

Draft Genome Sequence of *Acinetobacter bereziniae* HPC229, a Carbapenem-Resistant Clinical Strain from Argentina Harboring *bla*_{NDM-1}

Marco Brovedan,^a Patricia M. Marchiaro,^a Jorgelina Morán-Barrio,^a Santiago Revale,^b Marcela Cameranesi,^a Luciano Brambilla,^a Alejandro M. Viale,^a Adriana S. Limansky^a

Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina^a; Instituto de Agrobiotecnología Rosario (INDEAR), Rosario, Argentina^b

We report here the draft genome sequence of an NDM-1-producing *Acinetobacter bereziniae* clinical strain, HPC229. This strain harbors both plasmid and chromosomal resistance determinants toward different β -lactams and aminoglycosides as well as several types of multidrug efflux pumps, most likely representing an adaptation strategy for survival under different environments.

Received 26 January 2016 Accepted 29 January 2016 Published 10 March 2016

Citation Brovedan M, Marchiaro PM, Morán-Barrio J, Revale S, Cameranesi M, Brambilla L, Viale AM, Limansky AS. 2016. Draft genome sequence of *Acinetobacter bereziniae* HPC229, a carbapenem-resistant clinical strain from Argentina harboring *bla*_{NDM-1}. *Genome Announc* 4(2):e00117-16. doi:10.1128/genomeA.00117-16.

Copyright © 2016 Brovedan et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Adriana S. Limansky, limansky@ibr-conicet.gov.ar.

Acinetobacter bereziniae is isolated primarily from clinical specimens and health care-associated environments (1). Although most *A. bereziniae* isolates are susceptible to antimicrobials (2), clinical strains bearing metallo-beta-lactamase genes have just been reported (3–6). We recently described the sequence of a *bla*_{NDM-1}-harboring plasmid (pNDM229, KT072713.1) carried by a carbapenem-resistant *A. bereziniae* strain (HPC229) from Argentina (4). We report here the whole-genome sequencing (WGS) of HPC229, which constitutes the sixth *A. bereziniae* genome in databases. Genes involved in β -lactam and aminoglycoside resistance were found in the HPC229 genome. Moreover, genes involved in different efflux pump systems associated with resistance to antimicrobials and toxic compounds were identified (7, 8). These findings support the notion that *A. bereziniae* represents an environmental reservoir of resistance genes of clinical relevance.

HPC229 DNA was prepared using the Wizard genomic DNA purification kit (Promega) and subjected to 454 pyrosequencing (Roche Diagnostics Corporation) at INDEAR, Rosario. Data generated were assembled using Newbler v2.9, resulting in 134 contigs, 5 of them constituting the pNDM229 plasmid (4). The remaining 129 contigs have a length of 4,596,631 bp and a G+C content of 38.00%. Genome annotation was done using the NCBI Prokaryotic Genomes Annotation Pipeline (9), and eight contigs were left out for being shorter than 200 bp. The RAST server was employed for subsystem classification and functional annotation (10) and then the genome was manually curated. Complementary gene identification analyses were done using Mauve (11), ISFinder (12), Res-Finder 2.1 (13), and TCDB (14). A total of 4,124 protein-coding sequences, 4 rRNAs, and 62 tRNA genes were predicted by these analyses.

WGS analyses revealed genes associated with β -lactam resistance, including *bla*_{NDM-1} (4), *ampC*, a new *bla*_{OXA-229}-like (15) variant, and 3 other putative β -lactamase genes, as well as a new *carO* allele coding for an outer membrane protein associated with imipenem uptake (16). Other resistance genes included *aphA6* (4), and a putative phosphotransferase encoding resistance to aminoglycosides. Efflux

pump- and membrane-associated transporter genes of different superfamilies included (7, 8, 14, 17) ABC (MacA, MacB); BART (Acr3); IT (ArsB); MOP (NorM, AbeM); MFS (CraA, SmvA, MFS transporter, Bcr/CflA, MFS permease); DMT (AbeS, Qace Δ 1-like); MER (MerT, MerC); and RND (AdeABC, AdeIJK, AdeE, CzcABC, and CusABC). The latter operon, absent in the other *A. bereziniae* genomes, exhibits 95% nucleotide identity to a portion of the GI2 genomic island in *A. baumannii* LAC-4 (8). Downstream from *cusABC*, there are regions encompassing *ISAbA2*, *acr3*, and *feoAB* genes, with *acr3* showing the highest identity to its ortholog of *A. tandonii* DSM14970, whereas *feoAB* was identified in the *A. baumannii* LAC-4 GI2 immediately downstream of its *cusABC* operon. These observations suggest a chimeric construct in HPC229 derived from gene exchange among *Acinetobacter* species. WGS analyses will provide further evidence of the ability of *A. bereziniae* to act as a reservoir of resistance genes and may help to understand the adaptability mechanisms of *Acinetobacter* in response to environmental challenges.

Nucleotide sequence accession numbers. This WGS project has been deposited at DDBJ/EMBL/GenBank under the accession [LKDJ000000000](https://www.ncbi.nlm.nih.gov/nuccore/LKDJ000000000). We describe here the version LKDJ000000000.1.

