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Original article

Evaluation of Vitek-MS™ and Microflex LT™ commercial systems for identification of *Acinetobacter calcoaceticus–baumannii* complex

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ABSTRACT

Introduction: Acinetobacter is a genus that comprises a group of opportunistic pathogens responsible for a variety of nosocomial infections. The *Acinetobacter calcoaceticus–Acinetobacter baumannii* (Acb) complex includes some species of clinical importance, mainly *A. baumannii*, *A. pittii* and *A. nosocomialis*, which share phenotypic similarities that make it very difficult to distinguish between them using a phenotypic approach. The aim of this study was to evaluate two commercial matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems for the identification of different *Acinetobacter* species, with a special focus among those belonging to the Acb complex.
Methods: One hundred and fifty-six *Acinetobacter* spp. clinical strains, identified by amplified ribosomal DNA restriction analysis (ARDRA) and *rpoB* gene sequencing, were analysed by two different MALDI-TOF systems.
Results: Considering only the 144 strains of the Acb complex evaluated in this study, the Vitek-MS™ and Microflex LT™ systems correctly identified 129 (89.6%) and 143 (99.3%) strains, respectively.
Conclusion: After analysing 156 strains belonging to *Acinetobacter* spp., both Vitek-MS™ and Microflex LT™ proved to be rapid and accurate systems for the identification of Acb complex species showing a good correlation. However, both manufacturers should improve their databases to include new species in them.

Evaluación de los sistemas comerciales Vitek® MS y Microflex® LT para la identificación del complejo *Acinetobacter calcoaceticus-baumannii*

Palabras clave:
MALDI-TOF MS
Espectrometría de masas
Acinetobacter spp.
Bacilos gramnegativos no fermentadores

RESUMEN

Introducción: Acinetobacter es un género que comprende un grupo de patógenos oportunistas responsables de varias infecciones nosocomiales. El complejo *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) reúne algunas especies de importancia clínica, principalmente *A. baumannii*, *A. pittii* y *A. nosocomialis*, que comparten similitudes fenotípicas que hacen muy difícil poder discriminar entre ellas utilizando un enfoque fenotípico.

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El objetivo de este estudio fue evaluar 2 sistemas comerciales de espectrometría de masas de ionización por láser asistido con una matriz (MALDI-TOF MS) para la identificación de diferentes especies de *Acinetobacter*, con un enfoque especial entre los que pertenecen al complejo Acb.
Métodos: Analizamos 156 cepas clínicas de *Acinetobacter* spp., identificadas mediante análisis de restricción de ADN ribosomal amplificado (ARDRA) y secuenciación del gen *rpoB,* por 2 sistemas diferentes de MALDI-TOF.
Resultados: Teniendo en cuenta solo las 144 cepas del complejo Acb evaluadas en este estudio, los sistemas Vitek® MS y Microflex® LT identificaron correctamente 129 (89,6%) y 143 (99,3%) cepas, respectivamente.
Conclusión: Después de analizar 156 cepas pertenecientes a *Acinetobacter* spp., Vitek® MS y Microflex® LT demostraron ser sistemas rápidos y precisos para la identificación de especies del complejo Acb mostrando una buena correlación. Sin embargo, ambos fabricantes deberían mejorar sus bases de datos incluyendo nuevas especies en ellas.

Introduction

Acinetobacter baumannii belongs to the *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex that includes the most relevant nosocomial pathogens of genus *Acinetobacter*, which cause serious infections in critically ill and immunocompromised patients.[1] *A. baumannii* represents a major healthcare problem, being responsible for infections with high morbidity and mortality rates, particularly in intensive care units (ICU), because of its ability to persist in the environment and develop resistance to multiple antibiotics.[2–4] *Acinetobacter nosocomialis* and *Acinetobacter pittii*, two other important species of Acb complex, are also able to cause nosocomial infections, but they are more rarely associated with outbreak situations and usually display greater susceptibility to antimicrobial agents. An accurate identification of these microorganisms at the species level is, therefore, essential to minimize treatment failure or implement infection control measures.[5,6]

Since all *Acinetobacter* species included in the Acb complex are highly similar from a phenotypic point of view, as well as by DNA-DNA hybridization,[7] simple phenotypic tests and other available automated biochemical identification systems routinely used in clinical laboratories cannot distinguish among them.[8,9] In this context, Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) is a powerful tool that has been adapted for the identification of different microorganisms at the genus, species or subspecies level with high reliability.[10,11] Nevertheless, some studies have also highlighted the struggles of MALDI-TOF MS for discrimination between members of the Acb complex as well as additional *Acinetobacter* species, particularly since many novel species have been recently included in this taxon.[12–14]

There are several previous studies concerning identification, detection of antimicrobial resistance, and virulence among *Acinetobacter* species by MALDI-TOF MS. However, only a few of them have focused on determining the accuracy of this technique at the species level identification.[12,15–17] In addition, none of such studies have focused on comparing the performance of the two commercial MALDI-TOF MS systems most commonly used in clinical laboratories for the identification of isolates included in the Acb complex.

