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ORIGINAL ARTICLE

Impact of MALDI-TOF in the Routine Diagnostic Microbiology Laboratory in Alexandria University, Egypt

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ABSTRACT

Background: *In the last few years, matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) has emerged as a useful tool for identification of microorganisms. Identification of the various bacterial genera has been found to be challenging using the routine phenotypic tests or commercially available automated systems relying on the biochemical identification of bacteria.* ***Objectives:*** *In this study, we aimed to evaluate the possible role of the MALDI-TOF system with its Biotyper software as an accurate and rapid method of bacterial identification.* ***Methodology:*** *This study was conducted over a period of one month, where 299 isolates were retrieved from 523 clinical samples.* ***Results:*** *MALDI TOF identified 275 (92%) isolates to the species level, 287 isolates (96%) to the genus level and no reliable identification for 12 (4%) isolates using the direct formic acid application method. When protein extraction was applied, valid results for species and genus identification were obtained for 296 and 299 isolates respectively, so the overall rate of identification rose to 99% and 100% respectively. Discordant results between MALDI TOF and conventional methods were confirmed by Vitek 2 and 16s rRNA sequencing.* ***Conclusions:*** *MALDI-TOF is a rapid, accurate and inexpensive method for microbial identification that rivals other identification systems available. Its relative ease of use and rapid turnaround time makes it a powerful tool in the microbiology laboratory.*

INTRODUCTION

Rapid and accurate diagnosis of infectious diseases is critical for effective antimicrobial therapy, limitation of spread of bacterial resistance as well as infection control purposes. Phenotypic identification is the routinely used method in most clinical microbiology laboratories. [1] Unfortunately, they are costly, time consuming and of insufficient level of identification in many cases. [2] Molecular techniques such as real-time polymerase chain reaction (RT-PCR), 16S ribosomal RNA sequencing and microarrays can be used for faster and accurate diagnosis but these techniques are complex, expensive and require a workload that is difficult to adapt for routine use. [3]

Presumptive identification by simple conventional methods and commercially available identification systems is the routine done by most microbiology laboratories. However, misidentification by these methods may occur. Also, these methods are often expensive, time consuming and liable to errors.[4]

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Matrix assisted laser desorption ionization- time of flight (MALDI TOF) has been used for many years in the proteomics, toxicology and recently it has been applied to the field of microbiology for genus and species identification of bacteria. [5] There is also possible application for this technology in direct identification of bacteria from clinical samples without the need for culture. [6]

The potential for the identification of bacteria by their individual mass spectrometric "fingerprints" has long been appreciated. The adoption of MALDI-TOF MS in clinical microbiology laboratories is now possible because of available platforms with databases of bacterial whole-cell MALDI-TOF reference spectra. MALDI TOF mass spectrometry has been used as an effective tool for the identification of Gram-positive, Gram-negative, anaerobes, mycobacteria and fungi. [7, 8]

Aim:

Our aim in this study was to prospectively assess the performance of conventional phenotypic identification methods, and to evaluate the need for using MALDI-TOF MS for strain identification.

METHODOLOGY

1. Specimens

299 Clinical isolates were recovered from 523 routine clinical microbiology workflow specimens

submitted to Alexandria Main University Hospital Microbiology Laboratory for one month from November 2014 to December 2014.

Isolates were collected from fresh initial specimen cultures by Bench - by - bench approach. Blood and sterile body fluids, urine, wound, and respiratory specimens (sputum and minibal) were cultured and isolates were stored for further analysis. Microbial identification of clinical isolates was performed by:

2. Conventional protocol

Isolates were identified to the species level according to the standard operating procedures (SOPs) of the laboratory including Gram stain and biochemical reactions (catalase, coagulase, mannitol salt agar, bacitracin, optochin, bile esculin for Gram positive and Oxidase, TSI, MIO, citrate, urease for Gam negative microorganisms). [4]

3. MALDI - TOF PROTOCOL

MALDI-TOF MS UltraFlex system (Brüker Daltonik) for the MALDI MS identification was used, according to the following workflow:

a. Sample preparation: Using Direct transfer- formic acid method, bacteria were applied as a thin film onto a 384-spot polished steel target plate using a disposable loop and allowed to dry at room temperature. 1µl of 70% formic acid was added to the bacterial spot and allowed to air dry. Subsequently, 1µl of MALDI matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid [HCCA; Bruker Daltonics] in 50% acetonitrile and 2.5% trifluoroacetic acid) was applied onto the colony and allowed to dry. For the extraction method, 1 to 2 colonies (or a few colonies in the case of a small colony size) were suspended in 300 µl of molecular-grade water (Sigma-Aldrich, St. Louis, MO) and vortexed. Next, 900 µl of 100% ethanol (Sigma-Aldrich) was added, vortexed, and centrifuged (20,000 *g*) for 3 min. The supernatant was decanted, and the pellet was dried at room temperature. Fifty microliters of 70% formic acid (Fluka [Sigma-Aldrich], St. Louis, MO) and 50 µl of acetonitrile (Fluka) were added and thoroughly mixed by pipetting, followed by centrifugation (20,000 *g*) for 2 min. One µl of supernatant was spotted onto the plate and allowed to dry at room temperature before the addition of 1 µl of matrix.

