Review Article

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the detection of extended spectrum β-lactamases and carbapenemases among Gram-negative bacilli

Yuliya Zboromyrska[1], Jordi Vila[1,2]

[1]Department of Clinical Microbiology, School of Medicine, Biomedical Diagnostic Centre, Hospital Clínic, University of Barcelona, [2]ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic, University of Barcelona, Barcelona, Spain

ABSTRACT

In the last few years, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has become the new gold standard method for the identification of microorganisms. Nevertheless, other useful applications of mass spectrometry have recently been demonstrated including the detection of resistance mechanisms. The MALDI-TOF-MS hydrolysis assay allows rapid detection of β-lactamase activity with results available within a few hours. Rapid detection of β-lactamases is important since β-lactam antibiotics remains as the mainstay of the treatment of several community-acquired and nosocomial infections, and a steady increase has been observed of resistant microorganisms associated with the production of extended spectrum β-lactamases (ESBLs) and carbapenemases. Several studies have reported the high sensitivity and specificity of the MALDI-TOF-MS antibiotic hydrolysis assay for the detection of ESBLs - or carbapenemase-producing Gram-negative bacilli. In addition, this technique can be performed directly from positive blood culture, significantly shortening the time of resistance detection. However, the standardization of assay conditions as well as automatic analysis of the spectra obtained is required. The aim of this review was to summarize the currently available data regarding the accuracy of the detection of β-lactamase activity using the MALDI-TOF-MS antibiotic hydrolysis assay.

Key words: Carbapenemases, extended spectrum β-lactamases, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, resistance to β-lactam antibiotics

INTRODUCTION

In the last few years, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) has become the new gold standard method for the identification of microorganisms, greatly improving laboratory workflow, and shortening the time of microbiological response.[1,2] The microorganisms which can be identified by MALDI-TOF-MS include bacteria,

Address for correspondence:
Dr. Yuliya Zboromyrska, Department of Clinical Microbiology, Hospital Clínic, Barcelona, Spain.
E-mail: yzboromy@clinic.ub.es

Access this article online	
Quick Response Code:	Website: www.caijournal.com
	DOI: 10.4103/2225-6482.166061

How to cite this article: Zboromyrska Y, Vila J. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for the detection of extended spectrum β-lactamases and carbapenemases among Gram-negative bacilli. Community Acquir Infect 2015;2:65-70.

yeasts, and even viruses and parasites.[3-7] In addition, the identification can be performed not only from colonies but also directly from positive blood cultures and even from direct samples such as urine.[8-10] Although MALDI-TOF-MS has been widely demonstrated as an accurate method of microbial identification, several studies have recently been focused on other applications of mass spectrometry, such as bacterial typing, and the detection of resistance mechanisms and virulence factors.[11-13] Rapid detection of resistant microorganisms is extremely important due to the negative impact of inappropriate antibiotic treatment on the patients' outcome and survival.[14,15] To date, different approaches have been studied for this proposal, including comparison of mass spectra from resistant and susceptible strains or direct detection of the enzyme mass peak.[16,17] However, the MALDI-TOF-MS antibiotic hydrolysis assay is the most frequently studied approach and consists in the detection of the hydrolysis activity of β-lactamases. Rapid detection of β-lactamases is important since β-lactam antibiotics remains as the mainstay of the treatment of several community-acquired and nosocomial infections, and a steady increase has been observed of resistant microorganisms associated with the production of extended spectrum β-lactamases (ESBLs) and carbapenemases. Beta-lactamase production is the most frequent mechanism of resistance to β-lactam antibiotics, at least among *Enterobacteriaceae*. The aim of this review was to summarize the data currently available regarding the accuracy of β-lactamase activity detection using the MALDI-TOF-MS hydrolysis assay.

