Complete Genome Sequencing of *Acinetobacter* sp. Strain LoGeW2-3, Isolated from the Pellet of a White Stork, Reveals a Novel Class D Beta-Lactamase Gene

Ulrike Blaschke,[a] Evelyn Skiebe,[a] Michael Kaatz,[b] Paul G. Higgins,[c,d] Yvonne Pfeifer,[a] Gottfried Wilharm[a]

[a]Robert Koch-Institut, Bereich Wernigerode, Wernigerode, Germany
[b]Vogelschutzwarte Storchenhof Loburg e.V., Loburg, Germany
[c]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany
[d]German Centre for Infection Research (DZIF), Partner Site Bonn-Cologne, Cologne, Germany

ABSTRACT Whole-genome sequencing of *Acinetobacter* sp. strain LoGeW2-3, isolated from the pellet of a white stork (*Ciconia ciconia*), reveals the presence of a plasmid of 179,399 bp encoding a CRISPR-Cas (clustered regularly interspaced short palindromic repeats and associated genes) system of the I-F type, and the chromosomally encoded novel class D beta-lactamase OXA-568.

While studying the ecology of the nosocomial pathogen *Acinetobacter baumannii* (1), we isolated *Acinetobacter* sp. strain LoGeW2-3 from the pellet of a white stork (*Ciconia ciconia*) collected in Loburg, Germany, in the year 2015, following recently described protocols (2). Since partial 16S rRNA and *rpoB* gene sequencing (GenBank accession no. KT809317 and KT809318, respectively) did not indicate the strain's belonging to any described species, PacBio RS single-molecule real-time (SMRT) sequencing was commissioned at GATC (Konstanz, Germany). Genomic DNA was isolated as recently described (3). SMRT sequencing resulted in 84,981 reads with a total of 1,145,091,354 sequenced bases and 276-fold coverage. Genome assembly using the Hierarchical Genome Assembly Process (HGAP) version 3 yielded a circular chromosome with a size of 3,178,335 bp and a circular plasmid of 179,399 bp. NCBI Prokaryotic Genome Annotation Pipeline analysis revealed a total of 3,240 genes, including 3,122 coding sequences and 118 RNA genes, of which 93 define tRNAs, as well as 7 complete rRNA gene sets and 4 noncoding RNAs. PacBio modification and motif analysis identified *N*-6-methylated adenines in motifs TGAANNNNNCTG and CAGNNNNNTTCA and an unknown modification within the motif VNCGGTGTANND (modified bases underlined).

The plasmid encodes a CRISPR-Cas (clustered regularly interspaced short palindromic repeats and associated genes) system of the I-F type (4). CRISPRDetect version 2.1 (5) identified a CRISPR array ranging from nucleotides 50488 to 45838 in reverse orientation on the plasmid and harboring 77 spacer sequences with a predominant length of 32 nucleotides. Most of the spacer sequences (61%) show highest similarity to database entries of eukaryotic origin, casting into doubt a role in targeting plasmid and phage sequences.

Twenty putative genomic islands were predicted on the chromosome by at least one method applying IslandViewer version 4 (6).

Acinetobacter species found in diverse environmental habitats are considered to contribute to the mobilization of antibiotic resistance genes into clinically relevant *Acinetobacter* species (7–9). A search for putative resistance genes in the genome of *Acinetobacter* sp. strain LoGeW2-3 applying ResFinder version 3.0 (10) revealed the presence of a beta-lactamase gene with an overall identity of 79% to *bla*$_{OXA-363}$ of

Acinetobacter lwoffii. The novel allele received the designation $bla_{OXA-568}$ and the product was named class D beta-lactamase OXA-568. In its native background, no resistance phenotype could be attributed to $bla_{OXA-568}$ following standard procedures, as recently described (11).

Average nucleotide identity calculations based on BLAST+ (ANIb) analysis (12) indicate that *A. schindleri* CIP 107287 is the closest relative for which whole-genome data are available (87.11% identity with 80.65% of the chromosome of *Acinetobacter* sp. strain LoGeW2-3 aligned) and support our assumption that strain LoGeW2-3 is the first representative of a novel *Acinetobacter* species.

