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Genome Sequence of *Staphylococcus aureus* VC40, a Vancomycin- and Daptomycin-Resistant Strain, To Study the Genetics of Development of Resistance to Currently Applied Last-Resort Antibiotics

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The increasing emergence of multidrug-resistant *Staphylococcus aureus* is a problem of global importance. Here, we report the genome of *S. aureus* VC40, which is resistant to the last-resort antibiotics vancomycin and daptomycin. Its genome sequence will allow insights into the mechanisms that convey full resistance to these compounds.

The Gram-positive pathogen *Staphylococcus aureus* causes a wide spectrum of diseases ranging from complicated skin and skin structure infections (cSSSI) to life-threatening conditions such as pneumonia, endocarditis, or toxic shock syndrome (4, 7). In recent years, the alarming spread of antibiotic resistance has severely complicated the treatment of nosocomial and community-acquired infections, now substantially challenging our public health care systems in a hygienic, scientific, and economic way. Currently, the antibiotics vancomycin, linezolid, and daptomycin constitute the empirical therapy for serious infections caused by methicillin-resistant *S. aureus* (MRSA). However, the overuse of vancomycin has led to the emergence of vancomycin-intermediate (VISA; MIC, 2 to 8 μg/ml) and vancomycin-resistant (VRSA; MIC, $\geq$16 μg/ml) *S. aureus* strains (10, 14), and cross-resistance to daptomycin has also been observed in VISA (2, 15). Despite careful analyses of intermediately resistant clinical isolates, the explicit mode of resistance development to these antibiotics remains incompletely understood.

Here, we report the annotated genome of the vancomycin- and daptomycin-resistant *S. aureus* VC40, which had previously been generated by serial passage of *S. aureus* RN4220Δ*mutS*, a *mutS* gene deletion mutant of the parent strain RN4220 (1, 8), in the presence of vancomycin (11). *S. aureus* VC40 shows a vancomycin MIC of 64 μg/ml, which goes far beyond the MICs of the clinical VISA strains Mu50 and JH9 (MICs, 8 μg/ml) (5, 13). In addition, cross-resistance to daptomycin was also observed in strain VC40 with a MIC of 4 μg/ml in cation-adjusted Mueller-Hinton broth (A. Berscheid, unpublished data). *S. aureus* VC40 lacks a *vanA* gene cluster, the common VRSA resistance determinant (9), demonstrating that high-level vancomycin resistance occurs without horizontal gene transfer. The *S. aureus* VC40 genome will enable further investigations and mutational analyses on vancomycin and daptomycin resistance with the aim to gain deeper insights into the genetic mechanisms underlying resistance development in *S. aureus*.

Genomic DNA of *S. aureus* VC40 was extracted by using the Master Pure Gram-positive DNA purification kit (Epicentre Biotechnologies) and was fragmented by nebulization for pyrosequencing. A single 454 sequencing run based on the 454-FLX technology (Roche GS20 sequencer; MWG Biotech) was employed to generate the raw sequencing data sets for the genome, which was assembled using the Newbler assembly program provided with the 454 sequencing device. All steps were done according to the manufacturer's protocols and as previously described (12). Pyrosequencing resulted in 54 contigs which were assembled using *S. aureus* NCTC 8325 (accession number NC_007795) (3) as a reference. Gap closure was performed using PCR-based techniques followed by Sanger sequencing. Particular PCR products were subcloned into pJET vectors (Fermentas). The genome sequence of *S. aureus* VC40 (2,692,570 bp) was determined with >99.5% coverage. Annotation was performed using NCBI Prokaryotic Genomes Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html) (6) and was manually cured from errors. Single nucleotide polymorphisms (SNPs), insertions, or deletions that were detected in the genome sequence of strain VC40 but which were not present in the parent strains *S. aureus* RN4220Δ*mutS*, *S. aureus* RN4220, and *S. aureus* NCTC 8325 were confirmed by PCR followed by Sanger sequencing. Detailed whole-genome sequence comparison of these strains and other available *S. aureus* strains will be included in a future publication.

Nucleotide sequence accession number. The genome sequence of *S. aureus* VC40 was deposited in NCBI GenBank under accession number CP003033.

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