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Comparative performance analysis of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems for direct identification of urinary tract pathogens from clinical samples

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Ethics Committee Approval
The study was approved by the institutional ethics committee of Erciyes University Faculty of Medicine (approval number 2025/270).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Urinary tract infections (UTIs) are a significant global health concern that necessitates expedited diagnostic methods to guide appropriate antimicrobial treatment and mitigate the spread of multidrug-resistant organisms. This investigation was conducted to assess the effectiveness of two distinct matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) platforms for the direct identification of UTI-causing agents from urine samples: VITEK MS (bioMérieux, France) and MALDI Biotyper Sirius (Bruker Daltonics, Germany). The findings were evaluated by comparison of the outcomes with those of conventional urine culture, which is considered the diagnostic gold standard.
Methods: The study included 60 urine specimens consisting of 40 specimens from patients presenting with UTI symptoms and 20 specimens from a control group that showed no bacterial growth in culture. A differential centrifugation method was employed to prepare the samples, which were subsequently analyzed using both MALDI-TOF MS systems. Concurrently, all samples underwent conventional urine culture.
Results: Among the 40 culture-positive samples, the MALDI Biotyper Sirius system demonstrated an overall identification sensitivity of 69.2% and a specificity of 95.2%. The VITEK MS system showed a sensitivity of 79.5% and a specificity of 95.2%. For the 29 samples with a bacterial concentration of ≥1×105 colony-forming units per milliliter (CFU/mL), the sensitivity was 75.9% for the MALDI Biotyper Sirius system and 79.3% for the VITEK MS system. A statistical evaluation using McNemar's test determined that the difference in sensitivity between the two platforms was not statistically significant (P=0.125).
Conclusion: The findings suggest that both MALDI-TOF MS platforms have considerable promise for the rapid and direct identification of uropathogens, particularly in monomicrobial urine samples with a high bacterial load (≥1×105 CFU/mL). While the VITEK MS system exhibited a marginally higher overall identification sensitivity, the data indicate that both systems can be considered valuable assets in clinical diagnostic laboratories.

Keywords: urinary tract infection, MALDI-TOF MS, VITEK MS, MALDI Biotyper Sirius, uropathogen, rapid diagnosis

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Introduction

Urinary tract infection (UTI) is a frequently occurring bacterial condition that poses a substantial public-health challenge and impacts an estimated 150 million individuals globally each year. The clinical manifestations of UTIs vary widely, ranging from uncomplicated cases of cystitis to severe life-threatening conditions, such as uroseptic shock [1–3]. Uropathogenic *Escherichia coli* is the primary causative agent and accounts for over 80% of community-acquired UTI cases. Other significant pathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, and *Candida* species, of which the latter three are particularly relevant in healthcare-associated infections [3].

Conventional urine culture is the long-established benchmark for identifying urinary pathogens, but it is a labor-intensive and time-consuming process. In a typical microbiology laboratory, pathogen identification can take between 18 and 48 hours, and an additional 18 to 24 hours are required for antimicrobial susceptibility testing [3]. This extensive diagnostic timeline has direct clinical consequences and often leads to the prescription of empirical broad-spectrum antibiotics, which may not be appropriate for the specific pathogen. Over-prescription of such agents can delay effective treatment and, more critically, contribute to the selective pressure and proliferation of multidrug-resistant microorganisms. Consequently, there is an increasing necessity for expedited diagnostic techniques that can provide rapid pathogen identification and enable more timely antimicrobial susceptibility results [2,3].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a standard rapid method for bacterial identification from cultured colonies in many clinical microbiology settings. Furthermore, the technique has promise for direct identification from biological samples, such as urine. The direct analysis of clinical specimens is more challenging due to several inherent constraints. These limitations include the requirement of a sufficient number of microbial cells, an adequate sample volume, and a pre-analytical preparation protocol to address the presence of host cells, proteins, and other interfering biological components [4].

Despite these challenges, numerous studies have demonstrated that MALDI-TOF MS can provide reliable and swift detection of bacterial pathogens directly from urine [4–6]. The present study was designed to specifically evaluate the performance of two prominent commercial MALDI-TOF MS platforms, VITEK MS and MALDI Biotyper Sirius, for the direct identification of urinary tract pathogens from urine samples. The results were directly compared with those of conventional culture methods, which served as a reference standard.

Materials and methods

Study design and ethical considerations

Urine specimens were prospectively gathered from the Bacteriology Unit of the Central Laboratory at Erciyes University Faculty of Medicine. The sample cohort included 40 specimens from inpatients and outpatients with suspected UTI symptoms, as well as 20 control samples that were found to have no microbial growth in culture. For inclusion in the study, samples were required to contain bacteria or yeast and show no indication of polymicrobial growth, which was visually confirmed by Gram staining and microscopic examination. The study protocol was approved by the institutional ethics committee of Erciyes University Faculty of Medicine (approval number 2025/270) and was conducted with strict adherence to the Declaration of Helsinki. A waiver of informed consent was granted by the ethics committee as the study utilized de-identified residual clinical samples, which ensured complete patient privacy and confidentiality.

