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Reporting identification of *Acinetobacter* spp genomic species: A nationwide proficiency study in Spain



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ABSTRACT

Acinetobacter baumannii is the most important genomic species of *Acinetobacter* from a clinical and epidemiological point of view. Nevertheless, genomic species other than *A. baumannii* are increasingly recognized as nosocomial pathogens. Molecular methods of identification (genotypic and proteomic assays) are more accurate and reliable and have greater discriminatory power than phenotypic methods.

Eleven genomic species of *Acinetobacter* spp. (8 *A. baumannii*, 1 *A. pittii*, 1 *A. nosocomialis* and 1 *A. lwoffii*) with different antimicrobial resistance phenotypes and mechanisms of resistance to antimicrobial agents were sent to 48 participating Spanish centers to evaluate their ability for correct identification at the genomic species level. Identification of the genomic species was performed at the two Clinical Microbiology reference laboratories (Hospital Universitario Virgen Macarena, Seville, Spain; and Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain) by partial DNA sequencing of the *rpoB* gene and MALDI-TOF. The mean percentage of agreement was 76.1%. Fifty percent of CC-01 (*A. pittii*) and 50% of CC-02 (*A. nosocomialis*) identification results were reported as *A. baumannii*. Discrepancies by type of systems used for identification were: MicroScan WA (51.1%), Vitek 2 (19.5%), MALDI-TOF (18.0%), Phoenix (4.5%), Wider (3.8%) and API 20 NE (3.0%). In conclusion, clinical microbiology laboratories must improve their ability to correctly identify the most prevalent non *A. baumannii* genomic species.

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Capacidad de los laboratorios españoles para identificar las geno-especies de *Acinetobacter* spp.: estudio nacional multicéntrico español

RESUMEN

Acinetobacter baumannii es la geno-especie de *Acinetobacter* más importante desde el punto de vista clínico y epidemiológico. No obstante, otras geno-especies distintas de *A. baumannii* están adquiriendo cada vez mayor importancia como patógeno nosocomial. Los métodos moleculares (genotípicos y proteómicos) usados para la identificación de geno-especies de *Acinetobacter* son más precisos, fiables, y tienen mayor poder de discriminación que los métodos fenotípicos.

Palabras clave:

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En este estudio se incluyen 11 genopecies de *Acinetobacter* spp. (8 *A. baumannii*, 1 *A. pittii*, 1 *A. nosocomialis* y 1 *A. lwoffii*) con diferentes fenotipos de resistencia y mecanismos de resistencia antimicrobiana. Los aislados se enviaron a 48 centros españoles participantes para evaluar su capacidad para identificar correctamente la geno especie de *Acinetobacter*. Las identificaciones de referencia se realizaron en 2 Laboratorios de Microbiología Clínica (Hospital Universitario Virgen Macarena, Sevilla, España; Complejo Hospitalario Universitario de A Coruña, A Coruña, España) mediante secuenciación parcial del gen *rpoB* y MALDI-TOF. El porcentaje medio de concordancia fue del 76,1%. El 50% de los resultados de identificación del control CC-01 (*A. pittii*) y el 50% de los resultados de identificación del control CC-02 (*A. nosocomialis*) se informaron como *A. baumannii*. Considerando el tipo de sistema de identificación usado las discrepancias fueron: MicroScan/WA (51,1%), Vitek 2 (19,5%), MALDI-TOF (18,0%), Phoenix (4,5%), Wider (3,8%) y API 20 NE (3,0%). En conclusión, los laboratorios españoles de microbiología clínica deben mejorar su capacidad para identificar correctamente las geno especies de *Acinetobacter* distintas de *A. baumannii* que son más prevalentes.

