Supplemental Online Content

DNA sequencing, phylogenetic analyses, SNP determination, and enterococcal resistomes

Sample processing and sequencing

Genomic DNA was isolated with the QIAGEN QIAamp Mini Kit (Maryland, USA), and library preparation was performed using the Nextera DNA Flex Library Prep Kit (California, USA) with unique barcodes· Pooled isolates were sequenced with an Illumina MiSeq using 2x300 paired-end reads or an Illumina HiSeq 4000 with 2x150 paired-end reads· Sequencing data were processed and adapters and low-quality bases were trimmed with Trimmomatic[1] v0·361· Trimmed data were assembled using SPAdes[2] v3·11·12, and assembled contigs shorter than 500 bases were removed using a custom script·

Phylogenetic analysis

Genomes were annotated with Prokka[3] v1.14.5 to create GFF files. These were used to create core genome alignments with MAFFT[4] using Roary[5] v 3.13.0 with default parameters. Separate midpoint-rooted maximum-likelihood phylogenetic trees based on core genome alignment were created for *E· faecalis* and *E· faecium* using RAxML[6] v8·2·12 with 100 bootstrap iterations· Clade A and Clade B reference genomes (AUS004 and Com15, respectively) were included in the *E· faecium* tree to aid in determination of cladal division· Trees were visualized using iTOL[7]·

To determine relatedness and potential transmission events for *E. faecium* Clade A isolates, a SNP alignment was created with snp-dists (https://github.com/tseemann/snp-dists) using the core gene alignment from Roary, disregarding recombination events and non-coding sequences. A subtype was defined as organisms differing by < 20 single nucleotides, as confirmed by core gene alignment.[8] The heat map for visualization of the SNP matrix output from snp-dists was created with iTOL.

Resistome

Acquired resistance elements were searched with BLASTX[9] against the ResFinder database[10]· Hits were selected if they had an identity percentage higher or equal to 95% and a coverage of at least 80% of the target sequence· We determined fluoroquinolone resistance by identifying amino acid substitutions in GyrA (Ser83Ile/Arg, Glu87Gly for *E· faecalis* and Ser84Tyr/Leu/Ile/Arg and Glu88Lys/Gly for *E· faecium*, NCBI accession numbers: NP_813819·1, YP_006374612·1, respectively) in GyrB (Glu474Lys for *E· faecium* only· Accession: YP_006374611·1) and ParC (Ser80Arg/Ile and Glu84Lys for *E· faecalis* and Ser80Arg/Ile and Glu84Lys/Thr for *E· faecium*· Accessions: NP_815327·1, YP_006375753·1, respectively)[11, 12]· We investigated linezolid resistance associated mutation G2576T in genes encoding 23S rRNA (GeneID 13001435 for *E· faecium* and 1199161 for *E· faecalis*)[13] · Changes in protein L3 (substitutions between residues 127-174· Accession: AAO80075·1 for *E· faecalis* and AFK57681·1 for *E· faecium*) and L4 (substitutions between residues 65-72· Accession: AAO80076·1 for *E· faecalis* and AFK57682·1 for *E· faecium*) were also included[14]· Daptomycin (DAP) non-susceptibility was predicted by the identification of amino acid substitutions in LiaS (Thr120Ala· Accession: YP_006375543·1), LiaR (Trp73Cys· Accession: YP_006375544·1) and Cls (Asn13Ile, His215Arg, Arg218Gln, Asn237Asp, and Glu278Gln· Accession: YP_006375674·1) for *E· faecium*[15, 16]· Similarly, deletion of Ile117 in LiaF (Accession: AEA94900·1) and deletion of Lys61 in Cls (Accession: WP_002413481·1) for *E· faecalis* associated with DAP non-susceptibility[17] were identified· Prediction of ampicillin resistance for *E· faecium* was obtained using an artificial intelligence model[18] based on the sequence of the PBP5 S/R profiles[19]· The approach uses Random Forest over 100 decision trees trained with 42 genomes of isolates with known MIC to ampicillin (ranging MICs between 0·25-128 µg/ml) and tested on 208 genomes with different susceptibility profiles, obtaining predictions with 100% specificity and 96% sensitivity· The false negatives included 6 cases where the isolates were resistant but predicted to be susceptible[18]·

