SUPPLEMENTARY METHODS

Clinical Setting UMass Memorial Medical Center comprises two separate inpatient campuses that are approximately 1 mile apart, but which function as a single institution. It is considered a single hospital in relation to state licensure, accreditation, and by the Centers for Medicare & Medicaid Services. There are a single set of policies for the two campuses that include the infection control policies and protocols. Patients routinely go back and forth between the two campuses as necessary as certain services are predominantly located at one campus or the other.

Isolate extraction and sequencing. Isolates were received on Trypticase Soy Agar with 5% Sheep Blood from the clinical microbial diagnostic lab (Quest Diagnostics, Marlborough, MA). Bacterial mass for DNA extraction was obtained by cross-streak with a 1 ul disposable inoculating loop. Extractions were performed using the MagAttract Microbial DNA Kit (Qiagen, #27200-4; formerly MO BIO PowerMag Microbial DNA Kit #27200-4) on Retsch 96 Well Plate Shaker and Eppendorf epMotion 5075 Liquid Handler instrumentation as per manufacture protocols. DNA sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (illumina, #FC-131-1096) and Nextera XT Index Kit v2 Sets A-D as per manufacturer protocol. Multiplexed sample pools were sequenced as 2x150nt pair-end runs on an illumina NextSeq500 instrument using either NextSeq 500/550 High Output v2 kit (illumina, #FC-404-2004) or NextSeq 500/550 Mid Output v2 kit (illumina, #FC-404-2003). Sample read data was excluded from further analysis if the species composition was less than 80% of the target organism as assessed by MetaPhlan2 (database db_v20) (26418763-1).

Read processing and alignment. FASTQ files were processed using Trimmomatic (24695404-2). Trimmed reads were aligned using BWA (19451168-5) using the following reference genomes: Staphylococcus aureus NCTC 8325 (GenBank CP000253.1), Enterococcus faecium DO (GenBank CP003583.1), Klebsiella pneumoniae HS11286 (GenBank CP003200.1), and Pseudomonas aeruginosa PAO1 (GenBank AE004091.2). Sequence metrics were assessed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and SAMtools flagstat, and average sequence depth and coverage was calculated for each sample as well as across multilocus sequence typing (MLST) gene loci. PCR duplicates were removed using Picard markduplicates (http://broadinstitute.github.io/picard/). SAMtools (19505943-6) and VarScan2 (22300766-3) were used for single nucleotide variant detection and production of variant call files.

Multilocus sequence typing. Consensus fasta files generated by read alignment to a reference genome were used for multilocus sequence typing (MLST) analysis. Samples with less than 20X depth of coverage were removed prior to MLST analysis. BLAST+ (20003500-4) was used to identify the MLST assignment across allele definitions for all organisms curated by PubMLST (https://pubmlst.org/)

Additional references:

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3. Koboldt DC, Zhang Q, Larson DE, Shen D, McLellan MD, Lin L, Miller CA, Mardis ER, Ding L, Wilson RK. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012;22(3):568-76. doi: 10.1101/gr.129684.111. PubMed PMID: 22300766; PMCID: PMC3290792.
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6. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Genome Project Data Processing S. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078-9. doi: 10.1093/bioinformatics/btp352. PubMed PMID: 19505943; PMCID: 2723002.