HYDROLYSIS ASSAY BACKGROUND

The MALDI-TOF-MS hydrolysis assay is based on the detection of the hydrolysis of a specific β-lactam antibiotic in the presence of strains producing β-lactamases such as ESBLs or carbapenemases. In this case, the target to be analyzed by MALDI-TOF-MS is the antibiotic spectrum, in which hydrolyzed products can be detected after incubation with a resistant strain. Briefly, an established amount of bacteria is resuspended in antibiotic solution and incubated for the indicated time. Subsequently, this suspension is centrifuged to pellet the bacteria and the supernatant is analyzed by MALDI-TOF-MS [Figure 1]. During the incubation with β-lactamase-producing bacteria, the hydrolysis of the β-lactam ring of the antibiotic is produced leading to the molecular mass shift of the respective antibiotic. Apart from the intact and hydrolyzed forms of antibiotics, corresponding sodium and potassium adducts and additional degradation products can also be detected (deacetylated forms, hydrolyzed and decarboxylated forms, etc.). Therefore, the detection of enzymatic activity consists in the detection of changes in the mass peaks profile of the antibiotic in the presence of enzyme-producing strains. This approach requires different parameter settings of the flex control software of the MALDI-TOF-MS (Bruker Daltonik GmbH, Germany) as the lower mass spectrum range is analyzed (m/z between 100 and 1000). Moreover, a specific protocol for sample preparation and analysis is required.

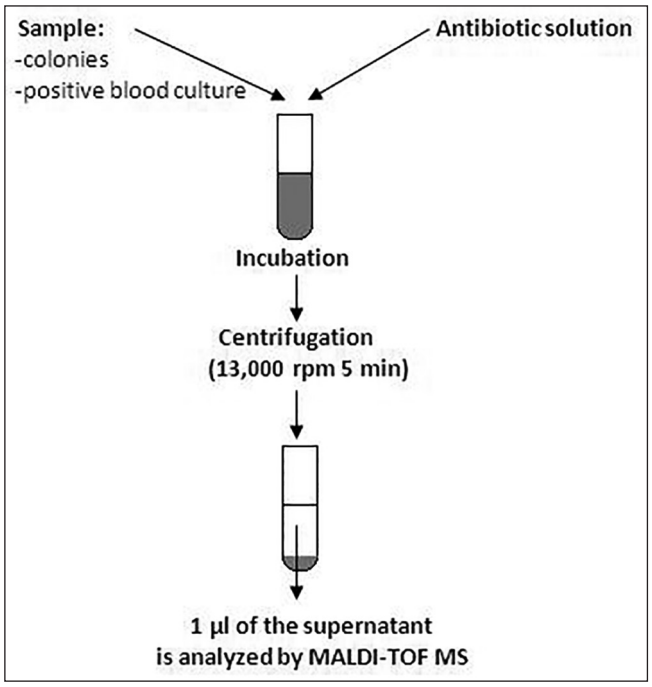

Figure 1: Scheme of the matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry hydrolysis assay

DETECTION OF CARBAPENEMASE-PRODUCING BACTERIA

The first report using MALDI-TOF-MS for the detection of resistance to β-lactam antibiotics based on the detection of hydrolysis was published in 2011. Hrabák *et al.* reported the detection of carbapenemase-producing strains following incubation with meropenem.[18] Three different organic matrix solutions were tested, with 2,5-dihidrobenzoic acid (DHB) diluted in 50% ethanol being considered the optimal solution, since less background was produced in the final spectrum. In order to perform the assay, an 8 McFarland suspension of the tested strain was prepared and then centrifuged to obtain bacterial pellet. Fifty microliters of 0.1 mM of meropenem in 20 mM Tris-HCL buffer was added to the pellet, and the mixture was incubated at 35°C for 3 h. Afterward, the mixture was centrifuged and 1 µL supernatant was used for analysis. The criterion of carbapenemase production was the lack of the peaks of intact meropenem after incubation. One hundred twenty-four strains of Gram-negative bacilli were tested, including 30 carbapenemase-producing bacilli. The meropenem hydrolysis assay showed a sensitivity and specificity of 96.67% and 97.87%, respectively. Figure 2 shows the spectrum obtained after 2 h of incubation of meropenem with carbapenemase-producing and noncarbapenemase-producing strains. In a later publication, the same authors

