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Evaluation of HB&L Uroquattro System for Rapid Antimicrobial Susceptibility Testing Directly From Positive Urine Samples

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Abstract

The aim of the study was to evaluate the concordance of the rapid antimicrobial susceptibility testing (AST) results obtained by the HB&L Uroquattro instrument performed directly from positive urine samples in comparison with the standard Kirby-Bauer disk-diffusion method performed with isolated colonies. We included 122 urine samples defined as positive by the HB&L system screening method. The AST was performed directly from them by the HB&L instrument. Simultaneously, all positive urine samples were cultured on CLED, MacConkey and Blood agar and incubated for 24h at 37°C. Species identification and AST of the pathogens isolated on solid media were done by the VITEK 2 automated system (bioMerieux) and respectively by the Kirby-Bauer disk-diffusion method. We defined one hundred and twenty-two positive urine samples:60 *Enterobacterales*, 42 *Enterococcus faecalis,* and 20 *Staphylococcus saprophyticus* isolates. A total of 690 susceptibility determinations were performed with a categorical agreement with the standard method of 94.8%. Thirty-six errors (5.2%) were found. Of these, only 5 very major errors were detected, associated with trimethoprime/sulfamethoxazole susceptibility testing among *Enterobacterales*, glycopeptides, and HLAR among enterococci and cefoxitin susceptibility among staphylococci. The turnaround time for AST directly from positive urine samples was between 3 and 5 hours versus 48 hours for the standard disk-diffusion method from isolated colonies.

The rapidity of the HB&L AST method and its very good categorical correlation with the standard disk-diffusion method determine the potential of the system for wide application in routine laboratory practice.
Keywords: urinary infections, HB&L, rapid antimicrobial susceptibility testing

Резюме

Целта на настоящето проучване е да се оцени съответствието на резултатите от бързото изпитване на чувствителността към антибиотици (AST), получени с HB&L Uroquattro апарата, извършено директно от положителни проби урина, в сравнение със стандартния дисково-дифузионен метод на Kirby-Bauer, извършен с изолирани колонии. В проучването са включени общо 122 проби урина, определени като положителни чрез скрининговата система HB&L. Бързото изпитване на чувствителността към антибиотици беше извършено директно от положителните проби урина с помощта на HB&L апарата. Едновременно с това всички положителни проби урина бяха култивирани върху CLED, MacConkey и кръвен агар и инкубирани за 24ч. на 37°C. Видовата идентификация и антибиотичната чувствителност на патогените, изолирани върху твърда среда, бяха определени чрез VITEK 2 (bioMerieux) автоматизирана система и съответно чрез дисково-дифузионния метод на Kirby-Bauer. Установени са 122 положителни проби урина, от които са изолирани 60 *Enterobacterales*, 42 *Enterococcus faecalis* и 20 изолата *Staphylococcus saprophyticus*. Извършени бяха общо 690 определяния на чувствителност към антибиотици с категорийно съответствие от 94.8% спрямо стандартния метод. Бяха установени 36 грешки (5.2%). От тях, само 5 бяха идентифицирани като много големи грешки, свързани с определянето чувствителността към trimethoprime/sulfamethoxazole сред *Enterobacterales*, към гликопептиди и HLAR сред ентерококите и към cefoxitin сред изолатите стафилококи. Времето за определяне на антибиотичната чувствителност директно от положителни проби урина беше между 3 и 5 часа в сравнение с 48 часа при стандартния дисково-ди-

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фузионен метод от изолирани колонии. Бързината на HB&L AST метода и неговата много добра корелация със стандартния дисково-дифузионен метод определят потенциала на системата за широко приложение в рутинната лабораторна практика.

Introduction

The alarming rise in antimicrobial resistance across the globe is a constantly growing threat to public health (Toosky *et al*., 2020). Mortality rates associated with drug-resistant microorganisms are expected to rise in the next decades. As stated by the National Institute for Health and Care Excellence, by 30 years up to 10 million people could be under death threat due to unsuccessful antibiotic treatment (Dadgostar, 2019).

