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ORIGINAL ARTICLE

AUTOMATED URINE SCREENING AND RESIDUAL ANTIMICROBIAL ACTIVITY TEST FOR RAPID DIAGNOSIS OF URINARY TRACT INFECTIONS IN AMBULATORY PATIENTS: A LABORATORY EVALUATION OF HB&L UROQUATTRO INSTRUMENT

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Abstract. Aim: *the aim of this study is to evaluate the accuracy of the HB&L Uroquattro instrument (Alifax, Italy) and the Residual Antimicrobial Activity test (RAA) for rapid and correct diagnosis of Urinary Tract Infections (UTIs) and to compare the results with those obtained with the classical cultural method.* ***Materials and methods:*** *A total of 1600 urine samples, collected prospectively from 842 ambulatory patients in Varna city, Bulgaria, were included in the study. All urine samples were tested for bacterial growth and for RAA by HB&L instrument (Alifax, Italy). Simultaneously, each sample was inoculated on Colorex TM Orientation agar and blood, CLED and MacConkey agars. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined.* ***Results:*** *Among the tested 1600 urine samples, the HB&L instrument detected 343 (21.4%) positive and 1257 (78.6%) negative urine samples. The culture-based method identified 1248 as negative (78%) and 352 urine samples (22%) as positive. The HB&L system correctly identified 343 samples as positive (97.4%) and 1248 samples as negative (100%). The PPV of the rapid automated screening was 100%, and the NPV – 99.3%. The overall accuracy was 99.4%. The positive RAA rate in the whole collection of 1600 urine samples was 5.7% and was detected in 91 patients, all with symptoms of UTIs and recent antimicrobial therapy. In the whole studied group (n = 842), a total of 113 patients reported recent antimicrobial treatment (13.4%). The cultural method demonstrated bacterial growth in 63 patients with positive RAA test, but no pathogens were isolated in 28 patients with RAA detected in their urine samples.* C***onclusions:*** *The screening system demonstrates excellent sensitivity and specificity and, compared to the classical cultural method, has a much faster turnaround time. The RAA test proved a valuable diagnostic tool, particularly in patients with bacteriuria who are under antimicrobial treatment.*

Key words: *Urinary tract infections, rapid diagnosis, Residual antibiotic activity*

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INTRODUCTION

Urinary tract infections (UTIs) are among the most common community and hospital-acquired infections. A large study in the WHO European region estimated 1.2 million deaths in 2019 involving one of 11 infectious syndromes as an underlying or an intermediate cause of death [1]. After the bloodstream, respiratory and intra-abdominal infections, UTIs take the fourth place, accounting for a total of 60 200 deaths in the EU in 2019. Among these, 48 700 (35 600-68 000) were associated with and 11 500 (8310-16 800) were attributable to antimicrobial resistance [1].

Due to the limited therapeutic options because of the constantly growing problem with antimicrobial resistance, nowadays the UTIs caused by multiple-drug resistant (MDR) organisms, especially Gram-negative bacteria, are of great concern [2]. Pathogen identification and susceptibility testing to appropriate antimicrobial agents in limited time is essential for the patient's adequate treatment and outcome [2-4]. Nowadays, new screening tools try to provide rapid pathogen identification utilizing existing molecular platforms such as multiplex PCR and mass spectrometry [3]. New emerging platforms for rapid antibiotic susceptibility testing using biosensors, microfluidics, real-time microscopy systems, and sequence-based diagnostics have been developed [3, 4]. In addition, automated instruments such as flow cytometers, light scattering instruments, and urine sediment analyzers have been proven to be useful screening tools with a high negative predictive value [3].

The culture-based microbiological method for the etiological diagnosis of UTIs is still acknowledged as the golden standard for laboratory diagnosis. Unfortunately, this approach usually takes 24 to 48 hours for initial identification, a time delay that compels clinicians to prescribe empirically antimicrobial agents [5]. The automated systems for rapid screening of urine specimens, based on light scattering technology (HB&L Uroquattro-Alifax, Italy; BacterioScan 216 Dx- BacterioScan Inc., USA) could be of great value in the diagnostic process of UTIs [6-8]. The rapid and easy to perform same day microbial growth screening, direct determination of antimicrobial susceptibility in only 6 hours, rapid phenotypic screening for MDR organisms, as well as the detection of residual antimicrobial activity (RAA) in the urine samples are among the major advantages of these instruments [9-11].

