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Draft Genome Sequences of *Acinetobacter baumannii* Isolates Recovered from Sewage Water from a Poultry Slaughterhouse in Germany

Mykhailo Savin,^a Marijo Parcina,^b Silvia Schmoger,^c Judith Kreyenschmidt,^a Annemarie Käsbohrer,^{c,d} Jens A. Hammerl^c

^aFaculty of Agriculture, Institute of Animal Sciences, University of Bonn, Bonn, Germany ^bUniversity Hospital, Institute of Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany ^cDepartment for Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany ^dInstitute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

ABSTRACT Acinetobacter baumannii is an important human pathogen usually associated with severe hospital-acquired infections. Here, we announce the draft genome sequences of two livestock-associated isolates recovered from sewage water from a poultry slaughterhouse in Germany. Short-read whole-genome sequencing was conducted to determine the genetic basis of their antimicrobial resistance phenotype.

A cinetobacter baumannii strains belong to the most critical pathogens for health care institutions, as they can efficiently incorporate antimicrobial resistance from the environment or other bacteria in their genomes (1, 2). Bacteria of this species are associated mainly with hospital-acquired pneumonia and sometimes with infections of the central nervous system, skin, or soft tissue (1, 3). Acinetobacter bacteria are Gramnegative, oxidase-negative, nonmotile, nonfermenting coccobacilli that are ubiquitously distributed in nearly all environmental habitats (i.e., water and soil) (2, 4). To assess the impact of livestock-associated *A. baumannii* strains on human health, selected isolates from sewage water from a poultry slaughterhouse were further characterized phenotypically and genotypically.

Two isolates exhibiting different colony morphologies (LWGS-03-02-11A and LWGS-03-02-11B) were obtained from process water of eviscerators from a German poultry slaughterhouse in 2018 by plating sample material on CHROMagar extended-spectrum beta-lactamase (ESBL) medium (Mast Diagnostica, Reinfeld, Germany). After incubation at 42°C for 24 h, cream-opaque Acinetobacter-like colonies were streaked onto 5% sheep blood agar (Mast Diagnostica) and confirmed by oxidase testing. Both isolates were assigned to the Acinetobacter calcoaceticus-A. baumannii complex using matrixassisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) employing a Vitek MS system (bioMérieux, Marcy l'Etoile, France). Antimicrobial resistance testing was conducted by broth microdilution using Mueller-Hinton broth, according to the recommendations of the CLSI guidelines, as previously described (5). Despite their different colony morphologies and Xbal pulsed-field gel electrophoresis (Xbal-PFGE) profiles, LWGS-03-02-11A and LWGS-03-02-11B exhibited identical MIC values, as shown in Table 1. To characterize the genetic basis of both isolates, genomic DNA (gDNA) was extracted from liquid cultures grown in lysogeny broth (LB) using the PureLink genomic DNA minikit (Invitrogen, Carlsbad, CA, USA). DNA libraries for wholegenome sequencing (WGS) were prepared using the Nextera XT DNA sample preparation kit. Short-read sequencing (MiSeq reagent v3 600-cycle kit) was conducted on a MiSeq benchtop sequencer (Illumina, San Diego, CA, USA), as previously reported (5). Raw reads were provided as quality-trimmed sequences and were further checked and

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Address correspondence to Mykhailo Savin, m.savin@uni-bonn.de, or Jens A. Hammerl, jens-andre.hammerl@bfr.bund.de.

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	Data for A. baumannii isolate ^a :	
Feature	LWGS-03-02-11A	LWGS-03-02-11B
Parameters		
No. of reads (total)	2,284,028	2,911,344
Avg read length (bp)	147.73	147.83
No. of contigs	90	103
/v ₅₀ (DP)	8	8
Genome coverage (\times)	>35	>45
Genome		
Size (bp)	3,983,337	3,982,846
GC content (%)	38.90	38.89
Genetic elements ^b (no.)		
Total genes	3,925	3,852
Total CDS	3,854	3,781
Coding genes	3,744	3,072
RNA genes	5,744 71	5,072 71
rRNAs (5S, 16S, 23S)	1, 1, 1	1, 1, 1
tRNAs	64	64
ncRNAs	4	4
Pseudogenes (no.)		
Total	110	109
Ambiguous residues	0	0
Frameshifted	41	40
Incomplete	60	60
Multiple problems	15	15
MLS1° Abaumanni1	ST-836	ST-836
Abaumanni2	ST-388	ST-388
Database accession no		
GenBank no.	RCUZ0000000	RCVA0000000
BioProject no.	PRJNA496252	PRJNA496253
BioSample no.	SAMN10237498	SAMN10237499
Genetic resistance		
determinants ^c		
Beta-lactams (%)	bla _{OXA-71} (99.88 [825/825]), bla _{ADC-25} (97.74 [1,152/1,152])	bla _{OXA-71} (99.88 [825/825]), bla _{ADC-25} (97.74 [1,152/1,152])
Phenotypic resistance		
(MIC, mg/liter) ^a		0
Ampicillin	8	8
Cefenime	 1	<u> </u>
Chloramphenicol	32	32
Ciprofloxacin	0.06	0.06
Colistin	≤1	≤1
Ertapenem	1	1
Cefotaxime	8	8
Cefoxitin	64	64
Gentamicin	I 0.25	1 0.25
Impenem	0.25	0.25
Nalidixic acid	<4	<4
Sulfamethoxazole	 ≤8	 ≤8
Ceftazidime	2	2
		(Continued on next page)

