





Complete Draft Genome Sequence of an Extended-Spectrum β -Lactamase-Producing *Citrobacter freundii* Strain Recovered from the Intestine of a House Sparrow (*Passer domesticus*) in Germany, 2017

Valerie Osieka,^a Mirjam Grobbel,^a Silvia Schmoger,^a Claudia A. Szentiks,^b ⁽ⁱ⁾ Alexandra Irrgang,^a Annemarie Käsbohrer,^{a,c} Bernd-Alois Tenhagen,^a Jens A. Hammerl^a

^aDepartment of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany ^bDepartment of Wildlife Diseases, Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany ^cInstitute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

ABSTRACT Here, we announce the genome of an extended-spectrum β -lactamaseproducing *Citrobacter freundii* strain isolated from the cecum of a house sparrow that was found dead in Berlin-Lichtenberg, Germany, in 2017. This isolate exhibits increased MICs for several antimicrobials and a comprehensive set of acquired resistance determinants potentially involved in horizontal gene transfer.

C*itrobacter freundii* is a Gram-negative, opportunistic pathogen of the family *Enter-obacteriaceae*. It is widely distributed in the environment and is also present in the intestine of wildlife, livestock, and humans. In the past, the pathogenic potential of this bacterium was considered low, but *C. freundii* is now recognized as an important nosocomial pathogen causing both superficial wound infections and life-threatening infections (1, 2), especially in neonates and elderly and immunocompromised persons (3). *C. freundii* has been shown to adapt efficiently to prevailing environmental conditions by acquiring genetic material from other bacteria. The uptake of various antimicrobial resistance determinants and the spread of multidrug-resistant (MDR) *C. freundii* clones, especially in health care units, are major public health issues (4). Further information on the biology and genetics will be needed to understand the evolution and dissemination of MDR *C. freundii* strains.

Extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* were recovered from fecal and cecal samples of German wildlife by cultivation on MacConkey agar supplemented with 1 mg/liter cefotaxime (5). Representative colonies of each sample were identified by a MALDI Biotyper (Bruker, Bremen, Germany). The isolates were tested for their susceptibility to antimicrobial substances using the broth microdilution method according to CLSI guideline M07 (CLSI M07-A10) (6) and assessed according to EUCAST ECOFFs (http://www.eucast.org). Among all isolated *C. freundii*, strain 236-17-2 attracted attention because of a comprehensive antimicrobial resistance pattern, with high MIC(s) for ampicillin (>64 mg/liter), chloramphenicol (>128 mg/liter), ciprofloxacin (0.5 mg/liter), nalidixic acid (>128 mg/liter), sulfamethoxazole (>1024 mg/liter), tetracycline (64 mg/liter), trimethoprim (>32 mg/liter), cefepime (0.5 mg/liter), ertapenem (0.12 mg/liter), cefotaxime (32 mg/liter), cefoxitin (>64 mg/liter), and ceftazidime (32 mg/liter). Here, we announce the draft genome of this isolate, which was recovered from the intestine of a house sparrow (*Passer domesticus*) in Berlin-Lichtenberg (Germany) in 2017.

Genome sequencing was performed using DNA extracted with the PureLink Genomic DNA minikit (Invitrogen, Carlsbad, CA, USA). Short-read sequencing (MiSeq

Received 29 May 2018 **Accepted** 30 May 2018 **Published** 28 June 2018

Citation Osieka V, Grobbel M, Schmoger S, Szentiks CA, Irrgang A, Käsbohrer A, Tenhagen B-A, Hammerl JA. 2018. Complete draft genome sequence of an extended-spectrum β-lactamase-producing *Citrobacter freundii* strain recovered from the intestine of a house sparrow (*Passer domesticus*) in Germany, 2017. Genome Announc 6:e00599-18. https://doi .org/10.1128/genomeA.00599-18.

Copyright © 2018 Osieka et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Jens A. Hammerl, jens-andre.hammerl@bfr.bund.de.

Reagent v3 600-cycle kit, Illumina, San Diego, USA) was conducted on a MiSeq benchtop sequencer using sequencing libraries prepared with the Nextera XT DNA sample preparation kit as previously described (6). De novo genome assembly was performed using the PATRIC database (https://www.patricbrc.org/) and resulted in 36 contigs with an average sequence coverage of 15 per consensus base. The resulting genome consists of 4,973,288 bp with an average G+C content of 51.51%. For initial genome annotation, the automated Prokaryotic Genome Annotation Pipeline of the NCBI database (7) was used, resulting in the identification of 4,856 genes, 4,753 coding sequences, 103 RNAs (16 rRNAs, 75 tRNAs, and 12 noncoding RNAs [ncRNAs]), and 130 pseudogenes. The antimicrobial resistance pattern of isolate 236-17-2 was partially confirmed by in silico detection of the acquired resistance genes for aminoglycosides (strA and strB), beta-lactams (bla_{CMY-67} and bla_{TEM-1A}), phenicols (catA1), sulfonamides (sul1), tetracyclines [tet(A)], and trimethoprim (dfrA1) using ResFinder3.0 (https://cge .cbs.dtu.dk/services/ResFinder/). Further analyses will be necessary to determine the locations of the resistance determinants on potential mobile elements (i.e., transposons and plasmids) and their role in the transmission to other bacteria of the Enterobacteriaceae.

Accession number(s). The complete genome sequence of the *C. freundii* strain 236-17-2 genome was deposited in GenBank under accession number PQFB00000000.

ACKNOWLEDGMENTS

We thank Zoltan Mezö (IZW) for his technical support.

This research was supported by grants from the German Federal Institute for Risk Assessment (43-001, 43-002, and 1322-648).

REFERENCES

- Lavigne J-P, Defez C, Bouziges N, Mahamat A, Sotto A. 2007. Clinical and molecular epidemiology of multidrug-resistant *Citrobacter* spp. infections in a French university hospital. Eur J Clin Microbiol Infect Dis 26:439–441. https://doi.org/10.1007/s10096-007-0315-3.
- Samonis G, Karageorgopoulos DE, Kofteridis DP, Matthaiou DK, Sidiropoulou V, Maraki S, Falagas ME. 2009. *Citrobacter* infections in a general hospital: characteristics and outcomes. Eur J Clin Microbiol Infect Dis 28:61–68. https://doi.org/10.1007/s10096-008-0598-z.
- Chen YS, Wong WW, Fung CP, Yu KW, Liu CY. 2002. Clinical features and antimicrobial susceptibility trends in *Citrobacter freundii* bacteremia. J Microbiol Immunol Infect 35:109–114.
- Lockhart SR, Abramson MA, Beekmann SE, Gallagher G, Riedel S, Diekema DJ, Quinn JP, Doern GV. 2007. Antimicrobial resistance among Gramnegative bacilli causing infections in intensive care unit patients in the United States between 1993 and 2004. J Clin Microbiol 45:3352–3359. https://doi.org/10.1128/JCM.01284-07.
- Beutlich J, Hammerl JA, Appel B, Nöckler K, Helmuth R, Jöst K, Ludwig M-L, Hanke C, Bechtold D, Mayer-Scholl A. 2015. Characterization of illegal food items and identification of foodborne pathogens brought into the European Union via two major German airports. Int J Food Microbiol 209:13–19. https://doi.org/10.1016/j.ijfoodmicro.2014.10.017.
- Clinical and Laboratory Standards Institute. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—tenth edition. CLSI M07-A10. Clinical and Laboratory Standards Institute, Wayne, PA.
- Hammerl JA, Irrgang A, Grobbel M, Tenhagen B-A, 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.