A new rapid method for detecting extended-spectrum beta-lactamase/AmpC-producing *Enterobacteriaceae* directly from positive blood cultures using the Uro4 HB&L™ system

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Abstract

Purpose To evaluate the performance of the Uro4 HB&L™ system in screening ESBL/AmpC-producing *Enterobacteriaceae* as compared with the resistant test by VITEK 2. The resistance profile of the ESBL-producing bacteria was also evaluated.

Methodology Blood culture samples were collected at bedside, inoculated directly into BD BACTEC™ culture bottles, and incubated. When microbial growth was found, samples were prepared for identification using MALDI-ToF. The Uro4 HB&L™ system was used for direct detection of ESBL/AmpC-producing bacteria compared with conventional methods.

Results A total of 103 positive blood cultures containing Gram-negative bacteria were tested. Uro4™ HB&L screening results were obtained in up to 6.5 h, with 91.3% concordance with the VITEK2 system, with 85% sensitivity, 95.2% specificity, and 91.1% positive and 90.9% negative predictive values. The resistance profile of ESBL-producing bacteria tested in the present study showed increased resistance ratio to ciprofloxacin, gentamicin, and trimethoprim/sulfamethoxazole. In parallel, susceptibility to amikacin and meropenem was not affected.

Conclusions The performance of the Uro4 HB&L™ system is acceptable for rapid screening ESBL/AmpC-producing *Enterobacteriaceae*. The data related to detecting ESBL-producing bacteria are crucial for managing antimicrobial therapy.

Keywords Blood culture · Extended-spectrum beta-lactamase (ESBL) · *Enterobacteriaceae* · Uro4 HB&L™ system

Introduction

Without appropriate treatment, bloodstream infections can have a high mortality rate. The mortality rate due to septic shock is estimated to be up to 54% among patients admitted to intensive care units [1]. Early initiation of appropriate antimicrobial therapy will improve the outcomes of these patients [2–4]. As identifying the agent and obtaining the antibiogram are time consuming, empirical treatment is administered when sepsis is recognized, and should be modified if necessary based on the antibiogram [5–8]. Although the effects of inappropriate empirical antimicrobial therapy are well-established [3, 9, 10], 30% of

mortality due to bloodstream infections results from Gram-negative bacteria, when inappropriate antimicrobial therapy was administered [5].

The most potent compounds for treating bloodstream infections are beta-lactam antibiotics alone or in combination with other antimicrobial agents. Therefore, rapid identification of the causative pathogen and detection of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* significantly decreased the time it takes to obtain results. Such results are essential for timely selection of appropriate antimicrobial therapy, which may result in better patient outcomes [11–14] and contribute to inhibiting antimicrobial resistance [15].

The introduction of matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI-ToF) decreased the time needed to identify microbes from positive blood culture broths, especially for *Enterobacteriaceae* [16–18]. However, the ability to rapidly detect resistant bacteria, such as ESBL-producing bacteria directly from positive blood cultures is crucial for managing antimicrobial therapy [19, 20].

Various methods have been suggested for detecting ESBL-producing bacteria. It has been shown that MALDI-ToF can be used to detect resistance against beta-lactam antibiotics

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[21] and using PCR-based molecular techniques on positive blood cultures has been proposed for rapid identification of ESBL-producing bacteria [22]. However, these require trained personnel and expensive materials. ESBL-producing bacteria can be identified rapidly with the ESBL Nordmann/Dortet/Poirel (NDP) test [23], which is based on biochemical detection of hydrolysis of the beta-lactam ring of cefotaxime. The double-disc synergy test (DDST) for phenotypic detection of ESBL producers was also suggested [24]. Because the DDST is performed in parallel with the antisusceptible test by VITEK 2, results are obtained 48 h later. The DDST and the VITEK 2 were considered to be the reference standard for detecting ESBL-producing bacteria [25].