The aim of this study was to evaluate the accuracy of the HB&L Uroquattro instrument (Alifax, Italy) and the residual antimicrobial activity test for rapid and correct diagnosis of UTIs and to compare the results with those obtained with the classical cultural method.

MATERIALS AND METHODS

A total of 1600 urine samples, collected prospectively from 842 ambulatory patients in Varna city, Bulgaria, during a seven-month period (October 2020 – April 2021), were included in the study. Among these, three patients were after renal transplantation, seven with recurrent bladder infections and urinary catheters and four patients – after surgical interventions and with nephrostoma. All patients filled in a questionnaire about the presence or absence of symptoms of UTIs, recent antimicrobial therapy and/or intake of urinary additives with antibacterial activity. All urine samples were tested for bacterial growth and for RAA by HB&L instrument (Alifax, Italy) following the manufacturer's instructions. Briefly, two vials (culture broth and RAA vial), each containing enriched medium to support the optimal microbial growth, were used. Upon arrival in the laboratory, 500 µl aliquots of each urine sample were inoculated both in the culture broth vial and in the RAA vial, the last containing 200 µl of Staphylococcus epidermidis ATCC 12228 suspension. The automated system monitors the growth phases of bacteria into the specific culture broth, providing real-time McFarland and quantitative bacterial count results in CFU/ml and, when testing for RAA, compares the S. epidermidis with the bacterial culture growth curves. For the purpose of bacterial growth screening, we used a protocol with a 4-hour incubation time and threshold of 800 CFU/ml. The RAA results were reported after 5 hours of incubation. No preserved boric acid urine samples were included. Simultaneously with the HB&L screening, each sample was inoculated on blood agar (bioMerieux), CLED (bioMerieux), Mac Conkey (bioMerieux) and Colorex TM Orientation agar (E&O labs, UK), using calibrated loop technique (1.76 µl). A culture of ≥ 176 colonies of one morphological type was interpreted as ≥ 105 CFU/ml; a colony number of 18-176 of one cultural morphological type was interpreted as 104-105 CFU/ml, and ≤ 18 colonies – as ≤ 104 CFU/ml.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated: Sensitivity = [number of true positive cases / (number of true positive cases + number of false negative cases)] x 100; Specificity = [number of true negative cases / (number of true negative cases + number of false positive cases)] x 100; PPV = [number of true positive cases / (number of true positive cases + number of false positive cases)] x 100; NPV = [number of true negative cases / (number of false negative cases + number of true negative cases)] x 100; Accuracy = [(number of true positive

cases + number of true negative cases) / (number of true positive cases + number of true negative cases + number of false positive cases + number of false negative cases)] x 100 (https://www.medcalc.org/calc/diagnostic_test.php, version 20.218).

In this study, the urine samples detected as positive by the HB&L instrument, which were also confirmed as positive by the cultural method, were defined as "true positive cases". Similarly, "true negative cases" were the HB&L negative urine samples, confirmed as negative by the cultural method.

RESULTS

Among the tested 1600 urine samples collected prospectively from 842 ambulatory patients during the seven-month study period, the HB&L instrument detected 343 (21.4%) positive and 1257 (78.6%) negative urine samples. The culture-based method identified 1248 as negative (78%) and 352 urine samples (22%) as positive. Contradictory results were found for nine urine samples obtained from nine patients: these samples were negative from the automated screening but demonstrated bacterial growth of < 103 CFU/ml on agar media: Candida spp., n = 3; Enterococcus spp., n = 2; Streptococcus agalactiae, n = 2; Aerococcus urinae, n = 1 and Pseudomonas aeruginosa, n = 1. The HB&L system correctly identified 343 samples as positive (97.4%) and 1248 samples as negative (100%). The PPV of the rapid automated screening was 100%, and the NPV – 99.3%. The overall accuracy was 99.4%.

