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ORIGINAL ARTICLE

The Identification of Microbial Pathogens Using Matrix Assisted Laser Desorption Ionization Time of Flight in Medical Intensive Care Unit Beni-Suef University Hospital

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ABSTRACT

Background: *Matrix Assisted Laser Desorption Ionization Time of Flight (MALDI-TOF) is a novel technique for identification of microbes. This new method led to a new era in microbial identification because of its rapid, accurate, valid, simple and relatively decreased cost.* ***Objectives:*** *The aim of this study was identification of predominant pathogens by MALDI-TOF technique.* ***Methodology:*** *Pathogens were identified by both conventional methods and MALDI-TOF.* ***Results:*** *From July till December 2018, predominant pathogens were Klebsiella pneumoniae (21%), Pseudomonas aeroguinosa and Candida each constitutes (17%), E-coli (10%), Staph. aureus (9%), Acinetobacter (9%). Identification of isolates (from September to December 2018) by MALDI-TOF revealed a total agreement of (94.1%) with conventional method at genus level, (88.2%) at level of species. Kappa agreement revealed almost perfect correlation between both techniques.* ***Conclusion:*** *The MALDI-TOF results might suggest that its' usage may be dependable for microbiological identification.*

INTRODUCTION

The microbial identification in the clinical laboratories have relied greatly on conventional phenotypic methods and gene sequencing till the last few years, where new techniques have been developed. Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) was mainly developed as a new and highly sensitive and specific technique for the differentiation, identification and classification of microorganisms both at the genus and the species levels[1,2]. It is understood that the concept of "laboratory time" is a particular factor that becomes even more important, considering the most important problem in modern healthcare such as the increasing rates of microorganisms' antibiotic-resistance[3].

This rapid new method led to a new era in the identification of pathogens in microbiology laboratories because of its rapid, accurate, valid, simple and relatively decreased cost[4]. Also, in septic patients, their management needs the initiation of empirical treatment as early as possible with broad spectrum antimicrobial drugs, which necessitates the early identification of the pathogen that will lead to more targeted treatment. Survival of these patients is very affected by delays in the final identification of causative pathogens and subsequent appropriate antimicrobial therapy[5,6,7].

According to these cited facts, this study was designed to compare between MALDI TOF technique and conventional methods regarding bacterial identification.

METHODOLOGY

Our study was performed at Medical Critical Care Unit of Beni-Suef University Hospital on all admitted patients prospectively. Identification of isolates (from September to December 2018) was done using both: conventional methods and MALDI-TOF[8].

Laboratory Testing:

All bacterial isolates were identified based on standard microbiological procedures in the microbiology laboratory of Beni-Suef University Hospital.

Further identification of the isolates was performed using:

MALDI-TOF MS:

Identification of the isolated micro-organism was done at (57357 Children's Cancer Hospital as an outsourcing).

Procedure:

The isolates from September till December2018 were sub cultured from glycerol on one blood agar plate and one MacConkey agar plate for MALDI-TOF and incubated at 35°C using an incubator StabiliTherm (ThermoScientific, Langenselbold, Germany). To identify a microorganism, the sample was mixed with 1 µL of matrix solution and placed on the steel surface of the target plate to dry. The matrix solution co-crystallizes with the sample on the target plate. The spectra were analysed on Vitek MS IVD system (bioMérieux; Marcy l'Etoile, France). Samples were performed in duplicate, with tests performed simultaneously on the same target slide. Part of single colony was transferred to an individual spot on the 48-well Vitek MS-DS disposable target slide. Each spot was covered with 1 µl ready-to-use Vitek MS alpha-cyano-4-hydroxycinnamic acid (HCCA) matrix (bioMérieux, France).The loaded target plate was inserted into the instrument, where it was transported to the measuring chamber. A high vacuum was continuously maintained inside the mass spectrometer. The individual samples were then subjected to short laser pulses. The energy of the laser then vaporized both the microorganism and the matrix, ionizing the ribosomal proteins. An electromagnetic field accelerated the ions before they enter the flight tube. The time taken for the flight of the analytes to reach the detector at the end of the flight tube was measured. Both the mass of the protein and degree of the ionization determined its individual time of flight. Based on this information, a characteristic spectrum was recorded and constitutes a sample "fingerprint," which was unique for any given species.

The target plate was read afterwards and then analyzed by the Vitek MS IVD system. In each specimen, the protein profile with *m/z ratio* of 3,000 to 15,000 was produced and profiles were then matched with the reference CE-IVD certified database Vitek MS (>20,000 spectra).

Matching of the results with confidence percentages of 90 to 98% had been considered for the genus level, while results of>98% confidence, had also been considered for the level of species, while results of <90% confidence were considered unacceptable. For specimens that showed different identification in the same genus, were identified only to the level of the genus, if greater than one genus or family identifications, these were considered unacceptable[9].

Statistical Analysis:

Agreement between conventional methods for the identification of microbes and MALDI TOF technique was done using Kappa agreement, interpretation was shown in table (1).

Table 1: Interpretation of Kappa statistics.

Kappa	Interpretation
< 0	No agreement
0.0 - 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

RESULTS

During the period from July till the end of December 2018, the predominant pathogens' distribution in the medical ICU is shown in fig. (1).

