

Rapid Identification and Susceptibility Testing of *Candida* Species, from Urine by MALDI-TOF Mass Spectrometry and VITEK 2

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Abstract: Urinary tract infection (UTI) specially with *Candida* Spp., are mostly common in females which are suffering from vaginitis or got the infection through usual gynecology clinic visits and as a nosocomial infection specially in case of using urinary catheterization. Early identification of *Candida* spp., is necessary especially in nosocomial UTI and it could help selecting appropriate antifungal for treatment. This study shows that Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) is a reliable tool for fast and accurate identification of *Candida* spp., 146(4.3%) positive urine samples for yeast infection out of 3,370 samples were submitted to Microbiology laboratory of Hail General Hospital and Hail Maternity Hospital from April 2014 to December 2015. Specimens were direct microscopically examined for fungal elements and cultured on Sabouraud's Dextrose agar (SDA), *Candida* Chromogenic agar and Blood agar as a routine work identification, isolates were analyzed using (MALDI-TOF MS) and Vitek 2 for identification and susceptibility testing. The results showed that more than 70% of the isolates were *C. albicans*, 10% *C. galabrata*, 8% *C. Kursi*, 5% *C. dublinsiensis*, 4% *C. lusitaniae* and 3% *C. tropicalis*. In conclusion Vitek 2 susceptibility system AST-YS07 cards and MALDI-TOF MS can be used for accurate routine clinical identification. Comparing to the Vitek 2, MALDI-TOF MS is easier, faster and more economic for each test but on the other hand unreliable identification due to mixed infection or the specimens containing $\leq 10^4$ CFU/ml. The databases currently available for both systems should be updated to enhance performance.

Keywords: *Candida* species, Identification, MALDI-TOF MS, Susceptibility, Urine, Vitek 2

1. Introduction

Because yeast in urine samples are often contaminants, confirmation of their pathogenicity should be the first step. To differentiate between contaminate and fungal infection obtaining of a second urine sample from the patient is important [1]. Clean mid-stream samples should be taken or using catheter specially in elderly [2]. Mostly candiduria patients are asymptomatic usually women and they noted when they have urinalysis or urine culture and commonly associated with vaginitis. Symptomatic patients sometimes showed cystitis, dysuria, suprapubic discomfort and when it's neglected can lead to flank pain, pyelonephritis, oliguria, painful urination, epididymo-orchitis, *Candida* prostatitis and perinephric abscess [3],[4],[5],[6]. Early identification is necessary and can be done by using Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) which can detect directly the weight of phenotypical proteins from urine samples as yeast pellets [7]. The present study was done to identify *Candida* species from urine samples by MALDI-TOF MS and Vitek 2 susceptibility system in order to evaluate both methods for rapid and accurate identification of yeast spp., 146 positive samples for fungal infection were investigated.

2. Materials and Methods

2.1 Urinalysis, Microscope and culturing

3,370 Clean mid-stream samples were collected from patients of Hail General Hospital and Maternity Hospital in Hail, KSA. Examination of urine samples for fungal elements by centrifugation and Gram stained, Microscopic

examination were done for the presence of budding yeast cells and hyphal elements. 1 μ L of well-mixed urine was inoculated and spread onto SDA, Blood agar and *Candida* Chromogenic agar plates (BBL CHROMagar *Candida*, Beckton Dickinson, France), for each sample using sterile plastic disposable loop (SPL Lifescience, Pocheon, Korea). Plates were incubated in an aerobic atmosphere at 37 $^{\circ}$ C for 18-24 hr. Growth were observed and the colonies on blood agar and SDA used for conventional methods of identification, colors on *Candida* Chromogenic agar were identified.

2.2 MALDI-TOF MS Identification

146 urine samples were Duplicated in order to use the same samples in both methods. Maldi BioTyper (Burker Daltonics, GmbH, Germany) were used and protocol by Ferreira *et al* [8] and Lavergne *et al* [9] for MALDI-TOF MS analysis were adapted, to remove leukocytes centrifugation of 5ml urine from each sample for 30sec were done, then the supernatant also centrifuged for 10 min to collect yeast cell, the sediment pellets were washed by distal water then 1.5ml of sodium dodecyl sulfate 0.1% was added in each tube and incubation 37 $^{\circ}$ C for 10 min then centrifuged again for 10 min at 13,000 rpm. The supernatant was removed and the pellets were dried and suspended into adjusted volume of 10 μ l 70% formic acid and equal volume of acetonitrile and centrifuged, 1 μ l of supernatant was pipetted and applied to the MALDI-TOF plates, about 1 μ l of matrix solution (α -cyano-4-hydroxycinnamic acid) HCCA in 50% acetonitrile and 2.5% trifluoroacetic acid (TFA) (sigma-Aldrich, France) were added to each well.

The following adjustments were used : delay,170ns;ion source 1 voltage, 20 kV ; ion source 2 voltage, 18.5 kV ;mass range, 2-20kDa. The resulting peaks were automatically acquired by the AutoXecute of the FlexControl v 3.3 software (Burker Daltonics, GmbH, Germany).

