

Original Research Article

Identification of bacterial urinary tract pathogens from urine samples using conventional methods and matrix-assisted laser desorption ionization-time of flight mass spectrometry

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ABSTRACT

Background: Early diagnosis of urinary tract infections (UTIs) is essential to avoid inadequate or unnecessary empirical antibiotic therapy. In this study, we evaluate the coincidence rate between conventional method for the diagnosis of UTIs (plate cultures and identification based on biochemical characteristics) and a fast method based on Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). In recent years, proteomic techniques have achieved a relevant role in the identification of microorganisms in the field of clinical microbiology. MALDI-TOF MS has been suggested as a fast and reliable method for bacterial identification.

Methods: Around 50 midstream urine samples submitted to Microbiology laboratory for Gram staining and bacterial culture were analyzed. Samples were microscopically tested, characterized, and identified using different media such as blood agar and Mac Conkey agar and by applying suitable biochemical tests. Urine specimens showing a significant bacteriuria on culture and single morphological type by Gram staining were then processed by MALDI-TOF MS.

Results: Of 50 specimens, colony growth was observed in 43(86%) specimens, and 38(88.3%) specimens had growth of single-colony morphological type in culture. 32(84.2%) of them had colony counts of $>10^5$ colony forming units (CFUs)/ml. 7(14%) samples were negative in culture, and all of them were also negative by MALDI-TOF MS. Microorganism identifications in this group were coincident at the species level in 28(87.5%) specimens. The most frequent microorganism identified was *Escherichia coli*, followed by *Klebsiella* species and *Acinetobacter baumannii*. MALDI-TOF MS identified *Providencia stuartii* in 3 samples and *Pseudomonas putida* in 1 of them; which were not in accordance with the conventional method used for identification.

Conclusions: Our results show that MALDI-TOF MS allows bacterial identification from infected urine in a short time, with high accuracy, and especially when uncommon uropathogens are involved.

Keywords: Gram negative bacilli; Gram positive cocci, MALDI-TOF MS; Urinary tract infections

INTRODUCTION

Urinary tract infections (UTIs) remain one of the most common bacterial infections and second most common infectious disease with 150 million diagnosed cases each

year.¹ Presence of more than 10^5 organisms per ml in a midstream urine sample refers to significant bacteriuria and caused mainly by normal bowel flora *Escherichia coli*, which is responsible for over >70% of the cases.^{2,3} Other common urinary pathogens include Gram negative

rods such as members of Enterobacteriaceae other than *E.coli*, *Pseudomonas spp.* and *Acinetobacter spp.*²

Nosocomial UTIs account for upto 40% of all hospital acquired infections and major risk factor being urinary catheterization.³ Early identification of uropathogens is necessary especially in nosocomial UTI to help selection of appropriate antibiotics for treatment.

Several tests are available for screening patients for UTIs, including urine dipstick testing, urinalysis, Gram staining, and urine culture. Urine culture is the “gold standard” for defining the diagnosis of UTIs, because it allows the quantification and identification of the uropathogenic species. However, this method is cumbersome and time-consuming. It requires 24-72hrs before results are available. Up to 70% of urine cultures are negative, with high costs for unnecessary testing.⁴ Therefore, clinical microbiology laboratories require rapid, reliable, and cost-effective methods for identification of potential pathogens in clinical samples. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been suggested as a fast and reliable method for bacterial identification, based on the characteristic protein profiles for each microorganism.⁵ Databases have been developed that include the main pathogenic microorganisms, thus allowing the use of this method in routine bacterial identification from plate cultures.⁶

The aim of the present study was to identify bacterial urinary tract pathogens from urine samples and to compare the results obtained by conventional method used for identification of bacterial urinary pathogens with that of MALDI-TOF MS based identification system.

METHODS

Data collection

A prospective study was conducted in the Department of Microbiology from a period of September 2016 to October 2016. The characteristics of patients whose samples were included in the study were analyzed by sex, age, department of origin, and urine collection technique.

Inclusion criteria

- Patients presenting with signs and symptoms of urinary tract infection.
- Patients whose microscopic urine examination indicates pyuria.

Exclusion criteria

- Patients with a history of UTI within the past two weeks and have taken antibiotics within two weeks at the time of recruitment.
- Patients who refuse to sign the informed consent forms.

Processing of urine samples and microbiological analysis

We analyzed 50 midstream urine samples submitted in Microbiology laboratory, Government Medical College, Amritsar from outpatients and in-patients with symptoms suggestive of UTIs for microscopy and bacterial culture for the conventional culture, 1µl of well mixed urine was inoculated and spread onto Blood agar and Mac Conkey agar plates using a sterile plastic disposable loop. Plates were incubated in an aerobic atmosphere at 37°C for 18-24hrs. Negative plates were incubated for a further 24h and, if they remained negative, were discarded. When there was bacterial growth, colonies from blood agar or Mac Conkey's agar plates were identified by conventional methods except when more than one colony morphology was found, in which case the urine sample was rejected.

