



Draft Genome Sequence of a Vancomycin-Resistant and Vancomycin-Dependent *Enterococcus faecium* Isolate

Marion Blaschitz,^a Sarah Lepuschitz,^a Laura Wagner,^a Franz Allerberger,^a Alexander Indra,^a Werner Ruppitsch,^{a,b} Steliana Huhulescu^a

Institute of Medical Microbiology and Hygiene, Austrian Agency for Health and Food Safety, Vienna, Austria^a; Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria^b

M.B. and S.L. contributed equally to this work.

Vancomycin-resistant enterococci have emerged as major nosocomial pathogens worldwide. While antimicrobial pressure promotes nosocomial colonization with these enterococci, prolonged exposure to vancomycin may foster the transition from vancomycin resistance to vancomycin dependence. Here, we report the draft genome sequence of a vancomycin-dependent *Enterococcus faecium* isolate showing partial teicoplanin dependence.

Received 13 January 2016 Accepted 24 February 2016 Published 7 April 2016

Citation Blaschitz M, Lepuschitz S, Wagner L, Allerberger F, Indra A, Ruppitsch W, Huhulescu S. 2016. Draft genome sequence of a vancomycin-resistant and vancomycindependent *Enterococcus faecium* isolate. Genome Announc 4(2):e00059-16. doi:10.1128/genomeA.00059-16.

Copyright © 2016 Blaschitz et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Werner Ruppitsch, werner.ruppitsch@ages.at.

Wancomycin-resistant enterococci (VRE) are major nosocomial pathogens worldwide. While antimicrobial pressure promotes nosocomial colonization with VRE, prolonged exposure to vancomycin may foster the transition from vancomycin resistance to dependence (1). Enterococci showing growth on medium containing 6 μ g/ml vancomycin and an MIC of >8 μ g/ml are considered vancomycin resistant. Strains unable to grow in the absence of 6 μ g/ml vancomycin, despite multiple subcultures, are considered vancomycin dependent (1). Vancomycin-dependent enterococcus (VDE) was first described in 1993 (2, 3). To our knowledge, only twenty-five cases of VDE have been described worldwide so far (4).

We report here the draft genome of a VRE/VDE isolate obtained in Austria in 2007 from an oncology patient 1 month after cessation of teicoplanin therapy (5). The described strain of VDE showed resistance to penicillin (MIC, 64 μ g/ml), ampicillin (MIC, 128 μ g/ml), amoxicillin-clavulanate (amoxicillin MIC, >256 μ g/ ml), erythromycin (MIC, 8 μ g/ml), clindamycin (MIC, 8 μ g/ml), ciprofloxacin (MIC, $\geq 2 \mu g/ml$), moxifloxacin (MIC, $\geq 2 \mu g/ml$), fusidic acid (MIC, 4 μ g/ml), and low-level resistance to gentamicin (MIC, 32 μ g/ml) when tested according to Clinical and Laboratory Standards Institute (CLSI) standards (6). The strain was susceptible to quinupristin-dalfopristin (MIC, $<0.5 \ \mu g/ml$), oxytetracycline (MIC, $<0.5 \ \mu g/ml$), linezolid (MIC, $2 \ \mu g/ml$), chloramphenicol (MIC, 8 μ g/ml), and mupirocin (MIC, 2 μ g/ ml). The strain showed intermediate resistance to teicoplanin when tested on Mueller-Hinton agar (bioMérieux, Marcy l'Etoile, France) using a 30-µg teicoplanin disc (Oxoid, Basingstoke, United Kingdom) or a teicoplanin Epsilon test strip (AB Biodisk, Solna, Sweden) (MIC, 8 µg/ml). It also showed partial teicoplanin dependence, i.e., it grew in the area of low concentration of teicoplanin (0.016 to 6 μ g/ml) but was inhibited in the area with high teicoplanin concentrations (8 to 256 μ g/ml). The strain showed high-level resistance to vancomycin (MIC, >256 μ g/ml) and complete vancomycin dependence when

tested on Mueller-Hinton agar (bioMérieux) with a $30-\mu g$ vancomycin disk (Oxoid) or a vancomycin Epsilon test strip (AB Biodisk). The strain also showed *in vitro* reversion to vancomycinnondependent vancomycin-resistant *Enterococcus faecium* mutants.

The MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, Hilden, Germany) was used to isolate genomic DNA from overnight cultures grown on Mueller-Hinton agar plates (bioMérieux) with a 30- μ g vancomycin disk (Oxoid) and a 30- μ g teicoplanin disk (Oxoid). The fragment library was prepared using the Nextera XT kit (Illumina, Inc., San Diego, CA) and 1 ng of genomic DNA. Paired-end sequencing $(2 \times 300 \text{ bp})$ was performed on a MiSeq (Illumina, Inc.), generating 1,365,744 reads from 333,173,006 unassembled nucleotides. Raw reads were de novo assembled into a draft genome using Velvet version 1.1.04 (7). Contigs were filtered for a minimum coverage of 5 and minimum length of 200 bp, which resulted in 232 contigs with a total of 2,949,766 nucleotides at a coverage of 95-fold. A total of 2,953 genes, 2,878 coding sequences, 95 pseudogenes, 7 rRNA genes, and 64 tRNA genes were identified using the NCBI Prokaryotic Genome Annotation Pipeline (http://www.ncbi .nlm.nih.gov/genome/annotation_prok/).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. LQRS00000000. The version described in this paper is the first version, LQRS01000000.

ACKNOWLEDGMENT

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Tambyah PA, Marx JA, Maki DG. 2004. Nosocomial infection with vancomycin-dependent enterococci. Emerg Infect Dis 10:1277–1281. http://dx.doi.org/10.3201/eid1007.030993.

- Fraimow HS, Jungkind DL, Lander DW, Delso DR, Dean JL. 1994. Urinary tract infection with an *Enterococcus faecalis* isolate that requires vancomycin for growth. Ann Intern Med 121:22–26. http://dx.doi.org/ 10.7326/0003-4819-121-1-199407010-00004.
- Green M, Shlaes JH, Barbadora K, Shlaes DM. 1995. Bacteremia due to vancomycin-dependent *Enterococcus faecium*. Clin Infect Dis 20:712–714. http://dx.doi.org/10.1093/clinids/20.3.712.
- Banerjee T, Anupurba S. 2013. Isolation and characterization of the first vancomycin dependent *Enterococcus* from India. Indian J Med Microbiol 31:91–92. http://dx.doi.org/10.4103/0255-0857.108747.
- 5. Huhulescu S, Schmid D, Mayer B, Stadlbauer S, Wewalka G, Allerberger

F, Dworzak M. 2007. Partial teicoplanin dependence in a vancomycindependent *Enterococcus faecium* isolated from an oncology patient 1 month after cessation of teicoplanin therapy, Austria. Int J Antimicrob Agents 31:76–90.

- 6. Clinical and Laboratory Standards Institute. 2007. Performance standards for antimicrobial susceptibility testing; approved standard, 17th informational supplement. CLSI document M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res 18:821–829. http:// dx.doi.org/10.1101/gr.074492.107.