

Complete Genome Sequence of *Staphylococcus aureus* Strain JKD6008, an ST239 Clone of Methicillin-Resistant *Staphylococcus aureus* with Intermediate-Level Vancomycin Resistance[∇]

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We report here the complete 2.92-Mb genome sequence of a clinical isolate of methicillin-resistant *Staphylococcus aureus* subsp. *aureus* that demonstrates intermediate-level vancomycin resistance. The strain, named JKD6008, belongs to multilocus sequence type 239 and was isolated from the bloodstream of a patient in New Zealand in 2003.

We have previously described the *in vivo* evolution of low-level vancomycin resistance in *Staphylococcus aureus* through comparative and functional genomic assessment of a pair of isogenic methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The vancomycin-susceptible *S. aureus* (VSSA) strain JKD6009 was a patient wound isolate, whereas vancomycin-intermediate *S. aureus* (VISA) strain JKD6008 was recovered from the bloodstream of the same patient after 42 days of vancomycin treatment (5). Comparison of the partially assembled genomes of the two isolates revealed a single-point mutation in the sensor region of the two-component regulatory gene *graS*, which caused a significant reduction in the vancomycin susceptibility of JKD6008 (6). Here we report the fully assembled and annotated genome of *S. aureus* JKD6008.

The genome sequence of *S. aureus* strain JKD6008 was determined by whole-genome shotgun sequencing using single-read 454 GS20 (Roche Diagnostics, Basel, Switzerland), Sanger (Applied Biosystems), and SOLiD (Applied Biosystems) sequencing technologies, producing approximately 20 times, 4 times, and 225 times coverage of the genome, respectively. GS20 reads were assembled using gsAssembler v2.0 software, resulting in 131 contigs (≥ 500 bp) totaling 2.83 Mbp (6, 10). Sanger paired-end reads (clone insert size, 3 to 5 kb) were combined with the GS20 contigs using Gap4 v4.11 software (3). Mate-pair SOLiD reads (3 to 5 kb) were aligned to the contigs using SHRiMP 1.3.2 software to identify and correct sequencing errors (11). Optical mapping produced a high-resolution XbaI chromosome restriction map, to which

the contigs were aligned using MapSolver 2.1.1 (OpGen) to determine misassemblies. Gap closures were performed by PCR, followed by Sanger sequencing and primer walking of amplification products (3730S DNA Analyzer sequencer; Applied Biosystems). The assembly of the completed genome was confirmed to be correct by reference to the XbaI optical map.

Protein-coding regions were predicted using GeneMarkS 4.6b software, tRNA genes using tRNAscan-SE 1.23, and rRNA genes using RNAmmer 1.2 (2, 8, 9). Gene products were assigned using HMMER 3.0 against the Pfam database (release 23) and BLAST 2.2.23 against RefSeq proteins (April 2010) and the Conserved Domain Database (v2.22) (1, 4). These automated analyses were followed by manual curation and comparisons with other completed *S. aureus* genomes.

The genome of *S. aureus* strain JKD6008 consists of a circular 2,924,344-bp chromosome with a 34% G+C content and no extrachromosomal elements. A total of 2,766 coding DNA sequences, 82 tRNA genes, and 5 rRNA loci were detected. Over 70% of genes were assigned to specific Clusters of Orthologous Groups (COG) functional groups, and 42% were assigned an enzyme classification number (12).

Initial analysis of the whole-genome sequence of JKD6008 confirmed it as a member of the ST239 complex, sharing 2,504 orthologous coding sequences (CDSs) with the recently described ST239 member TW20 (EMBL accession no. FN433596.1). There are 17 copies of IS256 and a type III staphylococcal cassette chromosome *mec* element (SCC*mec*). Comparisons with 19 published *S. aureus* genomes revealed 20 CDS not present in any other *S. aureus* genome, although some of these 20 CDS have orthologs in other *Staphylococcus* species. JKD6008 also harbors a 28-kb integrated pSK1-like plasmid that is predicted to confer resistance to aminoglycosides and trimethoprim, as well as efflux-mediated antiseptic and disinfectant resistance (7).

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