



# Direct Identification of Pathogens in Urine by Use of a Specific Matrix-Assisted Laser Desorption Ionization–Time of Flight Spectrum Database

Lucile Pinault,<sup>a,b</sup> Eric Chabrière,<sup>a,b</sup> Didier Raoult,<sup>a,b</sup>  Florence Fenollar<sup>b,c</sup>

<sup>a</sup>IRD, AP-HM, MEΦI, Aix-Marseille Université, Marseille, France

<sup>b</sup>IHU-Méditerranée Infection, Marseille, France

<sup>c</sup>IRD, AP-HM, SSA, VITROME, Aix-Marseille Université, Marseille, France

**ABSTRACT** Urinary tract infections are among the most common reasons for anti-microbial treatment, and early diagnosis could have a significant impact by enabling rapid administration of the adapted antibiotic and preventing complications. The current delay between sample receipt and pathogen identification is about 24 to 48 h, which could be significantly shortened by use of an accurate direct method. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is already used for the identification of pathogens in clinical laboratories and constitutes a promising tool for direct diagnosis. A simple preparation protocol was established for the processing of urine samples prior to MS analysis. MALDI-TOF spectra collected directly from 1,000 infected urine samples were used to create a specific reference database (named Urinf). A prospective study was then carried out to evaluate the Urinf database and compare the results obtained with the standard database provided by Bruker on the Biotyper Real Time Classification software. Seven hundred eighty urine specimens were processed and analyzed according to our method. Among them, almost 90% of 500 infected monobacterial samples could be correctly diagnosed with the Urinf database, compared to 50% using the standard database. The identification of *Enterobacteriaceae*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Enterococcus faecium* was greatly improved but not for *Staphylococcus epidermidis*. The creation of a database adapted to a particular type of clinical sample has great potential to increase both the rate and rapidity of pathogen identification. Sensitivity still remains to be improved for bacterial species that exhibit few specific peaks on mass spectra.

**KEYWORDS** bacteriological urine examination, MALDI-TOF, cystitis, diagnosis, urinary tract infection

Urine specimens are among the most processed samples in clinical laboratories. For instance, our laboratory of bacteriology (Marseille University Hospitals, France) receives approximately 62,050 urine samples per year, which represents about 17% of all biological samples analyzed. As a matter of fact, urinary tract infections (UTIs) are prevalent among bacterial infections (1). Rapid identification of pathogens responsible for UTIs has a significant impact, as it can avoid potentially fatal complications, such as septic shock (2). With the currently established routine methods, 24 to 48 h are required to correctly identify a pathogen present in a urine sample (3). This delay is due to the required culture step and often leads to unnecessary broad-spectrum treatment with an impact on microbiota and bacterial resistance (4). Reducing the delay between receipt of the sample and diagnosis could prevent these problems by allowing faster administration of the appropriate medication.

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Address correspondence to Florence Fenollar, [florence.fenollar@univ-amu.fr](mailto:florence.fenollar@univ-amu.fr).

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In recent years, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) mass spectrometry (MS) has emerged as a key tool in pathogen identification methods used in clinical laboratories (5). Although this is a revolutionary microbiological technique that allows for robust and rapid identification of bacteria, standard protocols do not have the need to culture the bacteria present in the sample. Indeed, they involve colony picking, and then MS analysis is performed. The generated spectra are compared to a database provided with the software, and the pathogen can be correctly identified in most cases, based on a score calculated between the most similar profiles.

Recent studies have demonstrated that MALDI-TOF MS has great potential to be part of a direct identification protocol performed on urine (6–10). However, we noted that the majority of the direct analysis protocols developed require a large volume of urine sample and a minimum of  $10^5$  CFU/ml in order to correctly identify the pathogen. It is not always possible to collect 10 ml or more of urine, especially from infants who are commonly affected with UTIs when diagnosed with a bacterial infection (11).

Therefore, we aimed to develop a direct analysis method that could provide rapid identification of a pathogen even with a small amount of sample (1 ml) and low bacterial counts. We realized that the use of the available commercial database generated from isolated colonies is not the most appropriate option for identifying microorganisms in a more complex environment such as urine. In this study, we present a direct method for identifying pathogens in urine using a specific MALDI-TOF spectrum database entirely created from infected urine specimens.

