Supplemental Contents

Supplemental Methods

Isolate collection

Diagnostic specimens (*e.g.* blood, urine, intra-abdominal sites, wounds) were cultured with specimen-specific standard protocols (1). Vancomycin-resistant enterococci (VRE) surveillance specimens (rectal swabs or stool) were cultured on selective and differential chromogenic medium, then subcultured onto blood agar plates. For each isolate, a sterile inoculating loop was used to collect multiple colonies from a pure subculture of the bacterial inoculation suspension used to setup VITEK 2 testing. The loop was placed directly into 100 µL of a Tris-HCl buffered Trizol lysis solution containing 200 mg of sterile 0.1 mm glass beads. For isolates collected after April 2017, the loop was first dipped into sterile LB-glycerol media prior to placement into the Trizol lysis solution to enable future phenotypic studies.

DNA extraction from isolates

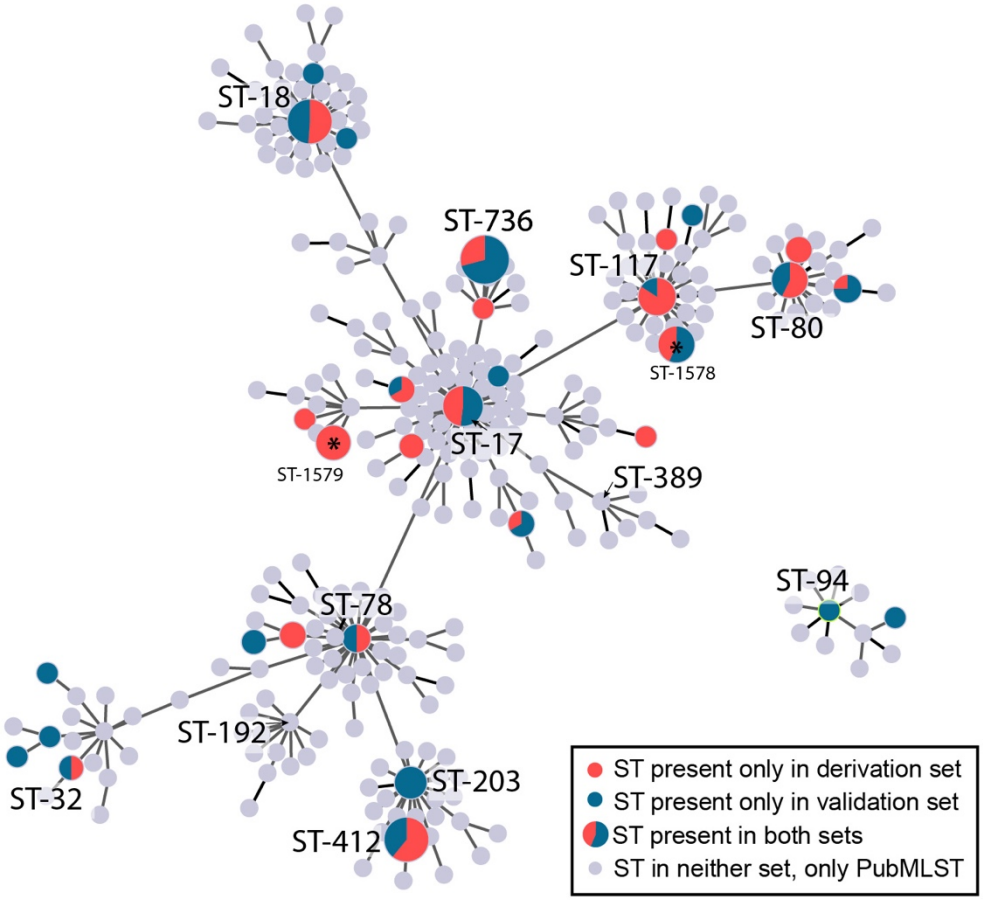
Bacterial isolates collected in 100 µL of buffered Trizol were mixed with 100 µL of Tris-HCl buffer with EDTA, 40µL of sodium dodecyl sulfate, and 100 µL of phenol-chloroform-isoamyl alcohol, incubated for 10 minutes at 4ºC, bead beat for one minute, and centrifuged at 2,800 x *g*. DNA was purified from 50 µL of the aqueous phase using the Qiagen QIAmp 96 DNA kit.

Details of rule-based method

Ampicillin resistance was predicted if *pbp5* did not contain 485M. Vancomycin resistance was predicted if *vanA* or *vanB* was present (determined with SRST2 criteria above). High-level gentamicin resistance was predicted if the complete *aac(6')-Ie-aph(2")-Ia* gene was detected (*e.g.* a common alignment containing 129 holes was not considered complete.) Ciprofloxacin and

levofloxacin resistance were predicted if mutations in *gyrA* 84S or *parC* 82S were present. Tetracycline resistance was predicted if *tetL, tetM,* or *tetS* were detected by SRST2 with any number of differences from the reference sequences. Doxycycline resistance was predicted if *tetM* was detected by SRST2 with any number of differences from the reference sequence. Linezolid resistance was predicted if 23S *rRNA* G2576T was present in at least 3 alleles. For each drug, if resistance criteria were not met, the isolate was considered susceptible to the drug.