Supplementary Figure Legends

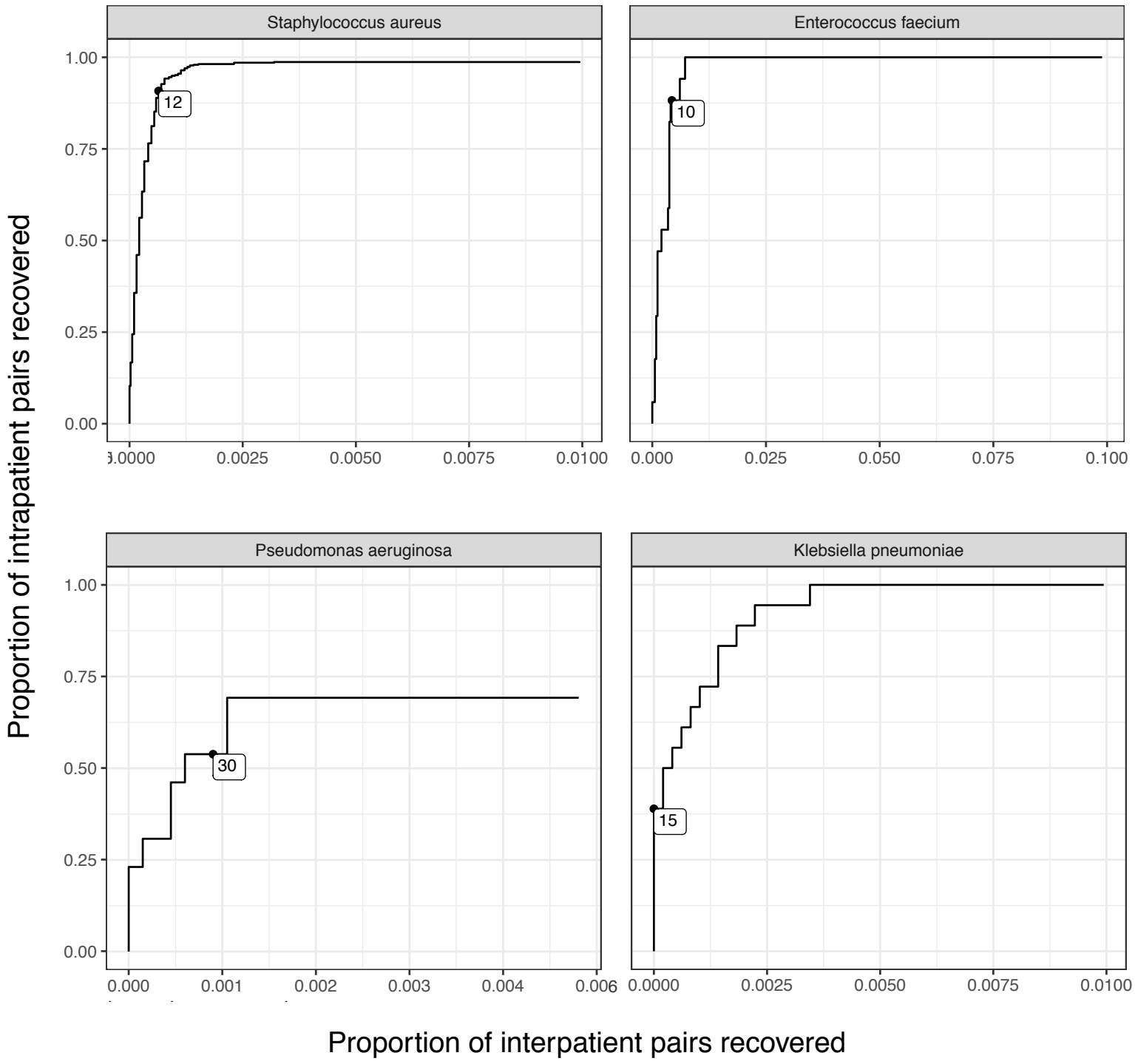
Supplementary Figure S1. ROC analysis of intra-patient and inter-patient SNV differences to establish informative thresholds to identify suspect inter-patient transmissions. ROC plots of inter-patient and intra-patient SNV distance were evaluated to identify optimal thresholds that would maximize discovery of transmissions while minimizing the number of false positive identifications. As the SNV threshold is raised, it passes a point in the curve where the number of false positives increases rapidly while the power to detect true positives falls off when too low of a threshold is selected.

Supplementary Figure S2. Characteristics of *S. aureus* Cluster 2. (A) Heatmap of pairwise SNV differences between isolates in this cluster. Boxes contain the number of SNV differences for the pair. Patient identifiers are on the horizontal and vertical axis. (B) Antibiogram profiles for the isolates tested. (C) Minimum spanning tree derived from SNV distances indicates the relationship between isolates in this cluster.