For each run, a bacterial test standard was included to calibrate the instrument and validate the run.

b. MALDI-TOF MS analysis: was operated in the positive linear mode (mass over charge ratio (m/z) ranging from 2,000 to 20,000) under control of FlexControl software. Each spectrum was obtained by averaging 240 laser shots in 40 shot steps acquired in automatic mode at the minimum laser power being necessary for ionization of the samples according to the manufacturer's suggested recommendations for interpretation: Spectra were compared to fingerprint database by using the Bruker Biotyper 3.1 software and library of 5,623 entries.

MALDI-TOF MS Identification score criteria used were those recommended by the manufacturer: a score of ≥2.000 indicated species-level identification, a score of 1.700 to 1.999 indicated identification to the genus level, and a score of <1.700 was interpreted as no reliable identification. Isolates that failed to produce a score of >1.700 were retested by protein extraction method.

4. 16s Ribosomal RNA sequencing

DNA was extracted from bacterial cultures using the GeneJet genomic DNA purification Kit (Thermo K0721). Genes coding for rRNA were amplified using the primers F: AGA GTT TGA TCC TGG CTC AG and R: GGT TAC CTT GTT ACG ACT T each at a concentration of 20µM. Maxima® Hot Start PCR Master Mix (2X) was used for DNA amplification in a 50µL reaction mixture. Reaction conditions included an initial denaturation cycle at 95ºC for 10 minutes, followed by 35 cycles of denaturation at 95ºC for 30 seconds, annealing for 1 minute at 65ºC and extension for 90 seconds at 72ºC. Final extension at 72ºC for 10 minutes was done.

PCR products were purified using GeneJET™ PCR Purification Kit (Thermo K0701) according to manufacturer's instructions.

DNA was sequenced by ABI 3730xl DNA sequencer. DNA sequences were analyzed by DNAMAN software.

RESULTS

This study was conducted over a one month period, during which 523 samples were submitted to the Microbiology lab for identification and antibiotic susceptibility testing. From them, 299 isolates were recovered, where 87(29.1%) isolates were from urine, 84 isolates from pus (28.1%), 76 from sputum (25.4%) and 52 isolates from blood and sterile body fluids (17.4%) (Table 1).

MALDI-TOF MS yielded accurate identification to the species level, where the score was >2 in 275 (92%) isolates, accurate identification to the genus level (1.7>x>2) in 287 (96%) isolates and no reliable identification (<1.7) for 12 (4%) isolates. These results were all obtained by the direct formic acid application method of the bacterial colonies, without prior pre-preparation. When protein extraction was applied, valid results for species and genus identification were obtained for 296 and 299 isolates respectively, so the overall rate of identification to the species level and genus level rose to 99% and 100% respectively.

Concomitantly, identification by routine lab techniques was recorded. The isolate identification results differed between routine techniques and

MALDI-TOF identification, identity confirmation was performed with the automated identification system VITEK 2 and/or 16s rRNA sequencing. Discordance of results was noted among 4 isolates. Two strains were confirmed by VITEK2 to be of similar identification as obtained by MALDI-TOF MS, where one was isolated from blood and routinely identified as Enterococcus was later identified as *Leuconostoc mesenteroides* by MALDI- TOF MS and confirmed by VITEK2. The other discordant strain was isolated from urine and diagnosed as *Candida* non-albicans and proved to be *Trichosporon asahii* by MALDI-TOF MS and VITEK 2.

The other two were confirmed by 16s rRNA sequencing because the VITEK2 results were different from that obtained by MALDI-TOF MS. The first, was an isolate from sputum, routinely identified as *Pseudomonas*, and later on identified as *Achromobacter ruhlandii* by MALDI TOF MS, while by VITEK 2 it was identified as *Pseudomonas fluorescens* (score of 99%). The second strain from blood was routinely identified as coagulase negative *Staphylococci*, and later on proved to be *Rothia amarae* by MALDI TOF MS. VITEK2 identified it as *Kocuria kristinae* (score of 98%). Identification results of the 16s rRNA sequencing confirmed the MALDI TOF identification.