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Introduction

The *Acinetobacter* genus includes more than 30 genomic species, of which *A. baumannii* (genomic species 2) is the most important from a clinical and epidemiological point of view.¹ Nevertheless, other genomic species are increasingly recognized as nosocomial pathogens.²

Part of the success of this nosocomial pathogen, particularly *A. baumannii*, has been attributed to the ease with which it has adapted to the nosocomial environment and its facility for acquiring resistance to antimicrobials, including extended-spectrum antimicrobials like carbapenems, colistin and/or tigecycline.^{1–4}

Correct identification of some genomic species of *Acinetobacter* spp. may be difficult because of the increasing number of genomic species recognized in the last years and the phenotypic similarity among some of these genomics species (i.e. *A. calcoaceticus*–*A. baumannii* complex). The problem of misidentification may have important consequences for the management of infections and outbreaks caused by these microorganisms because there are significant differences between them in terms of their pathogenicity, antimicrobial resistance and ability to cause outbreaks.

Phenotypic methods used for identification usually have insufficient discriminatory power, particularly for genomic species included in the *A. calcoaceticus*–*A. baumannii* complex.^{5–7} Molecular methods however are more accurate and reliable and have greater discriminatory power than phenotypic methods, as has been observed for some genotypic assays (i.e. ARDRA analysis and sequencing of the 16S–23S rDNA, *rpoB* and *gyrB*) and proteomic assays (MALDI-TOF mass spectrometry).^{7–15}

The aim of this study was to evaluate the ability of clinical microbiology laboratories in Spain to correctly identify the most clinically relevant genomic species of *Acinetobacter* beyond assigning them to the *A. calcoaceticus*–*baumannii* complex.

Materials and methods

Eleven *Acinetobacter* spp. isolates, coded CC-01 to CC-11, were selected for this study. The panel included 9 clinical isolates (CC-01 to CC-03, CC-05 to CC-09 and CC-11) with different mechanisms of resistance to carbapenems, one laboratory-derived mutant resistant to colistin (CC-10) and the reference strain ATCC 17978 (CC-04) (Table 1)^{16–22}. Isolates were sent in Amies transport medium to 48 participating hospitals in May 2014. The instructions specified that isolates should be treated as blood culture isolates.

Identification of the isolates was verified independently at the two reference laboratories used in this study: the Clinical Microbiology Laboratory, Hospital Universitario Virgen Macarena, Seville,

Spain, and the Clinical Microbiology Laboratory, Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain. Identification to the genomic species level was performed by partial DNA sequencing of the *rpoB* gene and MALDI-TOF mass spectrometry.^{11,13} Participating laboratories were requested to the (i) genomic species identified and (ii) the laboratory method used for identification. Identification was considered as concordant when the result obtained in the participating center was identical to that obtained in the reference center. A result identified as belonging to the *A. calcoaceticus*–*baumannii* complex rather than being assigned to a specific genomic species was considered discordant.

Results

Five hundred and eleven results were obtained using just one of the following semiautomated identification systems: MALDI-TOF (Bruker Daltonics, Bremen, Germany; 36%), MicroScan WalkAway (Dade Beckman Coulter Inc., CA, USA; 36%), Vitek 2 (bioMérieux, Marcy-l'Étoile, France; 15%), Wider I (Francisco Soria Melguizo, Madrid, Spain, 4%), Api 20NE (bioMérieux, 4%), Phoenix (BD Biosciences, Sparks, MD, USA, 3%) and DNA sequencing by the Sanger method (2%). Fourteen identification results were obtained using 2 or more of these semiautomated systems.

Overall mean concordance was 76.1%. Regarding the type of system used, discrepant results were obtained with the MicroScan WA (51.1%), Vitek 2 (19.5%), MALDI-TOF (18.0%), Phoenix (4.5%), Wider (3.8%) and API 20 NE (3.0%) systems. No discrepancies were observed for laboratories using DNA sequencing. Respect to type of isolate, overall concordance per isolate was 87.5% (CC-04, CC-05, CC-06 and CC-07), 85.4% (CC-11), 83.3% (CC-03, CC-09 and CC-10), 81.3% (CC-08), 37.5% (CC-01) and 33.3% (CC-02). Fifty percent of identification results for CC-01 (*A. pittii*) and 50% of results for CC-02 (*A. nosocomialis*) were reported as *A. baumannii*.

