I.
Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF
SCIENCE" at MU-Plovdiv; Regulations of MU-Plovdiv from 28.01.2021 and includes the
following documents:

- Application to the Rector of MU-Plovdiv for starting of the procedure for the defense of a
doctoral dissertation
- curriculum vitae in European format signed by the doctoral candidate

- a notarized copy of a higher education diploma
- orders for enrollment in doctoral studies and for completion with the right of defense
- order for conducting an according to the individual plan and the corresponding protocol for a passed exam or doctoral minimum in the specialty
- Minutes of the departmental council for the preliminary discussion of the dissertation and the decisions made for starting the procedure and for the composition of the scientific jury
- dissertation
- author's summary
- list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- declaration of originality and authenticity of the attached documents
- other documents related to the course of the procedure

The doctoral candidate has attached four publications.

I have no critical notes on the documents.

2. Biographical information for the doctoral student

Dr. Gergana Lengerova has worked as an assistant professor in the Department of Microbiology and Immunology "Prof. Elissay Yanev" since 2017. She has been an active participant in scientific forums, symposia, and conferences both nationally and internationally since she was a student at the Medical University – Plovdiv. She is also highly motivated to obtain new knowledge and exchange experience both nationally and internationally. The training and mobility events that the PhD student attended to serve as evidence of this. Her unceasing interest in scientific research is shown by the published scientific publications from various fields of medicine, as well as her work in scientific projects.

3. Relevance of the topic and appropriateness of the set aim and tasks

The topic of the dissertation is extremely relevant and interesting from the point of view not only of the science "Microbiology", but also for many clinical specialties. Of particular interest was the fact that this scientific work largely concerns the clinic I lead, where the main problem remains the treatment of critically ill patients with severe infections and sepsis. At the present moment, this is particularly evident in our patients, who after a prolonged illness from COVID-19 continue to develop life-threatening infections that require active treatment, part of which is the optimization of antimicrobial drug therapy according to microbiological results. Therefore, I think that this

scientific study is not only current and dissertation-worthy but also quite helpful for a variety of professionals, notably for us intense therapists and anesthesiologists.

4. Knowledge of the problem

Dr. Lengerova has prepared a literature review on the topic of the thesis based on a large number of literary sources: 375, including 16 in Bulgarian and 359 in English. The number of bibliographic titles from the past decade is 264, which accounts for 70% of all cited sources.

The topic that Dr. Lengerova selected is extremely relevant for microbiologists and clinicians. Early detection of the microbiological causative agent and its antimicrobial drug sensitivity, respectively drug resistance, are important factors for rapid diagnosis and initiation of effective antimicrobial therapy in bloodstream infections. The literature review observed the etiology of bloodstream infections, the antimicrobial resistance of various microorganisms, and the trends for its change, but the most important point is given to the modern aspects and challenges in the microbiological diagnosis of blood cultures. The advantages of rapid methods compared to conventional microbiological methods are highlighted, which has implications for the good work of microbiologists and clinicians. Based on this, Dr. Lengerova establishes a clear and specific aim: to investigate these methods and develop algorithms for the direct identification of pathogens in the bloodstream. There are 5 tasks to fulfill this aim, which are clearly formulated and outline the specific areas of research.

My overall impression of the dissertation is that it is excellently conceived, precisely executed at a prominent level of methodological quality, and excellently designed research, written very intelligently, with thorough information in all of its sections, and with essential characteristics and contributions. The thoroughness and finished look of the dissertation show that the doctoral student knows the problem very well and has penetrated deeply and with understanding into the matter that is considered in it. The dissertation's thoroughness and polished appearance show that the doctoral candidate has a thorough understanding of the problem and has delved into in-depth comprehension of the topic.

5. Research methodology

The research methods chosen by the doctoral student and his supervisor allow for achieving the set aim and obtaining an adequate answer to the tasks solved in the dissertation work. The study design met its needs and was implemented precisely. The equipment used and the microbiological methods derived from it are extremely modern, allowing accurate, fast and highly informative results.

