Journal of Medical Laboratory and Diagnosis Vol. 2(1) pp. 1-4, January 2011
Available online http://www.academicjournals.org/JMLD
ISSN 2141-2618 ©2011 Academic Journals

Full Length Research Paper

Comparison of matrix-assisted laser desorption ionization-time of flight mass spectrometry (maldi-tof ms) with conventional culture and biochemical method of bacteria identification to *species* level

Iroha Ifeanyichukwu Romanus[1]*, Ayogu Thomas Eze[2], Oji Anthonia Egwu[1], Afiukwa Felicitas Ngozi[1] and Nwuzo Agabus Chidiebube[1]

[1]Department of Applied Microbiology, Faculty of Biological Sciences Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria.
[2]Department of Food Technology, Institute of Management and technology, Enugu, P.M.B. 01079, Enugu State, Nigeria.

Accepted 28 November, 2010.

Identification of bacteria to species level is based on culturing in different media which takes 48 to 72 h from the time of isolation. Matrix-assisted desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a new technology in bacteria identification. We evaluated MALDI-TOF-MS system with 101 consecutively isolated bacteria colonies under routine laboratory conditions by comparing its identification efficiency with conventional culturing and biochemical tests. The MALDI-TOF MS identified bacteria colonies as *Escherichia coli* 44(43.5%), *Klebsiella pneumoniae* 56(55.4%) and *Stenotrophomonas maltophila* 1(1.4%) while cultural and biochemical method identified bacteria colonies as *E. coli* 43(42.5%), *K. pneumoniae* 56(55.4%) and failed to identify one bacteria colony which was identified as *S. maltophila* by MALDI-TOF MS. Our data suggests that although both methods are good for bacteria identification, MALDI-TOF MS is better than conventional culturing and biochemical tests because of its short turn around time with no loss of accuracy.

Key words: Bacteria identification, MALDI- TOF MS, culture, biochemical test, bacteria colony.

INTRODUCTION

Bacteria identification is crucial to perform in clinical specimen in order to identify pathogens and to guide antimicrobial therapy. Clinical laboratories develop ever more rapid, cost-effective and reliable methods for bacteria identification. Bacteria identification seeks to describe the diversity of bacteria species by naming and grouping organisms based on similarities such as cell structure, cellular metabolism, pigments etc while this scheme allows the identification and classification of bacteria strains, it was unclear whether these differences represent variations between distinct species or between strains of the same species. Culture method is designed to promote the growth and identity of particular bacteria while restricting the growth of other bacteria in the sample, often these techniques are designed for specific specimen and once an organism has been isolated it can be further characterized by its morphology, growth patterns, staining and biochemical test (Abe et al., 2009; De Cueto et al., 2004). These make the method cumbersome and time consuming and may not be useful in a situation where results are needed urgently for medical diagnosis (Abdessalam et al., 2010). Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a molecular analytical tool which may prove helpful diagnostically. This tool has been used extensively as a research tool for protein analysis and was applied recently to clinical microbiology (Marklein et al., 2009; Seng et al., 2009). The efficiency of mass spectrometry to identify crude bacteria was first established in non-medical microbiology laboratories and

*Corresponding author. E-mail: ifynero@yahoo.com.

was recently confirmed as the most promising technique for routine bacterial identification in clinical microbiology (Seng et al., 2009; Anhalt and Fenselau 1975; Degand et al., 2008; Carbonnelle et al., 2007). Compared with conventional phenotype MALDI TOF MS shows rapid turn around time, low sample volume requirements and modest reagent costs. The general work flow is straight forward starting with a single colony or other biological materials, samples can be analysed within few minutes with automated spectra acquisition completed in few seconds per sample. This method is specifically based on examining the protein contents and matching protein biomarkers that are unique to a specific bacterium. Assignment of specific mass peaks as species and even strain specific biomarkers for several micro-organisms has been reported (Haroun et al., 2001; Jones et al., 2003; Ricky et al., 2000; Catherine and Demirev, 2001). Several recent studies have shown that the obtained bacteria spectra depend on a number of instrumental factors and microbiological variables which include; choice of culture media and bacterial culture growth time. This study compared the identification accuracy and efficiency of MALDI-TOF MS with conventional culture and biochemical routine tests for bacteria species.