Patients with community-acquired urinary tract infections (CAUTIs) are among the top recipients of empirical outpatient antibiotic prescriptions (Grigoryan *et al*., 2014; Toosky *et al*., 2020). Therefore, clinicians commonly need to know their local laboratory's findings regarding antibiogram profile, especially for *Escherichia coli*, which causes around 75 - 95% of uncomplicated UTI episodes in women (Colganm *et al*., 2008; Hooton, 2012). Among hospital settings, UTIs account for almost 40% of all nosocomial infections and represent a major burden, given the associated morbidity and mortality (Antimicrobial Resistance Collaborators, 2022; Arienzo *et al.*, 2023).

The diagnosis of UTIs is often based on clinical symptoms and laboratory findings such as pyuria or bacteriuria, though the urine culture is the gold standard for the diagnosis, but with a turnaround time of 24 – 48 h. (Toosky *et al*., 2020). The development of rapid and accurate antimicrobial susceptibility testing (AST) lies at the core of concerted efforts to uphold sound antibiotic stewardship practices and prevent the spread of drug resistance (Toosky *et al*., 2020; Palmer and Buckley, 2021).

In recent years, many different methods for rapid identification and AST have been developed. Molecular approaches, such as multiplex PCR and mass spectroscopy, can be performed with isolated bacteria to identify strains and antimicrobial susceptibility (Machen *et al*., 2014; Czilwik *et al*., 2015; Park *et al*., 2016; Zhu Y *et al.*, 2016; Li *et al*., 2019). The genetic tests are reputably rapid when compared to the gold-standard culture method, but they do not always provide definitive results (Bard and Lee, 2018). The Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) - based rapid phenotypic AST method (MALDI AST) was extended to a new area of application in rapid AST for UTI pathogens directly from urine samples (Neuenschwander *et al*., 2023).

New approaches like biosensor platforms, digital microfluidics, and agarose microchannels have been developed to detect bacterial growth for AST (Li *et al*., 2019). Nevertheless, these techniques neither provide information about the bacterial species nor distinguish polymicrobial samples (Dupnik, 2017). Furthermore, most existing techniques require cultured isolates and are optimized based on a small panel of pathogens, thereby limiting their general applicability for infectious disease diagnostics (Li *et al*., 2019).

In this context, some automated instruments based on laser-scattering technology (HB&L Uroquattro-Alifax, Italy; BacterioScan 216 Dx- BacterioScan Inc., USA) offer a possibility for rapid affordable results. The HB&L Uroquattro is a CE-marked instrument designed for rapid microbial growth screening and for antimicrobial susceptibility testing directly from urine samples within 4 - 6 h (Sharma *et al*., 2023). This instrument can be easily combined with other systems used widely in the clinical microbiology laboratory such as MALDI-TOF MS and automated systems VITEK 2 system (bioMérieux, Marcy l'Etoile, France) and Phoenix (BD, USA) for direct urine analysis (Torres-Sangiao *et al*., 2022).

The objective of this study was to evaluate the concordance of the rapid AST results obtained by the HB&L instrument performed directly from positive urine samples and to compare with those obtained by the Kirby-Bauer disk-diffusion method with freshly isolated colonies.

Materials and Methods

This study was performed from October 2020 to April 2021. A total of 122 urine samples defined as positive by the screening HB&L system (screening time 4 hours, cut of > 10^3 CFU/ml) and associated with UTIs in 122 ambulatory patients, were included in the study. Urine samples positive for Gram-negative non-fermenters (n=5) and the episodes with polymicrobial bacteriuria (n=6) were excluded from the study. Based on the results from the Gram staining, performed directly from the positive urine samples, the HB&L instrument allows the selection of antimicrobial agents for testing.

