Journal of Microbiological Methods 105 (2014) 98–101

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Note

Rapid detection and identification of strains carrying carbapenemases directly from positive blood cultures using MALDI-TOF MS

Y. Hoyos-Mallecot [a,b], C. Riazzo [a,*], C. Miranda-Casas [a], M.D. Rojo-Martín [a], J. Gutiérrez-Fernández [a,b], J.M. Navarro-Marí [a]

[a] Department of Microbiology, University Hospital Virgen de las Nieves, Granada, Spain
[b] Clinical Medicine and Public Health, Postgraduate programme, University of Granada, Granada, Spain

ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form 12 July 2014
Accepted 14 July 2014
Available online 23 July 2014

Keywords:
MALDI-TOF
Carbapenemases
Blood culture

ABSTRACT

MALDI-TOF MS has been evaluated to detect carbapenemases activity and pathogen identification directly from positive blood cultures. 21 non-carbapenemase producers and 19 carbapenemase producers Enterobacteriaceae and *Pseudomonas aeruginosa* strains were included in the study. This technique is simple and detects carbapenemases in 4.5 h with high sensitivity and specificity.

In the last decade we have observed a rapid increase of infections due to carbapenem resistant bacteria (Cantón et al., 2012), putting in danger our antibiotic options. Carbapenems resistance is often due either to the amount of processes like efflux pumps, porin loss and hyperproduction of enzymes, or to acquisition of genes encoding enzymes capable of hydrolysing carbapenems (carbapenemases) (Walsh et al., 2005).

The use of mass spectrometry for detection of carbapenemase activity seems to be generally accepted (Hrabák et al., 2013). Matrix-assisted laser desorption–ionization time of flight (MALDI-TOF) equipment is increasingly used in microbiological laboratory (Clark et al., 2013) making this approach a rapid and easy way to detect carbapenemases. Early detection of these enzymes responds mainly to epidemiological studies, but for bacteriemic patients, choosing an inappropriate antimicrobial therapy can considerably worsen their prognosis (Qureshi et al., 2012), justifying the need of an accurate and rapid test to detect carbapenemases producing strains directly from positive blood cultures.

The goal of the present investigation was to apply mass spectrometry directly from positive blood cultures in order to detect and identify carbapenemases carrying strains.

A panel of 20 non-carbapenemase producing Enterobacteriaceae (n = 10) and *P. aeruginosa* (n = 10), and 19 carbapenemase producing Enterobacteriaceae (n = 11, 3 IMP, 4 VIM, 1 KPC and 3 OXA 48) and *P. aeruginosa* (n = 8, 6 VIM and 2 IMP) clinical strains from our laboratory collection were included in the study, all of them not sensitive to at least one carbapenem. *Escherichia coli* ATCC 25922 was used as a carbapenemase negative control strain (Table 1). The presence of carbapenemases was established on the molecular level for bla_{IMP}, bla_{VIM} and bla_{KPC} genes, as previously described (Hoyos-Mallecot et al., 2014). Additionally, a PCR with specific primers for bla_{OXA-48} (Poirel et al., 2011) was performed for all the isolates.

For each strain, BACTEC™ Plus Aerobic/F culture vials were inoculated with 10 mL of fresh sterile blood and supplemented with 1 mL suspension containing 10–100 CFU (colony-forming unit) of an 18–24 hour culture. The inoculum was prepared by doing serial dilutions of 0.5 McFarland (McFarland, 1907) in sterile H_2O. Immediately after inoculation, the bottles were loaded into the BACTEC™ 9240 blood culture system (BD Diagnostic System, Sparks, MD) until positivity was detected (Positivity time: 9.89–19.43 h). The inoculum (CFU/mL) was always checked after positivity (Table 1).

Positive blood cultures were processed as follow (Fig. 1): 8 mL sample of positive broth was extracted and centrifuged at 600 *g* for 10 min in order to remove blood cells. The supernatant was collected and centrifuged at 14,000 *g* for 1 min, then the supernatant was removed and the pellet was washed twice using 1 mL of sterile water. Finally a last centrifugation at 13,200 *g* for 1 min was performed in a 1.5 mL tube in order to pellet bacteria. Once microorganisms were pelleted, carbapenem hydrolysis assay was performed as previously described (Hoyos-Mallecot et al., 2014); briefly we resuspended the pellet with 50 μL of a ertapenem solution 0.25 $mg \cdot mL^{-1}$ (Invanz, Merck Sharp&Dohme, NJ, USA) diluted in reaction buffer: 20 $mmol \cdot l^{-1}$ Tris-HCl, 0.01% sodium dodecyl sulfate [SDS], pH 7.0 (Sigma-Aldrich). This suspension was