MATERIALS AND METHODS

Bacteria samples

A total of 101 clinical samples collected from urine n = 71, wound swab n = 13, HVS n = 17 from patients visiting University of Nigeria, teaching hospital Enugu were used for the study.

Bacterial identification

Culture and biochemical testing method

Urine, wound swab and HVS samples was inoculated on the surface of MacConkey, Cystine lactose electrolyte-deficient (CLED) and eosine methylene agar plates and were incubated at 37°C for 18 to 24 h. After incubation, the agar plates were carefully observed for colonies with uniform morphology and were selected for Gram staining and biochemical tests using standard Microbiology methods (Chessbrough, 2006).

Matrix preparation

The MALDI-TOF α cyano-4-hydroxycinnamic acid matrix was prepared daily as a saturated solution in 50% aceto-nitrile and 2.5% trifluoracetic acid (TFA).

Sample preparation for MALDI-TOF

A few colonies of fresh overnight cultures grown on blood agar plates at 37°C under aerobic conditions were suspended in 600 µl of 70% ethanol in eppidorff tube and vortex for 1 min to mix. This was centrifuged for 3 min at 13,000 rpm and the supernatant was poured away leaving the pellet. 20 µl of formate was added to the pellet mixed properly and vortex, 20 µl of aceto-nitrile was added into the mixture and vortex to mix. The mixture was centrifuged for 1 min at 13,000 rpm; after which 1 ml of the sample was gently dropped on the target plate and was allowed to air dry. After drying, 1 ml of the matrix solution was added and again air dried at room temperature.

MALDI-TOF MS measurement

MALDI-TOF measurements were performed with a bruker 2.0 Ultraflex II MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) instrument equipped with 200H_Z smartbeam laser technology was used for visual inspection of mass spectra. The whole process from MALDI-TOF MS measurement for identification was performed automatically. The spectra were recorded in the linear positive mode at a laser frequency of 20 Hz within a mass range of 2,000 to 20,000 Da with 50 shots per s from different positions of the target spot. The proteins flow to the reflector panel and are separated according to their sizes. As an integral part of the MALDI Biotype software, main spectra were used as reference libraries, containing information about averaged masses, average intensities and relative abundances in the measurements for all characteristics peaks. To identify unknown bacterial isolates, raw spectra were imported into MALDI Biotype software and analysed by the standard pattern matching algorithm against the library spectra using the standard settings. In pattern matching's, fingerprints of unknown samples were compared to all entries of the data base (Bruker Biotype database). Results of the pattern matching process were expressed as log (score) values ranging from 0 to 3. Values of > 1.7 generally indicate relationships on the genus level and log (scores) of > 2.0 relationships on the species level. The highest log (score) of a match against the database was used on the species identification. Both MALDI- TOF MS system result were compared to phenotypic bacteria identifications routinely performed, when MS and phenotypic identifications agreed at the species level, we consider MALDI TOF species identification correct.

RESULTS

Table 1 shows the mean log (score) value for identifying organisms to specie levels by MALDI- TOF MS method. Table 2 shows that culture and biochemical tests method identified bacterial colonies as *E. coli* and *K. pneumoniae* from clinical samples. Out of 71 urine samples 33(46.4%) were identified as *E. coil,* 38(53.5%) as *K. Pneumoniae* and bacteria colonies from one urine sample 1(1.4%) could not be identified to specie level. From swab samples 4(30.7%) of bacteria colonies were identified as *E.coli*, 9(69.2%) as *K. pneumoniae*, from HVS 7(37.5%) bacteria colonies were identify as *E. coli* and 10(62.5%) as *K. pneumoniae*. Bacteria identification using MALDI-TOF MS shows the same result as the culture method except that the sample which could not be identified by culture method was identified by MALDI TOF as *S. maltophila* (Table 3). Our findings show that MALDI TOF MS is a very good technology for bacterial identification when compared to culture method.