The AST was done directly from the positive urine samples by HB&L instrument strictly following the manufacturer's recommendations. After ob-

taining 0.5 McFarland in the screening vial, Gram staining microscopic examination directly from the positive vial was performed, and based on the result two antimicrobial panels were defined for rapid AST: panel 1 for Gram-negative bacteria: cefuroxime, ceftriaxone, meropenem, gentamicin, amikacin, ciprofloxacin, trimethoprime/sulfamethoxazole; panel 2 for Gram-positive bacteria: ampicillin, ciprofloxacin, gentamicin (high-level aminoglycoside resistance, HLAR), vancomycin, teicoplanin, cefoxitin. Briefly, 100 µl of each positive screening vial and 200 µl of each antibiotic (already regenerated) were added to an AST broth vial. Additionally, another 100 µl of the same positive screening vial were inoculated in an AST broth vial but with no antibiotic added (reference vial). The inoculum value of the reference vial was monitored to be ≥700.000 CFU/ml to ensure the correct performance of the AST. All vials were loaded on the HB&L system. After incubation of 3 hours for Gram-negative bacteria and 5 hours for Gram-positive ones, the instrument compared the growth curves of the tested bacteria in the antibiotic-containing vials to the reference control growth, calculating the percentage of inhibition of growth (PIG). The PIG was compared between control and antibiotic-containing vials and the result was reported as resistant, intermediate, and susceptible according to the range of inhibition: sensitive: growth inhibition > 65%; intermediate: inhibition between 65 and 50% and resistant: < 50%. In addition, species identification by VITEK 2 Compact System (bioMerieux) (GN-ID, GP-ID cards) was performed directly from each positive screening vial.

Simultaneously, all positive urine samples were cultured on CLED, MacConkey, and Blood agar and incubated for 24 h at 37°C. Species identification and antimicrobial susceptibility of the pathogens isolated on solid media were done by VITEK 2 automated system (bioMerieux) GN-ID (Gram-negative bacilli) and GP-ID (Gram-positive cocci & bacilli) cards and respectively by Kirby-Bauer disk-diffusion method, which was considered the standard for comparison. The susceptibility to vancomycin among staphylococcal isolates was determined by VITEK 2 GP-67/22226 cards. The results from AST were interpreted according to the EUCAST v.11.0, 2021 guidelines (EUCAST, 2021).

The categorial agreement (susceptible, intermediate, resistant) between the two susceptibility methods was determined. The types of errors were defined according to the ASM criteria (Clark, 2009): a very major error was considered when HB&L AST categorized the isolate as susceptible and the reference method as resistant; a major error was considered when HB&L AST categorized the isolate as resistant and the reference method as susceptible and a minor error - when HB&L or the standard method categorizes the isolate as intermediate and the other method as susceptible or resistant.

Results

From all 122 urine samples determined as positive by the HB&L instrument, a total of 60 Gram-negative and 62 Gram-positive bacterial isolates were identified by the automated VITEK 2 as follows: *E. coli*, n=46; *Klebsiella pneumoniae*, n=4; *Enterobacter cloacae* complex, n=4, *Proteus mirabilis,* n=3; *Morganella morganii*, n=1; *Citrobacter koseri,* n=1; *Serratia marcescens*, n=1; *Enterococcus faecalis*, n=42; *Staphylococcus saprophyticus*, n=20. We did not find any inconsistency in the species identification by VITEK 2 when it was performed directly from the positive urine sample and with the pure bacterial culture.

In this study, we compared 690 susceptibility determinations (HB&L AST directly from positive urine samples vs Kirby-Bauer disk diffusion from isolates). The categorial agreement was 94.8% and 36 errors were found (5.2%): 7 minor errors, 24 major and 5 very major errors.

Tables 1, 2, and 3 show the results of the correlation between the two susceptibility methods for the different bacterial groups. For *Enterobacterales* (n=60), the categorical agreement was 93.3% with 18 major errors, 5 minor errors, and only 1 very major error for trimethoprime/sulfamethoxazole. The lowest correlations in this group were for gentamicin and ciprofloxacin (91.7%). For all other antimicrobials, the correlations were greater than

Table 1. Correlation (%) between the susceptibility obtained by HB&L AST system and the reference Kirby-Bauer disk-diffusion method for 60 isolates of *Enterobacterales*

Enterobacterales (n=60)	CXM	CRO	MEM	G	AK	CIP	TMP-SMX
No. of comparisons	60	60	60	60	60	60	60
Correlation (%)	93.3	93.3	100	91.7	95.0	91.7	95.0

Abbreviations: CXM, cefuroxime; CRO, ceftriaxone; MEM, meropenem; G, gentamicin; AK, amikacin, CIP, ciprofloxacin; TMP-SMX, trimethoprime/sulfamethoxazole.

