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ORIGINAL ARTICLE

Direct MALDI-TOF MS-based method for rapid identification of microorganisms and antibiotic susceptibility testing in urine specimens

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ABSTRACT

Background and Objectives: Urinary tract infections (UTIs) are considered as a major public health issue, often causing complications. Although the traditional cultivation approach is reliable in diagnosis, it is time-consuming, leading to delay in treatment and contributing to antibiotic resistance due to suboptimal empirical treatments. This study aimed to evaluate the performance of a direct, rapid identification technique using MALDI-TOF MS for pathogen identification and antibiotic susceptibility testing in UTIs, aiming to reduce diagnostic time compared to standard culture methods.
Materials and Methods: In the span of a year, 458 monomicrobial urine samples were analysed using both the standard bacterial culture method and the direct MALDI-TOF MS-based method. Antibiotic susceptibility was directly tested on 20 samples using the disk diffusion method.
Results: The direct identification technique accurately identified 92.14% of microorganisms at the genus level and 60.92% at the species level within an hour, significantly faster than the 24 to 48 hours required by traditional culture methods. The direct antibiotic susceptibility test results were consistent with the standard post-culture method ranging from 60.00% to 100%.
Conclusion: Direct identification using MALDI-TOF MS can improve UTI management by enabling faster pathogen identification and targeted treatments, potentially reducing antibiotic resistance. Further studies are needed in terms of enhancing its clinical utility and reliability.

Keywords: Matrix-assisted laser desorption-ionization; Urine; Bacteria; Urinary tract infection; Antibiogram

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infections globally (1). In case of improper management, they can lead to serious complications such as recurrent infections, chronic kidney disease, sepsis and permanent kidney damage (2). These infections also have a significant social impact due to the costs associated with hospitalization and treatment (2).

Bacteria are the main cause of urinary tract infections with *Escherichia coli* being the most common agent (3). Urine culture is used to isolate bacterial colonies, which are then identified and tested for antibiotic susceptibility, allowing for optimal treatment to be selected (4). However, this method typically takes two to three days, which is often considered excessive given the speed at which the infection can progress and negatively impact a patient's health. In addition, the use of empirical antibiotic treatment while awaiting definitive results can lead to the development of bacterial antibiotic resistance, increasing the risk not only to the patient but also to others regarding to nosocomial infections (5).

With the advent of MALDI-TOF MS, identification can now be performed in minutes on isolated bacterial colonies after cultivation, reducing the identification time by 24 hours compared to traditional identification based on biochemical tests (6). This advancement is revolutionary, but it still requires culture time. This requirement could be bypassed if the identification by MALDI-TOF MS could be applied directly on urine samples, providing results within an hour following receiving the sample (7).

The aim of this study is to evaluate the performance of a rapid method of identification using MALDI-TOF directly on urine, as well as the performance of a direct technique which allows antibiotic susceptibility testing, in comparison with the standard method. The final objective is to evaluate the possibility of its implementation in routine practice to obtain preliminary results in less than an hour instead of the usual two to three days, which will contribute to better patient management, with targeted treatment, a reduction in hospital stay, and indirectly a reduction in bacterial resistance by reducing empirical treatments.

MATERIALS AND METHODS

Study design. This prospective study was conducted at the Central Laboratory of Bacteriology, Serology and Hygiene of the University Hospital Center IBN SINA of Rabat, from March 1, 2023, to March 1, 2024.

During the study period, 458 urine samples were selected based on the presence of bacteria. The urine samples were sent to the laboratory for routine cytobacteriological examination. Urine with bacteriuria underwent double analysis by means of standard culture, antibiogram method as well as direct identification.

Standard culture method. Based on our standard operating procedures, all received urine samples were cultured on differentiation agar media including, cystine-lactose-electrolyte-deficient (CLED) and blood agar and incubated aerobically at 37°C for 24-48 hours to obtain isolated colonies (4). Bacterial identification was then performed using MALDI-TOF MS, and antibiotic susceptibility testing was performed using the disk diffusion method by preparing a bacterial suspension with a turbidity equivalent to 0.5 McFarland standard, which was inoculated onto Muller Hinton agar and incubated at 37°C for 24 hours, with antibiotics tested as per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (8).