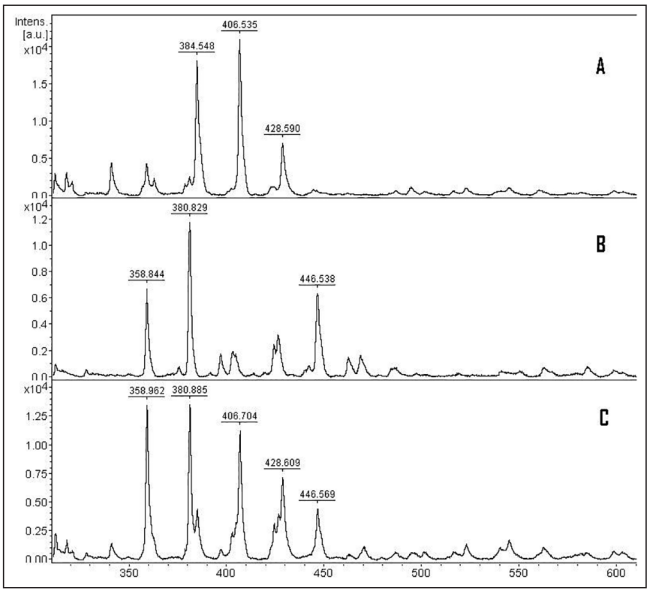

Figure 2: Mass spectra of the meropenem hydrolysis assay after 2 h of incubation — the spectrum after incubation with (a) noncarbapenemase-producing strain shows peaks of intact meropenem (m/z 384.5) and its sodium adducts (m/z 406.5 and 428.5); (b) with a carbapenemase-producing strain (KPC) - peaks of meropenem degradation products (m/z 358.8, 380.8, 446.5); (c) with a carbapenemase-producing strain (NDM) - peaks of intact meropenem adducts (m/z 406.7 and 428.6) and degradation products (m/z 358.9, 380.8 and 446.5).

modified the previously described protocol for the hydrolysis assay with the supplementation of the reaction buffer with 0.01% sodium dodecyl sulfate (SDS) to decrease the bacterial inoculum (3.0 McFarland) and shorten the incubation time (2 h).[19]

At almost the same time, Burckhardt and Zimmermann published a study using ertapenem instead of meropenem for the detection of carbapenemase producers.[20] In this study, α-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution was used. The criterion of carbapenemase production was also the lack of the peak of intact ertapenem and the detection of the hydrolyzed product. Interestingly, the time required for antibiotic degradation depended on the type of carbapenemases, being NDM-1 and IMP-1 the fastest in the study, with an average time of only 1 h being needed for complete ertapenem degradation. On the other hand, the authors did not observe a correlation between the minimum inhibitory concentrations of carbapenems and the time required for antibiotic hydrolysis. Another important finding was the detection of a peak of spontaneous hydrolysis in the spectrum of intact ertapenem even without having added any microorganisms. This should be taken into account at the time of analyzing the hydrolysis assay with ertapenem.

In 2012, Sparbier *et al.* published what could be considered as a basic guide to perform hydrolysis assays with MALDI-TOF-MS. They tested several β-lactamic antibiotics, including ampicillin, piperacillin, cefotaxime, ceftazidime, ertapenem, imipenem and meropenem[21] and provided the exact mass peaks of intact and hydrolyzed forms of each antibiotic. In addition, the inhibition of hydrolysis in the presence of clavulanic acid, tazobactam and 3-aminophenylboronic acid was studied depending on the antibiotic tested and resistance pattern. The authors also described the possibility to perform this assay directly from positive blood cultures.

