Supplementary Material

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Authors' contributions

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Performed initial identification and characterization of the BR-VRSA and BR-VSSA isolates, recovered the vancomycin-resistant *E. faecalis* from patient's rectum and collected all relevant clinical information.

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Confirmed the identification of the isolates and performed all experiments related to the molecular characterization of the isolates, molecular typing by pulsed field gel electrophoresis, MLST and *spa*, plasmid characterizations, mating experiments, minimal inhibitory concentration determinations and sequence analysis of pBRZ01.

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Performed whole genome sequencing and annotation of BR-VRSA and the VREfs isolate. Additionally, these investigators obtained, extracted and assembled the plasmid sequence of pBRZ01.

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Performed whole genome sequencing and annotation of BR-VSSA and carried out phylogenetic analysis of all *S. aureus* isolates and writing of the manuscript.

Barbara E. Murray, MD.

Contributed in the experiment design, analysis and writing of the manuscript.

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Principal investigator. Dr Arias designed the study, gathered the data, analyzed the data, wrote the manuscript and decided to publish the paper. Dr Arias vouches for the data and the analysis,

Supplementary Material and Methods

Genome sequencing

Genomic DNA was prepared from overnight cultures using DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genome libraries were prepped using the Nextera XT kit and sequenced using the Illumina platform (paired end reads of 250 bp). Genome assembly was done using ABySS [1] at multiple kmer sizes (kmer=23, 27, 31, 35, 39, 63), and assembly was done with the highest N50 value (kmer=39) for further analysis.

Phylogenetic and sequence analysis

Comparison of single nucleotide polymorphisms (SNP) between BR-VSSA and BR-VRSA was accomplished through the short read alignment to the *S. aureus* genome for strain TCH1516 as a reference using the Burrows-Wheeler Alignment (bwa) tool (http://bio-bwa.sourceforge.net). SNP calls were made using samtools (http://samtools.sourceforge.net) and SNPs were identified as "high quality" if they were unambiguous and had a q score greater than or equal to 20.

For the phylogenetic matrix, for assembled genomes we created single nucleotide polymorphism (SNP) phylogenetic dataset with reference to the *S. aureus* COL genome using the show-snps utility of NUCmer (http://mummer.sourceforge.net). Phylogenetic analysis was done using maximum likelihood (ML) framework with the RAxML v7.4.2 [2] We used the general time-reversible (GTR) substitution model [3] accounting for among-site rate heterogeneity using the Γ distribution and four rate categories [4] for ten individual searches with maximum parsimony random-addition starting trees. Node support was evaluated with 100 nonparametric bootstrap pseudoreplicates.

For restricting SNP analysis to the core genome, we excluded all regions of the genome that have been annotated as mobile genetic elements. The regions masked from the analysis are provided as supplementary information (Moblile_Elements_COL.xls, Mobile_Elements_TCH1516.xls)

Table S1. Minimal inhibitory concentrations of staphylococcal and enterococcal clinical isolates and transconjugants

Strain (date of isolation)	MICs (μg/mL)													
	OXA	**AMP**	**ERY**	**CLI**	**CIP**	**GEN**	**CHL**	**RIF**	**TET**	**MIN**	**TMP/SMX**	**LNZ**	**VAN**	**TEI**
Vancomycin-susceptible MRSA (07/16/2012)*	≥4	ND	≥8	≥8	≥8	≥16	ND	≤0.5	ND	ND	≥320	2	1	ND
Vancomycin-susceptible MRSA (07/24/2012)*	≥4	ND	≥8	≥8	≥8	≥16	ND	≤0.5	ND	ND	≥320	2	1	ND
Vancomycin-susceptible MRSA (BR-VSSA) (08/15/2012)	>64	ND	>64	>32	32	0.5	8	0.015	0.5	1	>64/1216	1	0.5	0.5
Vancomycin-resistant MRSA (BR-VRSA) (08/15/2012)	>64	ND	>64	>32	32	**32**	4	0.015	0.5	0.12	16/304	1	**>256**	**32**
BR-VSSA-FA[¶]	>64	ND	>64	>32	>32	1	8	0.015	0.5	0.25	>64/1216	2	1	0.5
BR-VSSA-FA[¥] Transconjugant 1	>64	ND	>64	>32	>32	**32**	8	0.015	0.5	0.25	>64/1216	2	**>256**	**32**
S. aureus RN4220-RF[¶]	0.25	ND	0.5	0.12	1	1	4	0.015	0.5	0.25	0.06/1.18	2	1	1
S. aureus RN4220 RF[¥¥] Transconjugant 2	0.25	ND	0.25	0.12	1	**64**	4	>8	0.5	0.12	0.06/1.18	1	**>256**	**32**
S. aureus COL-FA[¶]	>64	ND	0.25	0.12	1	0.5	8	0.015	0.5	0.12	0.25/4.7	1	0.5	0.5
S. aureus COL-FA[¥¥¥] Transconjugant 3	>64	ND	0.25	0.06	1	**64**	8	0.015	0.5	0.12	0.25/4.7	2	**>256**	**32**
Vancomycin-resistant *E. faecalis* (08/29/2012)[£]	ND	1	ND	ND	>32	>64	64	ND	ND	ND	ND	2	128	32