Recently, a new method was suggested for screening ESBL/AmpC-producing *Enterobacteriaceae* species. The assay was adopted from the description of Barnini et al. [26] to rapidly detect ESBL-producing bacteria from positive blood cultures. The system is CE approved and is called Alfred 60AST (Alifax, SpA, Polverara, Italy). The system was first used for screening urine and for antisusceptible bacterial isolates from urine [27]. Results were obtained in up to 6.5 h, which provided the clinician important data related to the properties of ESBL-producing/non-producing bacteria. In addition, testing for detecting ESBL-producing bacteria was performed directly using the Uro4 HB&L™ system (Alifax SpA). The principle of the method is based on laser light scattering to monitor the fluid culture for 6.5 h of incubation. A laser beam is passed through the glass bottles containing the reagent Uro4 HB&L™ ESBL/AmpC screening kit inoculated with bacteria and with added selective supplement-containing antibacterial and antifungal agents before starting the analysis. The broth, combined with the ESBL/AmpC supplement, inhibits Gram-positive bacteria and non-ESBL/AmpC-producing *Enterobacteriaceae* spp. The reagent is a broth culture enriched with an infusion of animal tissues, which allows the growth of most microorganisms present in the fluid of human pathological specimens. A magnetic stirrer is included in each vial to ensure homogenization of the suspension during analysis. The light scattering signals are detected and displayed as growth curves and a count evaluation is obtained by algorithmic calculation.

The aim of the present study was to evaluate the performance of the Uro4 HB&L™ system for ESBL/AmpC-producing *Enterobacteriaceae* species directly from positive blood cultures as compared simultaneously with the antimicrobial susceptibility test done by VITEK 2. The resistance profile of the ESBL-producing bacteria was also evaluated.

Materials and methods

Blood cultures This study was performed at the Clinical Microbiology Laboratory of Hillel Yaffe Medical Center, Hadera, Israel. Blood culture samples were collected at patients'

bedsides and inoculated directly into BD BACTEC™ culture bottles from April to June 2018. They were incubated in the automated BD BACTEC™ FX system (Becton Dickinson, Franklin Lakes, NJ, USA) for up to 5 days. When blood culture bottles signaled positive on the system (indicating microbial growth), aliquots of the blood culture were Gram stained and subjected to subculture for conventional identification. The blood cultures were simultaneously identified by MALDI-ToF and tested for detection of ESBL-producing bacteria by the Uro4 HB&L™ system and by the comparable conventional method VITEK 2.

Identification of bacteria by MALDI-ToF method Identification of microorganisms using the MALDI-ToF (Bruker Daltonics for Microflex LT spectrometer) technique was performed as described previously [17]. Briefly, 5 ml was transferred from the positive blood culture bottles to serum separator tubes (SST; BD Vacutainer systems). The tubes were centrifuged at 2000×g for 10 min and the bacteria were sedimented on the surface of the silicon layer. The sedimented bacteria were suspended with 1 ml of distilled water and transferred to an Eppendorf tube and centrifuged at 13,000 rpm for 2 min. The bacterial pellet was dried at room temperature and bacteria were then transferred from the pellet by direct loop onto the steel target plate for MALDI-ToF identification. One microliter of α -Cyano-4-hydroxycinnamic acid (HCCA) matrix was added to each spotted sample on the target plate and allowed to dry. The plate was then placed in the mass spectrometer MALDI-ToF system and genus and species were identified. The identification process was performed in real-time, as soon as the sample was analyzed by the spectrometer.

Identification and detection of ESBL-producing bacteria by VITEK2 Positive blood culture bottles signaled by the BACTEC™ FX instrument were Gram stained, subcultured onto appropriate solid medium (including Chromagar plate according the Gram stain), and incubated overnight at 37 °C. VITEK2 (VITEK 2 software, version 05.04; Advanced Expert System software, version 1.9.0; bioMérieux, Marcy Etoile, France) was used to identify ESBL-producing bacteria from the colonies grown after subculturing from the same positive blood culture that was used for detecting ESBL-producing bacteria by the Uro4 HB&L™ system. The cards used for identification and antisusceptible test were assessed weekly for quality control [12].

Direct detection of ESBL/AmpC-producing bacteria by Uro4 HB&L™ system The Uro4 HB&L™ system is designed to detect ESBL-producing bacteria from rectal swabs and we adopted it for screening positive blood cultures. The screening test was performed according to the manufacturer's instructions with minimal modifications. Briefly, 10 μ l of each positive blood culture sample of Gram-negative bacteria was

diluted 1:100 in saline. To a vial containing 2 ml HB&L broth (Alifax), 200 µl of the diluted bacteria was added with 200 µl of ESBL/AmpC supplement (Lyophilized antibiotic (Alifax), which was dissolved with 2 ml of regenerating solution and stored for no longer than 7 days after reconstitution at 4–8 °C). The vials were placed in the Uro4 HB&L™ instrument for 6.5 h. Bacterial growth was determined by reading the turbidity of the medium, which was displayed as a curve. Bacterial growth indicated that it was ESBL-producing. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as quality control strains. The lower limit of detection (LOD) as determined by the manufacture is < 50 Cfus/ml and was confirmed by us. Each test was performed twice and in some cases was repeated three times.