Kempf *et al.* focused on the detection of carbapenem resistance in *Acinetobacter baumannii* using MALDI-TOF-MS.[22] The antibiotic of choice in this study was imipenem, and a total of 106 A. *baumannii* strains were tested, including 63 carbapenem-resistant and 43 carbapenem-susceptible isolates. Interestingly, hydrolysis was detected in 2 h in 60 resistant strains, and only three strains required a prolonged incubation time (4 h). As imipenem showed weak spontaneous hydrolysis, the ratio between the peak for imipenem and its metabolite was used for interpretation of the results. The sensitivity and specificity of the assay was 100% after 4 h of incubation.

Although MALDI-TOF-MS seemed to be a quick and reliable tool for the detection of several types of carbapenemases, OXA-48 producers demonstrated weak hydrolysis, and therefore, frequently showed false negative results. To improve the detection of OXA-48 type carbapenemases Studentova *et al.* proposed the addition of NH_4HCO_3 to the reaction buffer, demonstrating an increased sensitivity for the MALDI-TOF-MS hydrolysis assay.[23]

After having demonstrated the accuracy of carbapenemase activity detection from the colonies, the possibility to perform this technique from positive blood cultures was investigated. In 2014, Carvalhaes *et al.* reported the detection of carbapenemase activity directly from positive blood culture using MALDI-TOF-MS.[24] A total of 100 blood cultures were tested, including 76 Gram-negative bacilli. Curiously, 29 out of 76 (38.2%) Gram-negative bacilli were carbapenemase-producing, including 15 *Klebsiella pneumoniae* and two *Enterobacter cloacae* harboring the bla_{KPC-2} gene, 10 A. *baumannii* strains with bla_{OXA-23}, one A. *baumannii* with bla_{OXA-72} and one *Pseudomonas aeruginosa* with bla_{SPM-1}. The MALDI-TOF-MS hydrolysis assay using ertapenem detected 21 out of 29 resistant strains directly from blood culture, greatly shortening the time for carbapenem resistance detection. The eight remaining isolates, all of which were A. *baumannii*, were positive for carbapenemase activity by only testing bacterial colonies. This may be explained by the weak hydrolysis activity of the respective carbapenemases and probably the lower amount of bacteria obtained from the positive blood cultures and used for the hydrolysis assay. The high sensitivity and specificity of MALDI-TOF-MS carbapenamase detection directly from positive blood culture have also been reported by Hoyos-Mallecot *et al.*[25]

Although the MALDI-TOF-MS carbapenamase detection assay provides results within 2-4 h, the reduction in the time to obtain results remains an important issue. Lasserre *et al.* proposed shortening the time of carbapenemase detection by using an established cut-off value of mass spectra ratio of intensities, metabolite/(metabolite + intact imipenem).[26] A ratio of ≥0.82 discriminated carbapenemase-producing and noncarbapenemase-producing bacteria in <30 min with a sensitivity and specificity of 100%.

With the recent development of several rapid tests for the detection of carbapenemases, comparative studies including MALDI-TOF-MS and alternative rapid techniques have been published. Chong *et al.* compared MALDI-TOF-MS detection of carbapenemases with the Carba-NP assay.[27] In this study a wide range of carbapenemase-producing bacteria were tested, and MALDI-TOF-MS showed a higher sensitivity (99%) but a lower specificity (94%) compared to the Carba-NP assay. In another study MALDI-TOF-MS and Carba-NP assay demonstrated the same sensitivity (87%) and specificity (100%).[28] Imipenem was the antibiotic of choice for the MALDI-TOF-MS hydrolysis assay in both studies.

As mentioned above, in the last 4 years several studies have evaluated MALDI-TOF-MS for the detection of carbapenemases. Almost all of these studies presented differences in testing conditions, including the amount of bacteria used for the test, type of antibiotic, matrix solution, time of incubation, the criterion of positivity, etc. [Table 1]. In addition, the interpretation of antibiotic degradation was done by visual detection, thereby introducing subjectivity in result evaluation. This highlighted the need for assay standardization and automated data analysis.