Supplemental Figure 1. GoeBURST visualization of isolates from this study superimposed on the background population structure of all 1,318 clinical *E. faecium* isolates deposited in PubMLST at the time of this analysis. Over 92% of derivation set isolates and 85% of validation set isolates belonged to CC17 and were most frequently ST-736, ST-18, ST-412, ST-17, and ST-117. Two locally common but novel STs, ST-1578 and ST-1579, are annotated with an asterisk. Group and sub-group founders are labeled with their sequence type (ST). Node colors represent whether the ST is present in the derivation set (red), validation set (blue), both sets (pie chart; *e.g.* 30% of ST-736 isolates were in the derivation set), or neither set (grey, exclusively found in PubMLST). Node size is proportional to the number of isolates belonging to that ST.



Supplemental Table 1. Novel MLST profiles, frequency, and source. SRST2 was used to assign allele numbers. Asterisks indicate the closest known allele (with at least 1 mismatch). When applicable, new MLST designations as assigned by PubMLST.org are shown.

***E. faecium* MLST allele call**							**Total Occurrences (Derivation, Validation)**	**Source (Occurrences)**	**New Official MLST Designation**	**Closest Existing ST Type (# of matching loci out of 7)**
atpA	**ddl**	**gdh**	**purK**	**gyd**	**pstS**	**adk**				
9	1	5	1	1	1	1	18 (7, 11)	Colonization (12), Infection (7)	ST-1578	
1	2	1	66	1	1	1	12 (12, 0)	Colonization (10), Infection (3)	ST-1579	
7	91*	1	1	5	1	1	2 (1, 1)	Colonization (2)		
70	1	1	44	12	20	1	2 (0, 2)	Colonization (1), Infection (1)	ST-2056	ST-412 (5/7)
13	8	8	8	23*	10	6	1 (1, 0)	Infection (1)		
9	2	1	3	1	1	5	1 (1, 0)	Infection (1)	ST-1577	
11	17	18	17	10	19	6	1 (0, 1)	Infection (1)	ST-2057	ST-107 (6/7) ST-116 (6/7) ST-931 (6/7)
15	91*	1	44	1	20	1	1 (0, 1)	Infection (1)		
149	8	116	23	6	27	6	1 (0, 1)	Infection (1)	ST-2059	ST-1696 in CC94 (6/7)
5	9	6	3	2	26	1	1 (0, 1)	Infection (1)	ST-2058	ST-929 (6/7)
1	1	1	66	1	1	1	1 (1, 0)	Colonization (1)		
1	91*	1	1	1	1	1	1 (1, 0)	Colonization (1)		
9	91	1	1	12	1	1	1 (1, 0)	Colonization (1)		

Supplemental Table 2. Van gene content of derivation and validation set isolates.

	Vancomycin phenotype	
Genotype:	**Susceptible (n=70)**	**Resistant (n=310)**
vanA	2*	300
vanB	1†	11
vanC	0	0
vanD	0	0
vanE	0	0
vanF	0	0
vanG	0	0
vanL	0	0
vanM	0	0
vanN	0	0
None of the above	67	0

* Comprised of one isolate in the derivation set (unable to re-phenotype), and the other isolate was a vancomycin-variable enterococcus (as described in the manuscript).

† Derivation set isolate, unable to re-phenotype.

Supplemental Table 3. Aminoglycoside resistance gene content of derivation and validation set isolates.

	High-level gentamicin phenotype	
Genotype:	**Susceptible (n=337)**	**Resistant (n=30)**
aac(6')-Ie-aph(2")-Ia	3*	28
aac(6')-Ii	337	30
ant(6')-Ia	252	26
aph(3')-Ia	1	0
aph(3')-IIIa	234	23
ant(6')-Ia -aph(3')-IIIa	0	0

* Present in derivation set, isolates unavailable for re-testing.

Supplemental Table 4. Linezolid resistance gene content of derivation and validation set isolates.

	Linezolid phenotype	
Genotype:	**Susceptible/ Intermediate (n=369)**	**Resistant (n=2)**
***23S rRNA* G2576T** in ≥3 alleles	1*	2
poxtA	1	0
cfr(A)	0	0
cfr(B)	1	0
cfr(C)	0	0
optrA	0	0
None of the above	366	0

* A derivation set isolate that was originally tested by a disk diffusion method was not available for re-phenotyping.

Supplemental Table 5. Sample type, MLST sequence type, phenotypic antimicrobial susceptibility testing results, antimicrobial resistance gene content, and genotypic predictions for isolates in the derivation and validation sets. ST: Sequence Type, MIC: Minimum inhibitory concentration, RIS: Resistant, intermediate, or susceptible categorical call, Depth: average read depth determined by SRST2, Diffs: any differences in the sample sequence compared to the reference allele.

References

1. Leber AL. Clinical microbiology procedures handbook. 2016.