ESBL detection by Clinical and Laboratory Standards Institute (CLSI) phenotype method In case of discrepancy between the results obtained from the Uro4 HB&L™ and the VITEK2, we used the NCCL ESBL phenotypic confirmatory test with ceftazidime (CAZ) and clavulanic acid (CA) by the disc diffusion method [25].

Statistical analysis The sensitivity, specificity, and predictive values were calculated for the screening tests, as previously described [28].

Results

A total of 103 positive blood cultures contained Gram-negative bacteria. The isolates were identified by MALDI-TOF and/or VITEK 2. The most common isolate was *E. coli* (62.1%), followed by *Klebsiella pneumoniae* (*K. pneumoniae*) (22.3%). *Proteus mirabilis* (*P. mirabilis*) was isolated at a rate of 11.65%. *Citrobacter koseri* and *Enterobacter cloacae* constituted 2.9% and 0.97%, respectively.

Table 1 summarizes the concordant and dis-concordant results for these organisms between Uro4 HB&L™ screening

system and VITEK2. As calculated, the concordant between methods of all microorganisms tested was 91.3%. When compared individually for each microorganism, the concordant was 96.9% for *E. coli*, 91.3% for *K. pneumoniae*, and 58.3% for *P. mirabilis*.

The calculations of specificity, sensitivity, positive predictive values (PPV), and negative predictive values (NPV) of Uro4 HB&L™ system are shown in Table 2. Analysis of the all isolates tested revealed excellent specificity (95.2%) and PPV (91.9%). The results of total sensitivity (85%) and NPV (90.9%) were very good. When calculated separately, *E. coli* had high sensitivity (96.4%), specificity (97.2%), PPV (96.34%), and NPV (97.2%). *K. pneumoniae* (100% sensitivity, 100% specificity, 77.8% PPV, and 100% NPV). Unexpected values were obtained with *P. mirabilis*. The sensitivity and the PPV were zero. The specificity and the NPV were 58.5% and 100%, respectively.

Table 3 shows the pattern of resistance to antimicrobial agents of ESBL-producing bacteria as compared with non-ESBL-producing bacteria. A total of 27 *E. coli* isolates and 7 *K. pneumoniae* were detected as ESBL-producing by the Uro4 HB&L™ system and VITEK2. In parallel, 35 *E. coli* isolates and 14 *K. pneumoniae* were non-ESBL-producing, as determined by the Uro4 HB&L™ system and the VITEK2. It was found that all isolates tested (*E. coli* and *K. pneumoniae*, ESBL-producing or non-producing) were sensitive to amikacin, ertapenem, and meropenem. All the ESBL-producing isolates were resistant to ampicillin, ceftriaxone, cefuroxime, and ceftazidime. In contrast, all the non-ESBL-producing isolates were sensitive to ceftriaxone, cefuroxime, and ceftazidime. Regarding ampicillin, 28.6% of *E. coli* and 100% of *K. pneumoniae* were resistant.

Nine (33.3%) isolates of ESBL-producing *E. coli* and 1 (14.3%) ESBL-producing *K. pneumoniae* were resistant to amoxicillin/clavulanate compared with none among the non-ESBL-producing strains.

Two (7.4%) isolates of ESBL-producing *E. coli* and 1 (14.3%) ESBL-producing *K. pneumoniae* were resistant

Table 1 Performance of Uro4 HB&L™ screening system for detecting ESBL-producing and non-ESBL-producing bacteria compared with VITEK2

	Concordant results		Dis-concordant results		Total isolates tested
	ESBL positive by Uro4 HB&L™ and VITEK2	ESBL negative by Uro4 HB&L™ and by VITEK2	ESBL positive by Uro4 HB&L™ and negative by VITEK2	ESBL negative by Uro4 HB&L™ and positive by VITEK2	
Total	34	60	3	6	103
<i>E. coli</i>	27	35	1	1	64
<i>Klebsiella pneumoniae</i>	7	14	2	0	23
<i>Proteus mirabilis</i>	0	7	0	5	12
<i>Citrobacter koseri</i>	0	3	0	0	3
<i>Enterobacter cloacae</i>	0	1	0	0	1