The overall positive RAA rate among the tested 1600 urine samples was 5.7%. The positive RAA tests were detected in 91 patients, all with symptoms of UTIs and recent antibiotic therapy and/or intake of urinary additives with antimicrobial activity. In the whole group of studied patients (n = 842), a total of 113 patients reported recent antimicrobial treatment (13.4%) (Table 1). The cultural method demonstrated bacterial growth in 63 patients with positive RAA test, but no pathogens were isolated in 28 patients with RAA detected in their urine samples (Table 1).

According to the provided information, among the patients with positive RAA test and positive culture, twenty-six patients (41.3%) were treated with antibacterial agents during the last 10 days before testing because of infections other than UTIs (mainly respiratory), twenty-seven patients (42.9%) were treated empirically for UTIs with antimicrobials administered by their general practitioner and ten patients (15.8%) were taking antimicrobial agents without prescription. Simultaneously with the antibiotic treatment, ten days of immunotherapy with preparations containing bacterial lysates was also reported by two patients.

DISCUSSION

According to reports in the scientific literature, more than 60% of the urine samples sent for microbiological examination in cases of suspected urinary tract infection remain sterile [12]. Therefore, performing an initial screening of samples for the presence of microorganisms using appropriate methods would reduce the time to result by identifying negative samples in a timely manner without missing the positive samples [13]. Last but not least, the approach using screening methods would also reduce the cost of laboratory consumables [13]. Screening systems for bacterial growth detection based on changes in the sample optical density have been in use since 1980 [14]. These systems use a small amount of urine and a laser detector to track the change in the optical density of the sample over a period of time. Improving integration and automation of this technology has led to systems that can detect bacteriuria in less than an hour [13]. Such an instrument is the HB&L Uroquattro (Alifax, Italy). It performs rapid testing of urine samples within 3 to 5 hours, enabling direct culture of the analyzed sample [7]. The urine samples with a high microbial count are determined as positive within the first hour of the testing, and the sterile samples are reported by the system as negative by the fourth hour.

The key element of each acceptable screening system is high sensitivity, which prevents the infections from being missed and sufficiently specificity,

Table 1. Results from the RAA test and classical cultivation method performed with urine samples of 113 patients with recent antimicrobial treatment or intake of urinary supplements with antimicrobial activity

Patients (n)	Result from the RAA test	Result from the culture-based method	%
n = 63	positive	positive	55.8%
n = 28	positive	negative	24.8%
n = 9	negative	positive	8%
n = 13	negative	negative	11.5%
Total, n = 113			100.0

decreasing the excessive follow-up testing [6]. Our study found a sensitivity of 97.4% and specificity of 100%, PPV and NPV of 100% and 99.3% for the HB&L automated screening method of 1600 urine samples collected from 842 ambulatory patients with symptoms of UTIs. These findings demonstrate the excellent performance of the HB&L instrument in the rapid detection of bacterial growth and a very good correlation with the results obtained with the classical cultural method. Our results are in concordance with other studies that report similar sensitivity, ranging from 93% to 99.8% and specificity – between 90% and 99.7% [3, 15, 16, 17]. We detected no false positive results. The instrument demonstrated excellent performance in predicting the negative urine samples (NPV 99.3%), which eliminates the need for further microbiological testing by the cultural method. Similarly, Hassan and Montgomery also reported very high NPV – 98.6% and 98.8%, respectively [8, 17].

In the present study, 9 samples were determined by the automated system to be false negative. This result should be taken into consideration because the microbial number detected in UTIs caused by particular bacterial species or slow-growing organisms (Actinotignum schaalii, Corynebacterium urealyticum, Aerococcus spp., Ureaplasma, Candida spp, etc.), as demonstrated in this study, is often lower (100-100 000) in comparison to classical UTI pathogens [18, 19]. The short operating protocol used by the system (3-4 hours) could be insufficient for their detection. Other potential sources for mistakes are the catheter urine samples, often containing more than one bacterial species, which the instrument reading may misinterpret as contamination. In addition, in some specific patient groups (children under 3 years, pregnant women, patients after kidney transplantation, urological surgery, catheterization), a lower microbial count (< 105) in the urine sample may be clinically significant and this should be taken into account to avoid misinterpretation of the samples as negative during the automated screening [20].