Sensitivity analysis

We used an inverse probability weighing (IPW) Cox analysis to evaluate the association of VRE with hospital mortality using the inverse of the propensity score as weights. We built a multivariate logistic

regression model for propensity score for VRE including age (continuous), sex (yes or no), history of previous hospitalization in the last year (yes or no), hospital unit of admission (ICU, Non-ICU), Charlson score (continuous) and history of bone marrow transplant (yes or no), neutrophil count < 500 cells/ml (yes or no) and history of living in nursing house facility (yes or no). From the estimated propensity score, we calculated the weights as 1/(propensity score) for the VRE group and 1/(1 − propensity score) for the VSE group. Extreme weights were trimmed by setting them to 10 (if >10) or 0.1 (if < 0.1). An IPW Cox regression model was used including VRE, Pitt bacteremia score, microbiological failure and urinary catheter as covariates, stratified by unit of admission and with robust SEs to account for institution. This analysis was also performed at 4, 7, 12, and 15 days after the index culture.

Table S1. Variables used to create propensity score

Variables
Age; years old median (IQR)
Gender, male (yes/no)
Intensive care unit admission (yes/no)
Reason of admission – medical (yes/no)
Length of hospitalization; days median (IQR)
Charlson comorbidity index; median (IQR)
Previous hospitalization within 1 year (yes/no)
Nursing home/Long term facility (yes/no)
Neutropenia, defined as <500 cells/microlitre (yes/no)

Table S2 Antibiotic resistance genes

Gene name	Predicted product	Antibiotic Class	Comments
gyrA	GyrA (DNA gyrase A subunit)	Fluoroquinolones	Ser83Ile/Arg, Glu87Gly for *E· faecalis* and Ser84Tyr/Leu/Ile/Arg and Glu88Lys/Gly for *E· faecium*
gyrB	GyrA (DNA gyrase B subunit)	Fluoroquinolones	Glu474Lys for *E· faecium* only
parC	Topoisomerase IV subunit C	Fluoroquinolones	Ser80Arg/Ile and Glu84Lys for *E· faecalis* and Ser80Arg/Ile and Glu84Lys/Thr for *E· faecium*
23s rRNA		Oxazolidinones	G2589T in *E· faecium* and G2587T in *E· faecalis*
rplC	L3 ribosomal protein	Oxazolidinones	Substitutions between residues 127-174 in both *E· faecium* and *E· faecalis*
rplD	L4 ribosomal protein	Oxazolidinones	Substitutions between residues 65-72 in both *E· faecium* and *E· faecalis*
liaF	LiaF	Daptomycin	Deletion of Ile177 in *E· faecalis*
liaS	LiaS	Daptomycin	Thr120Ala substitution in *E· faecium*
liaR	LiaR	Daptomycin	Trp73Cys substitution in *E· faecium*
cls	Cardiolipin synthase CLS	Daptomycin	Deletion of Lys61 in *E· faecalis*
pbp5	Penicillin binding protein 5 (PBP5)	Penicillins	S/R profiles for *E· faecium*
vanA	D-Ala-D-Lac ligase	Glycopeptides	
aadK	Aminoglycoside nucleotidyltransferase	Aminoglycosides	
aac(6')-Ie-aph(2")-Ia	Aminoglycoside acetyltransferase	Aminoglycosides	
ant(6)-Ia	Aminoglycoside nucleotidyltransferase	Aminoglycosides	
aph(2")-Ic	Aminoglycoside phosphotransferase	Aminoglycosides	
aph(3")-III	Aminoglycoside phosphotransferase	Aminoglycosides	
aadD	Aminoglycoside nucleotidyltransferase	Aminoglycosides	
spc	Aminoglycoside nucleotidyltransferase	Aminoglycosides	
cfrB	23S ribosomal RNA methyltransferase	Oxazolidinones	
ermA	23S ribosomal RNA methyltransferase	MLS_B	
ermB	23S ribosomal RNA methyltransferase	MLS_B	
ermT	23S ribosomal RNA methyltransferase	MLS_B	
lnuB	Lincosamide nucleotidyltransferase	MLS_B	
lsaA	ABC-F ribosomal protection protein	MLS_B	
mefA	ABC-F ribosomal protection protein	MLS_B	