Sample preparation and identification

A differential centrifugation protocol was employed to prepare the samples for mass spectrometry. First, 4 mL of each urine specimen were initially centrifuged at 2,000 × *g* for 30 seconds to separate host cells and cellular debris such as leukocytes into a pellet. This process led to enrichment with microbial cells with reduced host interference in the supernatant, which was collected and subjected to high-speed centrifugation at 15,500 × *g* for 5 minutes to concentrate the microorganisms into a pellet. The resulting microbial concentrate was then purified by a single wash cycle using deionized water.

For direct and parallel evaluation of the two systems, the isolated microbial pellet from each specimen was divided into two equal portions, and one was used for the VITEK MS system, while the other was used for the MALDI Biotyper Sirius system. This step was crucial to ensure a fair comparison by eliminating any pre-analytical variability between the two platforms. The rationale for the differential centrifugation protocol, particularly the initial low-speed centrifugation step, was to minimize the impact on the MALDI-TOF MS analysis by host cells and debris, such as leukocytes. While we did not perform a separate optimization study, this step is a key component of established protocols in the literature aimed at increasing the ratio of bacterial cells to host cells and improves the quality of the protein spectra and identification scores.

Identification with VITEK MS

A small portion of the prepared pellet was applied as a thin layer onto a VITEK MS target spot (bioMérieux, France) and allowed to air-dry completely. Following the initial drying step, 1 µL of an α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution was placed on top of the sample and left to dry at room temperature. The prepared target spots were then loaded into the VITEK MS instrument for analysis. Quality control and calibration were performed using the standard strain *E. coli* ATCC 8739.

Identification with MALDI Biotyper Sirius

A small quantity from the same microbial pellet was also applied as a thin film onto a MALDI Biotyper Sirius plate (Bruker Daltonics, Germany). After the sample had dried, 1 µL of a 70% formic acid solution was added to facilitate protein extraction and allowed to dry at room temperature. Next, 1 µL of the HCCA matrix solution was added. Once the spots had completely dried, the plates were loaded onto the MALDI Biotyper Sirius instrument for analysis. This system was also calibrated and subjected to quality control using the *E. coli* ATCC 8739 strain.

Table 1: Comparison of culture results and two different MALDI-TOF / MS system results for urinary tract pathogens identified from direct urine samples

