





First Report of a Whole-Genome Shotgun Sequence of a Clinical Enterococcus faecalis Sequence Type 6 Strain from South Africa

Nontombi Marylucy Mbelle,^{a,b} Nontuthuko Excellent Maningi,^a Vhudzani Tshisevhe,^{a,b} Lesedi Modipane,^a Daniel Gyamfi Amoako,^c Dohn Osei Sekyere^d

Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa^a; National Health Laboratory Service, Pretoria, South Africa^b; Biomedical Resource Unit, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa^c; Department of Pharmaceutics, Faculty of Pharmacy & Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana^d

ABSTRACT *Enterococcus faecalis* is a lactic acid-producing Gram-positive bacterium commonly found in the intestinal tract of humans and animals; it is implicated in multidrug-resistant nosocomial infections. The draft genome of this *E. faecalis* sequence type 6 (ST6) strain consists of 3,215,228 bp, with 37.20% GC content, 3,048 predicted coding sequences, and 61 RNA genes.

Interococcus faecalis is part of the human microbiota and is implicated in several fatal clinical infections, such as bacteremia and infective endocarditis (1). To our knowledge, this is the first draft genome sequence of a clinical *E. faecalis* strain, ST-6:CF006, from South Africa and the rest of Africa. This vancomycin-susceptible sequence type 6 (ST6) strain was isolated from the urine of a 41-year-old male patient hospitalized in Kalafong Hospital in Pretoria, South Africa, in 2013.

The strain was grown overnight anaerobically at 37°C in brain heart infusion (BHI) broth (Oxoid, UK) and was catalase negative but esculin hydrolysis and pyrrolidonyl arylamidase (PYR) positive. The identification was confirmed with Vitek 2 (bioMérieux, France). Genomic DNA was sheared to 200-bp libraries; 280-bp fragments were selected using 2% agarose gels and Pippin Prep (Sage Science, USA). Individual libraries were pooled to 100 pM and sequenced on the lon Proton (Thermo, Fisher, USA) at a coverage of 89.84×. The raw reads were *de novo* assembled using the SPAdes assembler (2).

The size, GC content, number of contigs, N_{50} , and L_{50} of the draft genome were 3,215,228 bp, 37.20%, 198, 104,004 bp, and 10 bp, respectively. Annotation with Rapid Annotations using Subsystems Technology (RAST) (3) and prokaryotic genome annotation pipeline (PGAP) (4) resulted in 3,048 protein-coding genes, 376 (10.98%) hypothetical proteins, 57 tRNAs, 3 rRNAs, and 4 noncoding RNAs. CRISPRFinder (5) predicted two clustered regularly interspaced short palindromic repeat 1 (CRISPR1) arrays each on nodes/contigs 3 and 59.

BLASTN analysis showed ST-6:CF006 to be closely related to the following *E. faecalis* strains with 99% nucleotide identity: V583 (GenBank accession no. AE016830), a clinical isolate from the United States; sorialis (accession no. CP015883), a fecal isolate from the United States; DD14 (accession no. CP021161), a meconium isolate from France; and L12 (accession no. CP018102) from swine in Brazil.

Resistome annotation with GoSeqlt and ResFinder (6, 7) showed aminoglycoside [aph(3')-III, ant(6)-Ia, aac(6')-aph(2'')], macrolide-lincosamide-streptogramin (isaA and mphD), and tetracycline (tetM) resistance genes. ST-6:CF006 was resistant (R) to genta-

Received 3 November 2017 Accepted 6 November 2017 Published 14 December 2017

Citation Mbelle NM, Maningi NE, Tshisevhe V, Modipane L, Amoako DG, Osei Sekyere J. 2017. First report of a whole-genome shotgun sequence of a clinical *Enterococcus faecalis* sequence type 6 strain from South Africa. Genome Announc 5:e01382-17. https://doi.org/10.1128/genomeA.01382-17.