DISCUSSION

The correct and fast identification of bacterial pathogens from clinical specimens and patients environments,

Table 1. Mean MALDI-TOF MS log (score) value for bacteria species identification.

Range	Description	Symbols	Colour
2.300 - 3.000	Highly probable species identification	+++	Green
2.000 - 2.999	Secure genus identification, probably genus identification	++	Green
1.700 - 1.999	Probably genus identification	+	Yellow
0.000 - 1.699	Not reliable identification	-	Red

Table 2. Bacteria identification to species level by culture and biochemical test.

Sample Nos	Organism identified	% of organism identified
Urine sample n=71	*E. coli* n=33	46.4
	K. pneumoniae n=38	53.5
	Un-identified n=1	1.4
Wound swab n=13	*E. coli* n=4	30.7
	K. pneumoniae n=9	69.2
HVS n=16	*E.coli* n=6	37.5
	K. pneumoniae n=10	62.5

Table 3. Mean MALDI-TOF MS log (score) identification values of 101 consecutive bacteria.

Sample Nos	Organism identified	Mean score value
Urine sample n = 71	*E. coli* n = 33	1.961 - 2341
	K. pneumoniae n = 38	2.196 - 2339
	S. maltophila n = 1	2.012
Wound swab n = 13	*E. coli* n = 4	2.035 - 2.922
	K. pneumoniae n = 9	2.334 - 3.037
HVS n =16	*E. coli* n = 6	1.992 - 2.633
	K. pneumoniae n = 10	2.336 - 2.994

especially in outbreak situation is of major concern for optimal patient management and effective measures for disease control. Molecular resolution approaches such as 16S rDNA or ITS DNA sequencing and real time PCR assays are expensive and time consuming.

In the present study MALDI-TOF MS proved to be a rapid and reliable procedure for the accurate identification of pathogenic bacteria with minimal cost and time and this potential method for bacterial identification has been well documented (Eric et al., 1999; Abdessalem et al., 2010; Dubois et al., 2010; Jackson, 2001; Guy et al., 2010). To assess the use of MALDI-TOF MS for identification of bacteria, the identity and spectra scores were determined for bacteria recovered in 101 clinical samples namely urine n = 71, wound swab n = 13, HVS n = 17. The spectra score range obtained from our data was 1.961 to 2.633 for *E. coli,* 2.196 to 2.341 for *K. Pneumoniae* and 2.012 for *S. maltophila*. 99% of bacteria identified by MALDI-TOF MS technology correspond with those identified by culture method.

MALDI- TOF MS has shown to be a very reliable method for bacteria identification than culture method because of the advantage of simple sample handling procedures, rapidity of analysis and species-specific patterns in the associated mass spectra. Although we could not resolve the discrepancies in the resolution by MALDI TOF MS in one of the sample as *S. maltophila* which we could not identify by culture method. We may say that this shows that MALDI- TOF MS has more resolution efficiency in bacteria identification than routine

culture and biochemical method. One major advantage of MALDI-TOF MS over culture method is that it can characterize organism to genus level based on log (score) value which culture method cannot. Our data show that MALDI- TOF MS will be a useful tool for research and will make vast improvements in the efficiency of clinical laboratories in the near future. This technology can identify a large number of isolates in less than 10 min per sample in a clinical bacteriology laboratory with less marginal cost than conventional methods and with equal and greater accuracy. Although MS equipment is expensive, it is comparable to that of other common bacteriology devices and the marginal costs are substantially less than those of conventional identification strategies. Therefore cost restriction, training and quality control requirements and the need for rapid turn around times makes MS quite appealing compared to conventional identifications. MALDI-TOF MS, however, currently does not provide adequate data on antimicrobial susceptibility and requires an isolate for starting material. Hence, there will be a continuing requirement for bacterial culture. It is likely that further database refinements of MS will allow rapid identification of antibiotic resistance characteristics dependent on the production of specific proteins or peptides. Database refinement and enrichment are essential elements of MALDI –TOF MS, which will allow the method to increase its power as it is used more frequently.