Microflex LT™ distinguishes the different *Acinetobacter* genomic species of the Acb complex. Vitek-MS™ globally identifies the Acb complex by versions v1.0, v2.0, and v3.0, while v3.2 distinguishes some of the different *A.* genomic species of the Acb complex, according to the manufacture's specifications.

This study aimed to evaluate the reliability of two systems, Vitek-MS™ (bioMérieux, Marcy l'Étoile, France) and Microflex LT™ (Bruker Daltonik GmbH, Bremen, Germany) for the identification of *Acinetobacter* spp. isolates belonging to the Acb complex. We have also compared four Vitek-MS™ database versions (v1.0, v2.0, v3.0, and v3.2), with two versions of Microflex LT™ (v6.0.0.0 and v8.0.0.0), to evaluate the evolution of such databases over time. In the case of Vitek-MS™, the software was used in IVD (In Vitro Diagnostic) mode instead of RUO (Research Use Only) mode to resemble clinical practice.

Material and methods

Bacterial strains

Eighty-four *Acinetobacter* spp. clinical strains recovered between 2004 and 2008 in the Microbiology Service of the University Hospital Marqués de Valdecilla, Santander, Spain, were chosen. Clonal relatedness among those strains belonged to the same *Acinetobacter* species were evaluated by repetitive extragenic palindromic PCR (REP-PCR) and by pulsed-field gel electrophoresis (PFGE) as reported previously.[18] For the global study, a collection of 72 strains, from the Hospital Clinic in Barcelona, Spain, of less frequent species of the Acb complex, previously identified by *rpoB* gene sequencing,[19] was also added in the analysis.

Species identification was performed by amplified ribosomal DNA restriction analysis (ARDRA) and rpoB gene sequencing

ARDRA was used as the reference method to confirm the identification of *Acinetobacter* species. The 16S rRNA gene was amplified for all *Acinetobacter* spp. strains using universal primers, and further digested separately with the restriction enzymes CfoI, AluI, MboI, RsaI, MspI and BfaI (Promega Biotech Ibérica, Madrid, Spain), as previously described.[18,20]

Any disagreements in the species identification results between ARDRA and MALDI-TOF MS systems, were analyzed by partial *rpoB* gene sequencing.[19] In these cases, DNA from those strains showing discordant results was extracted by InstaGene™ Matrix (Bio-Rad Laboratories, Hercules, CA, USA). The *rpoB* gene was amplified using specific primers and purified using NucleoSpin® Gel Kit (Macherey-Nagel, Duren, Germany) and sequenced with ABI PRISM™ 377 DNA sequencer (Applied Biosystems, Foster City, USA). The partial *rpoB* gene sequences obtained were compared to reference sequences available on GenBank database using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). To establish the correct identification, sequences of a given pairwise alignment with the lowest *E*-value and the highest number of identities (>98%) were selected as the most likely species.

The statistical analysis was done with the IBM SPSS Version 20.0 software including the analysis of ROC curves and the calculation of Pearson's kappa index of concordance.

MALDI-TOF MS Microflex LT™ system

All strains were subjected to analysis with Microflex LT™ system (Bruker Daltonik GmbH, Bremen, Germany) following the manufacturer's recommendations. The bacterial biomass was smeared onto a metallic MALDI-TOF MSP 96 plate (Bruker Daltonik GmbH, Bremen, Germany) and covered with 1 μL of saturated α-cyano-4-hydroxycinnamic acid–50% acetonitrile–2.5% trifluoroacetic acid. The MSP 96 plate was dried at room temperature for 5 min. Measurements were performed with the Bruker Microflex LT MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) using FlexControl software with Compass Flex Series version 1.3 software and a 60-Hz nitrogen laser (337 nm wavelength). The protein spectra ranging from the mass-to-charge ratio (*m*/*z*) 2 to 20 kDa were explored with the Bruker Biotyper 3.1 software package (Bruker Daltonik GmbH, Bremen, Germany) with default settings and compared with the reference spectra present in the database, showing the 10 most similar patterns for each isolate. The database for identification was the reference Biotyper library v6.0.0.0 and v8.0.0.0 MSP (Bruker Daltonik GmbH, Bremen, Germany), which included 6.903 and 7.854 species, respectively. The similarity of patterns was represented as a score (2.3 to 3, identification at the species level; 2 to 2.3, identification at the genus level; <1.700, no reliable identification). *Escherichia coli* ATCC 25922 was used as a standard for calibration and as a reference for quality control.[17,21]