msrC	ABC-F ribosomal protection protein	MLS_B	
*cat(*pC221)	Chloramphenicol acetyltransferase	Chloramphenicol	
cat	Chloramphenicol acetyltransferase	Chloramphenicol	
dfrG	Dihydrofolate reductase	Trimethoprim	
tetL	Tetracycline efflux protein	Tetracyclines	
tetM	Ribosomal protection protein	Tetracyclines	
tetS	Ribosomal protection protein	Tetracyclines	

Table S3. Estimated hazard ratios (HR) of in-hospital mortality when fitting an univariable and multivariate cox regression model.

	Unadjusted			Adjusted conventional §‡		
Variable	HR	95% CI	p value	HR	95% CI	p value
Age; (years)	0.99	0.97-1	0.172			
Sex; Male	1.2	0.64-2.26	0.577			
Intensive care unit	2.22	1.20-4.09	0.012			
Reason for admission (Medical)	2.4	0.57-10.14	0.23			
Length of hospitalization (days)	0.99	0.97-1.00	0.082			
Charlson score	0.93	0.80-1.08	0.328			
Bone marrow transplant	1.24	0.59-2.62	0.571			
Immunosuppressive therapy	0.9	0.49-1.68	0.744			
Previous hospitalization within a year	1.43	0.66-3.10	0.365			
Nursing home/long term facility	0.43	0.06-3.16	0.409			
Hemodialysis	1.15	0.56-2.42	0.663			
Recent surgical procedure	0.79	0.27-2.32	0.675			
Steroid use	1.63	0.84-3.15	0.15			
Pitt bacteremia score ≥ 2	2.72	1.52-5.14	0.001	1.83	1.47-2.28	<0.001
Neutropenia, defined as <500 cells/microlitre	2.78	1.50-5.14	0.001	3.13	2.89-3.39	<0.001
Central line placement	2.25	1.09-4.61	0.028			
Urinary catheter	2.17	1.17-4.02	0.014	1.85	1.17-2.93	0.009
Mechanical ventilation	3.15	1.60-6.10	0.001			
Polymicrobial infection	1.66	0.84-3.30	0.144			
VRE BSI	2.21	1.20-4.10	0.011	2.13	1.54-2.93	<0.001
Infectious diseases Consult ∞	1.96	0.60-6.38	0.261			
Central line infection	1.11	0.57-2.13	0.765			
Abdominal/gastrointestinal infection	0.94	0.45-1.98	0.879			
Unknown/primary source	1.06	0.56-1.99	0.853			
β-lactams†	0.51	0.22-1.22	0.131			
Daptomycin monotherapy	1.09	0.56-2.11	0.797			
Daptomycin monotherapy dose (mg/kg;continuous)	0.91	0.76-1.09	0.289			
Daptomycin plus other antibiotics	1.39	0.64-3.01	0.407			
BSI recurrence	1.02	0.39-2.70	0.962			
Microbiological failure	2.34	1.22-4.47	0.01	2.4	1.34-4.31	0.003

§ Inclusion of variables in the adjusted model were determined through purposeful variable selection. ‡A hospital specific random effect intercept was included in the model and were stratified by hospital unit of admission

Table S4 Evaluation the interaction between VRE and microbiological failure on in-hospital mortality

Variables	HR	0·95 (CI)	p value*
VRE	3·57	1·23-10·38	0·019
Microbiological failure	1·70	0·66-4·36	0·268
Microbiological failure / VRE (0:1)	**0·49**	**0·13-1·83**	**0·29**
	HR	0·95 (CI)	p value
VRE	1·76	0·83-3·76	0·141
Microbiological failure	3·45	1·38-8·62	0·008
Microbiological failure / VRE (1:0)	**0·49**	**0·13-1·83**	**0·29**
	HR	0·95 (CI)	p value
VRE	1·76	0·83-3·76	0·141
Microbiological failure	1·70	0·66-4·36	0·268
Microbiological failure / VRE (1:1)	**2·03**	**0·55-7·50**	**0·29**