Biochemical test

Selected colonies were identified and differentiated according to the culture characteristics, microscopic examination and microbiological analysis and were tested biochemically for further confirmation of isolated bacteria, such as; TSI, catalase, oxidase, indole production test, MR test, VP test, citrate utilization test, motility test and oxidative-fermentative test.⁷

MALDI-TOF mass spectrometry

For analysis by Bruker MALDI Biotyper 3.1 mass spectrometer, the growth of isolated colony from agar plate was taken and applied onto the Maldi plate. It was then mixed or coated with 1µl of matrix solution (saturated solution of HCCA [α -cyano-4-hydroxy cinnamic acid] inorganic solvent [50% acetonitrile and 2.5% trifluoroacetic acid]) and air dried. When the matrix crystallizes on drying, the sample entrapped within the matrix also co-crystallizes and is ionized in an automated mode with a laser beam. The protonated ions generated from analytes are then accelerated at a fixed potential, where these separate from each other on the basis of their mass-to-charge ratio (m/z) which is measured by determining the time required for it to travel the length of the flight tube. For microbiological applications mainly TOF mass analyzers are used and based on the TOF information, a characteristic spectrum called peptide mass fingerprint (PMF) is generated for analytes in the sample. In PMF matching, the MS spectrum of unknown microbial isolates is compared with the MS spectra of known microbial isolates contained in the database.

Result scoring

A score of 2.300-3.000 and 2.000-2.299 signifies highly probable and probable species identification, respectively. A score of ≤ 1.700 indicates no identification.⁸

RESULTS

Fifty urine samples were processed by both conventional semi-quantitative method and MALDI-TOF MS. The colony growth was observed in forty-three (86%) samples. No growth was observed in culture plates in seven (14%) samples and MALDI-TOF MS also did not identify any significant protein profile in any of these cases. 3 (6.9%) samples with morphology of more than two colonies, 2 (4.6%) samples with two colony morphology and 38 (88.3%) samples with single-colony morphology were obtained. Out of 38 single morphological types, significant bacteriuria ($\geq 10^5$ CFU/ml) was observed in 32 samples.

Among the urine samples containing CFU $\geq 10^5$ /ml, Gram negative bacilli (GNB) accounted for 87.5% and Gram positive cocci (GPC) accounted for 12.5% of the urinary pathogens. *Enterococcus faecalis* (6.25%) was the commonest Gram positive cocci. Among the GNBs, *Escherichia coli* was identified as the most common (46.4%) uropathogen, followed by *Klebsiella pneumoniae*, *Acinetobacter baumannii* (14.2% each) and *Pseudomonas spp* (10.7%).

Table 1: Comparison of bacterial isolates obtained using conventional method and MALDI-TOF MS.

No. of samples	Conventional method	MALDI-TOF MS	Coincidence rate
13	<i>Escherichia coli</i>	<i>Escherichia coli</i>	100%
4	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i>	100%
4	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	100%
3	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	100%
2	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas putida</i>	0% (at species level)
1	<i>Proteus mirabilis</i>	<i>Providencia stuartii</i>	0%
1	Non-Fermenting Gram Negative Bacilli (1)	<i>Acinetobacter calcoaceticus</i> (1)	0%
2	<i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i>	100%
1	<i>Staphylococcus saprophyticus</i>	<i>Staphylococcus saprophyticus</i>	100%
1	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	100%
Total	Gram negative bacilli =28	Gram positive cocci= 4	

The microorganisms identified by both conventional methods and MALDI-TOF MS are compared in Table 1. The detection rate by both conventional method and MALDI-TOF MS was 100% for *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

Pseudomonas aeruginosa, *Enterococcus faecalis* and *Staphylococcus saprophyticus*.

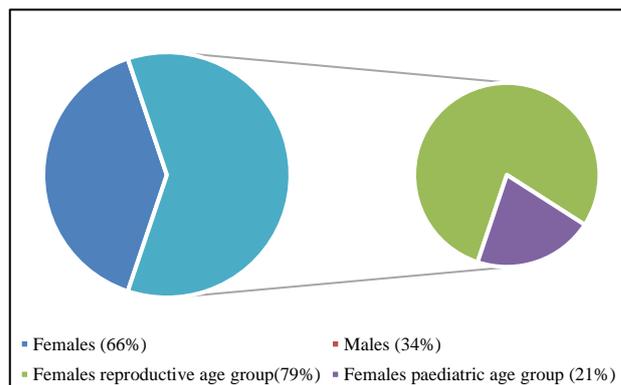


Figure 1: Distribution of samples according to age and gender.