## MATERIALS AND METHODS

**Urine sample collection.** Urine samples from patients with suspected UTI were collected at Marseille Public University Hospitals in France. In accordance with hospital procedures, urine specimens were transported to the core laboratory of bacteriology (Timone University Hospital, Marseille, France) in sterile containers (BD Vacutainer C&S boric acid kit and sodium borate/formate urine tubes; Becton, Dickinson, Franklin Lakes, NJ, USA). For pediatric patients, the samples were transported either in sterile containers with boric acid or in simple plastic sterile containers without boric acid. All aspects of the study were approved by the local ethics committee (agreement no. 2017-026). Overall, a total of 1,780 urine specimens were tested for this study. A urine sample was considered positive if at least one uropathogen could be clearly identified after a culture step with a colony count of at least  $10^4$  CFU/ml and mass spectrometry analysis. One thousand samples diagnosed as positive with a monobacterial infection were used to help create the Urinf database. We then evaluated our database using 500 samples reported as positive and monobacterial, 30 samples reported as positive and infected by 2 identified bacteria, and 250 samples reported as negative (including 170 “sterile” samples and 80 “nonsignificant microbial growth” samples).

**Routine urine sample processing for bacterial isolation and identification.** The urine samples that were selected for our study and that allowed the creation of our new database were all collected at the core laboratory of bacteriology. When received, each urine sample was analyzed with a Sysmex UF-500i flow cytometry system (bioMérieux, Marcy-l’Etoile, France) that allows the classification and enumeration of particles such as leukocytes or red blood cells. Samples were automatically inoculated on Columbia with 5% sheep blood (COS) agar plates (bioMérieux) with a Previ Isola system (bioMérieux) and then incubated at 37°C for at least 24 h, according to our standard urine culture protocol.

Colonies grown on plates were counted to determine the number of CFU. Bacteria were identified by MALDI-TOF MS on a microflex LT instrument (Bruker Daltonik GmbH, Bremen, Germany) using the Biotyper Real Time Classification 3.0 software and the MBT-BDAL-5627 MSP library (Bruker Daltonik).

**Optimized direct identification protocol for bacterial identification.** Each urine sample (1 ml) was vigorously mixed with 300  $\mu$ l of a 5% saponin solution (Sigma-Aldrich, Saint-Quentin Fallavier, France) and centrifuged at  $11,000 \times g$  for 5 min. The supernatant was discarded, and 500  $\mu$ l of a 10% trifluoroacetic acid solution (Sigma-Aldrich) was added to the resulting pellet. After vortexing, a new centrifugation step at  $11,000 \times g$  was performed for 5 min. The resulting pellet was washed with 1 ml high-performance liquid chromatography (HPLC)-grade water (VWR, Fontenay-sous-Bois, France) and centrifuged at  $11,000 \times g$  for 5 min. The supernatant was carefully removed, and 40  $\mu$ l of 50% HPLC-grade acetonitrile (VWR) as well as a microspatula covered with micro-glass beads ( $\leq 106 \mu$ m; Sigma-Aldrich) were added to the pellet. Several weighings were carried out to evaluate the quantity of glass beads for each sample, which was approximately 70 mg. Cells present were disrupted by lysis cycles on a FastPrep-24 instrument (MP Biomedicals, Santa Ana, CA, USA), which has been used as an alternative to the formic acid (FA) extraction generally recommended by the manufacturers after an accident involving FA and causing severe burns took place in our diagnostic laboratory (12). The obtained mixture was centrifuged at  $11,000 \times g$  for 5 min. Finally, 1  $\mu$ l of the supernatant was spotted in duplicate onto an MSP 96 target polished steel plate (Bruker Daltonik GmbH) and left to dry. Each dry spot was then covered with 1  $\mu$ l of the saturated  $\alpha$ -cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution (HCCA in

50% acetonitrile and 2.5% trifluoroacetic acid; Sigma-Aldrich). Protein analysis was performed with a microflex LT system (Bruker Daltonik GmbH) in the linear positive mode (electrode IS1, 20,00 kV; electrode IS2, 18,05 kV; focalization electrode, 6 kV; laser frequency, 60 Hz; detector gain, 8.8 $\times$ , post-ion-extraction [PIE], 120 ns;  $m/z$  range, 2,000 to 20,000 Da). Species identification was regarded as reliable when scores of  $\geq 1.9$  were obtained with 2 different spots from a single biological replicate.