Direct MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) identification. Urine samples with bacteriuria were detected using the URIT1280® Automated Urine Sediment Analyzer. Gram staining was then performed to confirm the presence of a single bacterial species in the urine (9).

For each confirmed positive monomorphic sample, 10 ml of urine was centrifuged for 1 minute at 1000 rpm to sediment the cells and other components present in the urine. The obtained pellet was discarded, and the supernatant was then centrifuged for 5 minutes at 13000 rpm to recover the new bacterial pellet.

The result was washed for the second time with distilled water and subjected to another high-speed centrifugation (13000 rpm for 1 minute). Afterwards, the supernatant was delicately removed and the pellet was collected. Using a 1 µL inoculation loop, a small amount of the resulting pellet was smeared onto an empty position on the MALDI target plate. The material was covered with 1 µL of 70% formic acid and dried at room temperature. Within 30 minutes after drying, the material was overlaid with 1 µL of al-

pha-Cyano-4-hydroxycinnamic acid (HCCA) matrix solution and dried again at room temperature (10).

Protein analysis was performed using the mass spectrometer MALDI-TOF Microflex LT (Bruker-DaltoniK GmbH, Bremen, Germany) with FLexControl v. 3.0 software (Bruker DaltoniK GmbH, Bremen, Germany) (10).

For each identification series, a BTS (Standard Bacterial Test) quality control was performed according to the manufacturer's instructions.

MALDI-TOF identification results were interpreted using the scores suggested by the manufacturer as follows: a score greater than 2 indicates species identification, a score in the range 1.7-1.99 indicates genus identification, and a score <1.7 indicates no identification (10).

Direct antibiotic susceptibility method. To perform the antibiogram susceptibility test directly on urine samples, a suspension with a turbidity equivalent to 0.5 McFarland standard was prepared from the pellet obtained previously by means of differential centrifugation. This suspension was then inoculated on Muller-Hinton agar medium and incubated at 37°C for 24 hours. The main antibiotics used for urinary tract infections (ampicillin, amoxicillin/clavulanic acid, cefixime, ceftriaxone, gentamicin, imipenem, amikacin, nalidixic acid, ciprofloxacin, fosfomycin, nitrofurantoin, cotrimoxazole) were tested as recommended by the EUCAST. Depending on the minimum inhibitory concentration (MIC) results obtained, the bacteria were categorized as "Susceptible" (S), "Intermediate" (I), or "Resistant" (R) (8).

To compare the two methods (standard and direct), the results of the comparison were classified as follows:

- Agreement: Indicates reliability and occurs when the results of both techniques are identical.
- Minor error: Indicates some deviation but is less critical. It occurs when the result for the direct method is "Intermediate," while the results of the standard method being "Susceptible" or "Resistant".
- Major error: Significantly impacts treatment efficacy and occurs when the result for the direct method is "Resistant" while the standard method indicates "Susceptible."
- Very major error: The most critical error, potentially leading to ineffective treatments. It occurs when the result for the direct method is "Susceptible" while the standard method indicates "Resistant" (11).

Ethical considerations. The study adhered to the ethical guidelines outlined in the Helsinki Declaration and did not require formal ethics approval or informed consent. It used anonymized urine samples intended for disposal, focusing on comparing two diagnostic methods without patient intervention or sharing results with treating physicians. The research aimed to improve diagnostic methods while ensuring patient anonymity.

RESULTS

During the study period, 458 urine samples were tested and found to be positive for the presence of bacteria and mono-bacteria in Gram staining. These samples were processed using both the direct method and the culture method. Table 1 summarizes the scored MALDI-TOF results obtained by both methods.

The culture method was superior in identifying microorganisms in urine samples, achieving a 100% identification rate. The direct method exhibited a significant failure rate, particularly in identifying Gram-positive bacteria (GPB).

For Gram-negative bacteria (GNB), the culture method achieved 100% identification rate (87.59% to species level), while the direct method achieved 94.40% identification rate (65.69% to species level) and a 5.60% failure rate (no identification).

For Gram-positive bacteria (GPB), the culture method achieved 100% identification rate (63.64% to species level), while the direct method achieved 72.73% identification rate (18.18% to species level) with a 27.27% failure rate.

For yeasts identified in our study, three Candida albicans species were found, all of which were identified by the culture method (66% to species level). The direct method identified 2 out of 3 (33% to species level).