DETECTION OF EXTENDED SPECTRUM B-LACTAMASES-PRODUCING BACTERIA

Another important application of MALDI-TOF-MS is the detection of ESBL-producing Gram-negative bacilli. The first report studying this possibility was published by Sparbier *et al.*[21] Since then most studies has been focused on the detection of carbapenemase-producing bacteria, since these strains represent an emerging public health problem. In 2014 Jung *et al.* used cefotaxime as the antibiotic of choice for the detection of ESBL-producing microorganisms.[29] The assay was performed directly with positive blood cultures and the cut-off value to interpret the strain as a β-lactamase producer was established separately for *Enterobacteriaceae* that constitutively expressed AmpC β-lactamases and those that typically express either chromosomally-or plasmid-encoded class A β-lactamases. Upon having determined the cut-off value, the validation of the assay was performed demonstrating 100% sensitivity for class A β-lactamases and a turnaround time of 2.5 h. Figure 3 shows the mass spectrum of cefotaxime alone or in combination with clavulanic acid after incubation with ESBL-producing and nonproducing strains after 2 h of incubation.

The accuracy of the detection of ESBL-producing bacteria from positive blood cultures was confirmed in another study, in which the authors performed visual detection of the hydrolysis product based on the appearance of the

Table 1: Comparison of the MALDI-TOF-MS hydrolysis assay parameters tested for the detection of carbapenemase activity among the different studies published

Assay parameters	Hrabák *et al.*	Burckhard *et al.*	Sparbier *et al.*	Kempf *et al.*	Lasserre *et al.*
Bacterial inoculum	8 McF	10 μL loop-sized amount	1 μL loop-sized amount	10 μL loop-sized amount	1 μL loop-sized amount
Antibiotic solution	50 μL meropenem (0.01-1 mM) in 20 mM Tris-HCl	Ertapenem (0.5 mg/mL) in 0.45% NaCl	Imipenem, ertapenem, and meropenem (0.5 mg/mL) in 10 mM ammonium hydrogen citrate	1 mL of imipenem in 0.45% NaCl (0.25-2 mg/mL)	20 μL imipenem (0.5 mg/mL) in water
Matrix solution	HCCA, DHB, DHAP	HCCA	HCCA	HCCA	HCCA
Maximum incubation time	3 h	2.5 h	3 h	4 h	30 min
Agitation	No	No	Yes	No	No
Carbapenemases tested	VIM, IMP, NDM, KPC	NDM, IMP, KPC, VIM	KPC	IMP, NDM, VIM, OXA-23, 24, 25, 58	IMI, IMP, KPC, NDM, NMCA, OXA-48 like, VIM
Instrument	Microflex LT mass spectrometer	Microflex LT mass spectrometer	Microflex LT mass spectrometer	Ultraflex I mass spectrometer	Microflex LT mass spectrometer
Interpretation of results	Disappearance of the peaks for meropenem and its adducts	Disappearance of the specific peak of ertapenem	Comparison of the intensities of distribution of the nonhydrolyzed and the hydrolyzed forms	Disappearance of the specific peak for imipenem The ratio between the peak for imipenem and its metabolite	The ratio metabolite/(metabolite+imipenem)

HCCA: α-cyano-4-hydroxycinnamic acid, DHB: 2,5-dihydroxybenzoic acid, DHAP: 2,5-dihydroxyacetophenone, MALDI-TOF-MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

