Rapid and accurate identification of genomic species from the *Acinetobacter baumannii* (Ab) group by MALDI-TOF MS

P. Espinal[1], H. Seifert[2], L. Dijkshoorn[3], J. Vila[1] and I. Roca[1]

1) *Department of Clinical Microbiology, Hospital Clinic of Barcelona, School of Medicine, University of Barcelona, IDIBAPS, and Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain,* 2) *Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany and* 3) *Department of Infectious Diseases, Leiden University Medical Center, Leiden, the Netherlands*

Abstract

The closely related members of the *Acinetobacter baumannii* (Ab) group (*A. baumannii, A. pittii* and *A. nosocomialis*) are difficult to identify with phenotypic tests in diagnostic laboratories. Genotypic identification methods require special skills and most do not provide rapid results. The aim of this study was to investigate the ability of MALDI-TOF MS to identify members of the Ab group. Sixty epidemiologically unrelated *Acinetobacter* spp. isolates were investigated by MALDI-TOF MS: 18 *A. baumannii*, 17 *A. pittii*, 18 *A. nosocomialis* and seven additional isolates representing other *Acinetobacter* spp. All strains were verified by ARDRA, rRNA intergenic spacer (ITS), *rec*A sequencing and $bla_{OXA\text{-}51}$. MALDI-TOF MS correctly identified all the genomic strains but erroneously identified *A. nosocomialis* as *A. baumannii* because there was no reference strain within the Bruker database. Peak analysis of individual spectra from representative strains of each member of *A. baumannii*, *A. pittii* and *A. nosocomialis* suggested enough differences between their protein signatures to allow accurate identification using MALDI-TOF MS. Inclusion of specific signature profiles for *A. nosocomialis* within the Bruker database allowed the correct identification of this genomic species. MALDI-TOF MS spectra can be used as a fast, simple and reliable method to identify members of the Ab group. The rapid and accurate identification of clinically significant *Acinetobacter* strains will improve insight into their epidemiology and allow for targeted therapeutic and infection control measures against clinically important strains.

Keywords: *Acinetobacter*, ARDRA, ITS, MALDI-TOF MS, *rec*A, sequence typing
Original Submission: 8 July 2011; **Revised Submission:** 27 September 2011; **Accepted:** 8 October 2011
Editor: G. Greub
Article published online: 13 October 2011
Clin Microbiol Infect 2012; **18:** 1097–1103
10.1111/j.1469-0691.2011.03696.x

Corresponding author: J. Vila, Department of Clinical Microbiology, Hospital Clínic, Villaroel, 170, Barcelona, Spain
E-mail: jvila@ub.edu

Introduction

The *Acinetobacter* genus comprises Gram-negative non-fermenting coccobacilli with 25 validly named species and nine genomic species defined by genomic DNA-DNA hybridization [1]. Among these, *Acinetobacter baumannii* constitutes the most important species causing nosocomial infections, particularly in intensive care units (ICU) [2], although *A. pittii* and *A. nosocomialis* (formerly *Acinetobacter* genomic species 3 and gen. sp. 13TU, respectively [3]) are emerging as important pathogens and have been involved in a number of outbreaks in ICUs [1]. *A. baumannii*, *A. pittii* and *A. nosocomialis* as well as the environmental species *A. calcoaceticus* are highly similar from a phenotypic point of view as well as by DNA-DNA hybridization, which has led to their inclusion in the *A. calcoaceticus-A. baumannii* (Acb) complex [4]. In fact, the three clinically important members of this group, also known as the *A. baumannii* (Ab) group [2], are so much alike that they cannot be differentiated by currently available identification systems and *A. pittii* and *A. nosocomialis* are often erroneously identified as *A. baumannii* by routine commercial systems such as API and VITEK (bioMérieux, Marcy l'Etoile, France) or PHOENIX (Becton Dickinson, Franklin Lakes, NJ, USA.) [5,6].

Genotypic methodologies to distinguish individual genomic species have been developed, including amplified 16S ribosomal DNA restriction analysis (ARDRA) [7], tRNA spacer fingerprinting [8] and selective amplification of restriction fragments (AFLP™) [9]. Specific gene sequences can also be

used, including the intergenic spacer (ITS) region between the 16S–23S rRNA genes [10], *recA* [11], *rpoB* [12] and *gyrB* [13], although not all of these methods successfully discriminate among members of the Ab group. In addition, most of these methodologies are laborious, time-consuming, require special skills and are unsuitable for use in routine clinical identification. Failure to correctly identify clinical isolates below the Ab group level may be misleading because infections caused by the different species within this group may differ in their clinical outcome [14]. Organisms belonging to these three species display different characteristics regarding colonization of human skin, antimicrobial susceptibility and mortality rates [14,15]. Therefore, a more rapid and accurate identification strategy is needed.