Copyright © 2017 Mbelle et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Nontombi Marylucy Mbelle, nontombi.mbelle@nhls.ac.za, or John Osei Sekyere, jod14139@gmail.com. Mbelle et al. genameAnnouncements"

micin, streptomycin, erythromycin (R > 8 μ g/ml), clindamycin (R > 8 μ g/ml), tetracycline (R > 168 μ g/ml), ciprofloxacin (R > 88 μ g/ml), and moxifloxacin (R > 88 μ g/ml) but susceptible to ampicillin (>2 μ g/ml), teicoplanin (\leq 0.5 μ g/ml), linezolid (2 μ g/ml), tigecycline (\leq 0.12 μ g/ml), and vancomycin (1 μ g/ml), in agreement with the resistome annotation. As no fluoroquinolone resistance genes were identified, mutations in a chromosome-borne DNA gyrase gene (qyrA) (8, 9) were further investigated using tBLASTn; fluoroquinolone-susceptible E. faecalis ATCC 29212 (accession no. CP008816) was used as the reference/wild-type strain to call single-nucleotide polymorphisms (SNPs). A Ser84lle mutation, previously implicated in fluoroquinolone resistance (8), was identified. Four plasmid replicon types, i.e., rep2, rep6, rep9, and repUS11, were identified with PlasmidFinder version 1.3 (10). The GoSeqlt VirulenceFinder database (6) discovered 19 virulence factor genes: collagen adhesion (ace), pheromone precursor lipoproteins (cad, camE, cCF10, and cOB1), cytolysin (cylA, cylB, cylL, and cylM), endocarditis- and biofilm-associated pili (ebpA, ebpB, and ebpC), endocarditis antigen A (efaAfs), enterococcal leucine-rich internalin-like protein A (elrA), gelatinase (gelE), hyaluronidase (hylA and hylB), sortase A (srtA), and thiolperoxidase (tpx), contributing to its ability to aggregate, adhere, lyse, and invade host tissues. Analysis of the genomes of E. faecalis will increase our insight into the factors that mediate its pathogenesis and antibiotic resistance, as well as establish a correlation between genomic and phenotypic data.

Accession number(s). This whole-genome shotgun project has been submitted to the National Center for Biotechnology Information GenBank database under the accession no. NXKG00000000. The version described in this paper is version NXKG01000000.

ACKNOWLEDGMENTS

We thank the staff of the Medical Microbiology Department of the Tshwane Academic Division of the National Health Laboratory Services.

This study was funded by grant 94445 from the NHLS.

REFERENCES

- Van Tyne D, Martin MJ, Gilmore MS. 2013. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. Toxins 5:895–911. https://doi.org/10.3390/toxins5050895.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- 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. https://doi.org/10.1186/1471-2164-9-75.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2015. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 43:D599–D605. https://doi .org/10.1093/nar/gku1062.
- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. https://doi.org/10.1093/nar/gkm360.

- Thomsen MC, Ahrenfeldt J, Cisneros JL, Jurtz V, Larsen MV, Hasman H, Aarestrup FM, Lund O. 2016. A bacterial analysis platform: an integrated system for analysing bacterial whole genome sequencing data for clinical diagnostics and surveillance. PLoS One 11:e0157718. https://doi.org/ 10.1371/journal.pone.0157718.
- 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.
- Aldred KJ, Kerns RJ, Osheroff N. 2014. Mechanism of quinolone action and resistance. Biochemistry 53:1565–1574. https://doi.org/10.1021/ bi5000564
- Osei Sekyere J, Amoako DG. 2017. Genomic and phenotypic characterisation of fluoroquinolone resistance mechanisms in *Enterobacteriaceae* in Durban, South Africa. PLoS One 12:e0178888. https://doi.org/10.1371/ journal.pone.0178888.
- Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/ AAC.02412-14.

Volume 5 Issue 50 e01382-17 genomea.asm.org **2**