Supporting Online Materials:

Materials and Methods

Bacterial strains: Vancomycin-resistant *S. aureus* HIP11714, MRSA (vancomycin-susceptible) *S. aureus* HIP11713, and vancomycin-resistant *Enterococcus faecalis* HIP11704 were obtained from the Michigan State Department of Health. *S. aureus* ATCC 12600 was purchased from the American Type Culture Collection. Staphylococcal and enterococcal cultures were grown in Todd Hewitt broth (THB, Difco), brain heart infusion broth (BHI) or on BHI agar (Difco).

Susceptibility testing: MICs were determined by broth microdilution using cation-adjusted Mueller Hinton broth (Difco) according to NCCLS guidelines (S1). Vancomycin was obtained from Lilly Research Laboratories (Indianapolis, IN) and teicoplanin from Aventis Pharmaceuticals, Inc. (Bridgewater, NJ).

Plasmid Isolation: *S. aureus* plasmids were isolated by following a user-modified protocol for the Midi- or Maxi-plasmid purification kits (QIAGEN, Valencia, CA). Cultures were grown in BHI broth with 10 \( \mu \text{g/ml} \) vancomycin to mid-log phase, harvested by centrifugation, and suspended in QIAGEN lysis buffer. Lysostaphin was added to a final concentration of 50 \( \mu \text{g/ml} \), and the cells were incubated 37 °C/30 min. before beginning the manufacturer’s protocol.

*E. faecalis* plasmids were isolated essentially as described by Clewell, *et al.*, (S2) with the following modifications. Cultures were grown in THB to mid-log phase. Glycine (Invitrogen, Carlsbad, CA) was added to a final concentration of 3%, and cells were harvested after one hour. Cells were incubated for 30 min./37 °C in 1 mg/ml lysozyme
Plasmid DNA was purified by two rounds of cesium chloride (CsCl)-ethidium bromide gradient centrifugation (S3) and extracted with water-saturated, CsCl-saturated isopropanol. The plasmid DNA preparation was diluted with TE (10 mM Tris, 0.1 mM EDTA, pH8) to prevent CsCl precipitation, and desalted by ethanol precipitation.

Isolated plasmids were digested with *Hind*III (New England Biolabs, Beverly, MA), and the resulting restriction fragments were separated on a 0.7% agarose gel in Tris-borate-EDTA (TBE) buffer at 50 V for 15 hrs. DNA fragment size standard: 1 Kb Plus DNA Ladder (Invitrogen). The gel and blot were photographed with ruler images to indicate relative band migration distances.

**Southern hybridization:** Plasmid DNA from a 0.7% agarose gel was transferred to BA-S reinforced nitrocellulose (Schleicher and Schuell, Keene, NH). The probe, which was hybridized at 65 °C, was a 1032-bp PCR product amplified from *vanA* using plasmid DNA from the vancomycin-resistant *E. faecalis* co-isolate as the template. The PCR product was generated by amplification with oligonucleotide primers *vanA1* (5’-ATGAATAGAATAAAAGTTGCAATAC-3’) and *vanA2* (5’-TCACCCCTTTAACCCTAATACGATC-3’) and labeled with ³²P-dATP by nick translation. Hybridizing bands were visualized by exposure to Kodak X-OMAT AR film at -20 °C for 21 hrs with intensifying screens.

**PCR and DNA sequence analysis for identification and characterization of the VRSA isolate:** 16S ribosomal DNA (rDNA) was amplified and sequenced as described by Ibrahim *et al.* (S4). Oligonucleotide primers for the *S. aureus* gyrA sequence were designed from sequence data published by Margerrison *et al.* (S5); The forward primer,
GYRA1 (5'- GGAAC{T}C{T}GATGGCTGA) and reverse primer, GYRA2R (5'-TGACG{G}C{T}C{T}TTTCATTAC) amplified a 484-bp gene fragment that includes the quinolone-resistance determining region (QRDR) and flanking nucleotide sequences of gyrA. PCR parameters: 95°C/5 min, followed by 35 cycles of: 95°C/45 s, 50°C/20 s, 72°C/30 s; then 72°C/7 min. using a GeneAmp 9700 thermal cycler (PE Biosystems, Foster City, CA). Oligonucleotide primers for gyrB were those described by Kaatz and Seo (S6). vanA was amplified as described by Clark et al. (S7).