*A p value < 0·05 is consider significant·
Three interaction models were tester 0=absence of the variable; 1= presence of the variable· Interaction terms are on bold·

	Day 4 of BSI Dead events =8			Day 7 of BSI Dead events =12			Day 10 of BSI Dead events =19			Day 12 of BSI Dead events =21			Day 15 of BSI Dead events =29		
Variables	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Neutropenia, defined as <500 cells/microlitre	3.26	3.12-3.40	<0 .001	3 .29	3.23-3.34	<0 .001	3 .36	3.20-3.53	<0 .001	3 .43	3.23-3.64	<0 .001	3 .82	3.65-3.99	<0 .001
Urinary catheter	1.83	1.22-2.74	0 .003	1 .78	1.20-2.63	0 .004	1.79	1.23-2.59	0 .02	1.83	1.29-2.60	0 .001	2 .00	1.51-2.64	<0 .001
Microbiology failure	2 .46	1.33-4.56	0 .004	2 .49	1.32-4.69	0 .005	2 .49	1.32-4.68	0.005	2 .48	1.34-4.64	0 .005	2 .49	1.28-4.82	0 .007
Pitt bacteremia score ≥2	1.80	1.42-2.27	<0 .001	1 .77	1.38-2.27	<0 .001	1 .75	1.34-2.30	<0 .001	1 .74	1.32-2.29	<0 .001	1 .72	1.27-2.34	0 .001
Time variable covariate															
VRE * time	1 .91	1.01-3.61	0 .046	1 .68	0.68-4.15	0 .260	1 .92	0.74-4.99	0 .178	2 .48	0.95-5.61	0 .066	7 .02	2.61-18.90	<0 .001

Table S5. Estimated hazard ratios adjusted by time for VRE in-hospital mortality at five period

A hospital specific random effect intercept was included in the model and was stratified by hospital unit of admission

Table S6 Estimated hazard ratios (HR) for in-hospital mortality among individual with *Enterococcus faecium* BSI

	Univariate			Adjusted ‡		
Variable	**HR**	**95% CI**	**p value**	**HR**	**95% CI**	**p value**
Age; (years)	0·99	0·96-1·01	0·349			
Sex; Male	1·14	0·53-2·47	0·733			
Intensive care unit	1·88	0·84-4·22	0·126			
Reason for admission (Medical)	2·46	0·52-11·71	0·258			
Length of hospitalization (days)	0·98	0·96-1·00	0·057	0·98	0·97-0·99	< 0·001
Charlson score	0·79	0·61-1·04	0·093	0·90	0·59-1·37	0·614
Bone marrow transplant	1·61	0·66-3·93	0·292			
Immunosuppressive therapy	0·56	0·26-1·21	0·143			
Previous hospitalization within a year	1·11	0·38-3·23	0·852			
Nursing home/long term facility	1·23	0·16-9·19	0·839			
Hemodialysis	0·79	0·30-2·07	0·633			
Recent surgical procedure	1·02	0·33-3·17	0·966			
Steroid use	1·37	0·61-3·08	0·444			
Pitt bacteremia score ≥2	1·75	0·81-3·79	0·154			
Neutropenia, defined as <500 cells/microlitre	1·46	0·67-3·17	0·34			
Central line placement	2·57	0·88-7·46	0·084	2·74	1·76-4·27	< 0·001
Urinary catheter	1·95	0·90-4·24	0·091	2·65	1·35-5·19	0·004
Mechanical ventilation	2·43	1·00-5·87	0·05	2·14	1·85-2·48	< 0·001
Polymicrobial infection	1·40	0·59-3·33	0·446			
VRE BSI	1·72	0·48-6·22	0·405			
Infectious diseases Consult ∞	0·99	0·23-4·23	0·987			
Central line infection	0·91	0·41-1·99	0·806			
Abdominal/gastrointestinal infection	1·43	0·61-3·34	0·407			
Unknown/primary source	0·83	0·33-2·08	0·689			
β-lactams†	0·58	0·14-2·49	0·465			
Daptomycin monotherapy	0·71	0·33-1·54	0·386			
Daptomycin monotherapy dose (mg/kg;continuous)	0·84	0·66-1·05	0·132			
Daptomycin plus other antibiotics	0·86	0·32-2·31	0·763			
Recurrence of BSI	0·69	0·23-2·07	0·504			
Microbiological failure	3·91	1·79-8·54	0·001	5·03	3·25-7·77	< 0·001

