AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

67 (2020) 3, 193–197

DOI:
10.1556/030.2020.01184

ORIGINAL ARTICLE

An alternative for urine cultures: Direct identification of uropathogens from urine by MALDI-TOF MS

ARZU AKŞIT İLKI*, SEVIM ÖZSOY, GULŞEN GELMEZ, BURAK AKSU and GÜNER SÖYLETIR

Department of Medical Microbiology, Faculty of Medicine, Marmara University, Istanbul, Turkey

Received: March 30, 2020 • Accepted: April 17, 2020
Published online: September 24, 2020

ABSTRACT

Urinary tract infections are one of the most common bacterial infections and rapid diagnosis of the infection is essential for appropriate antibiotic therapy. The goal of our study was to identify urinary pathogens directly by MALDI-TOF MS and to perform antibiotic susceptibility tests in order to shorten the period spent for culturing.

Urine samples submitted for culture to the Clinical Microbiology Laboratory were enrolled in this study. Urine samples were screened for leukocyte and bacteria amount by flow cytometry. Samples with bacterial load of 10^6–10^7/mL were tested directly by MALDI-TOF MS and antibiotic susceptibility tests (AST) were performed.

In total, 538 positive urine samples were evaluated in our study. MALDI-TOF MS identified the microorganism directly from the urine sample in 91.8% of these samples and the concordance rate of conventional identification and direct detection was 95.8% for Gram-negatives at the genus and species level. *Escherichia coli* (*n*:401) was the most frequently isolated microorganism, followed by *Klebsiella pneumoniae* (*n*:57). AST results were generated for 111 of these urine samples and the concordance was 90% and 87% for *E. coli* and *K. pneumoniae*, respectively.

Our results showed that screening of urine samples with flow cytometry to detect positive samples and identification of uropathogens directly by MALDI-TOF MS with an accuracy of over 90% can be a suitable method particularly for Gram-negative bacteria in clinical microbiology laboratories.

KEYWORDS

Urine, direct identification, flow cytometry, MALDI TOF MS

INTRODUCTION

Urinary tract infections (UTI) are one of the most common nosocomial and community acquired infections [1]. Rapid detection of causative bacteria in urinary tract infections and initiation of appropriate antibiotic therapy are very important in terms of time and cost. Urine culture is the gold standard for the microbiological confirmation of UTIs. However, conventional urine culture and antibiotic susceptibility testing can take 48–72 h, and delayed results lead to unnecessary or improper treatment of patients. For this purpose, many screening systems were introduced to detect the presence of bacteria in urine samples including urine dipstick testing, urinalysis [2–6]. Some of these systems allow the prompt prediction of negative samples, therefore automated analyzers for urine screening that rapidly identify the negative samples and detect the positive samples to be taken to conventional culture procedures became to be used in the laboratories.

This methodology has been successfully and routinely used to rapidly identify microorganisms from cultures. Direct detection of pathogens from samples can increase the usefulness of this method, since it can significantly shorten the identification time. The direct identification of uropathogens in urine by MALDI-TOF MS can significantly shorten the identification time from 24 to 48 h, using classical methods, to 30 min [7, 8].

*Corresponding author.
Tel.: +90 542 4354758.
E-mail: ailki@marmara.edu.tr

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Table 1. Comparison of direct MALDI TOF MS from urine and culture results

Culture	Agreement *n*(%)	Misidentified *n*(%)	Unidentified *n*(%)	Total *n*(%)
Pathogen	494(91.8)	13(2.4)	31(5.8)	538
Mixed	–	43(11.7)	322(88.3)	365
Sterile	–	5(12.8)	34(87.2)	39
Total	494(52.5)	61(6.5)	387(41)	942

In our study, our aim was to detect positive urine samples by flow cytometry and to identify bacteria directly by MALDI-TOF MS and perform antibiotic susceptibility testing (AST) in order to shorten the period spent on conventional culture (CC).

MATERIAL AND METHODS

Urine samples submitted for culture to the Clinical Microbiology Laboratory were enrolled in this study. Urine samples were analyzed for leukocyte and bacteria amount by flow cytometry (Sysmex UF-1000i, TOA Medical Electronics, Kobe, Japan) and samples which had a bacterial load of 10^6–10^7/mL were processed in two ways:

Direct identification by MALDI-TOF MS and direct antibiotic susceptibility testing

For this purpose, 4 mL of urine was centrifuged at 2,000×*g* for 1 min to remove cellular debris, leukocytes, and mucus. The supernatant was centrifuged at 15,500×*g* for 5 min to collect bacteria. After discarding the supernatant, the pellet was washed with deionized water. Residual water was removed by careful pipetting and pellets were spotted onto MALDI-TOF MS target plate and allowed to dry. It was overlaid with 1 µL of matrix solution (α-cyano-4-hydroxycinnamic acid solution) and air dried. Subsequently, for direct antibiotic susceptibility testing, positive urine samples pellets were used to adjust to the 0.5 Mac Farland standard and processed identically to the CC procedure.