Supplementary Figure S3. Characteristics of *S. aureus* Cluster 14. (A) Heatmap of pairwise SNV differences between isolates in this cluster. Boxes contain the number of SNV differences for the pair. Patient identifiers are on the horizontal and vertical axis. (B) Clinical characteristics associated with each isolate. (C) Antibiogram profiles for the isolates tested. (D) Minimum spanning tree derived from SNV distances indicates the relationship between isolates in this cluster.

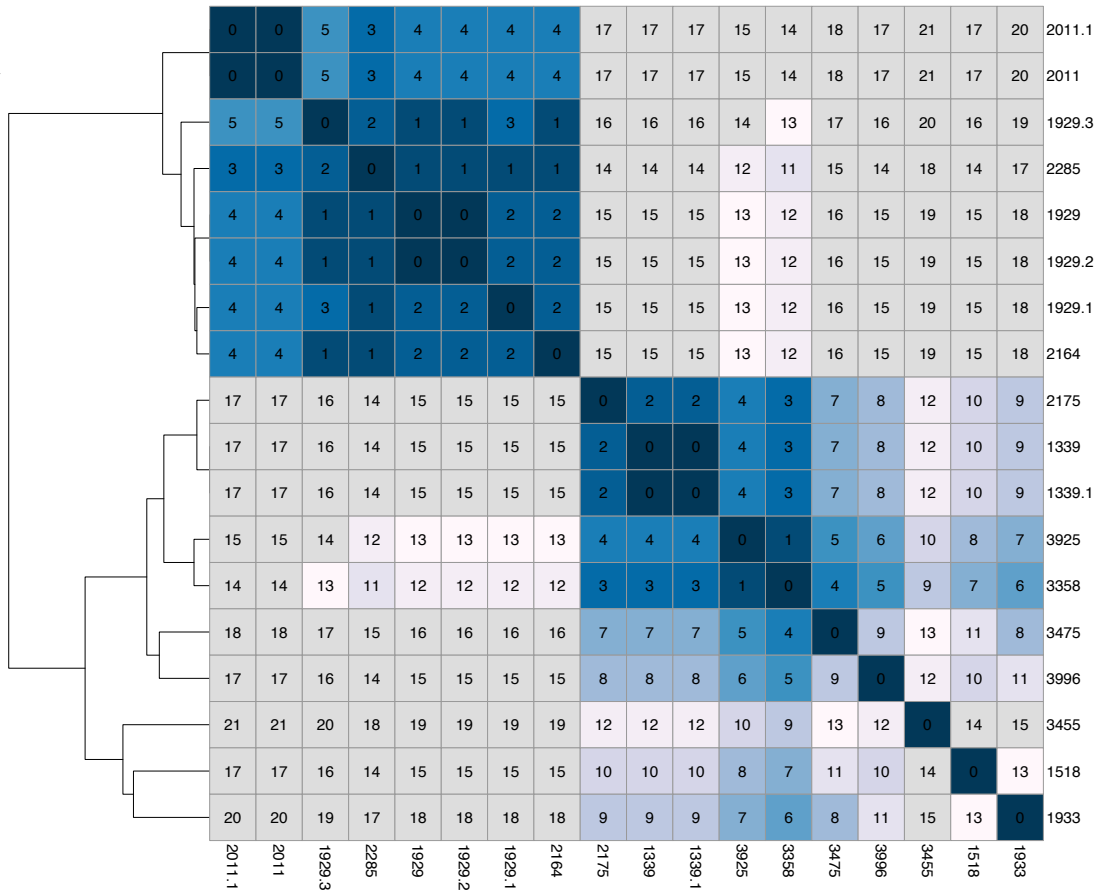
Supplementary Figure S4. Characteristics of an *E. faecium* cluster falling one SNV above the cutoff. (A) Heatmap of pairwise SNV differences between isolates in this cluster. Boxes contain the number of SNV differences for the pair. Patient identifiers are on the horizontal and vertical axis. (B) Antibiogram profiles for the isolates tested. (C) Minimum spanning tree derived from SNV distances indicates the relationship between isolates in this cluster. Analysis not shown indicates that vancomycin resistance resistance is conferred by the presence of a plasmid bearing the genes of the vanA operon; *vanHA, vanSA, vanYA, vanXA, vanZA, vanI, and vanRA.*