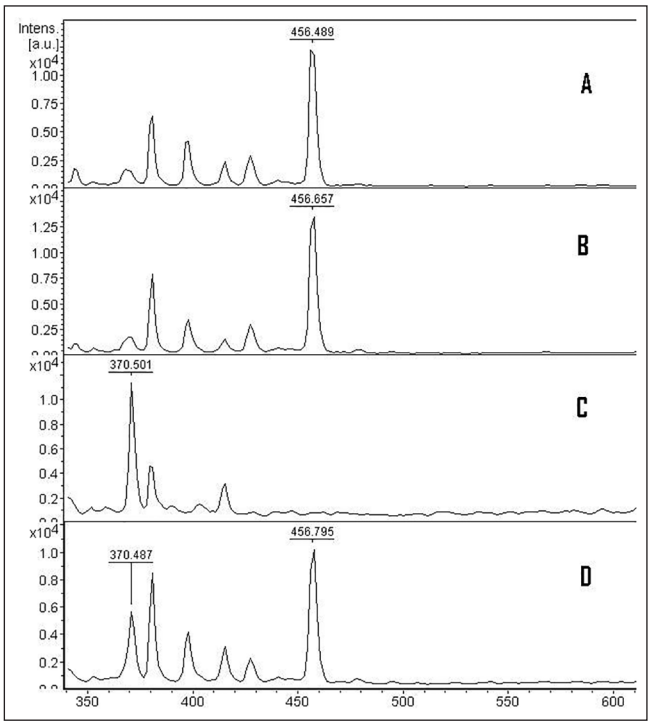

Figure 3: Mass spectra of the cefotaxime hydrolysis assay after 2 h of incubation — the spectrum after incubation of a nonextended spectrum β-lactamases-producing strain (a) with cefotaxime alone and (b) with cefotaxime plus clavulanic acid shows the peak of intact cefotaxime (m/z 456.6); (c) an extended spectrum β-lactamases-producing strain with cefotaxime alone - the peak of hydrolyzed cefotaxime (m/z 370.5) and (d) with cefotaxime plus clavulanic acid - a lower intensity peak of hydrolyzed cefotaxime (m/z 370.4) and the peak of intact cefotaxime (m/z 456.7) (inhibition of hydrolysis by clavulanic acid)

hydrolysis peaks[30] using cefotaxime and ceftazidime alone and in combination with clavulanic acid. The study showed 99% sensitivity for the detection of ESBL-producing bacteria from positive blood cultures.

Another approach to distinguish between ESBL-producing and nonproducing bacteria using MALDI-TOF-MS was proposed by Li *et al.*[31] These authors used ClinPro Tools software to generate algorithm models for rapid discrimination of sensitive and resistant strains. The rate of accuracy of the algorithms proposed varied from 82.4% to 88.2%.

CONCLUSION

MALDI-TOF-MS seems to be a rapid and accurate approach to detect β-lactamases. However, the main limitation of this technique is the lack of standardized assay conditions and special software for processing and automated analysis of mass spectra provided by the manufacturer. The first parameter to be chosen is bacterial inoculums for MALDI-TOF-MS, that could be achieved through McFarland suspension or using a calibrate loop. The test may be performed with an intact bacterial pellet or after bacterial lysis with lysozyme. Alternatively, SDS can be added to a reaction buffer to enhance hydrolysis. The next step is the selection of the antibiotic to be used: Ertapenem, meropenem or imipenem, for the detection of carbapenemase activity, and cefotaxime and/or ceftazidime for ESBL detection. The presence of spontaneous hydrolysis of some of the antibiotics used requires special attention when interpreting mass spectra. The bacteria tested can be resuspended in a variable amount of antibiotic solution: From 10 μL to 1 mL. The time of incubation is also variable and probably depends on the expected efficiency of β-lactamase to be detected. The introduction of agitation during incubation can also improve hydrolysis detection. Regarding sample preparation for MALDI-TOF-MS, different matrix solutions have been tested, including DHB, HCCA, and 2,5-dihydroxyacetophenone, diluted in a variety of solutions. The spectra were acquired manually or automatically depending on the study, and result interpretation also varied. Positivity for β-lactamase production was considered if the mass peak of intact antibiotic disappeared or decreased and/or the mass peaks of hydrolyzed products were present after incubation. Different ratios of intact antibiotic and their metabolites have also been used in several studies. Therefore, the implementation of MALDI-TOF-MS assay for the detection of β-lactamase activity currently requires the need to test and chose the optimal conditions to perform this test in each laboratory which could have a negative impact on the reproducibility and comparison of the results obtained. The other limitation of the MALDI-TOF-MS hydrolysis assay is that only one type of resistance mechanism is detected. For bacteria such as *Acinetobacter* spp. or *P. aeruginosa*, which frequently possess several resistance mechanisms, this method has limited usefulness since its results do not influence patient management and treatment.