93%. Total agreement was found for meropenem susceptibility (100%).

The categorical agreement for *E. faecalis* (n=42) was 95.2% with 6 major, 1 minor and 3 very major errors. The major errors affected gentamicin (HLAR) (n=2) and ciprofloxacin testing (n=4), and the very major errors were related to gentamicin (n=1) and glycopeptides (n=2).

Table 2. Correlation (%) between the susceptibility obtained by HB&L AST system and the reference Kirby-Bauer disk-diffusion method for 42 isolates of *E. faecalis*

E. faecalis (n=42)	AMP	CIP	G	VAN	TEI
No. of comparisons	42	42	42	42	42
Correlation (%)	97.6	90.5	92.9	95.2	95.2

Abbreviations: AMP, ampicillin; CIP, ciprofloxacin; G, gentamicin (high level aminoglycoside resistance to gentamicin, HLAR); VAN, vancomycin; TEI, teicoplanin.

The categorical agreement for *Staphylococcus saprophyticus* was 96.7%. One very major error for cefoxitin and one minor error for ciprofloxacin were identified. Total agreement for vancomycin susceptibility was found (100%).

The turnaround time for determination of antimicrobial susceptibility directly from positive urine samples by HB&L instrument was between 3 and 5 hours versus 48 hours for the standard disk-diffusion method.

Table 3. Correlation (%) between the susceptibility obtained by HB&L AST system and the reference Kirby-Bauer disk-diffusion method for 20 isolates of *Staphylococcus saprophyticus*

S. saprophyticus (n=20)	FOX	CIP	VAN*
No. of comparisons	20	20	20
Correlation (%)	95.0	95.0	100

Abbreviations: FOX, cefoxitin, CIP, ciprofloxacin; VAN, vancomycin; *the susceptibility was determined by VITEK 2 automated system.

Discussion

The present study compares the results obtained from the AST performed directly with 122 positive urine samples by the HB&L automated screening system with the AST results from the classical disk-diffusion method. A very good categorical correlation (94.8%) with the reference method was found. This is in concordance with studies that report similar or even higher correlation rates (93.4%; 94.9%; 95%; 97.1%) (Anton-Vazquez *et al*., 2019; Sánchez-Carrillo *et al*., 2019; Van den Poel *et al*., 2020; Cupaiolo *et al*., 2022). Our finding demonstrates that the implementation of the HB&L instrument for direct antimicrobial susceptibility testing in the routine laboratory practice could be of great value to decrease dramatically the time to the final microbiology result.

A total of 36 errors were found in this study. The most frequent were the major errors (66.7%) when the HB&L instrument categorized the isolate as resistant while the reference method defined it as susceptible. This is not a critical diagnostic issue as the treatment can be adjusted if necessary and does not pose a risk to the patient. Of course, if the error is related to antibiotics which are defined as a first choice for treatment of UTIs, this could result in a choice of a drug with a lower efficacy profile, a higher relapse rate, or more side effects.

