To the Chairman of the Scientific Jury,
Appointed by order P-109-469/10.12.2024
Of the Rector of MU-Varna

REVIEW

by Prof. Rumyana Donkova Markovska-Davidkova, MD, PhD

Department of Medical Microbiology,

Medical Faculty, Medical Univesity – Sofia

Regarding a dissertation on the topic: "Evaluation of the HB&L Automated System for Rapid Diagnosis of Urinary Tract Infections" by Dr. Victoria Snegarova-Toneva, submitted for the award of the educational and scientific degree "Doctor", **in the doctoral program "Microbiology"** in the field of higher education 4.0 Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences

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

Dr. Snegarova obtained her Master's degree in Medicine in 2007, having worked for several years as a full-time or honorary assistant at the Department of Microbiology and Virology, MU-Varna. She has two specialties – clinical microbiology (2014), clinical virology (2014). Her main place of employment is a private diagnostic laboratory in Varna, in which she has worked since 2013.

Relevance and significance of the selected scientific problem

Dr. Snegarova's PhD thesis is dedicated to a important problem in medical practice - urinary tract infections. This type of infection is very common, can be community-acquired and associated with medical care, and contributes greatly to the increase in antibiotic resistance. The PhD thesis includes an analysis of the etiological structure of urinary tract infections, the antibiotic resistance of the causative agents, as well as an evaluation of the methods for proving

the causative agents and determining their sensitivity. This work would be useful for doctors in outpatient practice. The emphasis of the work is an evaluation of the automated HB&L system for rapid diagnosis of urinary tract infections. This type of system accelerates the proof of the presence of urinary tract infection, the type of causative agent and its sensitivity. In addition, it determines residual antibiotic activity. These data would be useful for a faster and more accurate diagnosis and a better choice of therapy.

Structure of the dissertation

The PhD thesis is written on 143 pages, and is structured according to the generally accepted scheme: Abbreviations used - 1 pages; Introduction - 2 pages, Literature review - 35 pages, Aim and tasks - 1 page, Materials and methods - 15 pages, Results and discussion - 46 pages, Conclusions and contributions - 4 pages. The dissertation work is illustrated with 19 tables and 8 figures and one application. 655 sources were used, of which over 80% from the last 10 years. Two hundred eighty five sources were used, of which over 5 in Cyrillic and 280 in English. Over 50% (162 sources) are from the last 5 years, and 222 from the last 10 years

Evaluation of the qualities of the literature review

The literature review is written correctly. The classification and epidemiology of urinary tract infections (frequency, routes of occurrence, risk factors), the etiology of these infections and modern methods of microbiological diagnosis are discussed in detail. The frequency of urinary tract infections and their etiological spectrum are discussed on a global scale, and data for Bulgaria are also presented. Diagnostic methods are also presented in detail, describing the advantages and disadvantages of each method, including molecular-genetic methods and mass spectrometric methods. The review also includes data from global systems for monitoring the resistance of the main causes of urinary tract infections GLASS, SENTRY. They are compared with data from the national Bulstar system. The literature review ends with a conclusion, which logically justifies the need to develop a dissertation on such a topic.

Aim and tasks

The aim is clearly formulated, and the tasks (6 in total) correspond to the main aim and lead to its fulfillment.

Section "Materials and methods"

The section includes the methods used and a description of the isolates included. The criteria for inclusion or exclusion in the study are described. The PhD thesis included 842 outpatients, examined over a 7-month period in 2020/2021, with 1600 urine samples examined. The methodology for working with the HB&L Uro Quattro device for detecting bacterial growth and determining susceptibility directly from urine, as well as the methodology for determining residual antibiotic activity in urine samples, is presented in detail. The statistical methods used are appropriate. The studies conducted were approved by the Research Ethics Committee.

Section "Results and Discussion"

The section "Results and Discussion" is logically structured into six subsections. The study is prospective, with 1600 urines from 842 patients examined. It would be good to have a more detailed presentation of the data, apparently there are 2-3 urines examined from one patient. 352 non-repeating isolates were analyzed in the work. The inclusion of a larger number of examined isolates would enrich the data on the etiological structure of infections and the susceptibility of the isolates. In the etiological structure of urinary tract infections, the doctoral student established a frequency of Gram-negative microorganisms, in 80.1%. The rest are Gram-positive and fungi (Candida spp), in 0.9%. Dr. Snegarova proves the presence of *E. coli* – in 56.8%, followed by *K. pneumoniae* (11.4%) and *P. mirabilis* (7.4%). Of the Gram-positive, *E. faecalis* predominates, at 12.5%, and *S saprophyticus* (5.7%). The doctoral student compares the obtained data with international and Bulgarian data (Bulstar), to which its results are similar. The second subsection presents an assessment of the accuracy of the HB&L UROQUATTRO system. 842 outpatients and 1600 urines were examined. A problem is the lack of data on whether the urines from one patient were given consecutively on 2-3 consecutive days or over a longer period of time. The results presented by Dr. Snegarova show excellent sensitivity of the system (97.4%) and specificity (100%), with the time to obtain the result being 4 hours versus 24 hours. The discrepancies between the two methods (0.6%, 9 isolates not detected by the HB&L UROQUATTRO system) are associated with a low microbial count ($<10^3$ CFU/ml), slower growing microorganisms and *Candida* spp. isolates.

