

# Complete Genome Sequence of *Staphylococcus aureus* FCFHV36, a Methicillin-Resistant Strain Heterogeneously Resistant to Vancomycin

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**We report here the sequence of the entire chromosome of *Staphylococcus aureus* strain FCFHV36, a methicillin-resistant strain heterogeneously intermediate to vancomycin, bearing a type II staphylococcal chromosome cassette *mec* element (SCC*mec*), belonging to multilocus sequence type (MLST) 105, and isolated from a vertebra of a patient with osteomyelitis.**

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We present here the sequence of the entire chromosome of *Staphylococcus aureus* strain FCFHV36, recovered from a vertebral biopsy sample from a patient diagnosed with community-acquired osteomyelitis and under medical care in a hospital in the state of Santa Catarina, Brazil. FCFHV36 presents an MIC of vancomycin of 2 µg/ml, which classifies it as being susceptible to this antibiotic. The patient, however, did not respond to therapy with vancomycin. A heterogeneous resistance (heterogeneous vancomycin-intermediate *S. aureus* [hVISA]) profile was detected by the population analysis profile-area under the curve (PAP-AUC) technique (1), meaning that cell subpopulations of this strain present higher MICs than those of the overall cell population. The PAP-AUC ratio of FCFHV36 to hVISA type strain Mu3 was 1.02.

The total genomic DNA of FCFHV36 was used to construct a paired-end (PE) library and a mate-paired (MP) library, which were separately sequenced using the MiSeq platform (Illumina, Inc.). A total of 4,981,028 75-bp-long reads were generated for the paired-end library, and a total of 1,181,570 250-bp-long reads were generated for the mate-paired library, which had a mean insert distance of 2,700 bp. Both libraries were simultaneously used as input for *de novo* assembly using the A5 pipeline (2), generating 46 contigs (sum, 2.84 Mbp;  $N_{50}$ , 147.9 kbp; max length, 385.9 kbp). In order to build a scaffold, the contigs were ordered by synteny against a reference chromosome using Gepard (3). The reference genome chosen was the publicly available genome presenting the most similar *k*-mer spectrum to the contigs, as determined using KmerFinder (4), which was *S. aureus* JH1 (NCBI GenBank accession no. NC\_009632). Contigs pertaining to plasmids were separated. The contig order was verified by aligning mate-paired reads against the scaffold and verifying the existence of mate pairs straddling the gap close to the mean insert distance using Geneious 7 (5). The correct position of contigs without synteny to the JH1 reference genome was also determined by mate-paired distance information. Gaps were then filled with GapFiller

using reads from the paired-end library. After manual curation of gaps, the final circularized chromosome was annotated with Prokka (6), and features were manually curated by blasting against the GenBank nr database. Insertion sequences were found using the ISfinder database (7) and annotated manually using Artemis (8).

The chromosome of FCFHV36 carries 2,619 protein-coding sequences, 7 pseudogenes, 58 tRNA genes, and 16 rRNA genes. The *mecA* gene, which confers resistance to most β-lactams, is carried by a type II staphylococcal chromosome cassette *mec* element (SCC*mec*). *In silico* multilocus sequence typing (MLST) was able to attribute sequence type 105 (ST105) to the strain. Comparative genomics between this sequence and vancomycin-susceptible, VISA, and other hVISA strains will help determine the polymorphisms that correlate with decreased vancomycin susceptibility in *S. aureus*, which is hard to detect in the clinical laboratory.

**Nucleotide sequence accession number.** The complete genome sequence of *S. aureus* strain FCFHV36 has been deposited in DDBJ/EMBL/GenBank under the accession no. CP011147.

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We declare no conflicts of interest.

## REFERENCES

- Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP. 2001. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital.

- J Antimicrob Chemother 47:399–403. <http://dx.doi.org/10.1093/jac/47.4.399>.
2. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. *Bioinformatics* 31:587–589. <http://dx.doi.org/10.1093/bioinformatics/btu661>.
  3. Krumsiek J, Arnold R, Rattei T. 2007. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* 23:1026–1028. <http://dx.doi.org/10.1093/bioinformatics/btm039>.
  4. Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Pontén T, Aarestrup FM, Ussery DW, Lund O. 2014. Benchmarking of methods for genomic taxonomy. *J Clin Microbiol* 52:1529–1539. <http://dx.doi.org/10.1128/JCM.02981-13>.
  5. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <http://dx.doi.org/10.1093/bioinformatics/bts199>.
  6. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <http://dx.doi.org/10.1093/bioinformatics/btu153>.
  7. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 34:D32–D36. <http://dx.doi.org/10.1093/nar/gkj014>.
  8. Carver T, Berriman M, Tivey A, Patel C, Böhme U, Barrell BG, Parkhill J, Rajandream M-A. 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. *Bioinformatics* 24:2672–2676. <http://dx.doi.org/10.1093/bioinformatics/btn529>.