To the members of the Scientific Jury,
appointed by Order No. R-109-469/10.12.2024
of the Rector of the Medical University - Varna
for the procedure for defending a dissertation
for awarding the scientific degree "Doctor"
at the Department of microbiology and virology,
Faculty of Medicine, Medical University
"Prof. Dr. Paraskev Stoyanov" – Varna

Statement

by **Prof. Lyudmila Boyanova Georgieva, MD, PhD, DMSc,**
Professor at the Department of Medical Microbiology
"Corr. Member Prof. Dr. Ivan Mitov, DMSc", Medical
Faculty, Medical University-Sofia
e-mail: l.boyanova@hotmail.com

Regarding the PhD Thesis entitled:

EVALUATION OF HB&L UROQUATTRO AUTOMATED SYSTEM FOR RAPID DIAGNOSIS OF URINARY TRACT INFECTIONS

Scientific field 4. Natural sciences, mathematics and informatics, Professional direction 4.3. Biological sciences, Scientific specialty „Microbiology"

Author:

Viktoriya Snezhanova Snegarova-Toneva, MD

Research Supervisor:

Prof. Temenuga Stoeva, MD, PhD, DSc

I have no conflict of interest with Viktoriya Snegarova-Toneva, MD. The documents and materials given to me regarding the procedure are correct according to the requirements of the Development of the Academic Staff of the Republic of Bulgaria and the Rules for its application at Medical University (MU) „Prof. Dr. Paraskev Stoyanov" - Varna.

Brief biographical data about the candidate

Dr. Viktoria Snegarova-Toneva graduated with a master's degree from the University of Varna in 2007 and became a Master of Health Management in 2013. She obtained two medical specialties: "Microbiology" in 2014, and "Clinical Virology" in 2022, which shows her desire for constant development and self-improvement. She has been working as an assistant in the Department of Microbiology and Virology of MU-Varna since 2010.

She is a full-time doctoral student in the department. She has also been working as a microbiologist in "Laborexpres 2000", Varna since 2013.

Dissertation sections

The dissertation contains 134 pages, incl. Abbreviations used (1 p.), Introduction (2 pp.), Literature review (35 pp.), Aim and objectives (1 p.), Materials and methods (15 pp.), Results and discussion (46 pp.), Conclusions (2 pp.), Reference for contributions (2 pp.), and Scientific publications and announcements related to the dissertation work (2 pp.).

The dissertation is well illustrated with 8 figures, 19 tables and an appendix. It contains 285 references, most of which (280) are in Latin.

Relevance of the scientific topic

Urinary tract infections (UTIs) are some of the most common bacterial infections in the world, mainly in women, who have a lifetime risk of 50 to 70%, moreover, 20 to 30% of affected females develop recurrent infections. The most common causative agents are bacteria from the order *Enterobacterales*, less often other bacteria and fungi. However, microbial antibiotic resistance is constantly increasing and is a major cause of treatment failure.

Rapid and correct antibiotic therapy with automated methods can speed up and improve the detection of the common causative agents of UTIs and prevent complications such as chronification, sepsis and pyonephrosis. All these considerations determine the indisputable relevance of the present dissertation work.

Literary awareness of the problem

The author of the dissertation has a broad and up-to-date awareness of the problem of UTIs, antibiotic profiles of causative agents, and systems for rapid identification and antibiotic susceptibility testing. This is evidenced by the fact that about 80% of the references are from the last 10 years, and more than half (162) of the cited publications are from the last 5 years.

Aim and objectives

The aim of the present work was to investigate the benefits of the automated HB&L Uroquattro system directly from positive urine samples for the rapid UTI diagnosis and determining the antibiotic susceptibility of the causative agents. For comparison, the culture method and the automated VITEK 2 system of bioMérieux were used.

It was also important to detect the residual antibiotic activity in the samples with the HB&L apparatus and its importance in choosing the correct therapy. This method determines

the presence of antimicrobial substances in the clinical sample that can inhibit bacterial growth, e.g. in the case of unreported antibiotic intake.
For the set goal, 6 related tasks were correctly defined.

Evaluation of the materials and methods used

A wide range of methods was used in three main areas:

- screening of urine samples for growth and subsequent identification of infectious agents with the HB&L UROQUATTRO apparatus and standard culture examination,
- determining the antimicrobial susceptibility of microorganisms with 4 methods: the disk diffusion method, the method for broad-spectrum beta-lactamase (ESBL) detection, the automated system VITEK 2 Compact, and HB&L Uroquattro AST.
- Determination of residual antibiotic activity in urine samples with the HB&L apparatus.