6. Characterization and evaluation of the dissertation work

Dr. Gergana Lengerova's dissertation "Evaluation of modern microbiological techniques for rapid identification of microorganisms in patients with bacteremia and fungemia" is written on 197 pages and includes 14 tables, 67 figures, and 6 appendices. It is organized into 13 chapters. The bibliographic reference covers 375 literary sources, the majority of which were published after 2015. The dissertation fully complies with the structure and layout accepted for scientific work.

The literature review is well constructed, and the modern definitions and definitions of sepsis, bacteremia, and fungemia are methodologically correctly used and presented. All rapid diagnostic methods, their importance, advantages, and limitations are described in a good scientific and discussion style.

The aims and objectives of the study are logically formulated from the literature review. They are systematically correct without being overly descriptive, and they serve the scientific work in its entirety.

In the chapter "Materials and methods" the design of the study with its units of observation and the technical conditions for carrying out the studies are thoroughly and punctually presented. All microbiological diagnostic methods are described precisely and thoroughly, arranged chronologically, and their diagnostic-clinical value is discussed. The requirements for a detailed and accurate description of the object and units of observation, the selection of patients, the entire methodology from taking the samples to obtaining the result have been met. The statistical methods used are modern and completely adequately selected for this type of study.

The results of the conducted research are comprehensively and methodologically correctly presented, being divided according to the set scientific tasks. For me, as a clinician, working in the clinic of intensive care and critical care, the results of Task 4 - the evaluation of the economic effect of the applied methods in patients with bacteremia/fungemia, were of particular interest. I will not conceal the fact that, before becoming thoroughly acquainted with them, I was largely pessimistic about their appearance and significance. In terms of age, underlying disease, comorbidities, and therapeutic behavior, the population of critically ill patients with bacteremia/fungemia is extremely heterogeneous. This heterogeneity is likely the cause for the dearth of relevant clinical/pharmacoeconomic studies in this extremely uncertain area, such as the variable final prognosis and treatment duration. After carefully reading this section, I believe that it is of utmost importance to critical care professionals rather than microbiology majors. Indeed, the results in places seem insufficiently convincing, but the reasons for this should not be sought in the PhD student's methodology. In practice, Dr. Lengerova was able to use all the possible potential of the hospital information system and correctly divided the patients into "severe" and "very severe" ones,

having methodologically correctly calculated the cost of treatment and the economic effect of express microbiological diagnostics. The shortcomings and omissions are not so much in the field of microbiological work as in that of the clinicians, who for the most part have acted in a largely haphazard manner, guided solely by their own opinion, and consequently lacked stratification according to clinical scales, time after receiving clinical and paraclinical data of a new or worsening infection, the decline in organ functions, etc. From this perspective, I, as head of ICU, take much of the blame for the imperfect methodology. Despite all these limitations, the results show a positive economic effect of using the rapid methods for microbiological diagnosis of bacteremia/fungemia, regardless of their relatively high cost. It would be appropriate to continue the clinical-economic study, but already, as a prospective study, after the creation and implementation of a new design developed with the participation of the clinical units. In this aspect, the optimized diagnostic algorithm derived as a result of task №5 can be used for faster and more efficient diagnosis of positive blood cultures.

In the "Discussion" section, Dr. Lengerova's results are critically and motivated compared with contemporary scientific publications and their conclusions, paying special attention to the increasing resistance of isolated bacteria and fungi. Because of this resistance, the maximum early application of optimal antibiotic therapy is crucial for both individual patients with life-threatening infections and the entire population of patients with such infections. This, on the one hand, increases survival and, on the other, has a direct impact on the development of microorganisms resistant to antimicrobial therapy. In this section, the results of the economic effect, which I have already discussed in detail above, are also commented on.

The research student's conclusions are a logical summary of her findings, and I agree with them in principle. As a direct result of the overall scientific work, I fully accept the formulated conclusions and original and applied contributions.