§ Variables with a p value <0.1 were included into the adjusted models. ‡A hospital specific random effect intercept was included in the model and was stratified by hospital unit of admission. ∞ Defined as days from final report of blood culture to the day when the infectious diseases service was consulted. † β-lactams included ampicillin, ampicillin-sulbactam, ertapenem, amoxicillin-clavulanate, ceftriaxone or piperacillin/tazobactam.

Table S7· DOOR analysis for the entire VENOUS I population

Time	DOOR Probability (95% Confidence Interval)
4 days	0·404 (0·332, 0·473)
7 days	0·385 (0·311, 0·455)
10 days	0·396 (0·321, 0·468)
12 days	0·403 (0·328, 0·475)
15 days	0·413 (0·337, 0·486)

The Table shows the probability of a better clinical outcome within 4, 7, 10, 12, or 15 days from first positive blood culture for a randomly-selected patient with VRE vs· non-VRE BSI· Clinical outcomes ranked from best to worse are either 1) alive, 2) alive with microbiological failure/recurrent BSI, or 3) death· A probability of less than 50% – with a 95% confidence interval that excludes 50% – implies overall worse outcomes in VRE vs VSE BSI·

Table S8. Estimated hazard ratios in-hospital mortality at five period after adjusting using the inverse of the propensity score

	Day 4 of BSI Dead events =8			**Day 7 of BSI Dead events =12**			**Day 10 of BSI Dead events =19**			**Day 12 of BSI Dead events =21**			**Day 15 of BSI Dead events =29**		
Variables	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value	HR	95% CI	p value
VRE	2.92	0.69-12.35	0.145	3.39	0.97-11.85	0.055	3.39	0.97-11.85	0.055	1.59	0.61-4.19	0.344	1.16	0.50-2.67	0.73
Pitt bacteremia score ≥ 2	4.36	0.60-31.79	0.147	2.33	0.62-8.82	0.212	2.33	0.62-8.82	0.212	1.76	0.70-4.44	0.23	1.82	0.78-4.22	0.164
Urinary catheter	2.03	0.57-7.26	0.277	3.91	1.14-13.44	0.031	3.91	1.14-13.44	0.031	6.44	2.46-6.84	<0.001	5.27	2.10-13.21	<0.001
Microbiological failure	8.42	2.16-32.92	0.002	8.09	2.74-23.87	<0.001	8.09	2.74-23.87	<0.001	3.16	1.25-7.99	0.015	3.24	1.40-7.53	0.006

Figure S1. Flow-chart of patient inclusion in the VENOUS I study.

