AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Genomic Evolution of Two *Acinetobacter baumannii* Clinical Strains from ST-2 Clones Isolated in 2000 and 2010 (ST-2_clon_2000 and ST-2_clon_2010)

M. López,[a,h] A. Rueda,[b] J. P. Florido,[b] L. Blasco,[a] E. Gato,[a,h] L. Fernández-García,[a] L. Martínez-Martínez,[c,d,h] F. Fernández-Cuenca,[e,h] J. Pachón,[f,h] J. M. Cisneros,[f,h] J. Garnacho-Montero,[e,g] J. Vila,[g,h] J. Rodríguez-Baño,[e,h] A. Pascual,[e,h] G. Bou,[a,h] M. Tomás[a,h] on behalf of the Spanish Group of Nosocomial Infections and Mechanisms of Action and Resistance to Antimicrobials (GEIH-GEMARA) from the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) and the Spanish Network for Research in Infectious Diseases (REIPI)

Department of Microbiology, Complejo Hospitalario Universitario, A Coruña, INIBIC, La Coruña, Spain[a]; Bioinformatics, GBPA, Seville, Spain[b]; Department of Clinical Microbiology, Hospital Universitario Marqués de Valdecilla-IFIMAV, Santander, Spain[c]; Departament of Molecular Biology, University of Cantabria, Santander, Spain[d]; Unidad Intercentros de Enfermedades Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario Virgen Macarena, Seville, Spain[e]; Institute of Biomedicine of Seville (IBiS), University Hospital Virgen del Rocío/CSIC/University of Seville, Seville, Spain[f]; Department of Clinical Microbiology, Hospital Clínic, Barcelona School of Medicine, University of Barcelona, Barcelona and ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic, Barcelona, Spain[g]; Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015), Seville, Spain[h]

M.L. and A.R. contributed equally to this work.

***Acinetobacter baumannii* is a successful nosocomial pathogen due to its ability to persist in hospital environments by acquiring mobile elements such as transposons, plasmids, and phages. In this study, we compared two genomes of *A. baumannii* clinical strains isolated in 2000 (ST-2_clon_2000) and 2010 (ST-2_clon_2010) from GenBank project PRJNA308422.**

Received 30 August 2016 **Accepted** 31 August 2016 **Published** 20 October 2016

Citation López M, Rueda A, Florido JP, Blasco L, Gato E, Fernández-García L, Martínez-Martínez L, Fernández-Cuenca F, Pachón J, Cisneros JM, Garnacho-Montero J, Vila J, Rodríguez-Baño J, Pascual A, Bou G, Tomás M, Spanish Group of Nosocomial Infections and Mechanisms of Action and Resistance to Antimicrobials (GEIH-GEMARA) from the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC) and the Spanish Network for Research in Infectious Diseases (REIPI). 2016. Genomic evolution of two *Acinetobacter baumannii* clinical strains from ST-2 clones isolated in 2000 and 2010 (ST-2_clon_2000 and ST-2_clon_2010). Genome Announc 4(5):e01182-16. doi:10.1128/genomeA.01182-16.

Address correspondence to M. Tomás, ma.del.mar.tomas.carmona@sergas.es.

Acinetobacter baumannii is a successful nosocomial pathogen, especially in intensive care units (ICUs) (1, 2). This is due to the pathogen's ability to persist in the hospital environment for long periods of time by acquiring mobile genomic elements (transposons, plasmids, and phages) that are the main driving forces for the genome (3).

In this study, we sequenced two *A. baumannii* clinical strains (ST-2_clon_2000 and ST-2_clon_2010) from the GEIH-REIPI Spanish Multicenter *Acinetobacter baumannii* Study II (2000–2010) (GenBank accession no. PRJNA308422). Strain ST-2_clon_2000 (susceptible to carbapenems) compared with ST-2_clon_2010 (resistant to carbapenems) showed a clonal relation of 90% by pulsed-field gel electrophoresis, after a decade in the hospital environment (in the same ICU).

Next-generation sequencing of both strains was performed with a Roche 454 GS FLX+ sequencer according to the manufacturer's instructions (Roche 454 Life Sciences, Branford, CT, USA). Reads were assembled with Newbler. Putative open reading frames were predicted from assembled contigs with GeneMarKS (4), previously trained with the *A. baumannii* genome (GI: 83207914). Functional annotation of each predicted protein was carried out with Blast2Go (5) and RAST (6). The rRNA and tRNA were identified using RNAmmer (7) and tRNAscan-SE version 1.21 (8). The genomes of ST-2_clon_2000 and ST-2_clon_2010 were compared using Mauve (9).

ST-2_clon_2000 read assemblies generated 64 contigs, accounting for a size of 3,991,758 bp (mean coverage of 36.49-fold). BLAST analysis showed that 62 contigs were of chromosomal origin (3,922,379 bp) and that two contigs were plasmidic sequences with high similarity to *A. baumannii* TCDC-AB0715 plasmid p2ABTCDC0715 (6,650 bp). Assembly of the ST-2_clon_2010 reads produced 77 contigs representing 4,092,613 bp (mean coverage of 40.38-fold). BLAST similarity searches revealed that 74 contigs were part of the genomic chromosome (4,013,020 bp), two contigs were plasmidic with high similarity to *A. baumannii* TCDC-AB0715 plasmid p2ABTCDC0715 (70,004 bp), and one contig was a whole plasmid with very high similarity to *A. baumannii* pMMCU3 plasmid with the bla_{OXA-24} gene and AbkA/AbkB toxin/antitoxin system) (10, 11).