7. Contributions and significance of the development of science and practice

Significant amounts of valuable experimental data have been obtained. Significant scientific-theoretical and practical contributions have been achieved. Original contributions and enriching existing data contribution are essential, including:

1. A 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.
2. A comprehensive analysis (a six-year ambispective study) of the dynamics and trends in the etiology and antimicrobial drug resistance of isolates from positive blood cultures in the

largest university hospital in the country was performed, and the leading pathogens and types of antimicrobial drug resistance were identified.

3. The first financial analysis of the direct treatment costs of patients in the ICU diagnosed with bacteremia/fungemia during intensive care stay and evaluation of the effectiveness of the applied methods for diagnosing positive blood cultures was performed.

I also greatly value the following contributions of a confirmatory and scientific-practical character:

1. 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.
2. A modified algorithm was developed and implemented for the direct identification by MALDI-TOF MS of pathogens from blood cultures, immediately after their positivity.
3. For the first time in our country, the diagnostic significance and the economic effect of the application of rapid methods for the diagnosis of pathogens from positive blood cultures have been evaluated.

8. Evaluation of publications on the dissertation work

The doctoral student has 4 (four) publications on her dissertation work, all of which are in refereed journals, and she is the first author of three of them. She took part in 2 international and 11 national scientific forums and congresses. In addition, she has taken part in a total of 2 scientific projects, 1 intra-university and 1 national. She completed 3 (three) international trainings and 9 (nine) national trainings.

9. Personal participation of the doctoral student

The doctoral student is independently credited for the development of the entire study, the acquisition and processing of data, the study's design, and analysis of results, as well as the development of conclusions and scientific contributions. All tests were developed in whole or in large part by the PhD candidate in the Department of Medical Microbiology and Immunology „Prof. Dr. Elissay Yanev“ at the Faculty of Pharmacy and the Innovative Diagnostic Methods Unit at the Research Institute at the Medical University - Plovdiv (RIMU), as well as at the Laboratory of Microbiology at University Hospital „St. George“ - Plovdiv. Part of the results was confirmed at the National Center of Infectious and Parasitic Diseases - Sofia. The statistical analyzes were

prepared in the Department of Social Medicine and Public Health, Faculty of Public Health at MU-Plovdiv with the active participation of the PhD candidate.

10. Author's summary

The abstract is **formatted according to the requirements and reflects the main results achieved in the dissertation**. Written on 59 pages in understandable language and follows exactly the sections of the dissertation, illustrated with the necessary tables (5) and figures (39). The main developed algorithms are applied.

11. Critical remarks and recommendations

I have no critical remarks about the dissertation work. It was exceptionally planned, developed, and written, in my opinion. My recommendation is that Dr. Lengerova published the findings of her dissertation in an effort to optimize the diagnosis of bacteremia/fungemia in critically ill patients across the country and people.

12. Personal impressions

I have an excellent impression of Dr. Lengerova from her time as a student and our collaboration on the dissertation. Intelligent and motivated to advance as a clinical microbiologist, and always prepared to act when required.

CONCLUSION


The dissertation *contains scientific, scientific-practical and practical results, which represent an original contribution to science* and **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. The presented materials and dissertation results **fully** correspond to the specific requirements of the MU - Plovdiv.

The dissertation shows that the doctoral candidate Dr. Gergana Boteva Lengerova **has** in-depth theoretical knowledge and professional skills in the scientific specialty "Microbiology" by **demonstrating** qualities and skills for independent conduct of scientific research.

I confidently give my **positive assessment** of the conducted research presented in the above-reviewed dissertation, author's summary, achieved results and contributions, and **propose to the esteemed scientific jury to award, and deservedly so, the educational and scientific degree 'Doctor'** to Dr. Gergana Boteva Lengerova in a doctoral program in Microbiology.

12/15/2022

Reviewer:


Boris Hristov
MD, PhD, FRCR, FRCR
Prof. Chavdar Stefanov Stefanov, MD, Dsci

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