Correlation and prognostic analysis methods were used for statistical processing of the results.

Results and Discussion

A huge number of samples (1600) and outpatients (842) with symptoms of UTIs were studied, and the parameters of the rapid automated system HB&L Uroquattro were used and compared with those of the classical methods and the automated system VITEK 2. Similar detection of positive urine cultures was found with the automated HB&L system (21.4%) and the classical culture method (22%), indicating the applicability of the system in routine diagnostics. An important advantage is the short time to obtain the final result of the examination by HB&L (5 to 7 h) compared to 12-18 h using VITEK 2.

The causative agents of UTIs were identified and discussed according to patient and microorganism groups, and bacterial susceptibility to numerous antibiotics (14 even only for Gram-negative bacteria) was determined.

Bellow I present the most important contributions that this dissertation provides.

Assessment of scientific and applied contributions

I will focus on the contributions of an original character to the country and on the scientific and applied contributions that made the strongest impression on me.

Original scientific contributions to the country

- The etiological UTI profile of a large number of outpatients in the Varna region and their antibiotic susceptibility, including that for the less frequently studied antibiotics (nitrofurantoin, nitroxoline and fosfomycin) were determined. The data help guide the choice of empiric therapy for uncomplicated community-acquired UTIs.
- Benefits of the automated HB&L Uroquattro system for the rapid diagnosis of UTIs in the outpatient setting, as well as for the rapid antimicrobial susceptibility testing of infectious agents, were detected. This system counts only actively replicating microorganisms, eliminating the influence of non-living cells and blood clots in the samples.
- I find the study of the residual antimicrobial activity in the samples by the HB&L apparatus to be an important contribution, since detection of inhibitory antimicrobial substances in the urine can optimize the interpretations and prevent false-negative results and incorrect antibiotic treatment. This is of particular benefit to countries or regions with high antibiotic consumption, including our country.

Applied Scientific Contributions

- Rapid diagnosis and therapy of UTIs is particularly important due to their high frequency and potential serious complications. The presented algorithm for laboratory screening of

urine samples and evaluation of the antibiotic susceptibility of the causative agents directly from a clinical sample shortens the diagnosis by one to two days, which is its significant advantage. The limitations of this diagnosis are also determined, for example, in the case of low microbial counts, mixed infections and slow-growing bacteria and fungi.

- The HB&L Uroquattro Automated Rapid System demonstrates excellent (>99%) positive and negative predictive value and the advantages of the system make it fully applicable for routine diagnostics in laboratories.
- As mentioned above, the reading of residual antimicrobial activity in urine with the HB&L apparatus is of great benefit to patients with UTIs treated with antibiotics or who have not reported their use.

Publications and participation in scientific forums related to the dissertation

Dr. Snegarova-Toneva presents 4 publications in scientific journals, one in Bulgarian in the journal General Medicine and three in English in the journals Acta Medica Bulgarica, Acta Microbiologica Bulgarica and Scripta Scientifica Medica.

She has three participations in scientific forums, two posters and one oral presentation. The posters were presented at the 2nd International Electronic Conference on Antibiotics - Drugs for Superbugs: Antibiotic Discovery, Modes of Action and Mechanisms of Resistance, and are available online at Medical Sciences Forum (Med. Sci. Forum) 2022.

Dr. Snegarova-Toneva has also worked on a Science Fund Project, Medical University - Varna, on the topic "Evaluation of the automated system HB&L Uroquattro for rapid diagnosis of urinary tract infections" (2019–2023), which is one of the main focuses of her scientific interests.

Evaluation of the dissertation abstract:

The dissertation abstract is well presented and corresponds to the structure of the dissertation work.

Recommendations

I have some minor notes that do not affect the value of the thesis, e.g. "mid-stream catch" should be translated in Bulgarian. In Table 7, the bacteria should be in italics.

Conclusion

The dissertation work, its results, conclusions and contributions, which can speed up and improve both the diagnosis of urinary tract infections in microbiological laboratories, and the correct, faster and successful therapy of patients, give me full grounds for a positive evaluation, as well as to recommend to the respectable Scientific Jury **to award the educational and scientific degree "Doctor" (PhD) to Viktoriya Snegarova-Toneva, MD.**

Sofia
05 Feb. 2025

Prof. Lyudmila Boyanova Georgieva, MD, DMSc
Department of Medical Microbiology
"Corr. member Prof. Dr. Ivan Mitov, DMSc",
Medical Faculty, Medical University-Sofia