Supplementary Figure S1



Supplementary Figure 2

A



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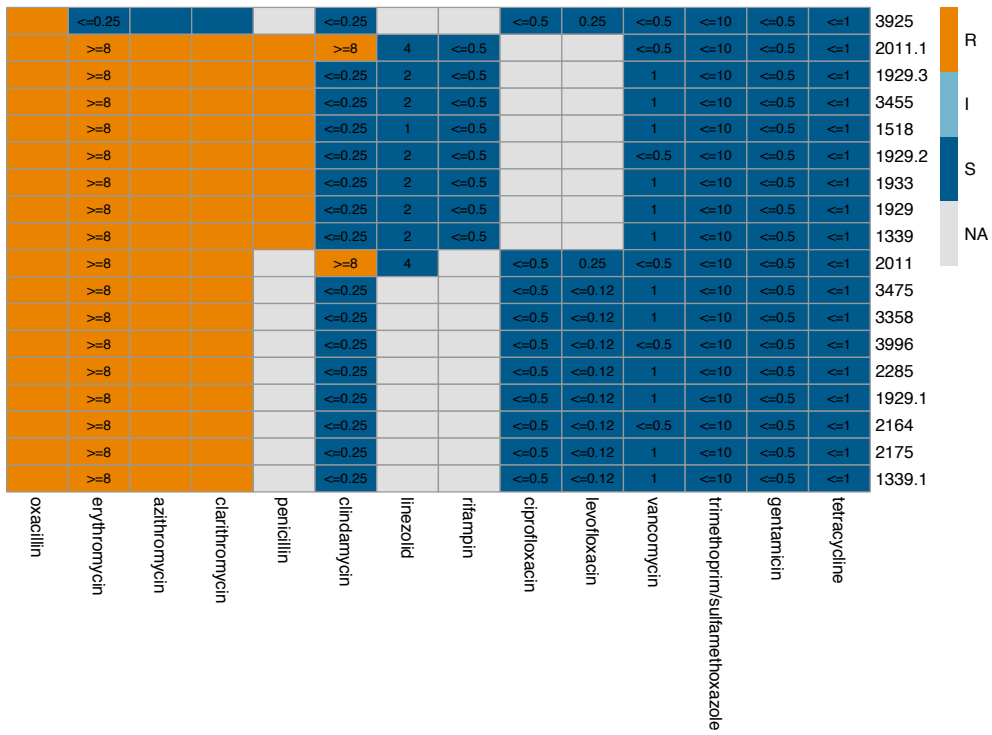