Discussion

The genus *Acinetobacter* includes more than 30 genomic species, of which *A. baumannii* is the most important from a clinical and epidemiological point of view.¹ Nonetheless, non-*A. baumannii* genomic species are increasingly being recognized as nosocomial pathogens, probably due to their ability to cause outbreaks and acquire resistance to multiple antimicrobials as *A. baumannii*.²

Genomic species identification of *Acinetobacter* can be carried out using phenotypic methods, but they have the drawback of limited reliability or discriminatory power, particularly for the genomic species belonging to the *A. calcoaceticus*–*A. baumannii* complex.^{5–7} The problem of misidentification may have

Table 1Panel of *Acinetobacter* spp. reference isolates sent to participating laboratories.

Isolate	Genomic species	Relevant phenotype of antimicrobial resistance	Main mechanism of antimicrobial resistance	Source
CC-01	<i>A. pittii</i>	Resistant to carbapenems	OXA-58 carbapenemase	16
CC-02	<i>A. nosocomialis</i>	Susceptible	none (wild type)	4
CC-03	<i>A. lwoffii</i>	Susceptible	none (wild type)	4
CC-04	<i>A. baumannii</i>	Susceptible	none (wild type)	ATCC 17978
CC-05	<i>A. baumannii</i>	Resistant to cephalosporins	ESBL (CTX-M-15)	17
CC-06	<i>A. baumannii</i>	Resistant to carbapenems	OXA-24 carbapenemase	18
CC-07	<i>A. baumannii</i>	Resistant to carbapenems	IMP-type carbapenemase	19
CC-08	<i>A. baumannii</i>	Resistant to carbapenems	Hyperproduction of OXA-51, Omp 33–36 porin loss	20
CC-09	<i>A. baumannii</i>	Resistant to carbapenems	OXA-24 carbapenemase and carbapenem heteroresistance	21
CC-10	<i>A. baumannii</i>	Resistant to colistin	OXA-24 carbapenemase and pmrAB mutation	22
CC-11	<i>A. baumannii</i>	Resistant to tigecycline	OXA-24 carbapenemase and overexpression of <i>adeABC</i>	18

important consequences for the management of infections and outbreaks caused by these microorganisms. In our study, the rate of erroneous identification was rather high, reaching almost 24% of all identification determinations performed, mostly of *A. nosocomialis* and *A. pittii*, which, together with *A. baumannii* and *A. calcoaceticus*, belong to the *A. calcoaceticus*–*A. baumannii* complex, as reported in previous studies.¹³ This may reflect the current inability of phenotypic systems to correctly identify the separate genomic species belonging to this complex.

Another source of error was the type of commercial system used, particularly the MicroScan WalkAway and Vitek 2 systems. This finding highlights the importance of using more than one phenotypic system for optimal identification of *Acinetobacter* genomic species. Real-time PCR may be useful for rapidly identifying some, but not all, *Acinetobacter* species. MALDI-TOF represents a promising method for the rapid and practicable identification of most *Acinetobacter* species and may, in a few years, prove to be an alternative to genotypic methods, including those based on DNA sequencing.¹⁵

An analysis of factors that contribute to discrepancies in the identification of *Acinetobacter* species should be a priority for clinical laboratories. One way to address this problem is to participate in quality control programs, which can be very helpful for detecting potential laboratory problems, as observed for antimicrobial susceptibility testing,^{23,24} and enabling corrective measures to be established for optimizing the process and the quality of the reports offered to clinicians.

In conclusion, this study clearly shows that microbiology laboratories need to improve their ability to correctly identify *Acinetobacter* genomic species.

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Conflict of interest

None to declare.