* Corresponding author at: Department of Microbiology, University Hospital Virgen de las Nieves, 18014 Granada, Spain. Tel.: +34 958 020465.
E-mail address: riazzoc@gmail.com (C. Riazzo).

http://dx.doi.org/10.1016/j.mimet.2014.07.016
0167-7012/

Table 1
Summary of the pathogen identification and ertapenem hydrolysis assay data performed directly from positive blood cultures.

Bacterial species	Molecular assay	CLSI susceptibility interpretation (MIC)			MALDI-TOF MS Pathogen identification (score)	MALDI-TOF MS Hydrolysis assay[1]	Cfu/mL 10^8
		Meropenem	Ertapenem	Imipenem			
Escherichia coli ATCC	ND	S	S	S	*E. coli* (2191)	−	3
Enterobacter asburiae	−	ND	S (≤0.5)	I (2)	*Enterobacter kobei* (1.73)	−	2
Enterobacter cloacae	−	ND	S (≤0.5)	I (2)	*E. cloacae* (2.115)	−	2.5
E. cloacae	−	ND	S (≤0.5)	I (2)	*E. cloacae* (2.287)	+	5
E. cloacae	−	ND	R (2)	S (≤1)	*E. cloacae* (2.236)	−	8
E. coli	−	S (≤1)	R (2)	S (≤1)	*E. coli* (2.299)	−	5
E. coli	−	S (≤1)	I (1)	S (≤1)	*E. coli* (2.37)	+	5
E. coli	−	S (≤1)	I (1)	S (≤1)	*E. coli* (2.348)	−	10
Klebsiella pneumoniae	−	S (≤1)	S (≤0.5)	I (2)	*K. pneumoniae* (2.286)	−	9
K. pneumoniae	−	S (≤1)	R (2)	S (≤1)	*K. pneumoniae* (2.122)	−	7
K. pneumoniae	−	ND	S (≤0.5)	I (2)	*K. pneumoniae* (2.129)	−	10
Pseudomonas aeruginosa	−	R (8)	ND	R (>8)	*P. aeruginosa* (2.161)	−	1.5
P. aeruginosa	−	R (>8)	ND	R (>8)	*P. aeruginosa* (2.148)	−	4
P. aeruginosa	−	R (8)	ND	R (8)	*P. aeruginosa* (2.01)	−	3
P. aeruginosa	−	R (>8)	ND	R (>8)	*P. aeruginosa* (2.271)	−	4
P. aeruginosa	−	I (4)	ND	I (4)	*P. aeruginosa* (2.34)	−	2
P. aeruginosa	−	R (>8)	ND	I (4)	*P. aeruginosa* (2.277)	−	2.5
P. aeruginosa	−	I (4)	ND	R (>8)	*P. aeruginosa* (2.08)	−	2
P. aeruginosa	−	R (8)	ND	R (8)	*P. aeruginosa* (2.06)	−	2.5
P. aeruginosa	−	R (>8)	ND	R (>8)	*P. aeruginosa* (2.328)	−	0.9
P. aeruginosa	−	R (8)	ND	R (8)	*P. aeruginosa* (2.052)	−	1
E. cloacae	IMP	R (>32)	ND	R (>32)	*E. kobei* (2.329)	+	6
Klebsiella oxytoca	IMP	R (4)	R (8)	R (4)	*K. oxytoca* (2.323)	+	5
K. pneumoniae	IMP	R (>32)	R (8)	I (2)	*K. pneumoniae* (1.99)	+	3
P.aeruginosa	IMP	R (>32)	ND	R (>32)	*P. aeruginosa* (2.115)	+	3
P. aeruginosa	IMP	R (8)	ND	R (>32)	*P. aeruginosa* (2.22)	+	1.5
E. cloacae	VIM	I (2)	R (4)	I (2)	*E. kobei* (2.239)	+	5
E. cloacae	VIM	R (>32)	R (16)	R (>32)	*E. cloacae* (2.342)	+	5
E. cloacae	VIM	R (32)	R (4)	ND	*E. kobei* (2.245)	+	10
K. pneumoniae	VIM	R (>32)	R (>32)	R (>32)	*K. pneumoniae* (2.334)	+	5
P. aeruginosa	VIM	R (>32)	ND	R (>32)	*P. aeruginosa* (2.352)	+	1.5
P. aeruginosa	VIM	R (>32)	ND	R (>32)	*P. aeruginosa* (2.292)	+	6
P. aeruginosa	VIM	R (>32)	ND	R (>32)	*P. aeruginosa* (2.355)	+	1
P. aeruginosa	VIM	R (>32)	ND	R (>32)	*P. aeruginosa* (2.3)	+	1.8
P. aeruginosa	VIM	R (>32)	ND	R (>8)	*P. aeruginosa* (2.2)	+	1.5
P. aeruginosa	VIM	R (8)	ND	R (>32)	*P. aeruginosa* (2.289)	+	4
K. pneumoniae	OXA-48	I (2)	ND	R (>32)	*K. pneumoniae* (2.212)	+	7
K. pneumoniae	OXA-48	S (1)	ND	R (4)	*K. pneumoniae* (2.175)	+	4
K. pneumoniae	OXA-48	S (1)	ND	R (16)	*K. pneumoniae* (2.159)	+	4
K. pneumoniae	KPC	R (>32)	R (>32)	R (>32)	*K. pneumoniae* (2.106)	+	6