OXA, oxacillin; AMP, ampicillin; ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; GEN, gentamicin; CHL, chloramphenicol; RIF, rifampin; TET, tetracycline; MIN, minocycline; TMP/SMX, trimethoprim/sulphamethoxazole; LNZ, linezolid; VAN, vancomycin; TEI, teicoplanin. FA, fusidic-acid resistant. Transferred resistances are shown in bold.

ND, not done

*Performed using only an automated method for MIC determination. Isolates are not available for further testing.

[¶] Fusidic Acid (FA) resistant variant used as recipient for mating experiments

[¥] Transconjugant obtained from a mating experiment between BR-VRSA and BR-VSSA-FA (fusidic acid resistant)

[¥¥] Transconjugant obtained from a mating experiment between BR-VRSA and RN4220-RF (rifampin and fusidic acid resistant)

[¥¥¥] Transconjugant obtained from a mating experiment between BR-VRSA and COL-FA (fusidic acid resistant)

[£] Isolate recovered from a rectal swab and exhibits high-level resistant to gentamicin and streptomycin.

Table S2. Transfer efficiency of the *vanA*-gene cluster between different staphylococcal and enterococcal strains

Donors	Recipients (all FA-resistant)	Efficiency (transconjugants per donor)
BR-VRSA	BR-VSSA	2.6×10^{-4}
BR-VRSA	*S. aureus* RN4220-RF	1.65×10^{-4}
BR-VRSA	*S. aureus* COL	6.0×10^{-5}
BR-VRSA	*E. faecalis* OG1RF	ND
VREfs*	BR-VSSA	ND
VREfs*	*S. aureus* RN4220-RF	ND
VREfs*	*E. faecalis* OG1RF	ND

All mating experiments were performed in brain heart infusion (BHI) agar in the presence of vancomycin (32 µg/ml) and fusidic acid (FA) (25 µg/ml) to select for transconjugants.

*VREfs, vancomycin-resistant *E. faecalis* isolate recovered from the patient using a rectal swab

ND, Not determined; colonies were not obtained, suggesting transfer below the limit of detection (10^{-9})

Table S3. Genetic determinants of antibiotic resistance present the BR-VRSA genome

Antibiotic	Minimal inhibitory concentration (µg/ml)	Genetic determinant
OXA	>64	*mecA*, encoding PBP2a
ERY	>64	*ermB,* encoding the rRNA methylase ErmB
CLI	>32	*ermB,* encoding the rRNA methylase ErmB
CIP	32	Substitutions in GyrA (Ser84→Leu) and GrlA (Ser80→Phe)
GEN	64	*aac(6)'aph(2)''*
TMP/SMZ	16/304	Substitution in the dihydropteroate synthase (Val26→Leu)
VAN	>256	*vanA* gene cluster
TEI	32	*vanA* gene cluster

Antibiotic susceptibilities were interpreted according to the Clinical Laboratory Standards Institute (CLSI) breakpoints. ERY, erythromycin; CLI, clindamycin; CIP, ciprofloxacin; GEN, gentamicin; TMP/SMX, trimethoprim/sulphamethoxazole; LNZ, linezolid; VAN, vancomycin; TEI, teicoplanin.