**Creation and incrementing of the specific urine spectrum database (Urinf database).** Immediately after going through the routine urine sample processing, all samples were stored at 4°C until culture results were available (24 to 48 h), as we had previously verified on 100 samples that this storage step did not affect identification results. Positive and monobacterial urine samples were selected for the creation of the urine spectrum database and submitted to our direct identification protocol. All spectra obtained from infected urine samples were evaluated; if the number of peaks and resolution were satisfactory, they were added to the database that we named the Urinf database. Each created reference is an average of 2 spectra collected from 2 deposits of a biological replicate and is named according to the bacterial species found and validated by the routine processing of urine sample at the core laboratory of bacteriology. The Biotyper 3.1 software was used to increment the database with the Biotyper MSP creation standard method.

For the Urinf database, spectral evaluation and processing were performed within the  $m/z$  4,000 to 16,000 Da range to virtually eliminate the  $\alpha$ -defensins triplet of intense peaks at  $m/z$  values of 3,371.0 Da, 3,442.5 Da, and 3,486.5 Da (13). Their presence constitutes an issue when confronting urine spectra to the Bruker library because those peaks do not appear in the standard spectra of isolated bacteria. It results in lowering the identification scores as they are in the range considered for their calculation. The Urinf database was incremented up to 1,000 references created from 1,000 different urine samples (Table 1). Our database contained a total of 47 different bacterial species, and the number of available references for each species depended on the number of cases observed throughout the study and the quality of the spectra obtained.

**Statistical analysis.** The Epi Info version 7 program (<http://www.cdc.gov/epiinfo/index.html>) was used for statistical analyses. A difference was considered statistically significant when  $P$  values were  $< 0.05$ .

## RESULTS

**Difference between empirical spectra and the commercial database.** Urine samples from our core laboratory of bacteriology underwent the developed preparation protocol for protein extraction and direct analysis by MALDI-TOF MS using the Biotyper Real Time Classification software. Although obtained spectra exhibited satisfactory numbers of peaks and resolution, poor identification scores were observed in some cases when matching with the Bruker database provided with the Biotyper software (data not shown). Our attention was then drawn to the fact that when looking at the same bacterial species, the spectra directly obtained from infected urine samples are drastically different from those obtained from isolated bacterial colonies on solid culture medium (Fig. 1). The disparity in terms of bacterial environment accounts for this observation and partially explains the lower identification scores obtained, as the standard Bruker database is generated from single-colony analyses. Additionally, the standard processing  $m/z$  range (3,000 to 15,000 Da) includes intense peaks of  $\alpha$ -defensins, abundant peptides involved in the host defense against microbial infections, that appear for urine spectra in the lower molecular weights ( $m/z < 4,000$  Da) (13, 14). The identification score is considerably reduced due to these peaks in a comparison of spectra with Bruker's standard database.