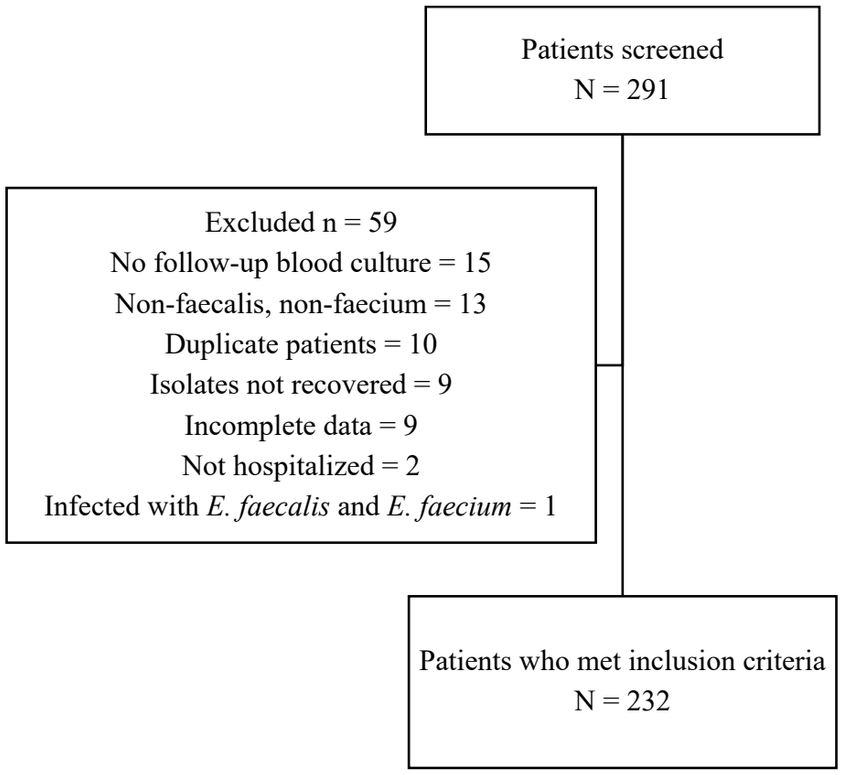
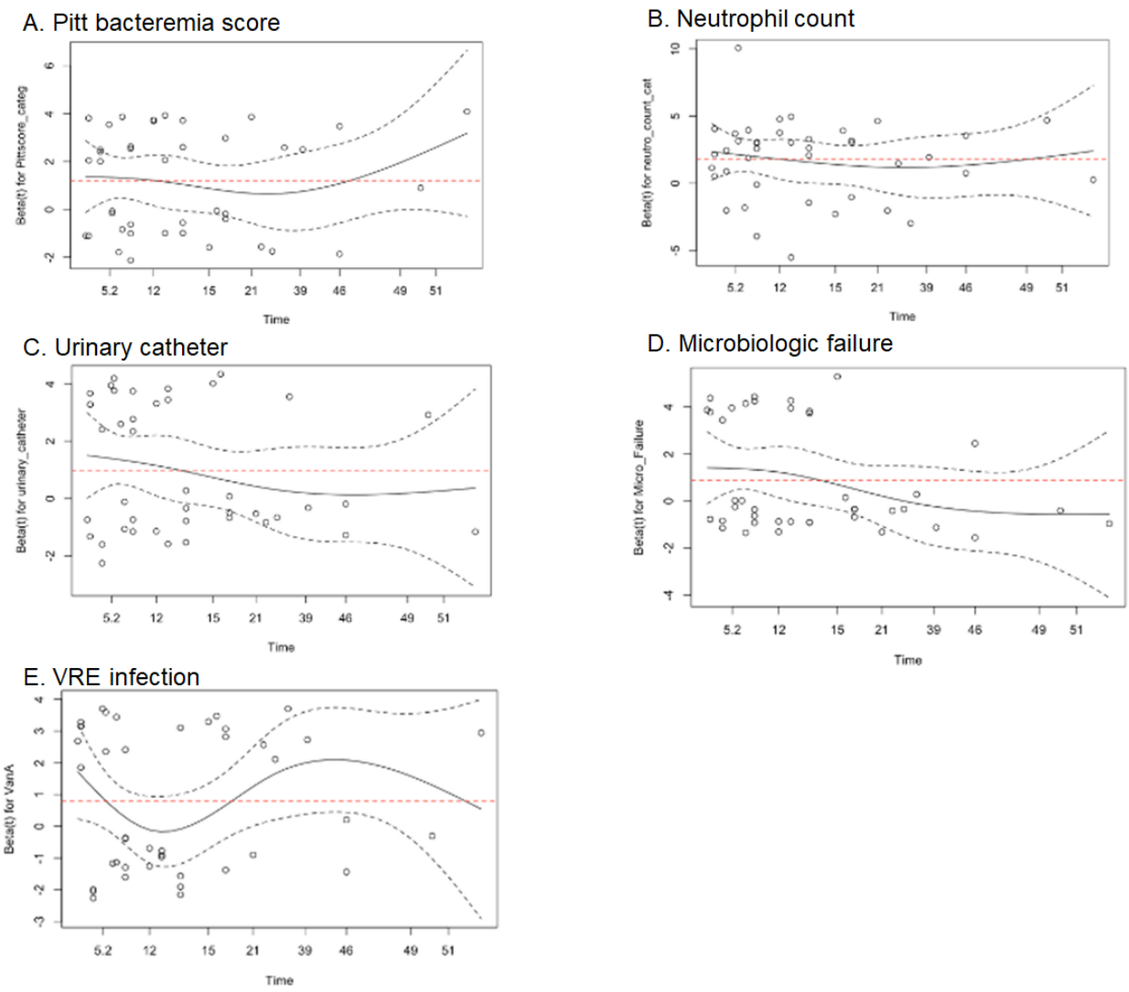