Table 2 The specificity, sensitivity, and positive/negative predictive values of ESBL-producing bacteria

Microorganism (no. of isolates)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
<i>E. coli</i> (64)	96.4	97.2	96.4	97.2
<i>Klebsiella pneumoniae</i> (23)	100	100	77.8	100
<i>Proteus mirabilis</i> (12)	0	58.5	0	100
Total number of isolates (99)	85	95.2	91.9	90.9

to piperacillin/tazobactam, as compared with none among the non-ESBL-producing *E. coli* and 7.1% *K. pneumoniae*.

Twenty-five (92.6%) ESBL-producing *E. coli* and 2 (28.6%) ESBL-producing *K. pneumoniae* isolates were resistant to ciprofloxacin, as compared with 10 (28.6%) of non-ESBL-producing *E. coli* and no non-ESBL-producing *K. pneumoniae*.

Regarding gentamicin, 9 (33.3%) ESBL-producing *E. coli* and 4 (57.1%) ESBL-producing *K. pneumoniae* were resistant. In contrast, no resistance to gentamicin was found among the non-ESBL-producing *K. pneumoniae* and 5 (14.3%) were found resistant to gentamicin among the non-ESBL-producing *E. coli*.

Resistance to trimethoprim/sulfa was found among 21 (77.4%) of the ESBL-producing *E. coli* and 7 (100%) of the ESBL-producing *K. pneumoniae*. In contrast, there was no resistance among the non-ESBL-producing *K. pneumoniae* and 7 (20.0%) of the non-ESBL-producing *E. coli* were resistant.

Discussion

The development of MALDI-TOF technology has allowed rapid identification of bacterial species. We took advantage

of the rapid identification by MALD-TOF to proceed with detecting ESBL-producing *Enterobacteriaceae*. We found that the Uro4 HB&L™ system directly on positive blood cultures identified ESBL-producing bacteria within 6.5 h. The system was able to provide results in real-time faster than the conventional method did. The combination of the MALDI-TOF technology with the Uro4 HB&L™ system provides the species of the pathogen and the partial antibiotic resistance profile such as ESBL-producing ability, the same day.

A total of 103 *Enterobacteriaceae* isolates from positive blood cultures were analyzed following Gram stain and direct identification by MALDI-TOF. Subsequently, testing for ESBL-producing bacteria was performed using the Uro4 HB&L™ system. This system was able to distinguish between ESBL-producing and non-ESBL-producing bacteria according to the growth of the bacteria inoculated in the vials containing the growth medium and selective antimicrobial agents. However, the assay did not determine the MIC of the antimicrobial agents tested and did not distinguish between ESBL and AmpC mechanisms.

The results displayed 91.6% concordance among all 103 isolates. Three isolates showed false positive results, which were detected by the VITEK2 system as non-

Table 3 The antimicrobial resistance profile of ESBL-producing bacteria

Antimicrobial agent	ESBL-producing <i>E. coli</i> (27) Resistant	Non-ESBL-producing <i>E. coli</i> (35) Resistant	ESBL-producing <i>K. pneumoniae</i> (7) Resistant	Non-ESBL-producing <i>K. pneumoniae</i> (14) Resistant
Amikacin	0	0	0	0
Amoxicillin/Ca	9 (33.3%)	0	1 (14.3%)	0
Ceftriaxone	27 (100%)	0	7 (100%)	0
Cefuroxime	27 (100%)	0	7 (100%)	0
Ciprofloxacin	25 (92.6%)	10 (28.6%)	2 (28.6%)	0
Ertapenem	0	0	0	0
Gentamicin	9 (33.3%)	5 (14.3%)	4 (57.1%)	0
Meropenem	0	0	0	0
Piperacillin/tazobactam	2 (7.4%)	0	1 (14.3%)	1 (7.1%)
Trimethoprim/Sulfa	21 (77.8%)	7 (20%)	7 (100%)	0
Ampicillin	27 (100%)	10 (28.6%)	7 (100%)	14 (100%)
Ceftazidime	27 (100%)	0	7 (100%)	0

ESBL-producing bacteria and detected by the Uro4 HB&L™ system as ESBL-producing bacteria. This discrepancy may be a result of the concentration of the bacteria drawn from the positive blood bottles to the vials of the Uro4 HB&L™ system. We speculate that the higher the inoculum concentration, the greater the likelihood of detecting ESBL-producing bacteria.