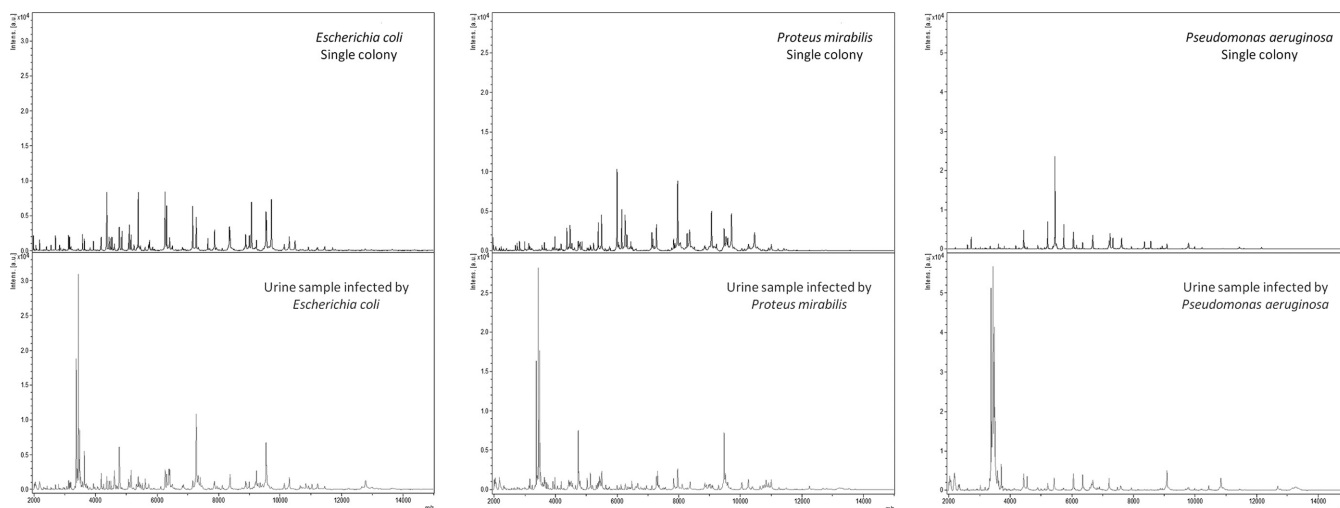
Based on these results, it was decided to create a specific database only with spectra obtained directly from urine samples, without a bacterial growth step, and to perform spectral processing over a narrower range of  $m/z$  values. The Urinf spectrum database contained 1,000 references created from 1,000 different infected urine samples.

**Evaluation of the Urinf database and comparison with the standard Bruker database.** Urine specimens from 500 new patients were selected, prepared, and analyzed according to the criteria and protocol detailed above. All the spectra obtained were confronted to both the standard Bruker database and the Urinf database. When we started evaluating the Urinf database, we observed that identification scores were either equal or greater than 1.9 with only correct identifications or lower than 1.4. Therefore, we established the cutoff score at 1.9 instead of the standard 2.0 for our study with both databases. Species identification was regarded as reliable when scores of  $\geq 1.9$  were obtained with 2 different spectra obtained with 2 different spots from a single biological replicate. The data collected were sorted according to the colony count determined on culture plates for all processed samples (Table 2). Our results were

**TABLE 1** Number of references incremented in the Urinf database for each bacterial species

Bacterium	No. of references
<i>Escherichia coli</i>	155
<i>Proteus mirabilis</i>	95
<i>Enterococcus faecalis</i>	85
<i>Klebsiella pneumoniae</i>	80
<i>Enterobacter cloacae</i>	80
<i>Pseudomonas aeruginosa</i>	76
<i>Staphylococcus aureus</i>	44
<i>Citrobacter koseri</i>	44
<i>Streptococcus agalactiae</i>	43
<i>Staphylococcus saprophyticus</i>	37
<i>Enterobacter aerogenes</i>	36
<i>Klebsiella oxytoca</i>	31
<i>Staphylococcus epidermidis</i>	30
<i>Enterococcus faecium</i>	29
<i>Morganella morganii</i>	24
<i>Citrobacter freundii</i>	20
<i>Serratia marcescens</i>	15
<i>Gardnerella vaginalis</i>	6
<i>Proteus vulgaris</i>	5
<i>Stenotrophomonas maltophilia</i>	4
<i>Streptococcus oralis</i>	4
<i>Hafnia alvei</i>	4
<i>Providencia stuartii</i>	4
<i>Acinetobacter pittii</i>	4
<i>Staphylococcus haemolyticus</i>	4
<i>Pseudomonas putida</i>	3
<i>Staphylococcus hominis</i>	3
<i>Enterobacter kobei</i>	3
<i>Raoultella ornithinolytica</i>	3
<i>Citrobacter amalonaticus</i>	3
<i>Aerococcus urinae</i>	3
<i>Acinetobacter baumannii</i>	3
<i>Enterococcus gallinarum</i>	2
<i>Staphylococcus capitis</i>	2
<i>Enterobacter asburiae</i>	2
<i>Streptococcus anginosus</i>	2
<i>Streptococcus mitis</i>	2
<i>Streptococcus pneumoniae</i>	1
<i>Acinetobacter nosocomialis</i>	1
<i>Providencia rettgeri</i>	1
<i>Corynebacterium amycolatum</i>	1
<i>Corynebacterium striatum</i>	1
<i>Corynebacterium tuberculostearicum</i>	1
<i>Pseudomonas plecoglossicida</i>	1
<i>Enterobacter ludwigii</i>	1
<i>Citrobacter braakii</i>	1
<i>Pseudomonas mosselii</i>	1