TABLE 1 Genetic features of Acinetobacter baumannii isolates LWGS-03-02-11A and LWGS-03-02-11B

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TABLE 1 (Continued)

	Data for A. baumannii isolate:	
Feature	LWGS-03-02-11A	LWGS-03-02-11B
Temocillin	>128	>128
Tetracycline	≤2	≤2
Tigecycline	≤0.25	≤0.25
Trimethoprim	16	16

^a Both isolates were obtained in 2018 from sewage water in Germany.

^b CDS, coding sequences; ncRNAs, noncoding RNAs.

^c In silico analysis was conducted using the Web-based tool MLST finder 2.0 (software version 2.0.1) (for MLST data) and ResFinder 3.1 (software version 3.1.0) (for genetic resistance determinants) of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). The percentages of nucleotide identity of the target sequence to the reference are given in parentheses, and the number of nucleotides covered by the identified resistance gene and that of the respective reference gene are given in brackets.

^d Resistance testing was conducted according to the guidelines of the CLSI.

verified by FastQC (version 1.0.4; https://www.bioinformatics.babraham.ac.uk/projects/ fastqc/). SPAdes *de novo* assemblies were conducted using PATRIC (version 3.5.21), while genome annotation was performed using PGAP of the NCBI database (6, 7). Default parameters were used for all software tools.

An overview of the genetic features and antimicrobial resistance profiles of both isolates is given in Table 1. The draft genomes of LWGS-03-02-11A and LWGS-03-02-11B exhibited little variability in their sizes (~3.983 Mbp), G+C contents (38.8 to 38.9%), and numbers of different genetic elements. Furthermore, the two isolates belong to the same sequence type (ST), ST-836 (Abaumanni1) and ST-388 (Abaumanni2), using the two available multilocus sequence typing (MLST) schemes for *A. baumannii* typing of MLST finder 2.0 (software version 2.0.1) (8). Bioinformatics analysis using ResFinder 3.1 (software version 3.1.0) (9) revealed that both genomes harbor bla_{OXA-71} (99.88% nucleotide identity to accession number AY750913) and bla_{ADC-25} (97.74% nucleotide identity to accession number EF016355) coding for beta-lactam antibiotics.

To assess the impact of livestock-associated *A. baumannii* isolates on human health, comprehensive data on their antimicrobial resistance development and their genetic basis will be needed. However, until now, *Acinetobacter* species of livestock and food origins have not usually been monitored.

Data availability. The draft genome sequences of LWGS-03-02-11A and LWGS-03-02-11B were deposited in GenBank under accession numbers RCUZ00000000 (Bio-Project number PRJNA496252) and RCVA00000000 (BioProject number PRJNA496253), respectively.

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REFERENCES

- Asif M, Alvi IA, Rehman SU. 2018. Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist 11:1249–1260. https://doi.org/10.2147/IDR.S166750.
- 2. Wareth G, Neubauer H, Sprague LD. 2019. Acinetobacter baumannii—a neglected pathogen in veterinary and environmental health in Ger-

many. Vet Res Commun 43:1-6. https://doi.org/10.1007/s11259-018 -9742-0.

- Harding CM, Hennon SW, Feldman MF. 2018. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol 16:91–102. https://doi.org/10.1038/nrmicro.2017.148.
- 4. Elhosseiny NM, Attia AS. 2018. Acinetobacter: an emerging pathogen with

a versatile secretome. Emerg Microbes Infect 7:33. https://doi.org/10 .1038/s41426-018-0030-4.

- Hammerl JA, Irrgang A, Grobbel M, Tenhagen BA, Käsbohrer A. 2018. Complete genome sequence of a bla_{CTX-M-1}-harboring Escherichia coli isolate recovered from cattle in Germany. Genome Announc 6:e01476-17. https://doi.org/10.1128/genomeA.01476-17.
- Hammerl JA, Jäckel C, Bortolaia V, Schwartz K, Bier N, Hendriksen RS, Guerra B, Strauch E. 2017. Carbapenemase VCC-1-producing *Vibrio cholerae* in coastal waters of Germany. Emerg Infect Dis 23:1735–1737. https:// doi.org/10.3201/eid2310.161625.
- 7. 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. https://doi.org/ 10.1089/omi.2008.0017.

- Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Ponten T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.06094-11.
- 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.