Supporting Information

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SI Materials and Methods

***Staphylococcus aureus* Isolates.** Eight *S. aureus* isolates were selected from among a collection of 284 CC30 patient isolates that were recovered over a span of 73 y (1935–2008) and from multiple geographical locations. Three isolates (21295, 22030, and 22251) are representative of the phage-type 80/81 clone, four MSSA isolates, and the EMRSA-16 reference strain are representative of contemporary CC30 hospital-associated *S. aureus*, and one isolate (22033) is representative of the Southwest Pacific clone that typically causes community-associated (CA)-MRSA infections.

Generation of Isogenic Mutant Phage-Type 80/81 Strains. The $agrC_{G55R}$ (G→A at position 2,184,961) and hla_{STOP} (G→A at position 1,181,065) mutations present in MRSA252 were created in the phage-type 80/81 clone 22030, according to a published method for allelic replacement (1), with the following modifications. PCR fragments containing the positions to be exchanged were amplified from chromosomal DNA of strain 3636, which contains these mutations, using primers introducing *att* sites for cloning into plasmid pKOR1 as follows: for the *hla* mutation, *hla*_MRSA252_Fwd_att1, 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTAATTTGTCATTTCTTCTTTTTCCCAATCGATTTTATA-3′ and *hla*_MRSA252_Rev_att2, 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTATGAAAACACGTATAGTCAGCTCAGTAACAACAAC-3′; for the *agrC* mutation: *agrC*_MRSA252_Fwd_att1, 5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTAGTCGTTTTTTATTCTTAACTGTAAATTTTTTTATGTT-3′ and *agrC*_MRSA252_Rev_att2, 5′-GGGGACCACTTTGTACAAGAAAGCTGGGTCTAGTTGTTAATAATTTCAACTTTTTGAATGAA-3′). The resulting pKOR1 derivatives were transformed first in strain RN4220, then in strain 22030, and the allelic replacement procedure was performed as described (1). The entire *agr* locus and/or *hla* was sequenced in the 22030 wild type and $agrC_{G55R}$ (22030$agrC_{G55R}$) and hla_{STOP} (22030hla_{STOP}) mutant strains to verify introduction of the 1-bp mutations and absence of unintended second-site mutations in the *agr* operon. Primers for sequencing of the *agr* operon were published previously (2).

Pulsed-Field Gel Electrophoresis (PFGE) and *spa* and SCC*mec* Typing. PFGE was performed as described previously (3). *Spa* typing (4) was performed using eGenomics software. Ridom spa types were assigned using the spa typing website developed by Ridom GmbH. SCC*mec* typing was performed using a molecular beacon real-time PCR (MB-PCR) assay as described elsewhere (5).

Genome Sequencing and Phylogenetic Analyses. Total bacterial DNA was isolated from overnight cultures using a DNeasy tissue kit (Qiagen) according to the manufacturer's instructions, except lysostaphin was added to samples and they were incubated for at least 3 h at 37 °C. Mate-pair libraries were prepared according to the manufacturer's recommendations and DNA sequencing was performed using the SOLiD 3 System (Applied Biosystems). Data generated by the SOLiD sequencer were analyzed using Corona-Lite (Applied Biosystems) and Zoom (Bioinformatics Solutions). This approach gave on average 100× sequence coverage and identified all single nucleotide polymorphisms (SNPs), small insertions/deletions (InDels, insertions up to 4 bp and deletions up to 11 bp), and large deletions. We note that this method is not optimal for identifying large insertions (e.g., mobil genetic elements, MGEs) in query genomes, as such DNA sequence would be absent from the reference strain.

Phylogenetic analyses were performed using a contiguous 1.4-Mb region of the genome identified by Mauve (University of Wisconsin Genome Center) or with 2,299 concatenated SNP nucleotides in the core genome of all eight query isolates compared with the EMRSA-16 reference strain MRSA252 (NC_002952). DNA sequences were aligned using Clustal W (6) and phylogenetic trees were generated by the neighbor joining method (7) using 1,000 bootstraps in the Geneious software package (New Zealand Genome Center).

Verification and Analysis of InDeLs and MGEs. InDels and MGEs were verified by PCR (Illustra PureTaq Ready-to-Go PCR Beads, GE Healthcare) and/or PCR-directed capillary DNA sequencing using oligonucleotide primers (Sigma Genosys) designed with Vector NTI software (Invitrogen). PCR products were purified using either the QIAquick 96-well PCR purification kit or MinElute PCR purification kit (Qiagen) and sequenced using an 3730XL DNA analyzer at the Genomics Core Facility, Research Technologies Section, Rocky Mountain Laboratories, as described previously (8).

Mouse Infection Studies. All animal studies conformed to National Institutes of Health guidelines and were reviewed, approved, and supervised by the institutional animal care and use committees at Rocky Mountain Laboratories (mouse bacteremia model) or the University of Chicago (mouse pneumonia model).