Figure 1 shows the age and gender wise distribution of various samples included in the study. Females in the reproductive age group of 18-40year constituted 79% of the total female population, while the large fraction in males (64.2%) was made up of elderly patients in the age group of 41-80years. Figure 1 shows the percentage distribution of samples among males and females.

DISCUSSION

Since urinary tract infections are among the most common nosocomial and community-acquired bacterial infections with varied etiology, a rapid diagnosis of UTI has a significant beneficial impact on patient's health. It reduces unnecessary or inadequate empirical antimicrobial therapy.⁹

Our study, however, showed that prevalence of urinary pathogens was not consistent across all age groups further divided by gender. Urinary tract infection was found to be less prevalent in the youngest female and male subjects (21% and 17.6%, resp.) and more frequent in female patients aged 18 years or older (66%). This is owing to the fact that women are more inclined to develop UTI due to their anatomical features like short urethra, and other factors like pregnancy, use of diaphragms and sexual activity.¹⁰ Among males (34%), a higher prevalence of UTI was seen in elderly patients 41 years and older owing to irregularities in the functioning of urinary tract like prostatic hypertrophy.¹⁰

Concerning the methods compared, in case of gram positive cocci, although the number of specimens was small (12.5%), the detection rate by both conventional method and MALDI-TOF MS was 100% but the coincidence rate between conventional culture and MALDI-TOF MS among GNBs was 85.7%. MALDI-TOF MS correctly identified *Escherichia coli* (most common identified pathogen), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.

It identified *Pseudomonas putida* in 2 samples, *Providencia stuartii* in 1 sample and *Acinetobacter calcoaceticus* in another 1 of them, which were not in accordance with the conventional method of identification. A report at the 48th ICAAC involving 37 urine samples revealed that MALDI-TOF MS correctly identified the etiological microorganism in 33 cases (17 *E. coli* cases, 4 *E. faecalis* cases, 6 *K. pneumonia* cases, 2 *P. aeruginosa* cases, 1 *S. anginosus* case, and 3 *S. agalactiae* cases), and failed in only 4 cases, all of which led to a mixed culture on conventional culture plates.¹¹

It has been reported that with MALDI-TOF MS, the best results are obtained when there is a high bacterial count (>10⁵CFU/ml) and the microorganism involved is Gram negative. The results obtained with clinical samples, when the bacterial count was <10⁵CFU/ml, were poorer than those obtained with high bacterial counts, and *in vitro* studies on *E. coli*, *E. faecalis*, and *P. aeruginosa* different inocula strengthen this impression, although some UTIs such as cystitis may emerge with quite lower bacterial counts (10² to 10³CFU/ml)⁶. A study done in Spain involving 260 samples, displayed similar results where growth of a single morphological type was obtained in culture in 235 samples. 220 of them showed bacterial growth of >10⁵CFU/ml. Microorganism identifications in this group were coincident at the species level in 202 cases (91.8%) and at the genus level in 204 cases (92.7%). The most frequent microorganism was *Escherichia coli* (173 isolates).⁶

Using the threshold proposed (single colony morphology on culture plate, associated with MALDI-TOF MS positivity when the CFU count is >10⁵CFU/ml), it almost guarantees that identification will correlate with the UTI⁶, and according to our results, allows the etiology of >90% of UTIs to be diagnosed in just a few minutes. Studies have described an excellent correlation between MALDI-TOF MS identification and conventional microbiological identification in clinical bacterial and fungal isolates. A study at AIIMS, New Delhi, India in 2014 involving comparative analysis of 82 clinical bacterial isolates using MALDI-TOF MS and conventional techniques was carried out. Amongst the clinical isolates, the accuracy at the species level for clinical isolates was 98.78%.¹² Direct analysis of clinical samples may further increase the usefulness of MALDI-TOF MS, because it allows clinically useful results to be obtained some minutes after sample reception in the laboratory, but there is scant information available about the use of MALDI-TOF devices for the direct identification of microorganisms growing in positive samples. MALDI-TOF MS allows the identification of the etiological agent, but antibiotic susceptibility should be studied by conventional methods. Thus, culture is still necessary, since bacterial growth is still needed for susceptibility studies.⁶ It allows the diagnosis of UTI and its etiology in minutes, thus affording the opportunity of guiding empirical treatment much more efficaciously.⁹

CONCLUSION

In conclusion, MALDI-TOF MS showed high rate of accuracy for the identification of GNBs and GPCs in urine specimens with ≥10⁵CFU/ml, without any major error. This could become a rapid and accurate diagnostic method useful in the diagnosis of UTI caused by single morphological type, and especially when uncommon urinary pathogens are involved.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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