No	Age	Gender	Service/Outpatient Clinic	Culture (CFU/mL)	MALDI Biotyper Sirius (Score)	Vitek MS (Score)
1	18	F	Emergency Medicine Outpatient Clinic	*E.coli* 100000	*E.coli* (2.29)	*E.coli* (99.9)
2	38	F	Urology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.29)	*E.coli* (99.9)
3	61	F	Nephrology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.28)	*E.coli* (99.9)
4	56	M	Urology Outpatient Clinic	*P.aeruginosa* 5.000	*M.morganii* (1.82)	Unidentified
5	80	F	Nephrology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.19)	*E.coli* (99.9)
6	9	M	Pediatric Nephrology Unit	*K.pneumoniae* 100000	*K.pneumoniae* (2.17)	*K.pneumoniae* (99.9)
7	1	F	Pediatric Nephrology Outpatient Clinic	*E.coli* 100000	Unidentified	Unidentified
8	65	F	Nephrology Outpatient Clinic	*E.coli* 50000	*E.coli* (1.88)	*E.coli* (99.9)
9	79	F	Oncology Service	*E.coli* 100000	Unidentified	Unidentified
10	84	F	Nephrology Service	*E.coli* 100000	*E.coli* (2.17)	*E.coli* (99.9)
11	55	M	Urology Service	*E.coli* 10000	*E.coli* (2.29)	*E.coli* (99.9)
12	7	F	Pediatric Nephrology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.36)	*E.coli* (99.9)
13	17	F	Pediatric Nephrology Outpatient Clinic	*E.coli* 100000	Unidentified	Unidentified
14	61	F	Nephrology Outpatient Clinic	*K.pneumoniae* 100000	*K.pneumoniae* (2.12)	*K.pneumoniae* (99.9)
15	76	F	Nephrology ICU	*C.albicans* 100000	Unidentified	*C.albicans* (99.9)
16	8	F	Urology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.24)	*E.coli* (99.9)
17	79	F	Oncology Service	*E.coli* 100000	*E.coli* (2.12)	*E.coli* (99.9)
18	81	M	Gastroenterology Service	*C.albicans* 100000	Unidentified	Unidentified
19	12	F	Pediatric Nephrology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.14)	*E.coli* (99.9)
20	64	F	Nephrology Outpatient Clinic	*E.coli* 100000	Unidentified	Unidentified
21	61	M	Internal Medicine ICU	*K.pneumoniae* 100000	*K.pneumoniae* (2.12)	Unidentified
22	66	F	Nephrology Outpatient Clinic	*E.coli* 50000	Unidentified	*E.coli* (99.7)
23	73	F	Gastroenterology Service	*E. faecium* 10000	Unidentified	Unidentified
24	44	F	Endocrinology Service	*E.coli* 100000	*E.coli* (2.20)	*E.coli* (99.9)
25	51	M	Urology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.17)	*E.coli* (99.9)
26	21	M	Infectious Diseases Outpatient Clinic	*E.coli* 100000	*E.coli* (2.26)	*E.coli* (99.9)
27	2	F	General Pediatrics	*P.mirabilis* 100000	*P.mirabilis* (2.21)	*P.mirabilis* (99.9)
28	54	F	Infectious Diseases Outpatient Clinic	*E.coli* 100000	*E.coli* (2.31)	*E.coli* (99.9)
29	29	F	Obstetrics and Gynecology Service	*P.mirabilis* 50000	*P.mirabilis* (2.01)	*P.mirabilis* (99.9)
30	70	F	Nephrology Outpatient Clinic	*E.coli* 1000	*E.coli* (2.26)	*E.coli* (99.9)
31	50	M	Urology Outpatient Clinic	*E.coli* 100000	*E.coli* (2.06)	*E.coli* (99.9)
32	5	M	Urology Service	*K.pneumoniae* 100000	*K.pneumoniae* (2.12)	*K.pneumoniae* (99.9)
33	73	M	Urology Outpatient Clinic	*K.pneumoniae* 100000	*K.pneumoniae* (2.01)	*K.pneumoniae* (93.1)
34	27	F	Infectious Diseases Outpatient Clinic	*E.coli* 5000	*E.coli* (2.35)	*E.coli* (99.9)
35	10	F	Pediatric Hematology and Oncology Service	*E.coli* 100000	*E.coli* (2.11)	*E.coli* (99.9)
36	76	M	Urology Outpatient Clinic	*A. xylosoxidans* 50000	*A. xylosoxidans* (1.91)	*A. xylosoxidans* (99.9)
37	7	M	Pediatric Nephrology Outpatient Clinic	*K.pneumoniae* 100000	Unidentified	*K.pneumoniae* (93.1)
38	73	M	Nephrology Outpatient Clinic	*E.coli* 100000	Unidentified	*E.coli* (99.9)
39	1	M	Pediatric Emergency	*E.faecium* 10000	Unidentified	*E.faecium* (99.9)
40	68	M	Urology Outpatient Clinic	*E.coli* 5000	Unidentified	*C.freundii* (99.5)

Urine culture

All urine specimens were concurrently subjected to conventional urine culture, which served as the reference method for pathogen identification. The reliability of the culture and identification procedures was ensured by using control strains, including *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (American Type Culture Collection, Manassas, VA, USA). The samples were incubated in an aerobic environment containing 5% carbon dioxide at 37°C for 18–24 hours. Any colonies on the plates that exhibited significant growth were then identified using the MALDI Biotyper Sirius system (Bruker Daltonics, Germany).

Statistical analysis

Statistical analyses were performed using the software SPSS Statistics version 20.0 (IBM, Corp., NY, USA). The performance metrics of the diagnostic tests included the sensitivity and specificity values, as well as the corresponding 95% confidence intervals (CIs). The paired diagnostic performance of the two MALDI-TOF MS systems was compared using McNemar's test.

Results

The study population comprised patients with an average age of 45.3 years. The most common bacterial species isolated from the samples was *E. coli*, which was identified in 26 patients. The overall performance of the two MALDI-TOF MS systems for direct pathogen identification was assessed against the conventional culture method. As shown in Table 1, the MALDI Biotyper Sirius system yielded a sensitivity of 69.2% (95% CI: 52.4–83.0%) and a specificity of 95.2% (95% CI: 76.2–99.9%). In contrast, the VITEK MS system demonstrated a sensitivity of 79.5% (95% CI: 63.5–90.7%) and a specificity of 95.2% (95% CI: 76.2–99.9%). The statistical comparison of the overall sensitivities using McNemar's test indicated that the observed difference was not statistically significant (P=0.125).

A subset analysis was conducted on the 29 urine specimens with a bacterial load of ≥1×105 CFU/mL. In this group, the identification sensitivity of the MALDI Biotyper Sirius system was 75.9% (95% CI: 56.4–89.7%), while that of the VITEK MS system was 79.3% (95% CI: 60.3–92.0%).