MALDI-TOF MS Vitek-MS™ system

Identification of strains with the Vitek-MS™ system (bioMérieux, France) was performed following manufacturer's recommendations. Cells from a single colony were directly applied onto the steel carrier overlaid with 1 μL of VITEK™ MS-CHCA ready-to-use-matrix solution (bioMérieux, France), and allowed to air-dry. Two samples of the same strain were applied and read in two rounds, as recommended. Measurement was performed with a Vitek-MS™ instrument supported by SARAMIS MS-IVD v1.0, v2.0, v3.0, and v3.2 databases (Anagnos Tee GMBH, BioMérieux) in positive linear mode, with a mass range of 2–20 kDa, using *E. coli* ATCC® 8739 as a molecular mass standard. The intensity of the 50-Hz nitrogen laser was under the control of the acquisition software. Only hits within the spectra with scores of 99.9% were accepted.

Results

A total of 144 *Acinetobacter* spp. strains belonging to Acb complex were evaluated, as follows: *A. pittii* (*n* = 54), *A. baumannii* (*n* = 47), *A. nosocomialis* (*n* = 16), *Acinetobacter dijkshoorniae* (formerly *A.* phenon 5 by ARDRA; *n* = 14), *Acinetobacter seifertii* (*n* = 12), and *Acinetobacter calcoaceticus* (*n* = 1). In addition, other 12 *Acinetobacter* spp. strains identified as belonging to other *Acinetobacter* species or genomic species (gen. sp.) not belonging to the Acb complex were also evaluated: *Acinetobacter bereziniae* (*n* = 5), *Acinetobacter* gen. sp. 16 (*n* = 1), *Acinetobacter gyllenbergii* (formerly *Acinetobacter* phenon 3 by ARDRA; *n* = 2), *Acinetobacter haemolyticus* (*n* = 2), and *Acinetobacter vivianii* (*n* = 2). In the case of the strains identified as *Acinetobacter* phenon 5 by ARDRA, they were reclassified as *A. dijkshoorniae* by *rpoB* gene sequencing.

Considering the 144 strains of the Acb complex evaluated in this study [*A. pittii* (*n* = 54), *A. baumannii* (*n* = 47), *A. nosocomialis* (*n* = 16), *A. dijkshoorniae* (*n* = 14), *A. seifertii* (*n* = 12) and *A. calcoaceticus* (*n* = 1)], Vitek-MS™ and Microflex LT™ systems correctly identified 129 (89.6%) and 143 (99.3%) strains, respectively. Two *A. baumannii* strains were misidentified as *A. nosocomialis* and *A. pittii* by Microflex LT™ v6.0 and v8.0, respectively. Since there is no reference spectrum for *A. dijkshoorniae* in the Vitek-MS™ v3.2 library, one of the strains of this species was misidentified as *Corynebacterium aurimucosum*, as well as one *A. nosocomialis* strain as *Burkholderia cenocepacia*.

In the non-Acb complex group, Vitek-MS™ v3.2 misidentified one *A. bereziniae* and one *Acinetobacter* gen. sp. 16 strains as *A. pittii* and *A. gyllenbergii*, respectively. In addition, for six strains of the control group (non-Acb complex), the obtained spectra were not included in the databases. Meanwhile, Microflex LT™ v8.0.0 misidentified one *A. bereziniae* as *A. pittii* (*n* = 1), two *A. vivianii* as *A. haemolyticus* and *A. haemolyticus/parvus* (*n* = 2), two *A. gyllenbergii* as *Acinetobacter proteolyticus* and *A. proteolyticus/haemolyticus* (*n* = 2), and one *Acinetobacter* gen. sp. 16 as *A. proteolyticus/haemolyticus* (Table 1). Vitek-MS™ v3.2 and Microflex LT™ showed a high sensitivity (>80%) and an area under the ROC curve (AUC) > 0.8, demonstrating that both systems are effective in discriminating the Acb complex from the other *Acinetobacter* species not belonging to the complex. On the other hand, according to the interpretation criteria proposed by Landis and Koch, the high positive Pearson Kappa index (>0.75) showed an excellent correlation with the identification data obtained by ARDRA plus *rpoB*. It is worth noting that Microflex LT™ v 8.0.0.0 showed the best results as a whole, comparing only the eighty-four *Acinetobacter* spp. strains from University Hospital Marqués de Valdecilla (Table 2).[22]

Table 1
Identification of *Acinetobacter* species with two MALDI-TOF MS systems.