For antibiotic susceptibility tests, the agreement between the two methods was limited to the two bacteria most implicated in urinary tract infections. The direct antibiotic susceptibility method was applied to 20 bacteria (15 *Escherichia coli* and 5 *Klebsiella pneumoniae*). The results of consistency between the two methods are indicated in (Tables 2 and 3).

DISCUSSION

The culture method achieved a 100% identification

Table 1. Score-ranked MALDI-TOF results obtained by culture and direct methods

Identified Micro-organisms	Standard MALDI-TOF method			Direct MALDI-TOF method		
	≥2.0 Species ID	1.7-1.99 Genus ID	<1.7 No ID	≥2.0 Species ID	1.7-1.99 Genus ID	<1.7 No ID
Escherichia coli	220	16	0	179	46	11
Klebsiella pneumoniae	52	14	0	38	23	5
Klebsiella oxytoca	16	3	0	10	8	1
Pseudomonas aeruginosa	15	4	0	12	6	1
Proteus mirabilis	14	2	0	8	7	1
Enterobacter cloacae	13	5	0	9	8	1
Acinetobacter baumannii	9	2	0	6	5	0
Morganella morganii	7	2	0	4	4	1
Citrobacter koseri	4	1	0	1	3	1
Citrobacter freundii	3	1	0	0	3	1
Acinetobacter pittii	2	1	0	1	2	0
Serratia marcescens	2	0	0	1	1	0
Providencia stuartii	2	0	0	1	1	0
Providencia rettregi	1	0	0	0	1	0
TOTAL GNB (89,74%)	360 (87.59%)	51 (12.41%)	0 (0.00%)	270 (65.69%)	118 (28.71%)	23 (5.60%)
Enterococcus faecium	8	4	0	3	6	3
Enterococcus faecalis	6	5	0	2	6	3
Staphylococcus aureus	5	2	0	2	4	1
Staphylococcus hominis	5	2	0	1	5	1
Staphylococcus haemolyticus	3	2	0	0	3	2
Streptococcus agalactiae	1	1	0	0	0	2
TOTAL GPB (9,60%)	28 (63.64%)	16 (36.36%)	0 (0.00%)	8 (18.18%)	24 (54.55%)	12 (27.27%)
Candida albicans	2	1	0	1	1	1
TOTAL MICRORORGANISMS: 458	390 (85.15%)	68 (14.85%)	0 (0.00%)	279 (60.92%)	143 (31.22%)	36 (7.86%)

rate. This high accuracy ensures reliable and definitive diagnostic information, however, it is time-consuming. the culture method can take up to 48 hours to yield results, which can be critical in acute or severe infections that require immediate treatment (12).

The direct technique, which identifies bacteria in urine specimens in less than an hour, allows early treatment by initiating targeted antibiotic therapy, which can improve patient outcomes and reduce the risk of complications. However, it has a lower overall identification rate than the culture method, with an identification rate of 92.14% and a failure rate of 7.86%.

The direct method also performs less effectively on Gram-positive bacteria (GBP), with a 72.73% identification rate and a 27.27% failure rate, which can be explained by the thicker peptidoglycan layer of Gram-positive bacteria, which limits effective cell lysis and protein extraction during the direct method (13). This structural barrier leads to insufficient generation of characteristic spectral peaks, reducing the accuracy of identification. Furthermore, the lower abundance of detectable proteins in Gram-positive bacteria and their less distinctive spectral profiles contribute to the increased difficulty in achieving reliable identification (14).

The results for yeast are based on a limited sample size. Inclusion of more samples in future studies would provide a more comprehensive understanding and ensure the relevance of the interpreted data.

Regarding the antibiotic susceptibility, the results were similar to other studies and indicated that the direct method is a promising tool for rapid antibiotic susceptibility testing with generally high accuracy (15, 16).

Regarding *Escherichia coli*, the overall agreement was generally high, ranging from 73.33% to 100%. Minor errors were present but relatively low, with a

Table 2. Results of *Escherichia coli* antibiotic susceptibility tests consistency between the two methods.