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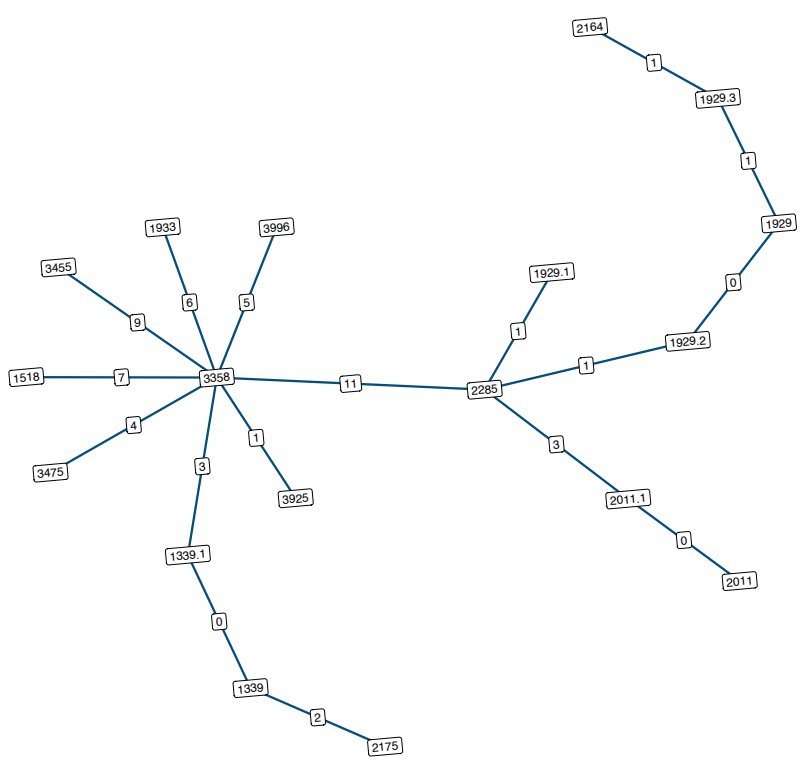
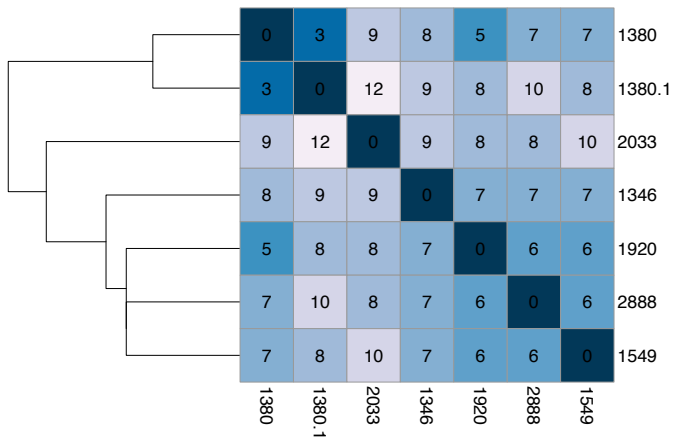


Supplementary Figure 3

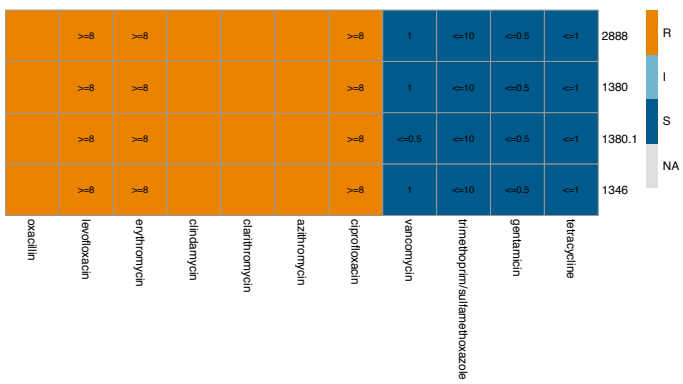
A



B

Patient	Characteristic	Specimen Source	Collection Day
1549	Hospital acquired	Nasal swab	1
1346	Hospital acquired	Sputum	27
1380	Hospital acquired	Surgical wound	9
1920	Healthcare associated	Nasal swab	99
2033	Hospital acquired	Tracheal aspirate	23
2888	Healthcare associated	Surgical wound	39