ARDRA/*rpoB* seq N° strains	Species	Vitek-MS™ v3.2 N° strains	Species	Microflex LT™ v8.0.0.0 N° strains	Species
54	*A. pittii*	55	+(*A. bereziniae*)	57	*A. bereziniae*
47	*A. baumannii*	47	*A. baumannii*	46	−1(*A. pittii*)
16	*A. nosocomialis*	15	−1(*B. cenocepacia*)	16	*A. nosocomialis*
14	*A. dijkshoorniae*	0	−13(P150[a])−1(*C. aurimucosum*)	14	*A. dijkshoorniae*
12	*A. seifertii*	12	*A. seifertii*	12	*A. seifertii*
1	*A. calcoaceticus*	1	*A. calcoaceticus*	1	*A. calcoaceticus*
5	*A. bereziniae*	0	−4(P150[a])−1(*A. pittii*)	4	−1(*A. pittii*)
1	*A.* gen. sp. 16	0	−1(*A. gyllenbergii*)	0	−1(*A. proteolyticus/haemolyticus*)
2	*A. gyllenbergii*	3	+1(*A.* gen. sp. 16)	0	−2(*A. proteolyticus*, *A. proteolyticus/haemolyticus*)
2	*A. haemolyticus*	2	*A. haemolyticus*	2	*A. haemolyticus*
2	*A. vivianii*	0	−2(P150[a])	0	−2(*A. haemolyticus*, *A. haemolyticus/parvus*)
	Kappa		0.81		0.93
	Sensitivity		98.6%		100%
	AUC		0.94 (0.83–1)		0.99 (0.98–1)

[a] P150: no result in the database for this spectrum was obtained. In bold were marked those species identified by ARDRA or *rpoB* gene sequencing belonged to Acb complex.

Table 2
Comparison of *Acinetobacter* species belonging to the Acb complex, from University Hospital Marqués de Valdecilla, identified by different database versions of Vitek-MS™ and Microflex LT™ system.

Identification	Vitek-MS™				Microflex LT™	
	v.1.0	v.2.0	v.3.0	v.3.2	v6.0.0.0	v8.0.0.0
Acb complex	76	76	75	ni	ni	ni
A. baumannii	ni	ni	ni	33	32	32
A. calcoaceticus	ni	ni	ni	1	1	1
A. nosocomialis	–	–	–	–	1	0
A. pittii	ni	ni	ni	37	39	39
A. dijkshoorniae	ni	ni	ni	ni	ni	2
Total	76	76	75	71	73	74
Kappa index	–	–	–	0.78	0.77	0.81
Sensitivity	97.3%	96.1%	92.4%	98.6%	98.6%	100%
AUC	0.89 (0.74–1)	0.84 (0.67–1)	0.69 (0.48–0.9)	0.94 (0.83–1)	0.94 (0.83–1)	0.99 (0.97–1)

Abbreviations: ni, not identified; AUC, area under the ROC curve.

Discussion

MALDI-TOF MS is a good methodology for the routine activity in clinical microbiology laboratories, since it rapidly identifies a wide range of bacterial and fungal species of clinical importance. In this study, both Vitek-MS™ and Microflex LT™ systems allowed acceptable identification of Acb complex. Our results demonstrated that ARDRA is still a powerful method that remains valid in those laboratories lacking a MALDI-TOF MS system; in addition, it is also becoming more cost-effective than conventional phenotypic based automated systems.[23]

Previous publications have already underlined some of the limitations we have found in this study, including the need to add newly recognized species in databases,[12] which has been considered in the latest available versions, or the difficulty of differentiating species from the haemolytic clade, a situation improved by using an alternative matrix solution (strongly acidified ferulic acid).[13] Although both Vitek-MS™ and Microflex LT™ systems recognize strains from other less common taxa as belonging to the genus *Acinetobacter*, identification at the species level using MALDI-TOF MS is recommended but with due caution for this purpose. Microflex LT™ discrepancies have minimal to no clinical impact on patient's antibiotic treatment, but Vitek-MS™ discrepancies, on the other hand, would have an enormous clinical impact. The current database of Vitek-MS™ v3.2, should be revised, as already done with previous versions, to include the new *Acinetobacter* species to force the system to simply identify at genus level, which is also of interest in routine practice. More studies, testing in IVD mode with higher number of strains, are still necessary in order to improve the databases of both MALDI-TOF MS systems, since non-*A. baumannii* species are important emerging nosocomial pathogens able to acquire antimicrobial resistance genes from *A. baumannii*[24] and having an actual clinical importance.

Transparency declarations

All authors have nothing to declare. This study has not been financially supported by any Diagnostic/Pharmaceutical company.

Ethical approval

Not applicable.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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