concordant with the literature, as we observed that our method was more effective with bacterial counts greater than  $10^5$  CFU/ml (92% identification with the Urinf database for bacterial counts of  $10^6$  CFU/ml or more), which is the case for most patients suffering from a UTI. When using the standard Bruker database, we obtained 26% of correct identifications for bacterial counts of  $10^4$  CFU/ml. However, for those same samples, an identification rate of 74% was achieved when matching spectra with the specific Urinf database ( $P = 0.001$ ). Overall, we observed that the use of the Urinf database enabled a significant improvement in identification scores (50% correct identifications with the Bruker database and almost 90% with the Urinf database;  $P < 0.0001$ ). A total of 19 different bacterial species could be found in the 500 infected urine specimens selected for evaluation of the Urinf database (Table 3). Infections caused by *Escherichia coli*, which represented more than 40% of studied cases, were correctly diagnosed with a 94% rate, versus 63% when using the standard database



**FIG 1** Comparison between MALDI-TOF MS spectra generated from bacterial colonies isolated from urine samples and spectra obtained from direct analysis of those same infected urine specimens.

( $P < 0.0001$ ). None of the *Proteus vulgaris* (1 case), *Staphylococcus saprophyticus* (6 cases), *Serratia marcescens* (4 cases), or *Streptococcus agalactiae* (10 cases) UTIs could be detected with the standard database, but 100%, 83%, 75%, and 40% of those samples were respectively identified using the Urinf database. In a comparison of the correct identifications of each bacterial species between the two databases, the results obtained were statistically significant ( $P < 0.05$ ) with the Urinf database for a vast majority of pathogens (96% patients of the study). For the remaining bacteria,  $P$  values  $>0.05$  could be explained by the small number of infected samples available (between 1 and 7 cases for each of those species in the study). The specimens infected with *Staphylococcus epidermidis* were the only ones that could not be diagnosed with our direct analysis protocol and database. Some samples reported as not infected or containing more than one microorganism were also analyzed to verify the robustness of our method. Indeed, 170 samples diagnosed as sterile and 80 specimens recorded as no significant microbial growth were confronted to the Urinf database and provided no positive result. Moreover, 30 specimens with 2 identified pathogens were processed and compared to the Urinf database. For 80% of them, one of the bacteria responsible for the infection was correctly identified and was the most prevalent in 1/3 of cases (for 1/2 of those specimens with a correct identification, both pathogens were estimated to be equally present in terms of CFU per milliliter).

Finally, the potential impact of external factors on identification results was taken into account. Indeed, 50 samples taken from the prospective study were submitted to the preparation protocol by two different operators and analyzed on two different microflex LT systems. A comparison between those results revealed that our method was neither operator dependent nor machine dependent. Moreover, leukocytes and red blood cell counts of the specimens studied were examined to determine if high

**TABLE 2** Evaluation of Urinf database based on percentage and number of correct identifications by colony count

Colony count (CFU/ml)	No. of cases	% identification (no. of identified cases) in:		P value
		Bruker database	Urinf database	
$10^4$	23	26 (6)	74 (17)	0.001
$10^5$	118	30 (35)	73 (86)	$<0.0001$
$\geq 10^6$	359	58 (207)	92 (330)	$<0.0001$
Total	500	50 (248)	87 (433)	$<0.0001$

counts had an impact on the identification rates obtained, but no clear trend could be determined.