In conclusion, use of MALDT-TOF MS will provide more reliable and faster bacteria species identification than conventional culture and biochemical methods. This is especially relevant in routine clinical diagnostic laboratories, since most results can be reported one day earlier. This method will probably replace conventional identification of bacteria but in the case of a mixture of species, only one is identified and false identification may occur.

REFERENCES

Abdessalam C. Jonathan H. Stephane E. Manuela T. Myriam G. Patrice F. Jacques S (2010). Comparison of two matrix-assisted laser desorption ionization time of flight mass spectrometry methods with conventional phenotypic identification for routine identification of bacteria to the species level. J. Clin. Microbiol., 48(4): 1169-1175.

Abe T. Tokuda Y. Ishimatsu S. Birrer RB (2009). Usefulness of initial blood culture in patients admitted with pneumonia from emergency department in Japan. J. Infect. Chemother., (15): 180-186.

Anhalt J. Fenselau C (1975). Identification of bacteria using mass spectrometry. Anal. Chem., (47): 219-225.

Carbonelle E. Beretti JL. Cottyn S. Quesne G. Berche P (2007). Rapid identification of Staphlococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J.Clin. Microbiol., 45: 2156-2161.

Catherine F, Plamen AD (2001). Characterization of intact micro-organisms by MALDI mass spectrometry. Mass Spect. Rev., 20(4): 157-171.

Damien D.David L. Jean-Paul C. Markus K. Pierre OS. Regime T, Richard B, Julien D (2010). Identification of a variety of Staphylococcus species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol., 48(3): 941-945.

De Cueto M, Ceballos E, Martinez-Martinez L, Perea EJ, Pascual A (2004). Use of positive blood cultures for direct identification and susceptibility testing with the vitek 2 system. J. Clin. Microbiol., (42): 3734-3734.

Degand N, Carbonnelle E, Dauphin B, Beretti JL, Le Bourgeois M, (2008). Matrix-assisted desorption ionization-time of flight mass spectrometry for identification of non-fermenting Gram-negative bacilli isolated from cystic fibrosis patients. J. Clin. Microbiol., (46): 3361-3367.

Eric CL, Ming-Chung C, Wen-Cherng T, Chau-Chung H (1999). Identification of Enterobacteriaceae bacteria by direct matrix-assisted laser desorption/ionization mass spectrometric analysis of whole cells. Rapid. Commnun. Mass Spec., (13):2022-2027.

Guy P, Alain B, Christian D, Jacques B, Gilbert G (2010). Matrix-assisted laser desorption ionization-time of flight mass spectrometry for direct bacterial identification from positive blood culture pellets. J. Clin. Microbiol., 48(4): 1481-1483.

Haroun NS, Carrina JK, Oliver S, Saheer EG (2001). Martix-assisted laser desorption/ionization time of flight spectrometry and proteomics: A new era in anaerobic microbiology. Clin. Infect. Dis., 35(suppl 1): S58-S64.

Jackson OL $_{Jr}$. (2001). MALDI-TOF mass spectrometry of bacteria. Mass Spectr. Rev., (20): 172-194.

Jones JJ, Stump MJ, Flemimg RC, Lay JO, Wilkins CL (2003). Investigation of MALDI-TOF and FT-MS techniques for analysis of *Escherichia coli* whole cells. Anal. Chem., 75: 1340-1347.

Marklein G, Josten M, Klanke U, Muller E, Horre R, Maier T, Wensel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG (2009). Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol., (47): 2912-2917.

Ricky DH, Fatemeh R, Thomas MH, John BSM, Kent JV, Jackson OL $_{jr}$ (2000). Matrix-assisted laser desorption/ionization time of flight mass spectrometric detection of bacteria. Rapid Commun. Mass Spectr., 14(10): 911-917.

Seng P, Drancourt F, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D (2009). Ongoing revolution in Bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time of flight mass spectrometry. Clin. Infect. Dis., (49): 543-551.