Urine culture evaluation and identification by MALDI-TOF MS-Conventional culture

Urine samples were Gram-stained for direct examination and were inoculated on 5% blood agar with a standard loop (10 µL) quantitatively and on chromogenic medium (ChromID CPS3; bioMérieux, France) by single colony method. After overnight incubation, positive plates were evaluated and the colonies were identified by MALDI-TOF MS according to the manufacturer's procedures. Antibiotic susceptibility testing was performed by both VITEK AST cards (bioMérieux, France) and the disk diffusion method.

Evaluation method

Three different categories were established to evaluate the identification results of direct urine specimens:

1. "agreement" represented concordance between identification results of direct identification and CC isolates at genus and species level:
2. "misidentified" represented the strain identification with direct identification bacteria which showed discrepant results at genus level:
3. "unidentified" represented no identification at all.

Susceptibility testing results of direct bacteria and CC isolates were evaluated per the EUCAST guidelines for agreement, minor errors, major errors, and very major errors when compared to that of disk diffusion testing as a reference method. Agreement represented similar results between test method and reference method. Minor errors conveyed the susceptible or resistant category for one system while intermediate for the other system. Major errors were identified when the results in the test system were resistant whereas they were susceptible using the reference methods. Very major errors were defined as results in the susceptible category by test system, while they are resistant by the reference method.

RESULTS

In total, 942 urine samples were evaluated in our study. Of these 538 were reported as positive, 365 as contamination and 39 were negative in plate culture. However, 494 (91.8%) were defined as positive and 387 were unidentified by direct identification with MALDI-TOF MS. Of these unidentified samples, 322 were reported as mixed flora and 34 as negative in urine culture results (Table 1). MALDI-TOF MS identified the microorganism directly from the urine sample in 91.8% of the isolates. Only 2.4% of the isolates were defined as misidentified by direct method.

By direct method, MALDI-TOF MS identified Gram-negative bacteria more reliably (Table 2). Of these samples, the coincidence rate of conventional identification and MALDI-TOF MS for Gram-negative bacteria was 95.8% at the genus and species level. Among the Gram-negative bacteria, the lowest identification rate detected was in *Proteus mirabilis*. The coincidence rate was also lower particularly in the identification of *Staphylococci* spp. (4.2%). As expected, *Escherichia coli* (*n*: 401) was the most frequently isolated microorganism, followed by *Klebsiella pneumoniae* (*n*: 57) whether by direct method or conventional cultures. However, three *E. coli* isolates were identified as *Klebsiella* spp. and 12 of them could not be identified. Correct

Table 2. MALDI-TOF MS versus conventional identification

Identification by conventional culture (no of isolates)	Correlation(%) at		Direct identification by MALDI-TOF MS (no of isolates)
	Species level	Genus level	
Escherichia coli(426)	94.1	94.1	*Escherichia coli(401) No reliable identification(25)*
Klebsiella pneumoniae(57)	100	100	*Klebsiella pneumoniae(57)*
Klebsiella oxytoca(4)	100	100	*Klebsiella oxytoca(4)*
Enterobacter cloacae(4)	100	100	*Enterobacter cloacae(4)*
Enterobacer aerogenes(1)	100	100	*Enterobacer aerogenes(1)*
Morganella morganii(1)	100	100	*Morganella morganii(1)*
Proteus mirabilis(16)	12.5	12.5	*Proteus mirabilis(2)*
Pseudomonas aeruginosa(3)	100	100	*Pseudomonas aeruginosa(3)*
Acinetobacter baumannii(2)	100	100	*Acinetobacter baumannii(2)*
Enterococcus faecalis(18)	100	100	*Enterococcus faecalis(18)*
Enterococcus faecium(1)	100	100	*Enterococcus faecium(1)*
Streptococcus agalactiae(2)	50	50	*Streptococcus agalactiae(1)*
Staphylococcus aureus(1)	0	0	*Unidentified*
Staphylococcus saprofiticus(1)	0	0	*Unidentified*
Staphylococcus epidermidis(1)	0	0	*Unidentified*
Total(538)			Total(520)

identification was highest (100%) for *K. pneumoniae*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, and *Enterococci* spp. with none in the unidentified category (Table 2).

AST results were generated for 111 of these urine samples and of these, 96 were *E. coli,* 11 *K. pneumoniae* and 4 *Enterobacter cloacae*. The concordance of antibiotic susceptibility testing was 90% and 87% for *E. coli* and *K. pneumoniae*, respectively.

Susceptibility test results of CC isolates revealed that agreement was >95% for tested antibiotics, with the exception of trimethoprim sulfamethoxazole between VITEK2 and disk diffusion results. Similar results, except for trimethoprim sulfamethoxazole were obtained when direct urine was used in VITEK2 system. VME was detected in three samples; two in ciprofloxacin and one in trimethoprim sulfamethoxazole in direct urine samples. Compared to disk diffusion, VITEK2 susceptibility results of direct urine displayed 0–2.7% and 0–3.6% major and minor error rates, respectively, similar to that of CC results (Table 3).