Based on all those combined results, we evaluated that our direct analysis protocol followed by comparison to the specific Urinf database had a positive predictive value (PPV) of 1 (457 out of 550 infected samples of the prospective study were detected as positive, and no false positives were observed) and a negative predictive value (NPV) of 0.8 (all 250 sterile and nonsignificant microbial growth specimens were determined to be negative, and 73 out of 550 infected samples were false negatives). The false negatives were all due to low-quality spectra, and no misidentification could be observed. Those numbers make it a reliable test for the rapid diagnosis of an infected sample. It was also noted that our method was more efficient for the identification of Gram-negative bacteria (90% correct identifications) than for Gram-positive bacteria (72% correct identifications).

## DISCUSSION

In this study, we sought to develop a direct analytical protocol that could be useful even for urine samples with low bacterial counts or available in small quantities and capable of detecting a wide variety of uropathogens without prior culture. When the first MALDI-TOF data were collected after submitting urine specimens to our preparation for direct analysis, identification scores obtained with the Biotyper software and the standard database were not satisfactory. For bacterial species such as *Streptococcus agalactiae*, *Staphylococcus saprophyticus*, *Serratia marcescens*, and *Proteus vulgaris*, no identification could even be achieved. While we did not change the acquisition parameters recommended by Bruker (2,000 to 20,000 Da), we tried comparing our collected spectra to the Bruker library only in the range 4,000 to 16,000 Da instead of the standard 3,000 to 15,000 Da. The objective was to eliminate the  $\alpha$ -defensin issue, but the identification results were modestly improved. We observed significant differences between spectra obtained with cultured colonies and spectra obtained with bacteria directly recovered from urine samples, which made us realize that using the standard database was not pertinent in our developed protocol. We therefore decided to create a specific database composed only of spectra generated after direct analysis of urine specimens. This choice proved to be more relevant since the identification

**TABLE 3** Evaluation of Urinf database based on percentage and number of correct identifications by bacterial species

Bacterium or bacterial group	No. of cases	% identification (no. of identified cases) in:		P value
		Bruker database	Urinf database	
<i>Escherichia coli</i>	208	63 (131)	94 (195)	<0.0001
<i>Klebsiella pneumoniae</i>	82	50 (41)	91 (75)	<0.0001
<i>Enterococcus faecalis</i>	45	51 (23)	84 (38)	0.0007
<i>Proteus mirabilis</i>	30	30 (9)	70 (21)	0.002
<i>Pseudomonas aeruginosa</i>	20	10 (2)	70 (14)	0.0002
<i>Enterobacter cloacae</i>	18	44 (8)	78 (14)	0.04
<i>Enterobacter aerogenes</i>	16	50 (8)	94 (15)	0.008
<i>Citrobacter koseri</i>	13	54 (7)	100 (13)	0.007
<i>Staphylococcus aureus</i>	12	25 (3)	75 (9)	0.02
<i>Klebsiella oxytoca</i>	12	50 (6)	92 (11)	0.03
<i>Streptococcus agalactiae</i>	10	0 (0)	40 (4)	0.04
<i>Staphylococcus epidermidis</i>	7	0 (0)	0 (0)	1
<i>Enterococcus faecium</i>	7	43 (3)	100 (7)	0.03
<i>Staphylococcus saprophyticus</i>	6	0 (0)	83 (5)	0.008
<i>Morganella morganii</i>	5	80 (4)	80 (4)	1
<i>Serratia marcescens</i>	4	0 (0)	75 (3)	0.07
<i>Citrobacter freundii</i>	3	67 (2)	100 (3)	0.5
<i>Raoultella ornithinolytica</i>	1	100 (1)	100 (1)	1
<i>Proteus vulgaris</i>	1	0 (0)	100 (1)	0.5
All Gram-negative bacteria	413	53 (219)	90 (370)	<0.0001
All Gram-positive bacteria	87	33 (29)	72 (63)	<0.0001
Total	500	50 (248)	87 (433)	<0.0001