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There are some inaccuracies in the presentation of the result – in the discussion it is written sensitivity of the system (97.4%) and specificity (100%), while in the conclusion and final conclusions - sensitivity of the system (100%) and specificity (99.3%). In my opinion, the first is correct, as the sensitivity of the system is 97.4%.

The antimicrobial resistance of the studied isolates is made and presented correctly in the third subsection. Data on enterobacteria for outpatient practice would be very useful. Their resistance to aminopenicillins is high, 30-40% are resistant to quinolones and under 16% to third-generation cephalosporins. As expected, considering the situation in hospitals, over half of the *K pneumoniae* isolates were producers of broad-spectrum beta-lactamases. Carbapenem-resistant isolates are missing. Resistance of *E. coli* to fosfomycin, 11.0%; nitrofurantoin, 8.0%; nitroxoline, 2.0% is very low. The picture is typical for outpatient isolates. Regarding Gram-positives, the numbers of *E. faecalis* (n=42) and *S. saprophyticus* (n=20) did not exceed 50, which makes the conclusions not enough statistically significant. Dr. Snegarova compares the obtained data on antibiotic resistance with data from other studies worldwide.

Dr. Snegarova evaluated the direct determination of antibiotic susceptibility of isolates with the HB&L AST Uroquattro system, with the sensitivity of 122 isolates determined by the system and by the disk diffusion method. The categorical compliance reported by Dr. Snegarova is 94.8% with 36 errors (5.2%). Of these, 5 are very large and 24 are large.

An interesting addition to the direct determination of antibiotic susceptibility is the use of the automated VITEK 2 system for determining antibiotic susceptibility, which works directly with the positive vials from the HB&L apparatus when reaching a turbidity of 0.5 Mc Farland. Dr. Snegarova obtains a categorical agreement of 97% compared to the standard method, with a total of 21 errors (3%). Of these, 3 are very major (14.3%) and 11 are major (52.4%). The presented laboratory algorithm, including screening of the urine sample, followed by microscopic examination and rapid antibiotic susceptibility testing directly from the clinical sample, dramatically shortens the diagnostic process by 24-48 hours. The speed of the HB&L AST method and its very good correlation with the standard disk diffusion method determine the potential of the system for widespread application in routine laboratory practice. The study proves a high relative proportion of urine samples with residual antibiotic activity (5.7%), which indicates the importance of this test.

As a result of the presented work, Dr. Snegarova formulated 10 main conclusions that meet the aims and objectives.

Evaluation of the contributions of the PhD thesis

From the results obtained and conclusions drawn, Dr. Snegarova formulated 10 contributions: 4 of an original nature, 3 of a confirmatory nature and 3 of a scientific-applied nature, which I fully accept. The most important contributions are:

1. The capabilities of the HB&L Uroquattro automated system for rapid and accurate diagnosis of urinary tract infections in outpatient settings were evaluated.

2. The capabilities of the HB&L Uroquattro automated system for rapid and accurate testing of susceptibility to antimicrobial drugs were evaluated.

3. The etiological spectrum of urinary tract infections in a large number of outpatients in the Varna region was studied, as well as the local susceptibility to antimicrobial drugs (including nitroxoline, nitrofurantoin and fosfomycin).

4. The possibilities of the residual antimicrobial activity test for correct interpretation of the result of the cultural microbiological examination and monitoring the effectiveness of therapy in cases of urinary tract infections have been evaluated. Of the other contributions, in my opinion, the indicated possibility of using this system in routine laboratory practice and for other biological materials obtained from primarily sterile anatomical areas in cases of invasive infections, for which the speed of microbiological examination is of critical importance, is important.

Scientific indicators

Dr. Snegarova has presented 4 full-text publications related to her dissertation, 3 of which have Q4. It is particularly impressive that she is the first author in all of them.

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 presented PhD thesis by Dr. Snegarova in terms of structure, content and volume meets the criteria in the ZRASRB and the Regulations of MU-Varna for acquiring the scientific degree "Doctor of Sciences". I give my positive assessment and recommend to the members of the Scientific Jury to vote for awarding the educational and scientific degree "Doctor of Sciences" in the scientific specialty "Microbiology" to Dr. Snegarova 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" in the scientific specialty "Microbiology" in the field of higher education 4.0 Natural Sciences, Mathematics and Informatics, professional field 4.3 Biological Sciences of Dr. Victoria Snezhanova Snegarova-Toneva.

7.02.2025г.   **Prepared the review:**

/ Prof. Rumyana Donkova Markovska-Davidkova, MD, PhD /