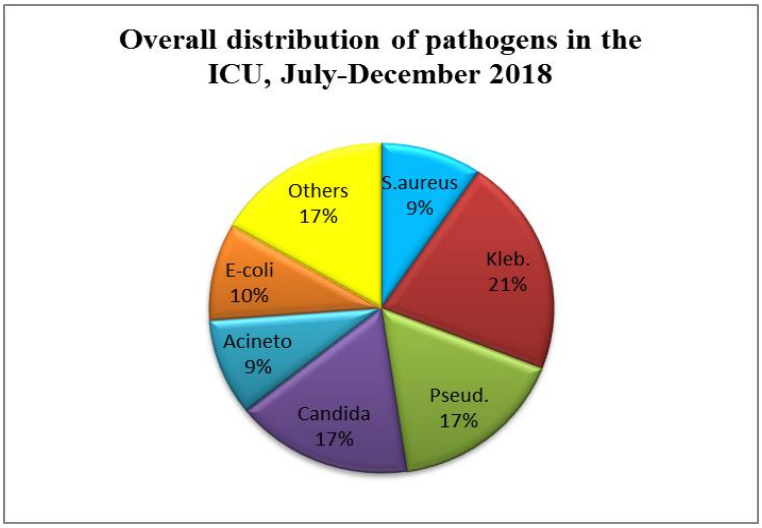

Fig. 1: The overall distribution of pathogens in the medical ICU, July –December 2018. *Klebsiella pneumoniae, pseudomonas, Acinetobacter baumannii, E-coli: Esherichia coli, S. aureus: Staph. aureus,* others: *Enterococci, Enterobacter, coagulase negative Staph.,Stenotrophomonas maltophilia*.

The most predominant pathogen is *Klebsiella pneumoniae* (21%), followed by *Pseudomonas aeroguinosa* and *Candida*, each constitutes (17%), then *E-coli* (10%), *Staph. aureus* (9%), and *Acinetobacter* (9%).

The identification of pathogens from September 2018 till December 2018, was also done using MALDI-TOF technique. The results are shown, table (2).

Table 2: Results of microbial identification by both conventional method and MALDI-TOF MS for pathogens in medical ICU from September till December 2018.

Serial No.	Conventional method	MALDI-TOF MS
1	*Candida species*	*Candida tropicalis*
2	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
3	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
4	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
5	*Staph. aureus*	*Staph. haemolyticus*
6	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
7	*Staph. aureus*	Not identified
8	*Acinetobacter baumannii*	*Acinetobacter baumannii*
9	*Klebsiella Pneumoniae*	Not identified
10	*Acinetobacter baumannii*	*Acinetobacter baumannii*
11	*Candida species*	*Candida tropicalis*
12	*Coagulase -ve staph.*	*Staph. haemolyticus*
13	*Klebsiella Pneumoniae*	*Klebsiella Pneumoniae*
14	*Enterobacter*	Not identified
15	*Pseudomonas aeruginosa*	*Ochrobactrum anthropi*
16	*E-coli*	*E-coli*
17	*Acinetobacter baumannii*	*Acinetobacter baumannii*
18	*Candida species*	*Candida albicans*
19	*Klebsiella Pneumoniae*	*Klebsiella Pneumoniae*
20	*E-coli*	*E-coli*

There was a total agreement of (94.1%) between MALDI-TOF and conventional method at the level of genus while it was (88.2%) at the level of the species. Three isolates have not been identified by MALDI-TOF technique. Kappa agreement revealed almost perfect correlation between conventional method and MALDI TOF, table (3).

Table 3: Kappa agreement between conventional method and MALDI TOF MS technique

		Value	Asymptotic Standardized Error[a]	Approximate T[b]	Approximate Significance
Measure of Agreement	Kappa	.806	.096	8.904	.000
N of Valid Cases		18			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Kappa value 0.806 and P value <0.001

DISCUSSION

The predominant organisms in this study are commonly found in patient care environment this may suggest that more effort needs to be done in environmental cleaning and disinfection, together with strict adherence to hand hygiene. These were *Klebsiella pneumoniae* (21%), followed by both *Pseudomonas aeruginosa* & *Candida species*, each constitutes (17%), then *E-coli* (10%), *Staph. aureus* (9%), and *Acinetobacter* (9%). This is somehow different from[10,11,12,13] where *Acinetobacter* was the most predominant pathogen. And all these are in contrast to other reports[14,15] where *Staph. aureus* was the most common pathogen.

The microbiological identification of isolates from clinical specimens of admitted ICU patients collected at microbiology lab of Beni-Suef University Hospital from September 2018 till December 2018, was done in this study using MALDI-TOF technique.

The results showed a total agreement of (94.1%) between MALDI TOF-MS and conventional methods at the level of the genus, while the total agreement was (88.2%) at the level of the species. Three isolates have not been identified by MALDI-TOF technique, this might be attributed to mixed samples. Kappa agreement revealed almost perfect correlation between conventional method and MALDI TOF. Our results agrees with a study done by Bizzini and Greub [16] where MALDITOF-MS identified correctly 84.1% to 93.6% of routine bacterial isolates to the level of the species[17]. Another report showed that out of 1200 examined isolates, (93.5%) of identifications made by MALDI-TOF MS matched the results of routine conventional methods[18].

Another study compared MALDI-TOF with VITEK 2 showed that from 1025 isolates, MALDI-TOF MS identified correctly (99.60%) at the level of the genus and also (93.37%) at the level of the species. This study also showed that 92.59% of isolates were completely matched by both techniques[19]. While in a second study comparing MALDI-TOF MS with VITEK 2 for the identification of aerobic gram positive and the yeast strains, all strains were identified accurately at genus level by MALDI-TOF MS[20]

CONCLUSION

The usage of a rapid sensitive technique for the pathogens' identification may affect treatment decisions greatly, especially in critical care settings e.g. CCU.

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Competing interests:

All authors approved the current version of the manuscript for submission.

Administrative and Ethical Issues:

Administrative approvals were taken from the head manager of Beni-Suef University Hospital, the head of Clinical Pathology Department and the head of the Medical Adult ICU and the Ethical Committee.

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