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ORIGINAL ARTICLE

A Comparative Study of MALDI-TOF MS and VITEK2 for Identification of Aerobic Gram Positive and Yeast Strains Isolated in Clinical Microbiology Laboratory

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ABSTRACT

Background: *Matrix-assisted laser desorption ionization- time of flight mass spectrometry (MALDI-TOF MS) has recently been used in microbiology laboratories for rapid and accurate identification of bacterial and yeast strains isolated from clinical samples.* ***Objective****: To evaluate the analytical and practical performance of MALDI - TOF MS (VILEK MS) system in comparison to conventional VITEK2 system for identification of aerobic gram positive and yeast strains.* ***Methodology:*** *A total of 416 isolates (340 aerobic Gram positive bacteria and 76 yeast strains) representing 5 genera and 30 species were analysed. Unmatched results were further investigated by 16s rRNA gene sequencing according to a reference method.* ***Results:*** *For 416 isolates, All strains (100%) were accurately identified at the genus level and 409 (98.3%) isolates at species level by using MALDI TOF MS. A total of 388 (93.26%) isolates were completely matched by both methods. There were 28 unmatched isolates, MALDI TOF MS made no errors at the genus level while VITEK 2 made 4 (0.96%) errors at the same level. At the species level the identification error rates of MALDI -TOF MS and VITEK 2 were 1.68% and 5.04% respectively.* ***Conclusion:*** *our findings demonstrated that MALDI -TOF MS has better performance than VITEK 2 in microbial identification. It is accurate, quick and cost- effective technique for bacterial identification in the Clinical Microbiology Laboratory.*

INTRODUCTION

Rapid microbial identification gives better management of antimicrobial therapies and infection control, especially with dramatic increase in drug resistance. Nowadays semi-autmated biochemical test platforms such as VITEK 2 (Biomerieux) or PHOENIX (BD Diagnostics) are commonly used in microbiology laboratories to complement the conventional cultured-based method for bacterial identification, reducing the mean time of identification to ~ 10 h [1,2].

Matrix – assisted laser desorption ionization- time of flight mass spectrometry (MALDI – TOF MS) has recently emerged in microbiology laboratories as a rapid, reliable and cost- effective alternative for bacterial and fungal identification. The technique is based on the production of microorganism protein fingerprints that are compard with those in a reference spectra database [3,4].

METHODOLOGY

The performance of MALDI -TOF MS system was evaluated at the Microbiology Laboratory of Zagazig University Hospital. The study design was approved by ethical committee, Faculty of Medicine Zagazig University.

Microbial Isolates:

During a 3 month period (April – June 2017) all aerobic Gram positive bacteria and yeast isolates from various clinical specimens were collected for this study. A total of 416 strains were isolated from 84 urine samples, 250 positive blood cultures, 48 pus samples, 22 sputum samples, 4 cerebrospinal fluid samples, 2 wound swabs, 1 vaginal swab, 2 mouth swabs, 1 nasal swab and 2 conjunctival swabs. (table l).

Table 1: Types of samples and number of isolates

Type of sample	Fungus	Gram +ve bacteria	No of Isolates
Urine	60	24	84
Blood	10	240	250
Pus	-	48	48
Sputum	2	20	22
Cerebrospinal	-	4	4
Wound swab	-	2	2
Vaginal swab	1	-	1
Mouth swab	2	-	2
Nose swab	1	-	1
Conjunctival swab	-	2	2
	76	340	416

Before MALDI -TOF MS analysis, all isolates were recovered on suitable media, blood agar for aerobic Gram positive and Sabouraud dextrose agar for yeast isolates under 35 c^0 -37 c^0 incubation for 24-48h in aerobic condition. Blood cultures were done using Bact/ALERT FA culture bottles and incubated for 7 days in the automated Bact/ALERT system (Biomerieux.Inc. Durham. USA).

Vitek 2 identification:

GP, YSI identification cards that were selected for identification analysis of Gram positive and yeasts isolates using Vitek 2 instrument (Biomerieux. Inc. Durham.USA) according to manufacture's instructions.

MALDI- TOF MS identification:

The isolates were identified by using the VITEK MS system (Biomerieuxinc.durham USA). A portion of a colony a freshly grown test isolates were picked up and smeared on the wells of disposable target slides to form thin layers of organisms, 1µL of VITEK MS CHCA matrix solution (cyano-4- hydroxycinnamic acid) was added to the sample and allowed to air dry for 1 to 2 minutes at room temperature. for yeasts, 0.5 µL of 25% formic acid was added to each sample on the target slide and allowed to air dry before adding 1 µL VITEK MS – CHCA matrix.