Annotation of strain ST-2_clon_2000 predicted 3,759 protein-coding sequences, 5S, 16S, and 23S rRNA genes, and 63 tRNAs, whereas annotation of ST-2_clon_2010 predicted 3,923 protein-coding sequences, 5S, 16S, and 23S rRNA genes, and 63 tRNAs.

Comparison of chromosomal sequences of strains ST-2_clon_2000 and ST-2_clon_2010 revealed the following: (i) 3,627 proteins were identical in both strains; (ii) 88 proteins were very similar; (iii) five proteins shared a similarity of less than 60%; (iv) 20 proteins were unique to ST-2_clon_2000; (v) 212 proteins were only present in ST-2_clon_2010, most of which were similar to

phage proteins; and (vi) the chromosome of strain ST-2_clon_2010 contains several bacteriophage sequences.

Finally, comparison of plasmidic sequences indicated the following: (i) 90 proteins were identical in both strains; (ii) three proteins showed a high degree of similarity (>60% similarity); (iii) 17 plasmidic proteins were found only in ST-2_clon_2010 (pMMCU3 harboring the bla_{OXA-24} gene and AbkA/AbkB toxin/antitoxin system) (10, 11); and (iv) ST-2_clon_2000 did not share any plasmidic proteins with ST-2_clon_2010.

Accession number(s). The ST-2_clon_2000 whole-genome shotgun (WGS) project has been deposited in DDBJ/ENA/GenBank under the accession number LJHA00000000. The version described in this paper is version LJHA01000000 and consists of sequences LJHA01000001 to LJHA01000064. The ST-2_clon_2010 WGS project has been deposited in DDBJ/ENA/GenBank under the accession number LJHB00000000. The version described in this paper is version LJHB01000000 and consists of sequences LJHB01000001 to LJHB01000077. Both WGS studies belong to the GEIH-REIPI Spanish Multicenter *Acinetobacter baumannii* Study II 2000–2010 project PRJNA308422.

FUNDING INFORMATION

This study was funded by grants PI10/00056 and PI13/02390 awarded to M. Tomás within the State Plan for R+D+I 2013-2016 (National Plan for Scientific Research, Technological Development and Innovation 2008-2011). It was cofinanced by the ISCIII-Deputy General Directorate of Evaluation and Promotion of Research-European Regional Development Fund "A Way of Making Europe" and the Instituto de Salud Carlos III FEDER, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015). M. Tomás was financially supported by the Miguel Servet Research Programme (C.H.U.A. Coruña and ISCIII).

REFERENCES

1. **del Mar Tomas M, Cartelle M, Pertega S, Beceiro A, Llinares P, Canle D, Molina F, Villanueva R, Cisneros JM, Bou G.** 2005. Hospital outbreak caused by a carbapenem-resistant strain of *Acinetobacter baumannii*: patient prognosis and risk-factors for colonisation and infection. Clin Microbiol Infect **11:**540–546. http://dx.doi.org/10.1111/j.1469-0691.2005.01184.x.
2. **Fournier PE, Richet H.** 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis **42:**692–699. http://dx.doi.org/10.1086/500202.
3. **Dijkshoorn L, Nemec A, Seifert H.** 2007. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol **5:**939–951. http://dx.doi.org/10.1038/nrmicro1789.
4. **Lukashin AV, Borodovsky M.** 1998. GeneMark.Hmm: new solutions for gene finding. Nucleic Acids Res **26:**1107–1115. http://dx.doi.org/10.1093/nar/26.4.1107.
5. **Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M.** 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics **21:**3674–3676. http://dx.doi.org/10.1093/bioinformatics/bti610.
6. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9:**75. http://dx.doi.org/10.1186/1471-2164-9-75.
7. **Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW.** 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res **35:**3100–3108. http://dx.doi.org/10.1093/nar/gkm160.
8. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res **25:**955–964. http://dx.doi.org/10.1093/nar/25.5.0955.
9. **Darling AC, Mau B, Blattner FR, Perna NT.** 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res **14:**1394–1403. http://dx.doi.org/10.1101/gr.2289704.
10. **Mosqueda N, Gato E, Roca I, López M, de Alegría CR, Fernández Cuenca F, Martínez-Martínez L, Pachón J, Cisneros JM, Rodríguez-Baño J, Pascual A, Vila J, Bou G, Tomás M, GEIH-GEMARA (SEIMC) and REIPI.** 2014. Characterization of plasmids carrying the blaOXA-24/40 carbapenemase gene and the genes encoding the AbkA/AbkB proteins of a toxin/antitoxin system. J Antimicrob Chemother **69:**2629–2633. http://dx.doi.org/10.1093/jac/dku179.
11. **Merino M, Acosta J, Poza M, Sanz F, Beceiro A, Chaves F, Bou G.** 2010. OXA-24 carbapenemase gene flanked by XerC/XerD-like recombination sites in different plasmids from different *Acinetobacter* species isolated during a nosocomial outbreak. Antimicrob Agents Chemother **54:**2724–2727. http://dx.doi.org/10.1128/AAC.01674-09.