The mouse bacteremia model was performed as described previously (9). *S. aureus* isolates were cultured to midexponential phase of growth (OD_{600} = 0.75) in TSB, washed twice with sterile Dulbecco's PBS (DPBS), and suspended in DPBS. Female CD1 Swiss mice (7–11 wk of age) (Charles River) were inoculated with 5×10^7 *S. aureus* in 100 μL of sterile DPBS or with sterile DPBS via the tail vein. Animals were killed if they were unable to eat or drink or they became immobile. In our experience, mice that are immobile die if not killed within 1–2 h. Therefore, near mortality was scored as death. All mice were killed by 14 d. Survival statistics were performed with a log-rank test (GraphPad Prism version 5.0 for Windows, GraphPad sofware).

The mouse pneumonia model was described previously (10–12). In brief, *S. aureus* strains were cultured at 37 °C in TSB to OD_{660} of 0.5 (exponential phase of growth). Culture aliquots of 50 mL were centrifuged, washed in PBS, and suspended in 750 μL PBS ($3–4 \times 10^8$ cfu per 30-μL volume). Seven-wk-old C57BL/6J mice (The Jackson Laboratory) were anesthetized and then inoculated with 30 μL of *S. aureus* suspension into the left nare. Animals were placed in a supine position for recovery and were observed for the duration of the experiment (48 h). A small percentage of animals typically succumb within the first 6 h after inoculation, likely from the combined effects of aspiration and anesthesia. These animals were not included in subsequent statistical analyses. Statistics for the mouse lung infection studies were performed using a one-way analysis of variance (ANOVA) with a Tukey's posttest to correct for multiple comparisons (GraphPad Prism version 5.0 for Windows).

Identification of hla_{STOP} and $agrC_{G55R}$ and Determination of Presence or Absence of *tst* or *lukF-PV*. The stop codon in *hla* was identified using duplex molecular beacon-based real-time PCR in a single reaction. One molecular beacon (*CGCGAT* GGTAGTTG-

CAACTATACCTTAA *ATCGCG*), labeled by 6-carboxyflurescein (FAM), was specific for the *hla* stop codon (TAG), and a second molecular beacon (*CGCGAT* GGTAGTTGCAACTGTACCTTAA *ATCGCG*), labeled with hexachlorofluorescein (HEX), was specific for the wild-type allele (CAG). PCR primers used for identification of the *hla* stop codon were 5′-GAAAGGTACCATTGCTGGTCA-3′ (forward) and 5′-CCGTTGAATCCATAAGTTAA-3′ (reverse). The region encompassing *agrC* was amplified with the primer set R55G-F (5′-TGGATGAAGCTGAAGTACCAAA-3′) and R55G-R (5′-TAATGTGTATTCATAATAGG-3′). Standard Sanger DNA sequencing was performed using the forward primer. Methods for detecting presence of genes encoding toxic-shock syndrome toxin-1 (*tst*) and the LukF subunit of PVL (*lukF-PV*) (Dataset S7) were described previously (13, 14).

Analysis of Hla and *RNAIII*. Overnight cultures of *S. aureus* were diluted 1:100 in 2 mL of TSB and subcultured to midexponential phase of growth (OD_{600} = 1.0). Bacteria were removed by centrifugation (16,000 × *g* for 10 min at 4 °C) and used for isolation of RNA (see below). A total of 200 μL of the culture supernatant was precipitated using TCA (10%, wt/vol) and proteins were resolved with 12% SDS/PAGE and transferred to polyvinylidene fluoride membranes. Membranes were incubated with rabbit anti-Hla (1:10,000 dilution, Sigma-Aldrich) for 2 h at room temperature, washed, and incubated with horseradish peroxidase-coupled goat antirabbit IgG (1:4,000 dilutions, Zymed-Invitrogen) for 1 h at room temperature. Hla was visualized with enhanced chemiluminescence (GE).

Bacterial pellets were treated with 100 μg/mL of lysostaphin (Sigma-Aldrich) at 37 °C for 30 min. RNA was isolated using RNeasy mini kit (Qiagen), according to the manufacturer's instructions. *RNAIII* and *gyrB* were quantified using real-time reverse-transcriptase PCR using a method published elsewhere (8).