ACKNOWLEDGMENTS

This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, Argentina); Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET); Ministerio de Salud and Secretaría de Ciencia, Tecnología e Innovación, Provincia de Santa Fe; and Secretaría de Salud Pública, Municipalidad de Rosario.

FUNDING INFORMATION

This work, including the efforts of Adriana S. Limansky, was funded by MINCyT | Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (PICT 2012-0680).

REFERENCES

1. Nemeč A, Musilek M, Sedo O, De Baere T, Maixnerová M, van der Reijden TJ, Zdráhal Z, Vanechoutte M, Dijkshoorn L. 2010. *Acineto-*

- bacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. *Int J Syst Evol Microbiol* 60:896–903. <http://dx.doi.org/10.1099/ijs.0.013656-0>.
2. Zander E, Seifert H, Higgins PG. 2014. Insertion sequence IS18 mediates overexpression of *bla*_{OXA-257} in a carbapenem-resistant *Acinetobacter bereziniae* isolate. *J Antimicrob Chemother* 69:270–272. <http://dx.doi.org/10.1093/jac/dkt313>.
 3. Lee K, Kim CK, Hong SG, Choi J, Song S, Koh E, Yong D, Jeong SH, Yum JH, Docquier JD, Rossolini GM, Chong Y. 2010. Characteristics of clinical isolates of *Acinetobacter* genomospecies 10 carrying two different metallo-beta-lactamases. *Int J Antimicrob Agents* 36:259–263. <http://dx.doi.org/10.1016/j.ijantimicag.2010.05.018>.
 4. Brovedan M, Marchiaro PM, Morán-Barrio J, Cameranesi M, Cera G, Rinaudo M, Viale AM, Limansky AS. 2015. Complete sequence of a *bla*_{NDM-1}-harboring plasmid in an *Acinetobacter bereziniae* clinical strain isolated in Argentina. *Antimicrob Agents Chemother* 59:6667–6669. <http://dx.doi.org/10.1128/AAC.00367-15>.
 5. Chagas TPG, Carvalho-Assef APDA, Martins Aires CA, Bertocini R, Asensi MD. 2015. Detection of an NDM-1-producing *Acinetobacter bereziniae* strain in Brazil. *J Glob Antimicrob Resist* 3:147–148. <http://dx.doi.org/10.1016/j.jgar.2015.03.005>.
 6. Jones LS, Carvalho MJ, Toleman MA, White PL, Connor TR, Mushtaq A, Weeks JL, Kumarasamy KK, Raven KE, Török ME, Peacock SJ, Howe RA, Walsh TR. 2015. Characterization of plasmids in extensively drug-resistant *Acinetobacter* strains isolated in India and Pakistan. *Antimicrob Agents Chemother* 59:923–929. <http://dx.doi.org/10.1128/AAC.03242-14>.
 7. Li X-Z, Plésiat P, Nikaido H. 2015. The challenge of efflux-mediated antibiotic resistance in gram-negative bacteria. *Clin Microbiol Rev* 28:337–418.
 8. Ou HY, Kuang SN, He X, Molgora BM, Ewing PJ, Deng Z, Osby M, Chen W, Xu HH. 2015. Complete genome sequence of hypervirulent and outbreak-associated *Acinetobacter baumannii* strain LAC-4: epidemiology, resistance genetic determinants and potential virulence factors. *Sci Rep* 5:8643. <http://dx.doi.org/10.1038/srep08643>.
 9. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. *Omics* 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
 10. 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>.
 11. 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>.
 12. Siguié P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 34:D32–D36. <http://dx.doi.org/10.1093/nar/gkj014>.
 13. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <http://dx.doi.org/10.1093/jac/dks261>.
 14. Saier MH, Reddy VS, Tamang DG, Västermark A. 2014. The transporter classification database. *Nucleic Acids Res* 42:D251–D258. <http://dx.doi.org/10.1093/nar/gkt1097>.
 15. Bonnin RA, Ocampo-Sosa AA, Poirel L, Guet-Revillet H, Nordmann P. 2012. Biochemical and genetic characterization of carbapenem-hydrolyzing β -lactamase OXA-229 from *Acinetobacter bereziniae*. *Antimicrob Agents Chemother* 56:3923–3927. <http://dx.doi.org/10.1128/AAC.00257-12>.
 16. Mussi MA, Limansky AS, Relling V, Ravasi P, Arakaki A, Actis LA, Viale AM. 2011. Horizontal gene transfer and assortative recombination within the *Acinetobacter baumannii* clinical population provide genetic diversity at the single *carO* gene, encoding a major outer membrane protein channel. *J Bacteriol* 193:4736–4748. <http://dx.doi.org/10.1128/JB.01533-10>.
 17. Chau S-L, Chu Y-W, Houang ETS. 2004. Novel resistance-nodulation-cell division efflux system AdeDE in *Acinetobacter* genomic DNA group 3. *Antimicrob Agents Chemother* 48:4054–4055. <http://dx.doi.org/10.1128/AAC.48.10.4054-4055.2004>.