1 − Ertapenem hydrolysis assay performing incubation with 50 μL of ertapenem solution 0.25 $mg \cdot ml^{-1}$ (Invanz, Merck Sharp&Dohme, NJ, USA) diluted in reaction buffer (20 $mmol \cdot l^{-1}$ Tris-HCl, 0.01% sodium dodecyl sulfate [SDS], pH 7.0; Sigma-Aldrich).

incubated in agitation at 37 °C for 4 h, (for *E. coli* ATCC 25922 and *Klebsiella pneumoniae* KPC we also checked hydrolysis at 1 and 2 h). The tubes were then centrifuged for 2 min at 12,000 *g*. Finally 1 μL of the supernatant was placed directly onto a MALDI-TOF target plate and dried at room temperature, subsequently adding 1 μL of HCCA Matrix solution (10 mg mL^{-1} cyano-4-hydroxycinnamic acid) and drying.

Mass spectra were acquired with a Microflex LT mass spectrometer using FlexControl 3.3 software (Bruker Daltonics GmbH, Bremen, Germany), following the protocol described by Sparbier et al (Sparbier et al., 2012). For spectrum analysis the FlexAnalysis 3.3 program (Bruker Daltonics GmbH, Bremen, Germany) was used. Peaks were compared with the molecular masses of ertapenem ($[M + H]^+$ at 476.5 Da, $[M + Na]^+$ at 498.5 Da, $[M + 2Na]^+$ at 520.5 Da, and $[M_{hydr./decarb.} + H]^+$ at 450.5 Da) with a tolerance of 0.5 m/z.

Carbapenemase producers were established using the following criteria: absence of all ertapenem intact peaks ($[M + H]^+$ at 476.5 Da, $[M + Na]^+$ at 498.5 Da and $[M + 2Na]^+$ at 520.5 Da) and presence of peak corresponding to hydrolysed, decarboxylated form $[M_{hydr./decarb.} + H]^+$ at 450.5 Da, this peak has been described in both carbapenemase and non-carbapenemase producers when done directly from plated microorganisms (Burckhardt and Zimmermann, 2011; Lee et al., 2013). This peak in fact is not really useful for discrimination purpose, but its absence may reveal interferences between ertapenem and blood cells, so when the peak corresponding to $[M_{hydr./decarb.} + H]^+$ at 450.5 Da disappeared, the assay was classified as invalid (Fig. 2). This problem was observed in 2 of the 40 strains (Table 1), however when the hydrolysis assay was repeated, both strains could be classified correctly. Using these criteria, we only found 2 discrepancies between PCR and this technique corresponding to one *Enterobacter cloacae* and one *E. coli*, which were classified wrongly as carbapenemase producers because of over expression of AMPc as previously described (Burckhardt and Zimmermann, 2011).

Identification was performed after hydrolysis assay through an in plate extraction (Hoyos-Mallecot et al., 2013) using the remaining pellet of the last centrifugation. FlexControl 3.3 and Maldi Biotyper 3.0 software (Bruker Daltonics) were used for the analysis. Identification of bacteria to species level was consistent with conventional identification for all the strains tested (Table 1) with the exception of *Enterobacter* sp., which species differentiation in the *cloacae* complex was nonconcordant as previously described (Risch et al., 2010).