C



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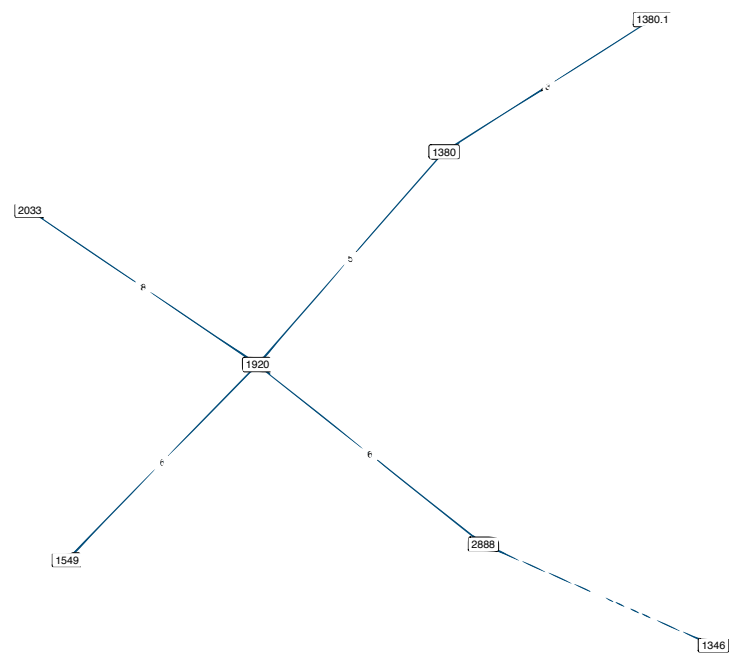
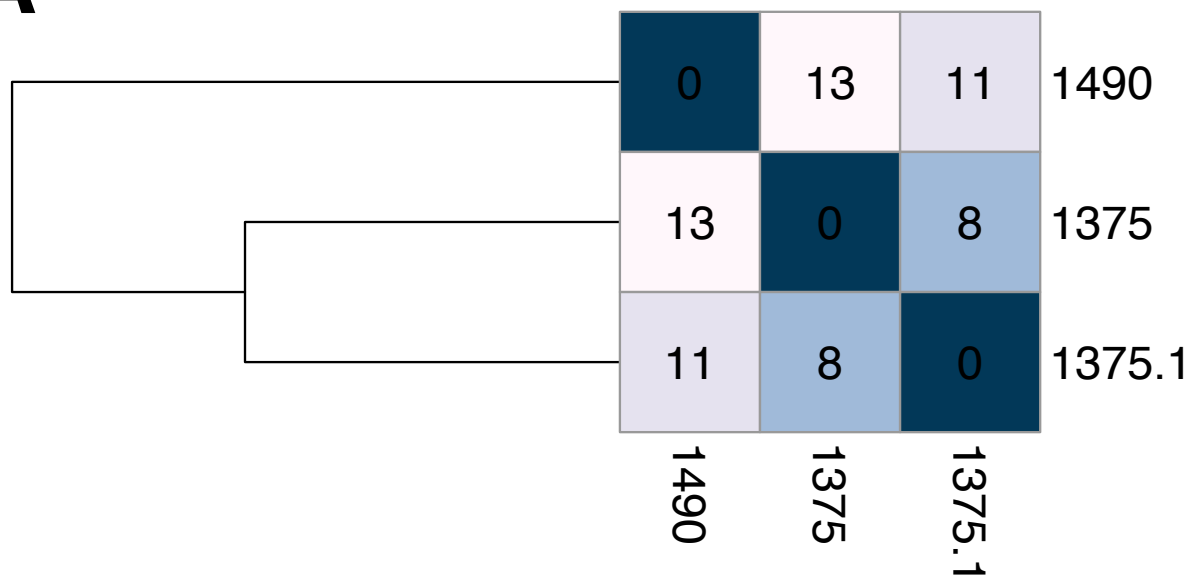