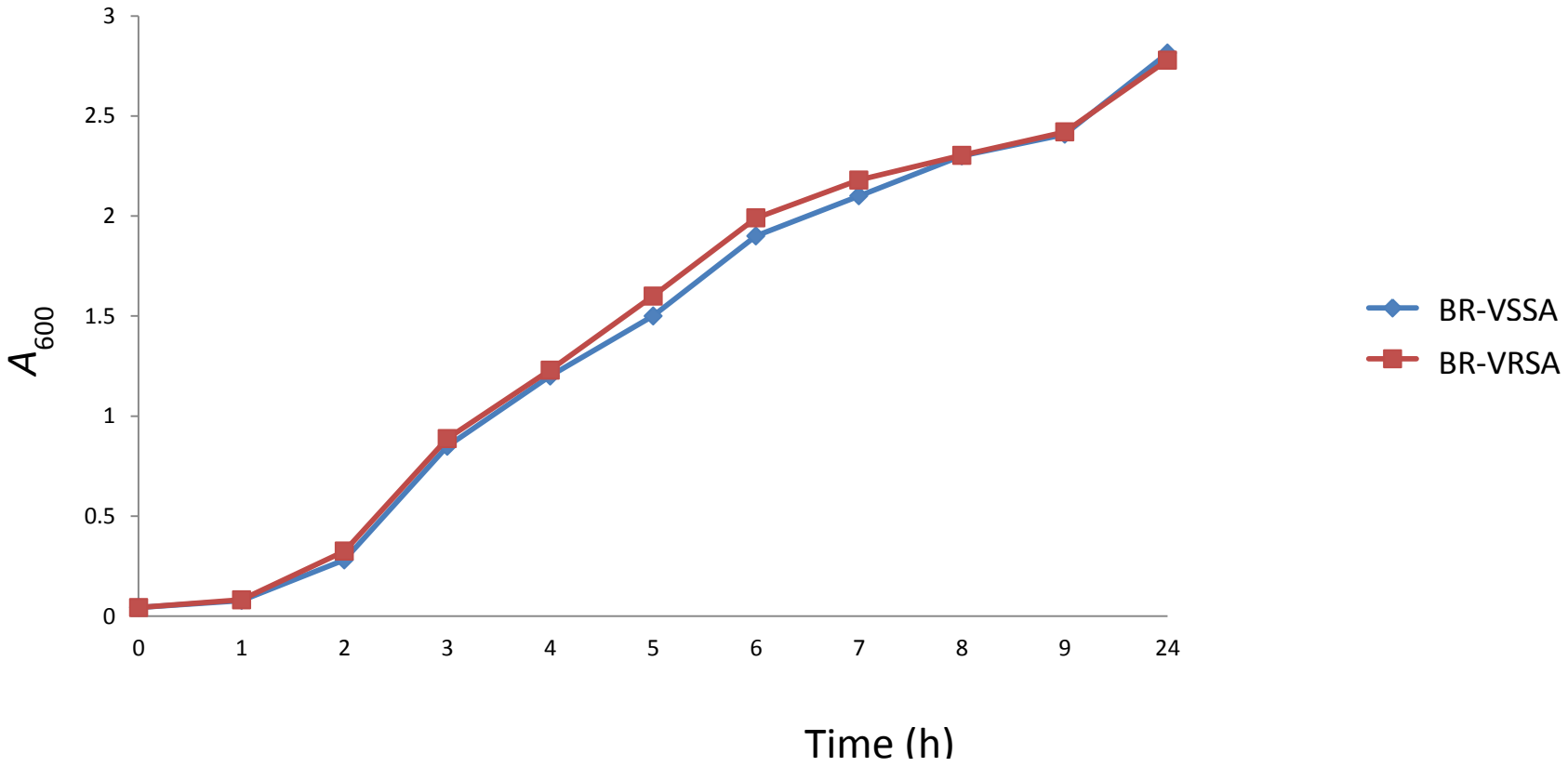

Figure S1. Growth curves of BR-VRSA and BR- VSSA in brain heart infusion (BHI) broth.
A_{600}, optical density at 600 nm; h, hours.