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Cajal (Madrid, Madrid; María Isabel Morosini), Hospital Universitario Insular de Gran Canaria (Las Palmas de Gran Canaria, Las Palmas; Antonio Manuel Martín Sánchez), Hospital de Jerez (Jerez de la Frontera, Cádiz; M Dolores López Prieto), Hospital de la Ribera (Alzira, Valencia, Javier Colomina Rodríguez), Hospital Universitario Fundación Alcorcón (Alcorcón, Madrid; Alberto Delgado-Iribarren), Hospital San Pedro de Alcántara (Cáceres, Cáceres; Jesús Viñuelas Bayón), Hospital Universitario Vall d'Hebron (Barcelona, Barcelona; Rosa Juve Saumell), Hospital Universitario Virgen del Rocío (Sevilla, Sevilla; Javier Aznar Martín), Hospital General Universitario de Albacete (Albacete, Albacete; Eva Riquelme Bravo), Hospital Universitario de Getafe (Getafe, Madrid; David Molina Arana), Consorcio Hospital General Universitario de Valencia (Valencia, Valencia; Nuria Tormo), Hospital Universitario Severo Ochoa (Leganés, Madrid; Pilar Reyes Pecharrmán), Hospital Virgen de las Nieves (Granada, Granada; Consuelo Miranda Casas), Hospital Universitario Virgen de la Arrixaca (El Palmar, Murcia; Genoveva Yagüe).