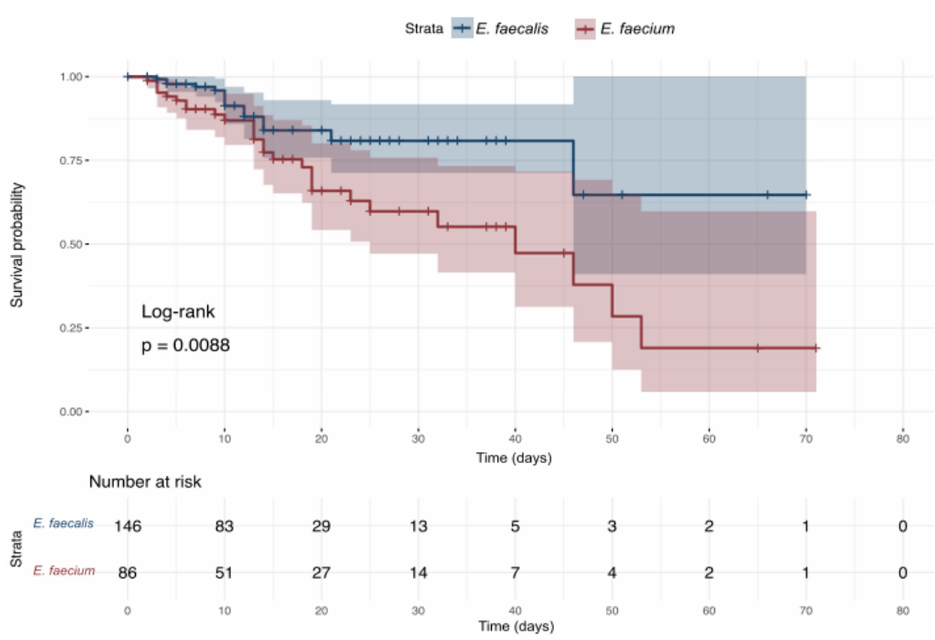
Figure S2 Schoenfeld residuals test for variables selected from purposeful selection method



Using the variables selected from the purposeful selection method, VRE BSI (E) shows evidence that the Schoenfeld residuals are not linear and appear to vary over time· Panels A, B, C and D do not show violation of the proportional hazard assumption.

Figure S3. Kaplan-Meier estimates. Survival curve of patients with enterococcal BSI by species. Panel A described the over-all in-hospital mortality; dotted line shows that the effect on mortality was not uniform throughout the observation period. Panel B shows the survival curve at day 2 of bacteremia. Curves are compared using the log-rank test and a value <0.05 was considered significant. Shade areas represents 95% confidence intervals