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has been adapted for the identification of different microorganisms at the genus, species or subspecies level and has proven to be a very user-friendly technique requiring tiny amounts of sample yet providing accurate results within minutes. MALDI-TOF MS generates protein fingerprint signatures that can rapidly be compared with those in a database of reference spectra [16]. Although MALDI-TOF MS has been applied to the identification of non-fermenting bacteria, including some *Acinetobacter* species [17–19], it has never been tested with different members of the Ab group.

The present study was undertaken to evaluate the use of MALDI-TOF MS in the identification of members of the Ab group and to compare its discriminatory power with that of ARDRA, *recA* and ITS sequencing.

Materials and Methods

Bacterial isolates

Sixty epidemiologically unrelated *Acinetobacter* spp. isolates were included in the study: a set of known clinical isolates from our collection, 16 *A. pittii*, 16 *A. nosocomialis* and 13 *A. baumannii*; and a set of reference strains, RUH 204 (*A. junii*), RUH 509 (*A. pittii*), RUH 503 (*A. nosocomialis*), RUH 44 (*A. haemolyticus*), RUH 45 (*A. lwoffii*), RUH 3517 (*A. radioresistens*), RUH 584 (*A. calcoaceticus*), RUH 875 (*A. baumannii*, European clone I), RUH 134 (*A. baumannii*, European clone II), RUH 5875 (*A. baumannii*, European clone III), 17BJ-209 (*Acinetobacter* gen. sp. 17), *A. haemolyticus*-JV, *A. baumannii* AYE strain, *A. baumannii* ATCC 19606 and *A. nosocomialis* ATCC 17903.

Amplification of the ITS region, *recA* and bla_{OXA-51}

Amplification of the ITS region, *recA* and bla_{OXA-51} was performed according to Chang *et al.* [10], Nowak *et al.* [20] and Ruíz *et al.* [21], respectively. Purified PCR products were analysed by gel electrophoresis or sent for sequencing and compared among all sequences and reference strains.

ARDRA

The complete 16S rRNA gene was amplified as described in [7]. Aliquots of the PCR product were independently digested with AluI, CfoI, MboI, MspI and RsaI (Promega Biotech Ibérica, Madrid, Spain). Restriction patterns were analysed by gel electrophoresis in 2.5% (w/v) agarose and patterns were compared with the library profiles described in [7].

MALDI-TOF MS

MALDI-TOF was conducted on a Microflex LT (Bruker Daltonics GmbH, Leipzig, Germany) benchtop instrument operated in linear positive mode under control of the FlexControl 3.0 software (Bruker Daltonics) at a laser frequency of 20 Hz within a range mass from 2000 to 20000 Da.

Mass spectra were processed using FlexAnalysis 3.0 software (Bruker Daltonics). External calibration was performed using the Bruker Daltonics Bacterial Test standard according to the manufacturer's instructions.

Bacterial extracts. Pure cultures were grown on Columbia sheep blood agar (Becton Dickinson) at 37°C for 24 h. One full 1 μL sterile loop of bacterial sample was suspended in 300 μL of sterile water and mixed with 900 μL of absolute ethanol. Samples were centrifuged at 12 000 *g* for 2 min and the supernatant was discarded. The pellet was resuspended in 50 μL of 70% formic acid (Sigma chemical Co., St Louis, MO, USA) and 50 μL of acetonitrile (Sigma) and centrifuged at 12 000 *g* for 2 min. The supernatant was collected and stored at −20°C.

Generation of a local database. One microlitre of each bacterial extract from three strains each of *A. baumannii* (RUH 875, RUH 134, RUH 5875), *A. pittii* (52, 60, 69) and *A. nosocomialis* (95, 192, 212), was spotted eight times onto a ground steel target and air-dried. Each sample was overlaid with 1 μL of a saturated matrix solution of α-cyano-4-hydroxy-cinnamic acid (Bruker Daltonics) in 50% acetonitrile (Sigma), 2.5% trifluoroacetic acid (Sigma) and air-dried. Each spot was measured three times. Every measurement was the sum spectrum accumulated from 250 laser shots (5×50 laser shots on different locations according to a predefined lattice raster). The resulting 24 spectra were carefully analysed using the FlexAnalysis software to yield a minimum of 20 accurate spectra that were uploaded onto the MALDI BioTyper 2.0 software (Bruker Daltonics) to create a single