Using MALDI-TOF MS for bacteria identification directly from blood cultures represents an advance in the treatment of septic patients (Clerc et al., 2013). Nevertheless, the prediction of resistance patterns in bacteria is not possible just with pathogen identification. For this purpose, MALDI-TOF has been evaluated recently for detecting resistance against 3rd generation cephalosporins and aminopenicillins in *Enterobacteriaceae* (Jung et al., 2014).

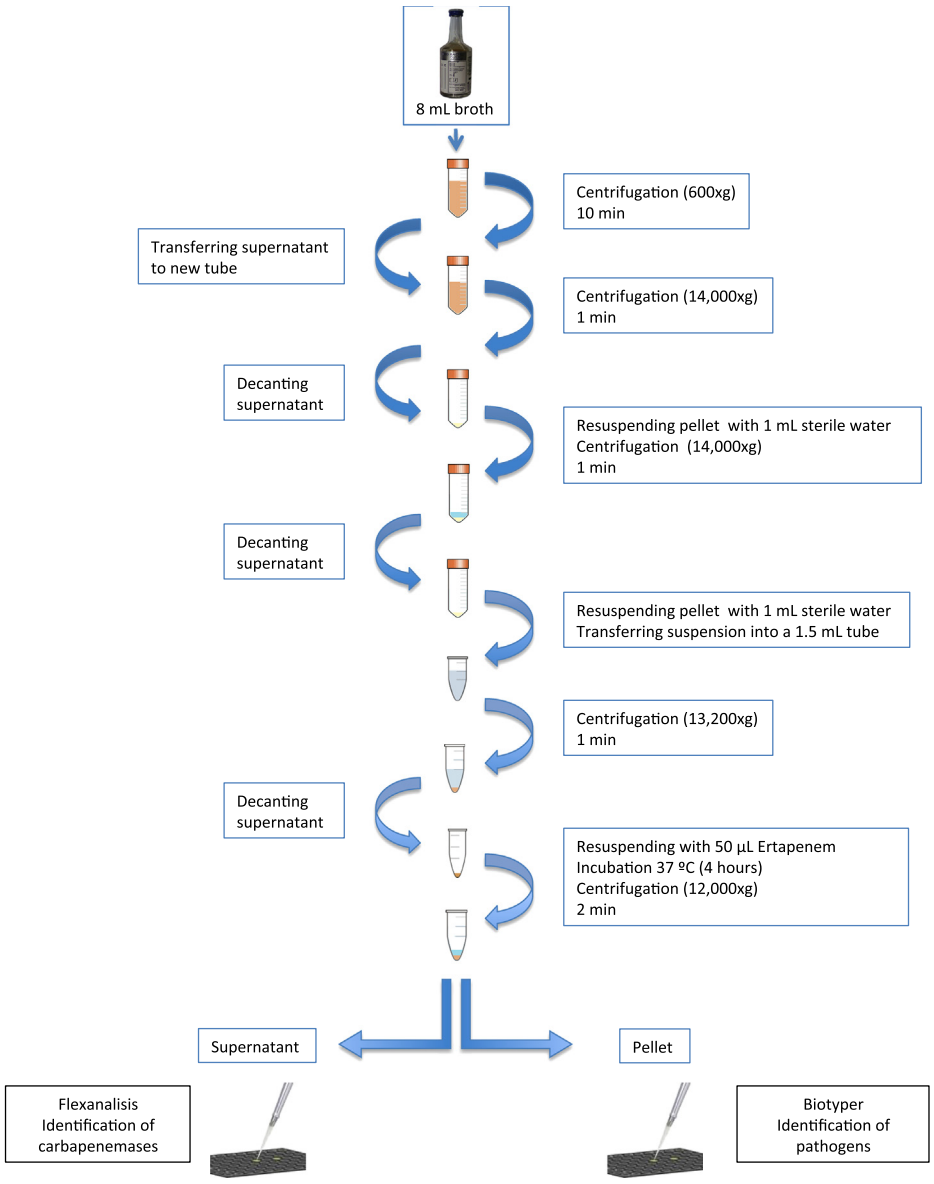

Fig. 1. Protocol for detection and identification of strains carrying carbapenemases directly from spiked blood cultures using MALDI-TOF MS.