Table 1: Distribution of isolated strains according to specimen type

Sample type	*Isolates No. (%)*
Blood	52 (17,4)
Urine	87 (29)
sputum and mini BAL	76 (25.4)
pus	84 (28)
Total	299

Table 2. Strains identified to the species level and genus level in this study

Strains identified to the species level	*No.*	*Percent*
Achromobacter ruhlandii	1	0.33
Acinetobacter baumanii	40	13.4
Burkholderia cenocepacia	10	3.3
Candida albicans	8	2.7
Candida glabrata	1	0.33
Candida tropicalis	15	5
Corynebacterium amycolatum	1	0.33
Corynebacterium striatum	1	0.33
Corynebacterium spp.	1	0.33
Enterobacter cloacae	3	1.0
Enterococcus faecalis	9	3
Enterococcus faecium	6	2
Enterococcus raffinosus	1	0.34
Enterococcus spp.	1	0.33
Esherichia coli	64	21.4
Klebsiella oxytoca	1	0.33
Klebsiella pneomoniae	28	9.4
Leuconostoc mesenteroides	1	0.33
Morganella morganii	3	1.0
Proteus mirabilis	15	5
Proteus vulgaris	1	0.33
Providencia stuartii	1	0.33
Pseudomonas aeruginosa	25	8.4
Rothia amarae	1	0.33
Serratia marscescens	1	0.36
Staphlococcus aureus	39	13
Staphlococcus epidermidis	6	2
Staphylococcus haemolyticus	7	2.3
Staphylococcus hominis	2	0.7
Staphylococcus saprophyticus	1	0.33
Streptococcus pneumoniae	1	0.33
Streptococcus pyogenes	1	0.33
Streptococcus salivarius	1	0.33
Streptococcus spp.	1	0.33
Trichosporon asahii	1	0.33
Total	**299**	**100.0**

Table 3. Identification of discordant strains

	Conventional	*MALDI-TOF MS*	*VITEK2*	*16s rRNA sequencing*
1	Pseudomonas	Achromobacter ruhlandii	Pseudomonas fluorescens	Achromobacter ruhlandii
2	coagulase negative Staphylococci	Rothia amarae	Kocuria	Rothia amarae
3	Enterococcus	Leuconostoc mesenteroides	Leuconostoc mesenteroides	ND
4	Candida non-albicans	Trichosporon asahii	Trichosporon asahii	ND

**ND: not done*

DISCUSSION

Alexandria Main University is a large tertiary hospital serving 3 of the largest governorates in Egypt; Alexandria, Beheira and Matrouh. The Medical Microbiology labroatory handles the microbiology specimens from all departments. The main methods used for identification depend on phenotypic characters of bacteria, such as Gram staining and biochemical reactions of microorganisms. With the development of new techniques that are rapid, cost effective and accurate, such as MALDI-TOF MS it was crucial to evaluate the routine used methods and foresee the need of other high-tech technologies. [9]

Accurate and timely identification of pathogens is central in any medical setting. It permits the clinicians to prescribe appropriate antibiotics, hence reducing mortality and morbidity rates, hospital stay time and health-care costs.

The conventional methods used in our laboratories do not satisfy all the needs required nowadays in modern institutions. These methods offer identification

often only to the genus level, and in genera with close biochemical reactions mis-identification can easily occur. Also, these methods are not helpful for epidemiologic studies and might hamper infection control practices.

MALDI-TOF MS offers a promising solution for these hurdles, by providing identification of bacterial isolates depending on the protein finger print of bacterial conserved proteins. In the present study, we used the MALDI-TOF MS along the routine biochemical identification methods for a period of one month, where 92% of the isolated strains were identified to the species level and 96% to the genus level and identification to the species and genus level was increased to 99% and 100% respectively after protein extraction. These rates were comparable to the levels obtained by others [10-16]. Guo et al. [17] identified 99.6% to the genus level and 93.37% to the species level. [14] Bizzini et al identified 95.1% of their samples to the species level.

Discordances between Vitek2 and MALDI-TOF were reported in several studies. Guo *et al* [14] reported that MALDI-TOF was more superior to Vitek2 in bacterial identification at both the genus and the species levels. Inability of the MALDI-TOF to identify a given strain was attributed to limitations in the database. Li *et al* reported the superiority of the MALDI-TOF in identifying anaerobic bacteria, where Vitek2 was unable to identify certain genera that were easily identified by MALDI-TOF [18]. In a study performed on enteropathogens there was no discrepancies between the Vitek2 and MALDI-TOF, however the authors demonstrated that the MALDI-TOF results were consistent with Vitek2 but results were obtained ten times faster and at much lower cost[19].

CONCLUSIONS

Our results signify that the use of a cost-effective and robust technique as the MALDI TOF is an essential tool in the diagnostic microbiology lab. The method is rapid, accurate and relatively cheap. It also offers detailed identification up to the species level, where other methods may be deficient.

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