Supplementary Figure S4

A



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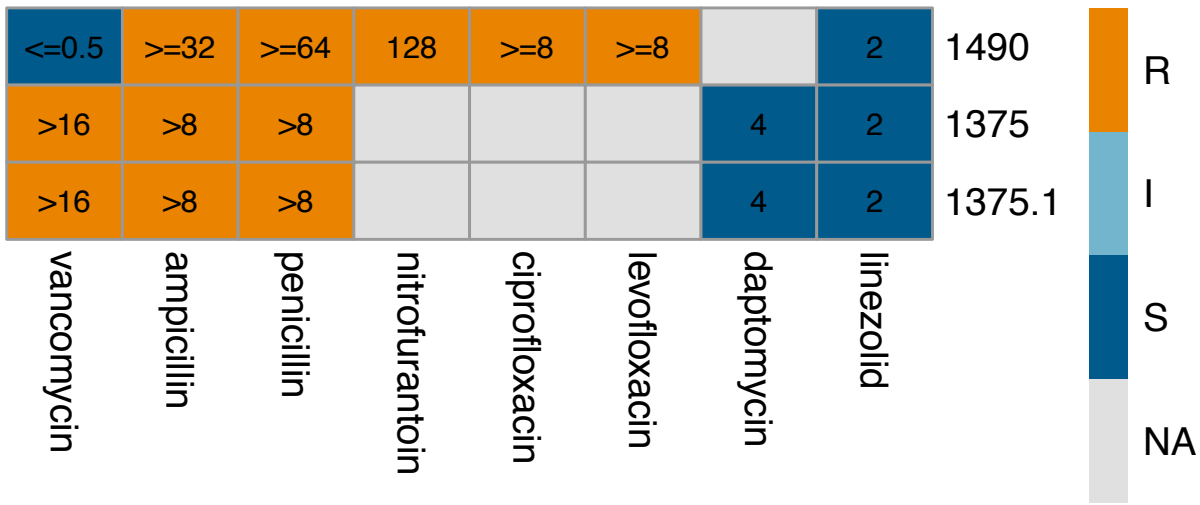

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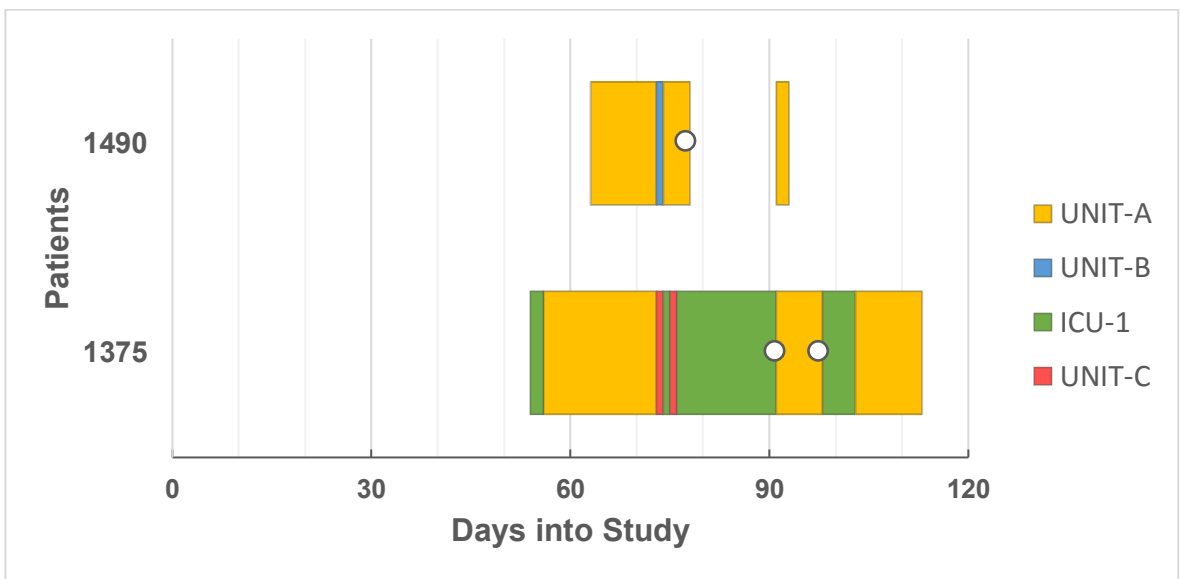

Supplementary Table S1: The number of genome assemblies used to compose the core genome, the resulting core genome size in base pairs and the corresponding SNV thresholds for genomic clustering for all ESKAPE pathogens.