Of greater concern are the false negative results obtained. Six isolates had false negative results which were detected by the VITEK2 system as ESBL-producing bacteria and detected by the Uro4 HB&L™ system as non-ESBL-producing bacteria. This discrepancy may be a result of the concentration of the bacteria drawn from the positive blood bottles to the vials of the Uro4 HB&L™ system. It is important to note that all the *P. mirabilis* isolates that were detected as ESBL-producing by the VITEK 2 system were detected as non-ESBL-producing by the Uro4 HB&L™ system. All discrepant results were confirmed by the disc diffusion method [25].

Compared with the VITEK 2 method for detection ESBL-producing bacteria, the Uro4 HB&L™ system had a sensitivity of 85% and a specificity of 95.2% for all isolates tested: *E. coli*, *K. pneumoniae*, and *P. mirabilis*. These findings are in concordance with the manufacturer's instructions. The relatively low sensitivity, compared with specificity, is related to the results of *P. mirabilis*, in which the Uro4 HB&L™ system did not detect ESBL-producing *P. mirabilis* (false negative). Although, the sensitivity for *E. coli* and *K. pneumoniae* was excellent (96.4% and 100%, respectively).

A study using MALDI-TOF for detecting beta-lactam resistance showed 100% sensitivity and 91.5% specificity, and the results were obtained within 2.5 h after the blood culture was found positive [29]. These results are reliable and rapid but can be expensive. Another study [22] showed that direct identification of bacteria by MALDI-TOF combined with NDP testing for detecting ESBL-producing bacteria was rapid. The result was obtained within 30 min. Moreover, the sensitivity and the specificity were 100%.

The false negative results/sensitivity is very important for patient safety. Accordingly, the Uro4 HB&L™ system is not appropriate for *P. mirabilis* because the sensitivity obtained for these bacteria was zero. Therefore, these data related *P. mirabilis* must be reported to the physician after confirmation by other method.

Since the Uro4 HB&L™ system is designed to detect ESBL-producing bacteria from rectal swabs and we adopted it for screening blood cultures, there are some limitations to this study. The proposed system could not detect ESBL-producing *P. mirabilis*. Moreover, the Uro4 HB&L™ system detects phenotypically ESBL-producing bacteria and did not explore the ESBL genes of the ESBL-producing bacteria such as CTX-M, SHV, and TEM.

Accordingly, using the Uro4 HB&L™ system to screen ESBL-producing bacteria directly from positive blood cultures is in experimental stages. Improvements to overcome these limitations are ongoing. Moreover, the procedure did not distinguish between ESBL-producing bacteria and AmpC isolates. Further tests needed to explore the mechanism of resistance.

In summary, the Uro4 HB&L™ system was able to detect resistance against beta-lactam antibiotics with high sensitivity for *E. coli* and *K. pneumoniae*, 24 to 48 h earlier than conventional methods would. This can make the test a valuable tool for early therapeutic guidance in critically ill patients. However, further studies are needed to test a larger number of isolates and to improve the detection of ESBL-producing *P. mirabilis*.

The resistance profile of the ESBL-producing or non-ESBL-producing bacteria provided information of cross-resistance with other antimicrobial agents. The analysis of the resistant profile of the ESBL-producing bacteria reveals that they were more resistant to ciprofloxacin, gentamicin, and trimethoprim/sulfamethoxazole. Therefore, the antimicrobial resistance profile of ESBL-producing bacteria should be considered to guide treatment.

Conclusions

The performance of the Uro4 HB&L™ system is acceptable for rapid screening ESBL/AmpC-producing *Enterobacteriaceae*. In addition, the combination of rapid identification of microbial pathogens by MALDI-TOF and the detection of ESBL-producing bacteria by Uro4 HB&L™ provide valuable information. The identity of the pathogen and whether or not it produces ESBL can be available the same day. This information allows the clinician to choose the most appropriate therapeutic management. Moreover, the antibiotic resistance profile addressed in this study shows that gentamicin, ciprofloxacin, and trimethoprim/sulfamethoxazole might not be effective. Although the ability to detect *E. coli* and *K. pneumoniae* as ESBL-producing is very good, detection of *P. mirabilis* needs improvement.

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Author contributions All authors have seen and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement Not applicable.

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