References

1. Peleg AY, Breij A, Adams MD, Cerqueira GM, Mocali S, Galardini M, et al. The success of *Acinetobacter* species: genetic metabolic and virulence attributes. *PLoS ONE*. 2012;7:e46984.
2. Park KH, Shin JH, Lee SY, Kim SH, Jang MO, Kang SJ, et al. The clinical characteristics, carbapenem resistance, and outcome of *Acinetobacter* bacteremia according to genospecies. *PLoS ONE*. 2013;8:e65026.
3. Jawad A, Seifert H, Snelling AM, Heritage J, Hawkey PM. Survival of *Acinetobacter baumannii* on dry surfaces: comparison of outbreak and sporadic isolates. *J Clin Microbiol*. 1998;36:1938–41.
4. Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *J Hosp Infect*. 2012;80:56–60.
5. Bernards AT, van der Toorn J, van Boven CP, Dijkshoorn L. Evaluation of the ability of a commercial system to identify *Acinetobacter* genomic species. *Eur J Clin Microbiol Infect Dis*. 1996;15:303–8.
6. Zbinden A, Böttger EC, Bosshard PP, Zbinden R. Evaluation of the colorimetric VITEK 2 card for identification of gram-negative nonfermentative rods: comparison to 16S rRNA gene sequencing. *J Clin Microbiol*. 2007;45:2270–3.
7. McCarron AJ, Xu J, Armstrong C, Glynn G, Millar BC, McClurg RB, et al. Comparison of the identification of *Acinetobacter* spp. with API20NE and 16S rRNA gene sequencing techniques. *Br J Biomed Sci*. 2011;68:994–7.
8. Ehrenstein B, Bernards AT, Dijkshoorn L, Gerner-Smidt P, Towner KJ, Bouvet PJ, et al. *Acinetobacter* species identification by using tRNA spacer fingerprinting. *J Clin Microbiol*. 1996;34:2414–20.
9. Dijkshoorn L, Van Harsselaar B, Tjernberg I, Bouvet PJ, Vaneechoutte M. Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. *Syst Appl Microbiol*. 1998;21:33–9.
10. Zarrilli R, Giannouli M, di Popolo A, Tomasone F, Chu YW, Vaneechoutte M, et al. Identification of *Acinetobacter* genomic species 13TU by sequence analysis of the 16S-23S rRNA gene spacer region. *J Clin Microbiol*. 2009;47:1281–2.
11. Gundi VA, Dijkshoorn L, Burignat S, Raoult D, La Scola B. Validation of partial rpoB gene sequence analysis for the identification of clinically important and emerging *Acinetobacter* species. *Microbiology*. 2009;155:2333–41.
12. Lee MJ, Jang SJ, Li XM, Park G, Kook JK, Kim MJ, et al. Comparison of rpoB gene sequencing, 16S rRNA gene sequencing, gyrB multiplex PCR, and the VITEK2 system for identification of *Acinetobacter* clinical isolates. *Diagn Microbiol Infect Dis*. 2014;78:29–34.
13. Espinal P, Seifert H, Dijkshoorn L, Vila J, Roca I. Rapid and accurate identification of genomic species from the *Acinetobacter baumannii* (Ab) group by MALDI-TOF MS. *Clin Microbiol Infect*. 2012;18:1097–103.
14. Kishii K, Kikuchi K, Matsuda N, Yoshida A, Okuzumi K, Uetara Y, et al. Evaluation of matrix-assisted laser desorption/ionization-time of flight mass spectrometry for species identification of *Acinetobacter* strains isolated from blood cultures. *Clin Microbiol Infect*. 2014;20:424–30.
15. Mari-Almíral M, Cosgaya C, Higgins PG, Van Assche A, Telli M, Huys G, et al. MALDI-TOF/MS identification of species from the *Acinetobacter baumannii* (Ab) group revisited: inclusion of the novel *A. seifertii* and *A. dijkshoorniae* species. *Clin Microbiol Infect*. 2017;23, 210.e1–210.e9.
16. Marti S, Sánchez-Céspedes J, Blasco MD, Ruiz M, Espinal P, Alba V, et al. Characterization of the carbapenem-hydrolyzing oxacillinase oxa-58 in an *Acinetobacter* genospecies 3 clinical isolate. *Antimicrob Agents Chemother*. 2008;52:2955–8.
17. Beceiro A, Fernández-Cuenca F, Ribera A, Martínez-Martínez L, Pascual A, Vila J, et al. Spanish Group for Nosocomial Infection (GEIH). False extended-spectrum beta-lactamase detection in *Acinetobacter* spp. due to intrinsic susceptibility to clavulanic acid. *J Antimicrob Chemother*. 2008;61:301–8.
18. Rumbo C, Gato E, López M, Ruiz de Alegría C, Fernández-Cuenca F, Martínez-Martínez L, et al. Spanish Group of Nosocomial Infections and Mechanisms of Action and Resistance to Antimicrobials (GEIH-GEMARA); Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC); Spanish Network for Research in Infectious Diseases (REIPI). Contribution of efflux pumps, porins, and β -lactamases to multidrug resistance in clinical isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2013;57:5247–57.
19. Cornaglia G, Riccio ML, Mazzariol A, Lauretti L, Fontana R, Rossolini GM. Appearance of IMP-1 metallo- β -lactamase in Europe. *Lancet*. 1999;353:899–900.
20. Mar Tomás M, Beceiro A, Pérez A, Velasco D, Moure R, Villanueva R, et al. Cloning and functional analysis of the gene encoding the 33- to 36-kilodalton outer membrane protein associated with carbapenem resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2005;49:5172–5.
21. Fernández-Cuenca F, Tomás-Carmona M, Caballero-Moyano F, Bou G, Martínez-Martínez L, Vila J, et al., grupo del proyecto GEIH-REIPI-Ab 2010. In vitro activity of 18 antimicrobial agents against clinical isolates of *Acinetobacter* spp.: multicenter national study GEIH-REIPI-Ab 2010. *Enferm Infecc Microbiol Clin*. 2013;31:4–9.
22. Beceiro A, Moreno A, Fernández N, Vallejo JA, Aranda J, Adler B, et al. Biological cost and impact on virulence of different mechanisms of colistin resistance in *Acinetobacter baumannii*. *Antimicrob Agents Chemother*. 2013;58:518–26.
23. Jones RN, Glick T, Sader HS, Flamm RK, Ross JE, Rhomberg PR, et al. Educational antimicrobial susceptibility testing as a critical component of microbiology laboratory proficiency programs: American Proficiency Institute results for 2007–2011. *Diagn Microbiol Infect Dis*. 2013;75:357–60.
24. Fernández Cuenca F, Tomás M, Caballero Moyano FJ, Bou G, Pascual A. Reporting antimicrobial susceptibilities and resistance phenotypes in *Acinetobacter* spp: a nationwide proficiency study. *J Antimicrob Chemother*. 2017. <http://dx.doi.org/10.1093/jac/dkx464>.