The target slides were then loaded into the VITEK MS system to acquire the mass spectra of whole microbial cell protein. Lastly, the mass spectra obtained for each sample were compared to the known mass spectra contained in the database and given a confidence score according to how close the acquired spectra matched those contained in the database. The matches that produced results with values exceeding 90% were considered significant and displayed. Identification was done using a new database (v2.0) and MYLA software developed for in vitro diagnostic (IVD) use. For calibration and internal identification control, *The Escherichia coli* ATCC 8739 strain was used.

It was inoculated on the calibration spots of each acquisition group, in addition *Staphylococcus aurues* ATCC 25923 and *Candida glabrata* ATCC 2950 were used as the external positive control for Gram- positive and yeast respectively. For quality control purposes, these external positive controls and the bacteria- free VITEK MS Matrix solution which served as a negative control, were analysed on each day of testing.

Final reference identification and discrepancy resolution:

When the VITEK MS system proposed a species-level identification that was completely matched with that provided by VITEK 2, the results were considered as the final reference identification and no further investigation was performed.

In cases where species- level identification was not available from either systems or if there was a mismatch between the two systems, microbial DNA was extracted for gene sequencing.

Gene sequencing:

Discrepancies between MALDI - TOF MS system and Vitek 2 were investigated by 16s rRNA gene sequencing , using FAST micro SEQ ® 500 16s rDNA Bacterial indenfication Kit and FAST Micro SEQ ™ D2 rDNA fungal identification Kit. DNA was extracted, 16s ribosomal RNA genes were amplified then analysed using Micro SEQ® ID Analysis software that compared them to sequences found in the GenBank database. A correct identification was determined as any result from MALDI -TOF MS and VITEK2 concordant with the final reference identification at the species level and/ or genus level, whereas a misidentification result was denoted when the identifications obtained from VITEK MS and VITEK 2 did not match with the final reference microbial identification [5].

RESULTS

- Microbial Identification by MALDI – TOF MS

A total of 416 microbial isolates were analysed in this study. These included 340 aerobic Gram-positive bacteria and 76 yeasts, representing 5 genera and 30 species.

MALDI TOF MS correctly identified 416/416 (100%) isolates to the genus level and 409/416 (98.3%) to the species level. The remaining 7/416 (1.7%) were misidentified.

Gram positive bacteria

Among the 340 Gram positive isolates; Staphylococci (n=254), Streptococci (n=30) and Enterococci (n=56) were the collected pathogens in the clinical laboratories during the study period. MALDI – TOF correctly identified 98.2% (334/340) of the isolates to the species level (3 genera and 21 species). All the 254 Staphylococci isolates were correctly identified to species level by VITEK MS. Two *Enterococcus Faecium* isolates 2/56 (3.6%) were correctly identified to the genus level as multiple choices of species level identification were given.

VITEK MS provided correct species level identification for 54/56 (96.4%) of Enterococci. The species level identification of Streptococci were 26/30 (86.6%). In particular, only 2/6 of *Streptococcus mitis* were correctly identified to the species level. (tabl 2). VITEK MS reported a split identification between *S.pneumoniae* and *S.mitis* group.

Yeasts

A total of 76 yeast isolates (2 genera and 9 species) were analysed.

Of the 74 candida isolates, all but one was correctly identified to the species level by VITEK MS. The missing isolate were *Candida Albicans* 1/74 (1.3%) which was only correct to the genus level. The two *Cryptococcus neoformans* isolates were correctly identified to species level 2/2 (100%).

- Matched results by the two identification methods

Identification results of 388 (93.2%) isolates, were in agreement using both methods the result are shown in table2.

Table 2: Matched results by both MALDI -TOF and VITEK 2

Identified strains	Number of strains
Gram positive bacteria	
Entercoccus flavus	3
E . faecalis	14
E . gallinarum	1
E . Faecuim	32
E . hirae	4
Staphylococcus aureus	108
Staphylococcus dpidermidis	51
Staphylococcus hominis	52
Staphylococcus saprophyticus	2
Staphylococcus haemolyticus	19
Staphylococcus cohnii	2
Staphylococcus capitis	7
St pneumoniae	5
St . mitis	2
St . parasanguinis	2
St . gordonii	1
St . gallolyticus	1
St . sailvarius	1
St . agalactiae	8
St . sanguinis	1
St . pyogenes	2
Yeasts	
C . albicans	36
C. glabrata	18
C. krusei	6
C. dubliniensis	1
C. tropicalis	4
C. lusitaniae	3
C. guilliermondii	1
C. parapsilosis	1
Total No	388 (93.27)

- Unmatched results by the two identification methods

Among the 416 isolates, 28(6.7%) Strains produced discordant results between the two identification methods. VITEK MS made no error at the genus level while VITEK 2 made 4 (0.96%) errors at the genus level. At species level the identification error rates of the two methods were 1.68% and 5.04% for VITEK MS and VITEK 2 respectively. (Table 3).