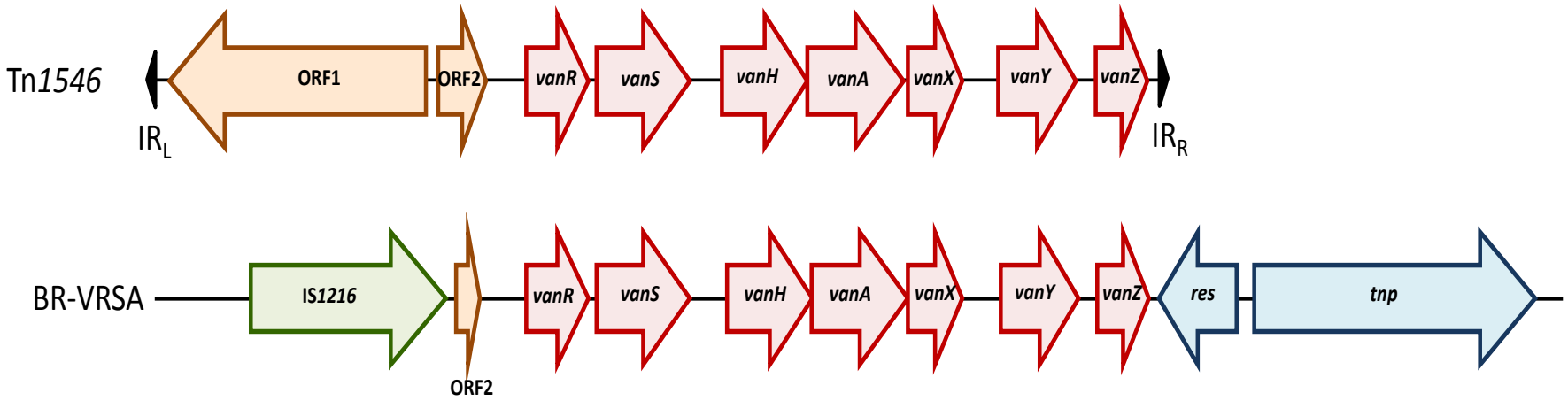

Figure S2. Genetic organization of Tn*1546* and Tn*1546*-derivative from BR-VRSA. Tn*1546* from *Enterococcus faecium* BM4147 (Accession number M97297)[5]. Open arrows represent coding sequences, IRL and IRR, inverted left and right repeats, respectively. ORF, open reading frame; *res*, resolvase gene; *tnp*, gene encoding a transposase from the family Tn*3*.

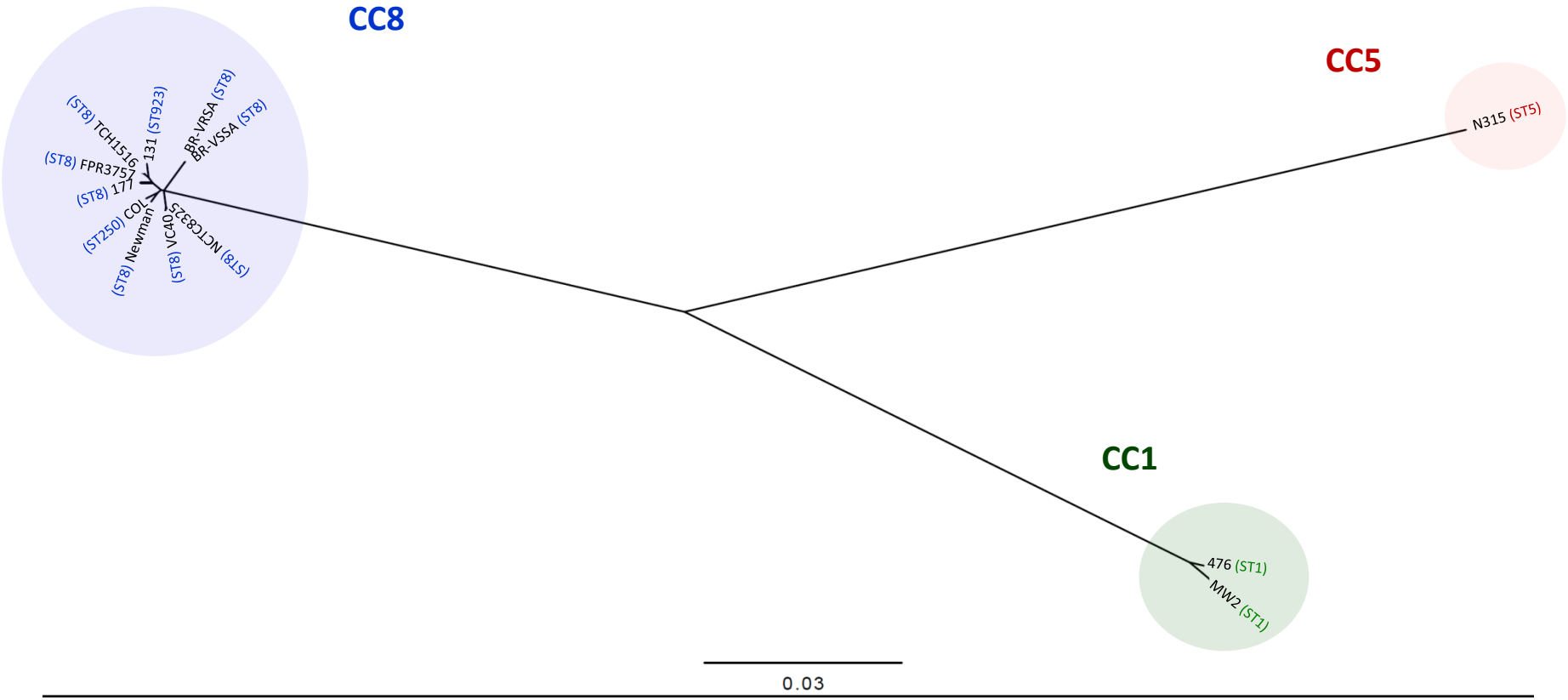

Figure S3. Phylogenetic tree based on core genome single nucleotide polymorphisms, generated using the maximum likelihood optimality criterion. Branch lengths are proportional to the inferred number of evolutionary changes. All nodes have 100% boostrap support. Sequence type (ST) and clonal complex (CC) of the *S. aureus* strains are indicated.

References

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