The determination of residual antimicrobial activity in the urine samples is especially important for the correct interpretation of culture results in cases with no reported antimicrobial therapy to avoid false negative results and inappropriate antimicrobial treatment [21]. The present study identified 5.7% of the urine samples positive for residual antimicrobial activity. The RAA rate varies between different studies and strongly depends on the method applied. For example, using a modified urine antibacterial substance assay, Wilson et al. detected much lower rates of RAA (2.6%) among 14 680 urine samples collected from ambulatory and hospitalized patients in Qatar [22]. Research performed in hospital settings in Sri Lanka reported a 19.2% RAA rate, and about 20% of the specimens contained antibiotics that interfered with the culture result [23]. Both researchers used manual techniques for RAA measurement. A study conducted by Kussen compared a manual method and the automated instrument Alfred-60 (Alifax, Italy) to detect RAA in the urine samples of hospitalized patients [24]. The author reported a sensitivity of 71.4% for the manual technique and 92.8% for the automated approach [24] and explained the lower sensitivity of the manual technique with the small sample volume in the used agar diffusion method [24, 25].

The diagnosis of UTI can be complicated by the presence of antibiotics in urine specimens submitted for culture, particularly in countries where they are purchased over the counter, without prescriptions [22]. Regarding the RAA testing performed in our study, among the patients who reported symptoms of UTIs and recent antimicrobial treatment, a very high proportion of both RAA and culture-positive patients were detected. This result always necessitates further evaluation of the patient clinical status (incl. administration of additional laboratory tests) simultaneously with the re-evaluation of the current antimicrobial therapy to assess its adequacy. This is because bacterial counts can be temporarily reduced by antibiotics, causing a transient remission of clinical symptoms but leading to complications like chronic or recurrent asymptomatic infections [22].

This study also identified an unexpectedly high rate of culture-negative but RAA-positive urine samples in a cohort of ambulatory patients with UTIs. This result is significantly above the rates reported by G. Wilson et al. (0.04%) [22], Botão et al. (1%) [24], Cardozo et al. (7.45%) [25], Suresh et al. (15%) [26]. The significant differences in the rates of the RAA (+)/culture (-) samples in the studies mentioned above can be explained by the sample origin (hospitalized or ambulatory patients), with the used laboratory method, as well as with differences in some healthcare practices [22, 27]. Analyzing the medical information provided by the included patients, our result confirmed the findings of Wilson et al., who identified that the prior intake of antibiotics for infections other than UTIs are among the major reasons for positive RAA tests [27]. This fact demonstrates the importance of the history-taking process, especially in outpatients, which can have a profound impact on the quality of the laboratory report, follow-up care of the patient and on inap-

propriate antibiotic use [22]. Also, this result reflects the situation in Bulgaria regarding antimicrobial consumption in the community and the hospital sector during the last few years. It should be mentioned that our study was conducted during the COVID-19 pandemic. According to the ECDC (European Centre for Disease Prevention and Control) report for 2020 and 2021, Bulgaria is among the European countries with the highest total antimicrobial consumption in the region for that period (22.7-24.4 DDD/1000 population per day) [28].

CONCLUSION

To the best of our knowledge, this is the first study in Bulgaria that compares the HB&L Uroquattro automated urine screening with the classical cultural method for diagnosing UTIs and detecting RAA. The screening system demonstrates excellent sensitivity and specificity and, compared to the classical cultural method, has a much faster turnaround time. Some slow-growing organisms could be a potential source for diagnostic mistakes at the time of the screening procedure. The RAA test performed by the HB&L Uroquattro instrument detected 5.7% of the urine samples positive for RAA and proved to be a valuable diagnostic tool for UTIs, particularly in patients with bacteriuria who are under antimicrobial treatment.

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