Accession number(s). The complete genome sequence of *Acinetobacter* sp. strain LoGeW2-3 has been deposited at GenBank under the accession no. CP024011 (chromosome) and CP024012 (plasmid).

ACKNOWLEDGMENT

REFERENCES

1. Wilharm G, Skiebe E, Higgins PG, Poppel MT, Blaschke U, Leser S, Heider C, Heindorf M, Brauner P, Jäckel U, Böhland K, Cuny C, Łopińska A, Kaminski P, Kasprzak M, Bochenski M, Ciebiera O, Tobółka M, Żołnierowicz KM, Siekiera J, Seifert H, Gagné S, Salcedo SP, Kaatz M, Layer F, Bender JK, Fuchs S, Semmler T, Pfeifer Y, Jerzak L. 2017. Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen *Acinetobacter baumannii* to lineages spread in hospitals worldwide. Environ Microbiol 19:4349–4364. https://doi.org/10.1111/1462-2920.13931.
2. Poppel MT, Skiebe E, Laue M, Bergmann H, Ebersberger I, Garn T, Fruth A, Baumgardt S, Busse HJ, Wilharm G. 2016. *Acinetobacter equi* sp. nov. isolated from horse faeces. Int J Syst Evol Microbiol 66:881–888. https://doi.org/10.1099/ijsem.0.000806.
3. Blaschke U, Wilharm G. 2017. Complete genome sequence of *Acinetobacter* sp. strain NCu2D-2 isolated from a mouse. Genome Announc 5(4):e01415-16. https://doi.org/10.1128/genomeA.01415-16.
4. Koonin EV, Makarova KS, Zhang F. 2017. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol 37:67–78. https://doi.org/10.1016/j.mib.2017.05.008.
5. Biswas A, Staals RH, Morales SE, Fineran PC, Brown CM. 2016. CRISPRDetect: a flexible algorithm to define CRISPR arrays. BMC Genomics 17:356. https://doi.org/10.1186/s12864-016-2627-0.
6. Dhillon BK, Laird MR, Shay JA, Winsor GL, Lo R, Nizam F, Pereira SK, Waglechner N, McArthur AG, Langille MG, Brinkman FS. 2015. IslandViewer 3: more flexible, interactive genomic island discovery, visualization and analysis. Nucleic Acids Res 43:W104–W108. https://doi.org/10.1093/nar/gkv401.

7. Yoon EJ, Grillot-Courvalin C, Courvalin P. 2017. New aminoglycoside-modifying enzymes APH(3')-VIII and APH(3')-IX in *Acinetobacter rudis* and *Acinetobacter gerneri*. J Antibiot (Tokyo) 70:400–403. https://doi.org/10.1038/ja.2016.144.
8. Yoon EJ, Goussard S, Touchon M, Krizova L, Cerqueira G, Murphy C, Lambert T, Grillot-Courvalin C, Nemec A, Courvalin P. 2014. Origin in *Acinetobacter guillouiae* and dissemination of the aminoglycoside-modifying enzyme Aph(3')-VI. mBio 5(5):e01972-14. https://doi.org/10.1128/mBio.01972-14.
9. Yoon EJ, Goussard S, Nemec A, Lambert T, Courvalin P, Grillot-Courvalin C. 2016. Origin in *Acinetobacter gyllenbergii* and dissemination of aminoglycoside-modifying enzyme AAC(6')-Ih. J Antimicrob Chemother 71:601–606. https://doi.org/10.1093/jac/dkv390.
10. 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. https://doi.org/10.1093/jac/dks261.
11. Savov E, Pfeifer Y, Wilharm G, Trifonova A, Todorova I, Gergova I, Borisova M, Kjoseva E. 2016. Isolation of *Acinetobacter radioresistens* from a clinical sample in Bulgaria. J Glob Antimicrob Resist 4:57–59. https://doi.org/10.1016/j.jgar.2015.10.008.
12. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931. https://doi.org/10.1093/bioinformatics/btv681.