2.3 Direct susceptibility testing by Vitek 2

Yeast cell pellets were dissolved in 0.45% saline solution to prepare suspension with McFarland turbidity 1.8 : 2.2 recommended by Vitek 2 (bioMérieux, Marcy l’Etoile, France)[10]. Yeast suspensions were directly inoculated into Vitek 2 susceptibility testing device using AST-YS07 cards (bioMérieux, Marcy l’Etoile, France) .The AntiFungal Susceptibility Test (AFST) results were for Amphotericin B, Voriconazole, Fluconazole, Caspofungin and Flucytosine. MICs value were used to assign isolation susceptible(S), intermediate (I) and resistant (R) depending on Vitek 2 breaking point setting “ EUCAST 2013 and CLSI 2013 D” . according to Table (1).

Table 1: Minimum Inhibitory Concentrations (MICs)

Antifungal	S	I	R
Amphotericin B	≤ 1	-	≥2
*Fluconazole	≤2	=4	≥8
Voriconazole	≤ 0.125	-	≥0.25
Caspofungin	≤ 2	-	-
Flucytocine	≤4	8≤ and ≤16	≥32

*Except for *C.glabrata* [S ≤1, 2≤I≤ 32, R≥ 64].

3. Results

146 (4.3%) out of 3,370 were positive samples to fungal infection depending on direct microscopic examination of urine samples, which showed yeast cells and budding . On CHROMagar different colors were present as *C.albicans* greenish colony, *C.glabrata* creamy color, *C.Kursi* pink color , *C. dublinsiensis* greenish blue ,*C. lusitaniae* white to mauve and *C.tropicalis* were metallic blue. Results showed isolates were distributed as the following 70%*C.albicans* ,10%*C.glabrata* ,8%*C.Kursi* , 5%*C.dublinsiensis* ,4%*C. lusitaniae* and 3% *C.tropicalis*.

3.1 Direct MALDI-TOF MS identification results

Species identification was (91.8%) (134/146) of the samples The results were compared to the results of conventional method as in Table(2).

Table 2: Candida species identification rate by using MALDI-TOF MS, n=146

species	Total number of samples	Number of identified samples	Number of unidentified Samples *
<i>C.albicans</i>	102	99	3
<i>C.glabrata</i>	15	13	2
<i>C.kursi</i>	12	10	2
<i>C.dublinsiensis</i>	7	5	2
<i>C.lusitaniae</i>	6	4	2
<i>C.tropicalis</i>	4	3	1
Total	146	134	12

*No reliable identification insufficient peak due to urine samples mixed with bacterial infection or containing ≤ 10⁴

(Colony Forming Units) CFU/ml.

3.2 Direct susceptibility testing by Vitek 2 results

100% agreement of species identification of the samples with conventional methods. Table (3) showing the results of antifungal susceptibility test by direct inoculation method. The duration of susceptibility testing in Vitek 2 was 23hr. *C.glabrata* showed resistant to Fluconazole along with Voriconazole.

Table 3: results of antifungal susceptibility testing

Antifungal/species (No.of isolates)	Direct method				
	Category			MIC (µg/ml)	
	S	I	R	50%	90%
Amphotericin B	146	0	0	≤0.25	0.5
Fluconazole	131	0	15*	2	4
Voriconazole	132	0	14*	≤0.12	0.25
Caspofungin	146	0	0	≤0.25	≤0.25
Flucytocine	146	0	0	≤1	≤1

**C.glabrata* ,MIC₅₀ and MIC₉₀were not calculated for single species due to low number of isolates .

4. Discussion

Candida is a very important pathogen , infection and dissemination can happen with serious complications .The reliability of yeast identification results in significantly improving treatment .

In the past 10 years *Candida* spp. are between fourth and sixth most common nosocomial infection specially with catheterization [11],[12].Previous studies have shown excellent correlation between Vitek 2 susceptibility testing, MALDI-TOF MS and conventional yeast identification [13],[14] .Based on the present work results showed higher rate of identification of specimens containing ≥ 10⁵CFU/ml than that containing ≤ 10⁴CFU/ml which showed insufficient peaks so considered as unreliable identification which corresponds with [15],[16].Vitek2 susceptibility testing results have been shown a reliable performance[17],[18], however Fluconazole and Voriconazole showed resistant with *C.glabrata* and *C.kursi*. The prevalence of *Candida spp* according to previous studies in the Kingdom of Saudi Arabia , Brazil and India[19],[20],[21] showed some variation of the percentage of each *Candida spp* .This study show that *C.albicans* 70% of the isolates followed by 10% *C.glabrata*,8%*C. Kursi*, 5%*C.dublinsiensis*,4%*C. lusitaniae* and 3% *C.tropicalis*.

In conclusion many *Candida spp* ; *C.albicans*, *C.glabrata*, *C. Kursi*, *C.dublinsiensis*, *C. lusitaniae* and *C.tropicalis* have been recorded from patient admitted to Microbiology laboratory of Hail General Hospital and Hail Maternity Hospital between April 2014 and December 2015and farther investigation has to be done in different regions in the Kingdom of Saudi Arabia in order to monitor *Candida spp* distribution .

MALDI-TOF MS showed higher results when the specimens contain ≥ 10⁵CFU/ml and it’s fast ,easier and more economic , Vitek 2 AST-YS07 card AFST showed high

performance but for Fluconazole and Voriconazole were suboptimal and subculture remain indispensable to validate the results also the databases currently available for both systems should be updated to enhance performance.

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