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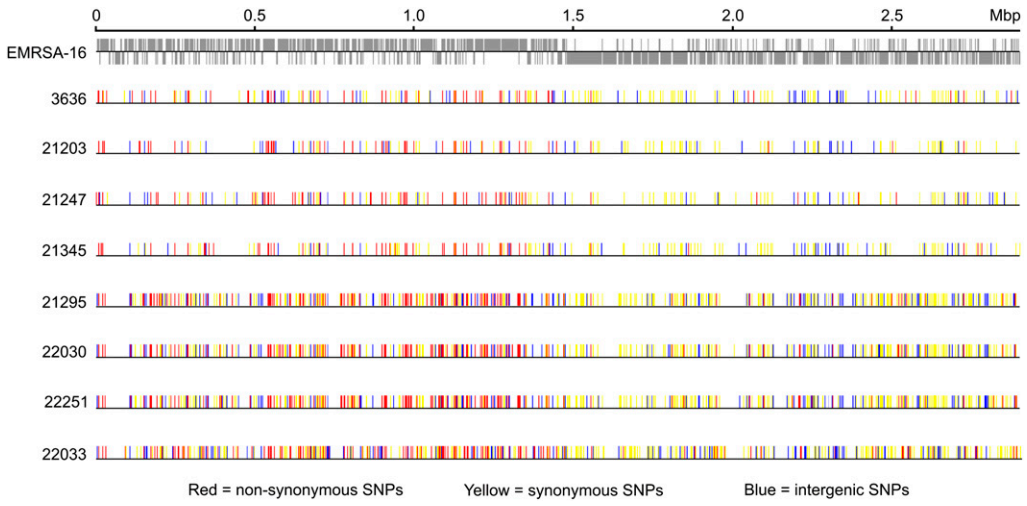

Fig. S1. Distribution of 2,299 SNPs in the core genome of CC30 isolates. Nonsynonymous SNPs (red lines), synonymous SNPs (yellow lines), and SNPs in intergenic regions (blue lines) were mapped using Genespring GX 7.3 (Agilent Technologies).

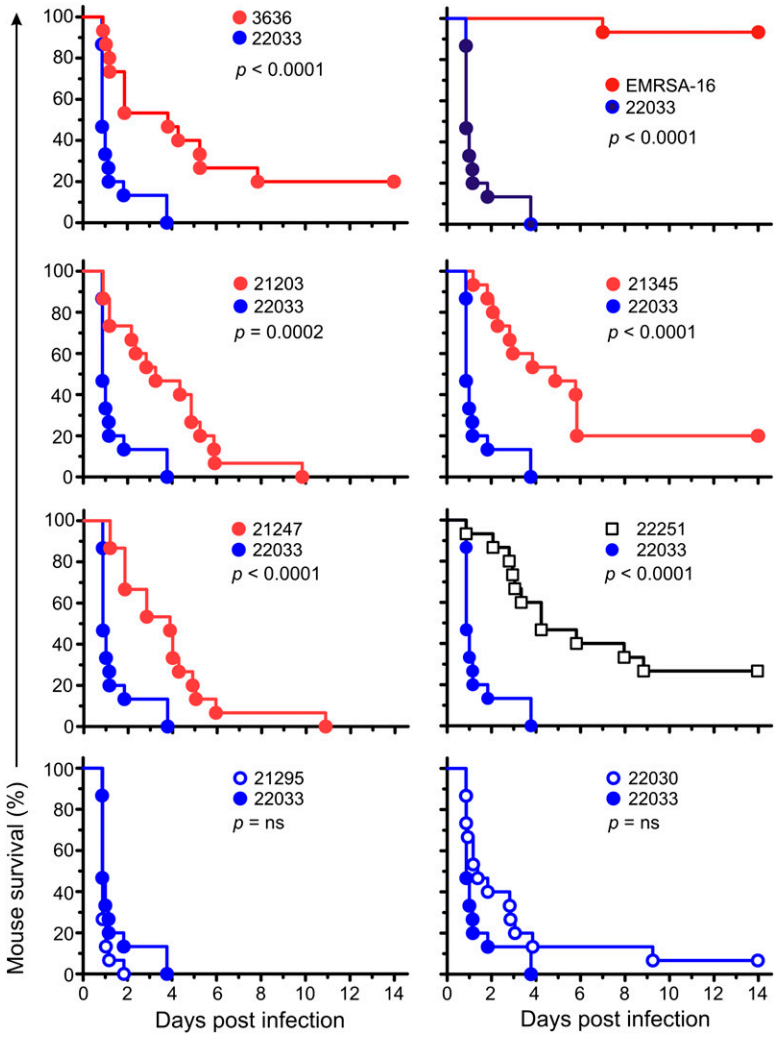

Fig. S2. Virulence of CC30 isolates in a mouse sepsis model. Mice (15 mice per strain) were infected with 5×10^7 cfu of *S. aureus* and animal health was monitored for up to 14 d as described in *SI Materials and Methods* (above).

Other Supporting Information Files

Dataset S1 (XLS)
Dataset S2 (XLS)
Dataset S3 (XLS)
Dataset S4 (XLSX)
Dataset S5 (XLS)
Dataset S6 (XLS)
Dataset S7 (XLS)