Table 3: Unmatched results compared with species detected by gene sequencing

Species identified by gene sequencing	Number of strains	MAIDI – TOF		VITEK 2	
		generic errors	species errors	generic errors	species errors
E. faecium	2		2		
Staphylococcus aureus	5			2	3
Staphylococcus hominis	6				6
Staphylococcus epidermidis	2				2
St. mitis	4		4		4
St. agalactiae	3				3
Candida albicans	1		1		
c. tropicalis	2				2
c. guilliermondi	1				1
Cryptococcus neoformans	2			2	
	28(6.7%)		7(1.68%)	4(0.96%)	21(5.04%)

DISCUSSION

The identification of microorganisms including bacteria and fungi becomes efficient quick and inexpensive by using MALDI-TOF which is compatible with wide range of culture media and culture conditions and it is considered the fastest mean to detect microbes in positive blood culture [6].

Amongst some gram positive bacteria, identification to the species level is difficult by the conventional methods [7]. It has been demonstrated that the MALDI-TOF MS system provides an accurate and reliable platform for good identification of Gram- positive aerobic bacteria [8,9]. In agreement with the findings of these studies. we also experienced a significantly better performance of VITEK MS than VITIEK 2 in species identification of Gram- positive bacteria, especially Staphylococci (100%) Enterococci (96.4%) and Streptococci (86.6%). This is important for the diagnosis of sepsis and meningitis in which identification to the species level is necessary.

Previous studies done by Bizzini et al.,[10]; Cherkaoui et al.,[11]; Neville et al.,[12] and McElvaniaTekippe et al.,[13] reported that misidentifications of *S. mitis* group strains as *S. pneumoniae* by MALDI BIOTYPER. However this problem appears to have been overcome in the Bruker Biotyper software (Version 3.1; MBT-BDAL-5627 MSP library; Bruker Daltonics) [14].

Yeast infections cause great morbidity and mortality. So, the rapid identification of yeast species is essential for initiation or modification to any antifungal treatment due to species specific susceptibility patterns [15]. The most commonly recovered yeasts in Clinical Microbiology Laboratories are Candida species including *C. albicans*, *C. glabrata*, *C. Krusei*, *C. tropicalis, and C. parapsilosis*. However, some of other species and genera are being seen at increasing frequency [15,16].

Cendejas et al.[17] reported that conventional phenotypic laboratory techniques for yeast identification are slow (24-72h) and unable to identify less clinically common species. The study compared between phenotypic and molecular methods in identification of rare pathogenic yeast species, only 25% were correctly identified by conventional methods and that most of the erroneously identified isolates showed a resistant antifungal profile.

MALDI- TOF correctly identified 98.7% (75/76) of yeast isolates at the species level compared to 97.4% (74/76) by VITEK 2. Our results showed that MALDI - TOF correctly identified 98.64% (73/74) of Candida spp. and 100% of the two *Cryptococcus neoformans* isolates analyzed in our study. The candida strains number reflects the high prevalence of these isolates in Microbiology Laboratories and confirms that non-candida species are minority.

A study by Wesblade et al.[18] reported that MALDI - TOF correctly identified 91.6% of 226 yeast like isolates to the species level including all 35 isolates of *Cryptococcus neoformans.* In addition Mancini et al., [19] reported that 72.5% of non-candida yeast like fungi including 10 isolates of *Cryptococcus neoformans* were correctly identified with MALDI -TOF MS.

In contrast, Chen et al., [20] showed that only 35% of the 20 yeast like fungi studied can be identified by MALDI - TOF MS and only one of seven *Cryptococcus neoformans* was correctly identified to the species level.

Marked decreases in the cost and time of identification to a few minutes (15 minutes) for one isolate and (50 min) for 16 isolates by MALDI- TOF MS are the major advantages in yeast identification [4]. Therefore, rapid species identification method for the diagnosis of yeast infections will make correct patient treatment possible by selection of a more species-specific antifungal therapy.

Conclusion: MALDI- TOF MS is rapid, easy, relatively inexpensive and more accurate than VITEK 2.

Due to these advantages and with continued development of MALDI-TOF MS technology and its clinical knowledge base, MALDI TOF MS becomes a rapid routine method for identifying bacteria and fungi isolated in Microbiology Laboratory.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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