Despite the limitations mentioned, MALDI-TOF-MS has important advantages, at least for the detection of β-lactamase activity among *Enterobacteriaceae*. First, this method is inexpensive and is relatively easy to perform. Second, the results are available within a few hours and this can potentially lead to adjustment in empirical treatment and may have an impact on the patients' outcome. In addition, this method can be directly applied to positive blood cultures without the need to wait for colony growth.

Financial support and sponsorship

This study was supported by the Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, co-financed by the European Regional Development Fund (ERDF) "A Way to Achieve Europe," the Spanish Network for Research in Infectious Diseases (REIPI RD12/0015), and by grant 2014SGR0653 from the Departament d'Universitats, Recerca i Societat de la Informació, of the Generalitat de Catalunya. Yuliya Zboromyrska was supported by the Hospital Clínic of Barcelona.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Welker M. Proteomics for routine identification of microorganisms. Proteomics 2011;11:3143-53.
2. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: A fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547-603.
3. Vila J, Juiz P, Salas C, Almela M, de la Fuente CG, Zboromyrska Y, *et al.* Identification of clinically relevant *Corynebacterium* spp. *Arcanobacterium haemolyticum*, and *Rhodococcus equi* by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:1745-7.
4. Tudó G, Monté MR, Vergara A, López A, Hurtado JC, Ferrer-Navarro M, *et al.* Implementation of MALDI-TOF MS technology for the identification of clinical isolates of *Mycobacterium* spp. in mycobacterial diagnosis. Eur J Clin Microbiol Infect Dis 2015;34:1527-32.
5. Pinto A, Halliday C, Zahra M, van Hal S, Olma T, Maszewska K, *et al.* Matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. PLoS One 2011;6:e25712.
6. Calderaro A, Arcangeletti MC, Rodighiero I, Buttrini M, Gorrini C, Motta F, *et al.* Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry applied to virus identification. Sci Rep 2014;4:6803.
7. Calderaro A, Piergianni M, Buttrini M, Montecchini S, Piccolo G, Gorrini C, *et al.* MALDI-TOF mass spectrometry for the detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar*. PLoS One 2015;10:e0122448.
8. La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One 2009;4:e8041.
9. Juiz PM, Almela M, Melcion C, Campo I, Esteban C, Pitart C, *et al.* A comparative study of two different methods of sample preparation for positive blood cultures for the rapid identification of bacteria using MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 2012;31:1353-8.
10. Ferreira L, Sanchez-Juanes F, Gonzalez-Avila M, Cembrero-Fucinos D, Herrero-Hernandez A, Gonzalez-Buitrago JM, *et al.* Direct identification of urinary tract pathogens from urine samples by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:2110-5.
11. Novais Â, Sousa C, de Dios Caballero J, Fernandez-Olmos A, Lopes J, Ramos H, *et al.* MALDI-TOF mass spectrometry as a tool for the discrimination of high-risk *Escherichia coli* clones from phylogenetic groups B2 (ST131) and D (ST69, ST405, ST393). Eur J Clin Microbiol Infect Dis 2014;33:1391-9.
12. Hrabák J, Chudácková E, Walková R. Matrix-assisted laser desorption ionization-time of flight (maldi-tof) mass spectrometry for detection of antibiotic resistance mechanisms: From research to routine diagnosis. Clin Microbiol Rev 2013;26:103-14.
13. Gagnaire J, Dauwalder O, Boisset S, Khau D, Freydière AM, Ader F, *et al.* Detection of *Staphylococcus aureus* delta-toxin production by whole-cell MALDI-TOF mass spectrometry. PLoS One 2012;7:e40660.
14. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, *et al.* Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
15. Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, *et al.* Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 2009;136: 1237-48.
16. Wybo I, De Bel A, Soetens O, Echahidi F, Vandoorslaer K, Van Cauwenbergh M, *et al.* Differentiation of cfiA-negative and cfiA-positive *Bacteroides fragilis* isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:1961-4.
17. Camara JE, Hays FA. Discrimination between wild-type and ampicillin-resistant *Escherichia coli* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal Bioanal Chem 2007;389:1633-8.
18. Hrabák J, Walková R, Studentová V, Chudácková E, Bergerová T. Carbapenemase activity detection by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:3222-7.
19. Hrabák J, Studentová V, Walková R, Zemlicková H, Jakubu V, Chudácková E, *et al.* Detection of NDM-1, VIM-1, KPC, OXA-48, and OXA-162 carbapenemases by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2012;50:2441-3.
20. Burckhardt I, Zimmermann S. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 hours. J Clin Microbiol 2011;49:3321-4.
21. Sparbier K, Schubert S, Weller U, Boogen C, Kostrzewa M. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against beta-lactam antibiotics. J Clin Microbiol 2012;50:927-37.
22. Kempf M, Bakour S, Flaudrops C, Berrazeg M, Brunel JM, Drissi M, *et al.* Rapid detection of carbapenem resistance in *Acinetobacter baumannii* using matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLoS One 2012;7:e31676.
23. Studentova V, Papagiannitsis CC, Izdebski R, Pfeifer Y, Chudackova E, Bergerova T, *et al.* Detection of OXA-48-type carbapenemase-producing *Enterobacteriaceae* in diagnostic laboratories can be enhanced by addition of bicarbonates to cultivation media or reaction buffers. Folia Microbiol (Praha) 2015;60:119-29.
24. Carvalhaes CG, Cayô R, Visconde MF, Barone T, Frigatto EA, Okamoto D, *et al.* Detection of carbapenemase activity directly from blood culture vials using MALDI-TOF MS: A quick answer for the right decision. J Antimicrob Chemother 2014;69:2132-6.
25. Hoyos-Mallecot Y, Riazzo C, Miranda-Casas C, Rojo-Martín MD, Gutiérrez-Fernández J, Navarro-Marí JM. Rapid detection and identification of strains carrying carbapenemases directly from positive blood cultures using MALDI-TOF MS. J Microbiol Methods 2014;105:98-101.
26. Lasserre C, De Saint Martin L, Cuzon G, Bogaerts P, Lamar E, Glupczynski Y, *et al.* Efficient detection of carbapenemase activity in *Enterobacteriaceae* by matrix-assisted laser desorption ionization-time of flight mass spectrometry in less than 30 minutes. J Clin Microbiol 2015;53:2163-71.
27. Chong PM, McCorrister SJ, Unger MS, Boyd DA, Mulvey MR, Westmacott GR. MALDI-TOF MS detection of carbapenemase activity in clinical isolates of *Enterobacteriaceae* spp. *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* compared against the Carba-NP assay. J Microbiol Methods 2015;111:21-3.
28. Knox J, Jadhav S, Sevior D, Agyekum A, Whipp M, Waring L, *et al.* Phenotypic detection of carbapenemase-producing *Enterobacteriaceae* by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry and the Carba NP test. J Clin Microbiol 2014;52:4075-7.
29. Jung JS, Popp C, Sparbier K, Lange C, Kostrzewa M, Schubert S. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid detection of ß-lactam resistance in *Enterobacteriaceae* derived from blood cultures. J Clin Microbiol 2014;52:924-30.
30. Oviaño M, Fernández B, Fernández A, Barba MJ, Mouriño C, Bou G. Rapid detection of *Enterobacteriaceae* producing extended spectrum beta-lactamases directly from positive blood cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Clin Microbiol Infect 2014;20:1146-57.
31. Li B, Guo T, Qu F, Li B, Wang H, Sun Z, *et al.* Matrix-assisted laser desorption ionization: Time of flight mass spectrometry-identified models for detection of ESBL-producing bacterial strains. Med Sci Monit Basic Res 2014;20:176-83.