More worrying is the very major errors because they could result in an initiation of a wrong antibiotic therapy and treatment failure, and in cases such as recurrent infections or urosepsis can lead to serious complications (Gajic *et al*., 2022). We identified 5 very major errors (1 in *Enterobacterales*, 3 in *Enterococcus* spp., and 1 in *Staphylococcus* spp.). The single major error among *Enterobacterales* was for trimethoprime/sulfamethoxazole. This should be taken into consideration as trimethoprime/sulfamethoxazole is one of the agents of first choice in the treatment of uncomplicated UTIs in areas where the resistance is below 20%. Among *Enterobacterales*, the lowest correlation rate was found for quinolones (ciprofloxacin) and aminoglycosides (gentamicin) but was associated with mostly minor errors. Of the β-lactam antibiotics tested (cefuroxime, ceftriaxone), a correlation with the culture method was found in 93.3% and the identified inconsistencies were mainly related to major errors. The β-lactam group is a frequent choice in outpatient treatment in different age groups. The detected correlation rate is relatively high compared to other studies which report more discrepancies (Boland *et al*., 2019). For example, a study conducted by Boland reported a correlation rate of 85.3 % for ceftazidime (Boland *et al*., 2019). Because of subtherapeutic plasma concentrations, oral β-lactams are not recommended as a first choice in cases of uncomplicated urinary tract infections, except in childhood and pregnancy (Hooton, 2012).

Among the enterococcal isolates, the lowest categorical agreement was found for ciprofloxacin (90.5%), followed by gentamicin (HLAR) (92.9%) and glycopeptide antibiotics (95.2%). In this bacterial group, 3 very major errors were found: one for gentamicin (HLAR) and 2 for the glycopeptide group. Other authors report even lower cor-

relation for aminoglycosides (HLAR) (87.5%) but a higher correlation rate for vancomycin (100%) (Sánchez-Carrillo *et al*., 2019). In the research of Anton-Vazquez, the correlations for teicoplanin (92%) and vancomycin (90%) were lower than our result (Anton-Vazquez *et al*., 2019). We obtained an excellent categorical agreement for ampicillin (97.6%). This result is similar to that reported by Sánchez-Carrillo, who found a total agreement between the two methods used (100%) (Sanches-Carrillo *et al*., 2019).

Among the staphylococcal isolates, a high correlation was found for ciprofloxacin (95%) and vancomycin (100%). In contrast, other authors report significant differences when comparing resistance to glycopeptides with different methods, including the detection of very major errors (Rybak *et al*., 2013; Sánchez-Carrillo *et al*., 2019). Regarding cefoxitin, although a categorical agreement of 95%, we detected one very major error, and this should be taken into consideration. In a similar study, the highest agreement was observed for cefoxitin and teicoplanin (92%) and the lowest for vancomycin (80%) (Anton-Vazquez *et al*., 2019). Similar to our result, the authors also report very major errors associated with cefoxitin susceptibility (Anton-Vazquez *et al*., 2019). Another study on coagulase-negative staphylococci (including *S. saprophyticus*) found mainly major errors in testing this susceptibility (Boland *et al*., 2019).

In our study, no discrepancies in bacterial identification by VITEK 2 were found when it was performed directly with a positive urine sample and with freshly isolated colonies. Combining the HB&L Uroquattro and Vitek 2 systems can achieve simultaneously rapid microbial identification and antimicrobial susceptibility by direct inoculation from positive urine samples thus yielding a result on the same day of the sample arrival.

The introduction of genetic methods in recent years in laboratory practice also significantly shortens the time to result (Davenport *et al*., 2017; Li H *et al*., 2018; Toosky *et al*., 2020; Burg *et al*., 2022; Gajic *et al*., 2022). However, an important advantage of the HB&L system is the ability to test the susceptibility to a large number of antimicrobials, as well as to select the agents according to the isolate and the specificities of the particular patient (Sánchez-Carrillo *et al*., 2019).

Conclusion

To the best of our knowledge, this is the first study in Bulgaria that evaluates the HB&L Uroquattro for rapid antimicrobial susceptibility testing directly from positive urine samples. The instrument demonstrated a very good correlation with the standard Kirby-Bauer disk-diffusion method, performed with freshly isolated colonies. The presented laboratory algorithm, including urine screening, followed by microscopic examination and rapid antimicrobial susceptibility testing directly from the clinical sample, dramatically shortens the diagnostic process by 24 - 48 hours. These major advantages of the HB&L instrument determine its potential for wide application in routine laboratory practice.

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Ethics

This study was approved by The Ethics Committee of Medical University - Varna (protocol No92/02.04.2020).

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