DISCUSSION

For clinical microbiology laboratories, rapid and accurate identification of urine samples are very important, since these comprise the largest workload of laboratories. Screening urine samples with flowcytometry can help to

Table 3. Comparison of Vitek2 antibiotic susceptibility results of direct urine and conventional culture by disk diffusion method

Antibiotics	Direct urine				Conventional culture			
	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)	Agreement (%)	Minor error (%)	Major error (%)	Very major error (%)
Ampicillin	100	0	0	0	96.4	0	3.6	0
Amoxicillin Clavulante	100	0	0	0	98.2	0	0.9	0.9
Amikacin	99.1	0.9	0	0	98.2	1.8	0	0
Cefixime	100	0	0	0	100	0	0	0
Ciprofloxacin	95.5	2.7	0	1.8	93.7	2.7	0	3.6
Ceftriaxone	100	0	0	0	99.1	0	0.9	0
Cefuroxime	100	0	0	0	96.4	0	2.7	0.9
Fosfomycin	97.3	2.7	0	0	93.7	2.7	3.6	0
Nitrofurantoin	99.1	0	0.9	0	99.1	0	0.9	0
Gentamycin	99.1	0	0.9	0	98.2	0	1.8	0
Imipenem	100	0	0	0	98.2	0	1.8	0
Meropenem	98.2	1.8	0	0	98.2	0	1.8	0
Trimetoprim/ Sulfamethoxazole	93.7	2.7	2.7	0.9	94.6	2.7	0.9	1.8
Piperacililn/tazobactam	96.4	3.6	0	0	95.5	4.5	0	0

eliminate the negative urine samples and detect positive samples [9]. Previous studies have shown that MALDI-TOF MS is a useful method for bacterial identification from culture, therefore direct analysis may further increase the usefulness of MALDI-TOF MS to detect positive urine samples in a few minutes time on the day of administration. For this purpose, we screened urine samples with flow cytometry and detected the samples with a bacterial load of 10^6–10^7/ml for direct identification of uropathogens with MALDI-TOF MS. When compared with urine culture results, the correct identification rate was 91.8%. The MALDI-TOF MS identified *E. coli* (91.8%), *K. pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, *A. baumanii*, and *Enterococcus faecalis* positively. In accordance with previous studies, the identification of Gram-negative bacteria has provided better results than Gram-positive bacteria and yeast [10–12]. We did not detect any yeast, however the identification rate for Gram-positives were 83.3%. Among Gram-positive *Enterococci* spp, either *E. feacalis* or *Enterococcus faecium* could be identified directly with 100% identification; however, *Staphylococci* spp. could not be identified (Table 2). In our study, five positive samples (*E. coli* and *P. aeruginosa*, and *E. coli* and *E. faecium*) contained colonies with two different morphologies on the culture plate; however, MALDI-TOF MS detected only one of the two microorganisms Very similarly, Wang et al. analyzed urine specimens containing two microorganisms in different ratios two types of bacteria were simultaneously detected in a mixture at a ratio 1:1 or 1:2 [13]. In the study of Inigo et al. analysis of 75% of the polymicrobial urine samples provided the correct identification of one microorganism. This might be related to the low bacterial amounts in those samples [14].

Screening urine samples with flow cytometry to detect positive samples and identification of uropathogens directly by MALDI-TOF MS can process a large number of urine samples in 30 min per sample with an accuracy of over 90%. However, the antibiotic susceptibility results for these pathogens is still required. Therefore, in the second part of our study, antibiotic susceptibilities of direct urine samples were evaluated according to CC results. ASTs of direct urine samples performed by VITEK2 displayed >95% for tested antibiotics, except for trimethoprim sulfamethoxazole between VITEK2 and disk diffusion results (Table 3). Ampicillin, amoxicillin/clavulanate, cefixime, ceftriaxone, imipenem designated no error, in agreement with both methods. Remarkably, first choice antimicrobials used in UTI including fosfomycin, ciprofloxacin, nitrofurantoin and gentamycin demonstrated high (>95%) percentages of agreement. Similar results were evaluated in a few previous studies in which aliquots of urine were used directly for antibiotic susceptibility and the results were comparable by disk diffusion [3, 15]. Susceptibility results indicated that direct urine can be performed for the determination of the antibiotic susceptibilities of Enterobacteriaceae, especially for *E. coli* and *Klebsiella* spp. which are the most common pathogens in UTIs.

In conclusion, MALDI-TOF MS allows bacterial identification directly from urine in a short time, with high accuracy. Particularly MALDI-TOF MS used together with flow cytometer methods, seems to be a reliable system to obtain urine microbiological results in a timely manner. Direct urine identification and AST could also reduce the use of empirical and/or inappropriate antimicrobials, resulting in cost effectiveness.

Conflict of interest: The authors declare no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by a grant from Marmara University Scientific Research Projects Commission with a grant project number of SAG-A-100713-0294.

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