When considering bacteriemia due to carbapenemase producers almost all β-lactams are not useful against these isolates. Therefore, having a short-term tool to predict the presence of carbapenemases would be very useful for an early and appropriated treatment. Both real-time PCR (Francis et al., 2012) and carba NP test (Dortet et al., 2013) have showed to be reliable techniques for rapid detection of carbapenemases directly from positive blood cultures. Our protocol not only detected carbapenemase producing strains with excellent values of sensitivity (100%) and specificity (90%), but also identified the pathogen involved in bacteriemia. Moreover, although we used a 4 h incubation time because it was suitable for our laboratory routine, this test can be performed even in less time as the process of pelleting microorganisms takes 20 min and the detection of hydrolysis could be as short as 1 h for KPC producing *K. pneumoniae* (data not shown), allowing carbapenemase detection and pathogen identification in a maximum of 4.5 h.

We conclude that this technique is simple, fast and reliable for detection of carbapenemases directly from positive blood cultures; however additional studies with more strains should be performed in order to confirm sensitivity and specificity.

References

Burckhardt, I., Zimmermann, S., 2011. Using matrix-assisted laser desorption ionization-time of flight mass spectrometry to detect carbapenem resistance within 1 to 2.5 h. J. Clin. Microbiol. 49, 3321–3324.

Cantón, R., Akóva, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M., Livermore, D.M., Miriagou, V., Naas, T., Rossolini, G.M., Samuelsen, Ø., Seifert, H., Woodford, N., Nordmann, P., European Network on Carbapenemases, 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin. Microbiol. Infect. 18, 413–431.

Clark, A.E., Kaleta, E.J., Arora, A., Wolk, D.M., 2013. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin. Microbiol. Rev. 26, 547–603.

Clerc, O., Prod'hom, G., Vogne, C., Bizzini, A., Calandra, T., Greub, G., 2013. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with Gram-negative bacteremia: a prospective observational study. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 56, 1101–1107.

Dortet, L., Bréchard, L., Poirel, L., Nordmann, P., 2013. Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures. Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis. 4, 340–344.

Francis, R.O., Wu, F., Della-Latta, P., Shi, J., Whittier, S., 2012. Rapid detection of Klebsiella pneumoniae carbapenemase genes in enterobacteriaceae directly from blood culture bottles by real-time PCR. Am. J. Clin. Pathol. 137, 627–632.

Hoyos-Mallecot, Y., Cabrera-Alvargonzalez, J.J., Miranda-Casas, C., Rojo-Martín, M.D., Liebana-Martos, C., Navarro-Marí, J.M., 2014. MALDI-TOF MS, a useful instrument