Inconsistent results were noted in several samples, particularly those with a lower bacterial count. For instance, a sample with 5,000 CFU/mL of *P. aeruginosa* (Sample 4) was not identified by the VITEK MS system, while the MALDI Biotyper Sirius system incorrectly identified it as *Morganella morganii*. Similarly, in a sample with 5,000 CFU/mL of *E. coli* (Sample 40), the MALDI Biotyper Sirius system failed to provide an identification, whereas the VITEK MS system provided an erroneous identification of *Citrobacter freundii*.

The differential centrifugation protocol proved effective and enabled direct identification of the most common UTI pathogens with both systems. While both platforms performed similarly on high-titer samples (≥1×105 CFU/mL), the VITEK MS system demonstrated a marginally higher overall identification sensitivity across the entire sample set. The complete comparative data for all samples are presented in Table 1.

Discussion

The central objective of this study was to perform a direct comparative evaluation of the performance of two widely used commercial MALDI-TOF MS systems, the MALDI Biotyper Sirius and the VITEK MS, for the rapid identification of bacterial pathogens directly from urine samples. Previous research has already established the better performance of MALDI-TOF MS over conventional methods for identifying microorganisms from colonies grown on culture media [7–10], but the present work provides a focused head-to-head comparison of these two major platforms under identical clinical conditions.

Our findings confirm the robust performance of both systems, particularly for the identification of Gram-negative bacteria, which are the most frequent causative agents of UTIs. High sensitivity of 79.3% for the VITEK MS system and 75.9% for the MALDI Biotyper Sirius system was obtained in monomicrobial urine samples with a high bacterial load of ≥1×105 CFU/mL. These results are consistent with the range of 67% to 86.6% reported in the literature for direct urine analysis using this technology [5,11–13]. Our data support the findings of Zboromyrska et al. [4], who reported a similar success rate of 72.8% in a multicenter study, which further supports the clinical utility of this approach. The alignment of our findings with previous research validates the method's potential as a reliable tool for quick UTI diagnosis, particularly for specimens with a high concentration of microorganisms.

This study also highlights several persistent limitations of direct MALDI-TOF MS analysis that have been documented previously. A significant technical constraint is the dependency on a sufficient microbial load. The performance of both systems was noticeably decreased when analyzing samples with low bacterial density. This observation reinforces the findings of other researchers who have proposed that direct identification from urine is most successful when the bacterial concentration exceeds 1×105 CFU/mL [14]. Given these outcomes, the bacterial count of ≥5,000 CFU/mL used in this study could serve as a practical threshold for pre-selecting samples for rapid analysis, as suggested by other investigators [3,4,15]. This approach would not only optimize the method's sensitivity but also streamline laboratory workflows by prioritizing the most promising samples for rapid testing.

Another critical challenge was the difficulty in identifying yeast species. This is consistent with prior research reporting low identification rates for yeasts [6,16]. In our analysis of two urine samples that were culture-positive for *Candida albicans*, only one was correctly identified by the VITEK MS system, and neither was identified by the MALDI Biotyper Sirius system. In such instances, MALDI-TOF MS may provide identification at only the genus level or no result at all, thus providing limited useful information for clinicians. This enduring challenge suggests that the unique protein profiles or cell-wall compositions of yeasts may be less amenable to current direct-identification protocols and highlights the continued necessity of conventional culture methods for the definitive diagnosis of yeast-related UTIs [3].

Conclusions

This study has demonstrated that MALDI-TOF MS using the VITEK MS and MALDI Biotyper Sirius systems provides a valuable and rapid alternative for the direct identification of uropathogens from high-titer urine samples. Both platforms exhibited acceptable sensitivity in monomicrobial samples with a bacterial load of ≥1×105 CFU/mL. While the VITEK MS system showed a slightly higher overall identification performance, the statistical analysis determined that the difference was not significant. From a clinical perspective, this non-significant difference suggests that the selection between these two powerful systems for a laboratory setting would likely be based on practical considerations beyond performance, such as cost per test, ease of integration with existing systems, or the size and scope of their respective protein databases.

The findings affirm the clinical utility of direct MALDI-TOF MS analysis as a powerful tool for accelerating UTI diagnosis, particularly for cases with a high bacterial count. However, the persistent limitations of this method in identifying pathogens in low-density samples and the consistent challenges with yeast species underscore key areas for future research. Future studies should concentrate on optimizing the sample-preparation protocols by exploring advanced enrichment or concentration techniques to enhance sensitivity across all bacterial loads. Furthermore, continued efforts to improve the protein databases and algorithms will be essential to expand the routine use of MALDI-TOF MS for a broader spectrum of microorganisms, which could ultimately improve the speed and accuracy of UTI diagnosis.

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