Antibiotic	Agreement (%)	Minor Error (%)	Major Error (%)	Very Major Error (%)
Ampicillin	93.33	0.00	6.67	0.00
Amoxicillin/Clavulanic Acid	93.33	6.67	0.00	0.00
Cefixime	100.00	0.00	0.00	0.00
Ceftriaxone	80.00	6.67	13.33	0.00
Gentamicin	93.33	0.00	6.67	0.00
Imipenem	93.33	6.67	0.00	0.00
Amikacin	86.67	6.67	6.67	0.00
Nalidixic Acid	93.33	6.67	0.00	0.00
Ciprofloxacin	93.33	0.00	6.67	0.00
Fosfomycin	73.33	6.67	20.00	0.00
Nitrofurantoin	93.33	0.00	6.67	0.00
Cotrimoxazole	100.00	0.00	0.00	0.00

Table 3. Results of *Klebsiella pneumoniae* antibiotic susceptibility tests consistency between the two methods.

Antibiotic	Agreement (%)	Minor Error (%)	Major Error (%)	Very Major Error (%)
Ampicillin	100.00	0.00	0.00	0.00
Amoxicillin/Clavulanic Acid	100.00	0.00	0.00	0.00
Cefixime	100.00	0.00	0.00	0.00
Ceftriaxone	80.00	0.00	20.00	0.00
Gentamicin	100.00	0.00	0.00	0.00
Imipenem	80.00	0.00	20.00	0.00
Amikacin	100.00	0.00	0.00	0.00
Nalidixic Acid	100.00	0.00	0.00	0.00
Ciprofloxacin	100.00	0.00	0.00	0.00
Fosfomycin	60.00	0.00	40.00	0.00
Nitrofurantoin	100.00	0.00	0.00	0.00
Cotrimoxazole	80.00	20.00	0.00	0.00

maximum of 6.67% for several antibiotics. Major errors were notable for ceftriaxone (13.33%) and fosfomycin (20.00%), indicating occasional incorrect resistance classification, and not very major errors were detected, indicating no misclassification of resistant bacteria as susceptible.

For *Klebsiella pneumoniae*, the overall agreement ranged from 60.00% to 100%. Minor errors were minimal, with only cotrimoxazole showing a 20.00% minor error rate. Major errors were significant for ceftriaxone (20.00%), imipenem (20.00%), and fosfomycin (40.00%). Very major errors were not detected, indicating no misclassification of resistant bacteria as susceptible.

It is important to note that interpretation of these results must take into account of the limited number of bacteria tested for antibiotic susceptibility in this study due to financial limitations and the unavailability of reagents.

These results were consistent with similar studies which had tested the performance of other identification techniques using MALDI-TOF directly from samples without the need for a culture step (17, 18). Some of these techniques involve the use of different lysis solutions, prefiltration steps, or short incubation cultures (19, 20). The direct method proposed in this study, in addition to giving satisfactory results, has the advantage of being simple and inexpensive.

It also requires no additional reagents beyond those already routinely used for standard identification by MALDI-TOF.

We recommend improving the direct method to reduce non-identification rates and to increase species-level identification accuracy by using lysis solutions (saponin, sodium dodecyl sulfate…) prior to the MALDI-TOF MS identification step. For antibiotics with higher error rates, careful consideration and possible confirmatory testing is recommended to ensure effective treatment decisions.

Given the strengths and weaknesses of each method, a combined approach may be advantageous. Initial screening with the direct method allows rapid identification of potential pathogens and initiation of empiric treatment, especially in emergency or acute care settings. This approach leverages the speed advantage of the direct method to ensure timely clinical intervention. Confirmation with the culture method follows to confirm the initial findings and provide definitive identification, resolving any discrepancies or missed identifications by the direct method and improving overall diagnostic accuracy.

CONCLUSION

The direct method has limitations, including errors in antibiotic susceptibility testing and lower accuracy for certain bacteria, particularly Gram-positive bacteria (BGP). These issues necessitate further refinement and confirmatory testing to ensure reliable results, highlighting the continued importance of the gold standard culture method for comprehensive diagnostics. However, the direct method offers significant benefits with its rapid turnaround, providing results approximately 24 hours faster than traditional culture methods. This speed enables earlier decisions on antibiotic therapy, enhances treatment efficacy, and reduces illness duration. Integrating the direct method's efficiency with the culture method's accuracy can provide a balanced and effective approach to patient care.

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