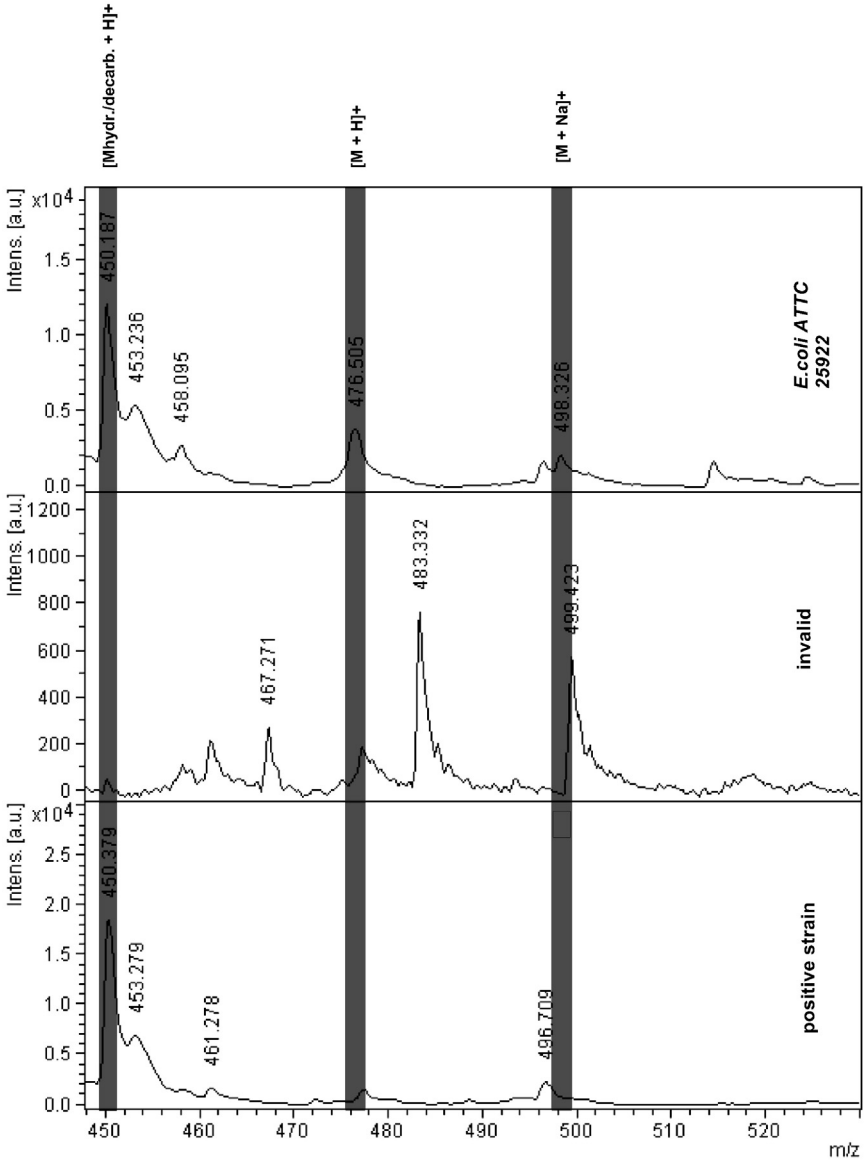

Fig. 2. Mass spectra of the ertapenem hydrolysis.

for differentiating metallo-β-lactamases in Enterobacteriaceae and *Pseudomonas* spp. Lett. Appl. Microbiol. 58 (4), 325–329.

Hoyos-Mallecot, Y., Miranda-Casas, C., Cabrera-Alvargonzalez, J.J., Gómez-Camarasa, C., Pérez-Ramirez, M.D., Navarro-Marí, J.M., 2013. Bacterial identification from blood cultures by a rapid matrix-assisted laser desorption-ionisation time-of-flight mass spectrometry technique. Enferm. Infecc. Microbiol. Clin. 31, 152–155.

Hrabák, J., Chudácková, E., Walková, R., 2013. 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. 26, 103–114.

Jung, J.S., Popp, C., Sparbier, K., Lange, C., Kostrzewa, M., Schubert, S., 2014. Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid detection of β-lactam resistance in enterobacteriaceae derived from blood cultures. J. Clin. Microbiol. 52 (3), 924–930.

Lee, W., Chung, H.-S., Lee, Y., Yong, D., Jeong, S.H., Lee, K., Chong, Y., 2013. Comparison of matrix-assisted laser desorption ionization-time-of-flight mass spectrometry assay with conventional methods for detection of IMP-6, VIM-2, NDM-1, SIM-1, KPC-1, OXA-23, and OXA-51 carbapenemase-producing *Acinetobacter* spp., *Pseudomonas aerug.* Diagn. Microbiol. Infect. Dis. 77 (3), 227–230.

McFarland, J., 1907. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index for vaccines. JAMA 49, 1176–1178.

Poirel, L., Walsh, T.R., Cuvillier, V., Nordmann, P., 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis. 70, 119–123.

Qureshi, Z.A., Paterson, D.L., Potoski, B.A., Kilayko, M.C., Sandovsky, G., Sordillo, E., Polsky, B., Adams-Haduch, J.M., Doi, Y., 2012. Treatment outcome of bacteriemia due to KPC-producing Klebsiella pneumoniae: superiority of combination antimicrobial regimens. Antimicrob. Agents Chemother. 56, 2108–2113.

Risch, M., Radjenovic, D., Han, J.N., Wydler, M., Nydegger, U., Risch, L., 2010. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. Swiss Med. Wkly. 140.

Sparbier, K., Schubert, S., Weller, U., Boogen, C., Kostrzewa, M., 2012. Matrix-assisted laser desorption ionization-time of flight mass spectrometry-based functional assay for rapid detection of resistance against β-lactam antibiotics. J. Clin. Microbiol. 50, 927–937.

Walsh, T.R., Toleman, M.A., Poirel, L., Nordmann, P., 2005. Metallo-beta-lactamases: the quiet before the storm? Clin. Microbiol. Rev. 18, 306–325.