A



B

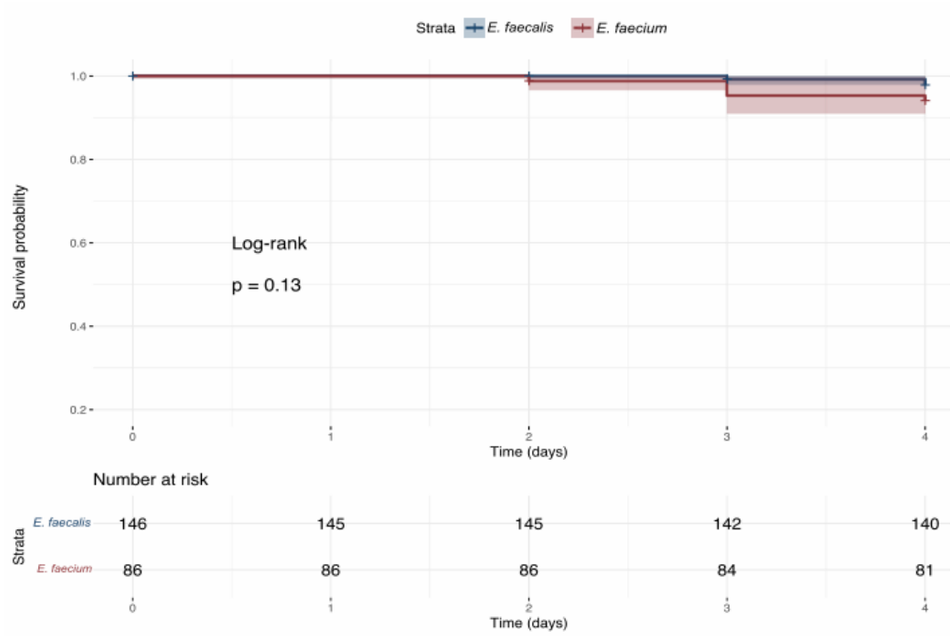
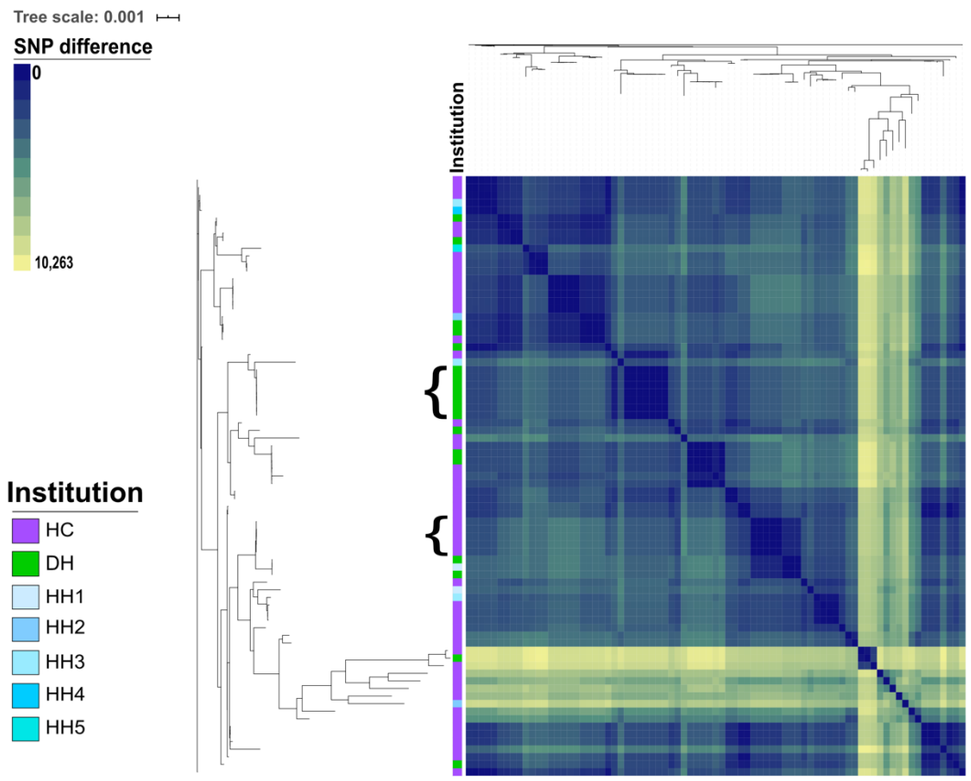


Figure S4· *E· faecium* Clade A core genome SNP distance matrix heat map

E. faecium Clade A core genome SNP distance matrix heat map (n=79). SNP differences ranged from 0 (dark blue) to 10,263 (light yellow). Location of isolate collection is denoted by a color strip to the left of the matrix, and brackets indicate clusters of ≥5 isolates that differ by <20 SNPs, indicating clonality. HC = Houston cancer center; DH = Detroit hospital; HH = Houston hospital



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