DNA sequences of each gene were determined by using the forward and reverse primers with independent PCR-amplified gene fragments as DNA templates. The sequences were compared with analogous sequences from the S. aureus type strain, ATCC 12600. The rDNA sequence was consistent with S. aureus. The VRSA gyrA contained a single nucleotide substitution in the codon for Ser-80, a mutation associated with fluoroquinolone resistance. The gyrB sequence was identical to that of the S. aureus type strain. Amplification of enterococcal ligase genes was as described by Dutka-Malen et al. (S8).

**Sequencing, assembly, and annotation of pLW1043.**

Cloning, sequencing, and assembly were performed following the standard strategy for TIGR microbial genome projects (S9). One small insert (2-3 kb) and one large insert (~10 kb) shotgun libraries were constructed in the pHOS2 vector (a pBR derivative) after random, mechanical shearing (nebulization) of plasmid DNA. Sequencing of the small and large insert libraries was achieved at a success rate of 90%, with an average read length of ~ 850 nucleotides. Random sequences were assembled using TIGR assembler (S10) into a single molecule of 57,889 kb. The coverage criteria were that every position
required at least double-clone coverage and either sequence from both strands or with two different sequencing chemistries. The sequence was edited manually with the TIGR Editor. Open reading frames (ORFs) likely to encode proteins were predicted by Glimmer (S11) a program based on interpolated Markov models. Glimmer was trained with ORFs previously identified from \textit{S. aureus} and \textit{E. faecalis} plasmids available in GenBank. All predicted proteins larger than 30 amino acids were searched against a nonredundant protein database. Alignments were evaluated and database matches selected for genes with significant similarity scores. The sequence was deposited in GenBank, with an accession number of AE017171.

**Filter mating.** \textit{E. faecalis} 11704 (donor) and \textit{E. faecalis} JH2-2 (recipient) were grown in BHI with 50 $\mu$g/ml vancomycin or 25 $\mu$g/ml fusidic acid, respectively, to maintain selective phenotypes. Overnight cultures were diluted 1:500 and incubated at 37 $\°C$ /5 hr. Cells from the mating mix (200 $\mu$l), consisting of a 20:1 ratio of donor/recipient cells, were collected on a 0.2 $\mu$m, 115-ml Nalgene filter unit (Nalgene, Rochester, NY) by vacuum filtration and incubated on BHI agar, 37 $\°C$ /16 h. Cells were recovered by mincing the filter and washing the pieces in 5 ml BHI broth. Serial dilutions were plated on BHI agar containing either 50 $\mu$g/ml vancomycin, 25 $\mu$g/ml fusidic acid, or both. Conjugation efficiency was approximately 3 x $10^{-4}$ transconjugants per recipient.

\textit{S. aureus} 11714 (donor) and COL (recipient) were grown in BHI broth with 50 $\mu$g/ml vancomycin or 4 $\mu$g/ml tetracycline, respectively. Each of the overnight cultures was diluted 1:500 in fresh BHI broth and incubated at 37 $\°C$ /5 hr. One hundred microliters of a 1:10 mixture (donor to recipient) was collected on a 0.2 $\mu$m Nalgene filter (as above). The filter was incubated for 37 $\°C$ /16 hr on BHI agar. Cells were washed from the filter,
plated on BHI agar containing 4 µg/ml tetracycline plus 4 µg/ml erythromycin, and incubated 37 °C /48 hr. Individual colonies were selected and screened for vancomycin resistance on BHI agar containing 50 µg/ml vancomycin.

**Fig. S1. Pulsed-field gel electrophoresis (PFGE).** Genomic DNA was digested with *Eag*I. Lanes: 1, DNA size marker (*S. aureus* 8325/SmaI digest); 2, VRSA; 3, MRSA; 4, *S. aureus* COL; 5, transconjugate of the COL strain with the VRSA plasmid. The arrow indicates the additional band seen in the transconjugate genomic DNA when compared with the *S. aureus* COL (host) strain. This band, also visible in the VRSA, hybridized with a *vanA* probe (Data not shown) (S12). PFGE was performed as described by McDougal *et al.* (S13).
Supporting Online References:
S1. NCCLS. M7-A5, 2000, National Committee for Clinical Laboratory Standards, Wayne, Pa.).
S12. Southern hybridization with probe for vanA was performed as described above.