Species	Number of assemblies	Core size (bp)	Threshold (SNV)
Enterococcus faecium	782	1,700,924	10
Staphylococcus aureus	2000	1,284,348	12
Klebsiella pneumoniae	2876	3,952,452	15
Pseudomonas aeruginosa	1000	3,707,173	30

Supplementary Table S2: The number of samples and patients (in parentheses) used for determining SNV thresholds and optimizing core genome definition. As samples from both current study and external sources were used, we have split the numbers.

Species	Current study
Enterococcus faecium	84 (69)
Klebsiella pneumoniae	100 (82)
Pseudomonas aeruginosa	116 (101)
Staphylococcus aureus	935 (599)

Supplementary Table S3

Cluster	Species	Days	Isolates	Patients	Min SNV	Median SNV	Mean SNV	Max SNV	Clinical Support	Clinical Characteristics†
1	*S.aureus*	6	13	2	2	7.5	7.9	15	Yes	HA-1,2
2	*S.aureus*	321	21	13	0	13	11.6	22	Yes/No	HCA (2 patients, transplant); CA-3 (6 patients); CA-4 (5 patients)
3	*S.aureus*	224	5	3	3	6.5	6.4	10	No	-
4	*S.aureus*	184	7	3	0	5	3.4	6	No	-
5	*S.aureus*	84	2	2	8	8	8	8	No	-
6	*S.aureus*	2	3	3	7	8	8	9	No	-
7	*S.aureus*	4	3	2	4	5	4.7	5	Yes	HA-1
8	*S.aureus*	103	7	3	0	2	3.1	8	No	-
9	*S.aureus*	312	9	3	0	8	5.4	11	No	-
10	*S.aureus*	76	2	2	4	4	4	4	No	-
11	*S.aureus*	102	3	2	0	5	3.3	5	No	-
12	*S.aureus*	54	4	4	4	9	9	13	No	-
13	*S.aureus*	301	12	3	0	6.5	7.3	21	No	-
14	*S.aureus*	192	7	6	3	8	7.6	12	No	-
15	*S.aureus*	139	2	2	2	2	2	2	Yes	CA-3
16	*S.aureus*	266	6	6	1	12	10.5	15	No	-
17	*S.aureus*	47	6	2	0	3	2.3	4	Yes	CA-3
18	*S.aureus*	23	2	2	5	5	5	5	Yes	HA-1,2
19	*S.aureus*	9	4	2	2	3	3.2	4	No	-
20	*S.aureus*	2	2	2	12	12	12	12	No	-
21	*S.aureus*	50	2	2	4	4	4	4	Yes	HA-1
22	*E. faecium*	147	2	2	4	4	4	4	Yes	HCA-5,6,7
23	*E. faecium*	1	2	2	9	9	9	9	No	-
24	*E. faecium*	122	7	4	0	8	8.7	16	No	-
25	*E. faecium*	60	3	3	1	1	1.3	2	No	-
26	*P. aeruginosa*	12	3	2	8	22	20	30	No	-
27	*S.aureus*	214	2	2	11	11	11	11	No	-
28	*S.aureus*	179	3	3	5	7	8	12	No	-
29	*S.aureus*	205	4	2	0	4.5	4.5	9	No	-
30	*S.aureus*	217	3	2	1	12	8.7	13	No	-
31	*S.aureus*	25	2	2	11	11	11	11	No	-
32	*S.aureus*	268	3	3	6	9	10	15	No	-
33	*S.aureus*	197	2	2	10	10	10	10	No	-
34	*E. faecium*	70	3	2	4	5	4.7	5	Yes	HA-2,5

†Clusters are categorized as **HA**, Hospital Acquired (evidence supporting transmission during hospitalization); **HCA**, Healthcare Associated (evidence supporting transmission through healthcare connection); **CA**, Community Acquired, or "-" when there was not an obvious clinical association. When clusters contain multiple categorizations the number of patients in each category is provided. The nature of the clinical support is coded as a number corresponding to the values: **1**, same inpatient unit or inpatient ward; **2**, concurrent hospitalization; **3**, shared IVDU risk factor; **4**, no obvious association; **5**, same clinical service (e.g. cardiology service, renal service); **6**, same outpatient clinic; **7**, same physician