scores obtained were significantly improved with use of the Urinf database. Some of the recently published protocols require tedious prehandling, such as a preincubation step (15, 16), dual filtration (16), or diafiltration (17), that would be difficult to implement in a clinical laboratory because of the substantial amount of incoming samples. It was estimated that only 40 to 60 min was necessary to carry out our entire procedure on up to 20 samples and to perform bacterial identification. Evidently, this number could be broadly increased by processing more samples at the same time when implementing this method in a clinical laboratory. Performing Gram stain on a similar number of specimens would require approximately the same amount of time, between slide preparation, fixing, coloration, observation, and interpretation. Therefore, our developed method could provide a feasible substitute and has the significant advantage of allowing the diagnosis of the species of uropathogen responsible for UTI, while Gram staining only allows the classification of bacteria into the Gram-negative or Gram-positive group.

Moreover, several studies also recommend a preselection of samples depending on a minimum bacterial load (13, 18–21), which does not always meet clinical recommendations (22), and are performed on a limited diversity of microorganisms (17). The use of our specific Urinf database, which currently contains spectra of 47 different microorganisms, enables the identification of a large variety of pathogens that can even be broadened by the addition of new references. Additionally, an identification rate of 74% was achieved for patients with lower-level bacteriuria ( $<10^5$  CFU/ml). In order to verify the reproducibility of our method, some of the samples used for the evaluation of the Urinf database were pretreated by different operators and analyzed on two microflex LT systems. Based on those results (data not shown), we determined that the elaborated procedure was neither operator dependent nor machine dependent. Furthermore, the analysis of noninfected samples gave no false-positive result and confirmed that we developed a reliable detection method.

The most common bacteria found in UTIs are *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, and *Enterococcus* spp., which are involved in 80 to 95% of both complicated and uncomplicated UTIs (1, 23, 24). With our developed method and based on the identification rates obtained, infections caused by those predominant uropathogens could be correctly diagnosed with an identification rate of about 92%. Therefore, our direct analysis protocol is appropriate for the detection of prevalent microorganisms in community-acquired UTIs.

It is important to note that results reported as incorrect were all due to low-quality spectra and that no misidentification could be observed. We examined red blood cell and leukocyte counts in infected specimens that could not be diagnosed with our protocol, as it was previously reported that they could alter the quality of generated spectra when present in large amounts (19). However, the proportion of patients with a high number of blood cells was roughly the same in a comparison of correctly identified and nondetected specimens, which suggests that they are not correlated with unsatisfactory identification scores. We also compared the proportion with high bacterial load ( $>10^5$  CFU/ml) in cases with positive or negative test results. This led us to the observation that 57% of infected patients that could not be diagnosed exhibited bacterial counts of  $10^5$  CFU/ml or less, versus 28% for patients who were correctly diagnosed, which could partially account for low identification rates.

Sensitivity remains an issue for bacterial species such as *Staphylococcus epidermidis* that could not be detected. *Staphylococcus epidermidis* infections are nosocomial and mostly affect catheterized patients (25). Our result could be explained by the spectra obtained for specimens infected with that pathogen. Indeed, only a few specific peaks could be found in the processing range of  $m/z$  4,000 to 16,000 Da, which can account for the low matching scores obtained in a comparison to the Urinf database. Incrementing the Urinf database with more *Staphylococcus epidermidis* references or developing a bioinformatics tool to improve spectrum processing could help solve this issue.

In conclusion, our developed screening method enables the rapid identification of uropathogenic bacteria. As a first step for incorporating this technology into a clinical

laboratory, our protocol could be implemented for specific patient populations with a need for rapid diagnosis, such as kidney transplant patients or pediatric newborns. Besides, a combined procedure could be created by coupling it with a direct antibiotic susceptibility test (AST), as suggested by Zboromyrska et al. (26), as soon as a positive result is reported. Possibilities of incrementation of the Urinf database with new references are virtually infinite and could continuously improve sensitivity and identification scores.

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D.R. and F.F. conceived and designed the study. L.P., E.C., and F.F. analyzed and interpreted data. All authors drafted the manuscript and/or worked on critical revisions, and all authors read and approved the final manuscript.

The preparation protocol and the Urinf database are the objects of a deposited patent (Publication number EP3212801) for which all authors are listed as inventors.

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