**SUPPLEMENTARY MATERIALS**

**MANUSCRIPT TITLE:**

Whole-genome sequencing surveillance of vancomycin-resistant *Enterococcus faecium* (VRE) detects hospital outbreaks and identifies the post-anesthesia care unit as a transmission locus

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**CONTENTS:**

* Supplementary methods
* Table s1: legend & separate Microsoft Excel file
* Table s2: legend & separate Microsoft Excel file
* Table s3: legend & separate Microsoft Excel file
* Figure s1: legend & figure
* Figure s2: legend & figure

**SUPPLEMENTARY METHODS**

**Additional details on study setting and Brigham and Women’s Hospital (BWH) vancomycin-resistant enterococci (VRE) screening protocols during the period of this study**

Per BWH infection control policy, all adult patients admitted to intensive care units (ICUs) and oncology units are screened for VRE via rectal swab or stool sampling upon admission and weekly thereafter. Patients known to be colonized with VRE are considered persistently colonized unless cleared by three sets of negative cultures (including rectal swab or stool and from any previously positive sites except urine or blood) and are therefore exempt from screening. A patient not admitted to an ICU or oncology unit can also be screened for VRE at the discretion of infection control or the clinical team caring for a patient. BWH has 11 dedicated oncology units and ten adult ICUs, including two medical ICUs, two surgical ICUs, one combined medical and surgical ICU, two neurologic/neurosurgical ICUs, one cardiothoracic ICU, one cardiac ICU, and one cardiac surgical ICU. Patients in ICUs and most oncology patients are housed in single-occupancy rooms; a minority of oncology patients stay in double-occupancy rooms with one bathroom per room.

VRE screening is conducted by the nursing staff via either rectal swab or stool sample. All specimens are submitted to the BWH clinical microbiology laboratory for processing. Perirectal sampling and environmental sampling are not routinely performed as part of VRE surveillance programs at BWH. During the period over which the vancomycin-resistant *Enterococcus faecium* (VREfm) isolates included in this study were obtained (June 2022-September 2023), the VRE screening protocols described above were continuously in place across all intensive care and oncology units. Compliance with VRE screening protocols is not monitored by infection control personnel.

Most of the isolates sequenced for this study (143/159, or 91.7%, including 129 isolates from rectal screens and 14 isolates from diagnostic specimens) were obtained from June 2022 to January 2023. The 129 isolates obtained from screening specimens represented 60.8% of the total number of isolates obtained from screening specimens from unique patients during this period and are best classified as a convenience sample.

**BWH contact precautions and environmental disinfection protocols for patients known to be colonized or infected with VRE**

Patients for whom screening or diagnostic cultures are positive for VRE have a flag placed in the EHR to indicate the need for appropriate precautions. Per BWH infection control protocol, adult patients known to be colonized or infected with VRE must be housed in a private room or with another patient known to be colonized or infected with VRE. All individuals must disinfect their hands with an alcohol-based hand sanitizer before entering and upon leaving the room, and gowns and gloves must be worn while in the room per BWH contact precautions. Patients must don a clean cover gown and disinfect their hands with an alcohol-based hand sanitizer prior to transport within the hospital. Reusable patient care equipment must be disinfected with a quaternary ammonium disinfectant before use on another patient. Daily and terminal environmental disinfection consists of wipe down of high-touch surfaces (daily) or all surfaces (terminal) with a quaternary ammonium disinfectant applied to a microfiber cloth.

To discontinue precautions, cultures can be obtained from patients who have no known VRE-positive cultures in the previous 90 days and have not received antibiotics effective against VRE in the past 48 hours (linezolid, daptomycin, tigecycline, quinupristin-dalfopristin, telavancin). Cultures must be performed from rectal swab or stool and from any previously positive site except for urine and blood. If three sets of cultures obtained on different days are negative for VRE, the patient can have the VRE flag removed by infection control and is eligible for inclusion in routine screening protocols.

**Infection control surveillance methods**

Positive culture screening results are routinely reviewed by infection control staff. The colonization status of other patients on the unit is reviewed to determine whether transmission from another patient on the unit might have occurred. During the study period, infection control personnel could request sequencing of isolate genomes if there was concern for a cluster of healthcare associated cases (*e.g.*, positive screens from three or more patients on the same unit within a 14-day period).

**Table s1. Hospital unit designations and descriptions.** Designation in figures and text: the abbreviation used to refer to the unit in the manuscript figures and text. Intensive care units (ICUs) and dedicated oncology units (onc) are specified and assigned arbitrary numbers. All other main hospital units are given arbitrary unit numbers. Units at an affiliated rehabilitation (rehab) hospital and an affiliated full hospital (AH) are designated separately. Unit description: the medical specialty to which patients on the unit are admitted. Included in BWH VRE screening protocol?: whether or not patients on the unit are screened for VRE via the protocols described in the supplementary methods.

TABLE IN SEPARATE FILE: Table\_s1.xlsx

**Table s2. Vancomycin-resistant *Enterococcus faecium* isolates included in this study.** Isolate ID: the number given to the isolate and used throughout the manuscript to refer to it. NCBI BioSample ID: an identifier assigned by the NCBI Pathogen Detection website that can be used to retrieve the isolate in the Pathogen Detection database (<https://www.ncbi.nlm.nih.gov/pathogens/>). Source: sample type from which the isolate was recovered. IC request: whether sequencing of the sample was requested by infection control (IC). ST: multilocus sequence type. Genomic + epi cluster: the putative transmission cluster to which the isolate belongs based on genomic relatedness and epidemiologic (epi) links. Associated figure(s): the figures in which the isolate appears in the manuscript. Isolates separated by ≤ 15 SNPs (CFSAN, SKA, or CFSAN & SKA): isolates that are ≤ 15 single nucleotide polymorphisms (SNPs) away from the isolate by referenced-based alignment (CFSAN) or split k-mer analysis (SKA), ordered by increasing SNP distance (in parenthesis; in the “CFSAN & SKA” column, the CFSAN SNP distance is listed first). Isolates for which epidemiologic links were identified are in bold. N50: a measure of contiguity defined as the length of the shortest contig that must be included along with contigs of equal or greater length to achieve 50% coverage. Coverage at 12x: percent of the reference covered at a minimum depth of 12 reads. Average depth at min 12x: average coverage at a minimum depth of 12 reads. Maximum depth: maximum number of reads. Across all isolates, the average coverage at a minimum depth of 12 reads was 94% of the reference (minimum 80.02%). Excluding loci with read depth < 12, the average read depth was 122.72 (minimum 30.65).

TABLE IN SEPARATE FILE: Table\_s2.xlsx

**Table s2.** **Single nucleotide polymorphism (SNP) distances for all pairwise comparisons between the 156 isolates in this study.** In the column “Isolate pair”, isolate pairs are formatted with the two isolate IDs in numerical order separated by an underscore. The column “CFSAN” contains the SNP distance calculated by reference-based alignment. The column “SKA” contains the SNP distance calculated by split k-mer analysis. The column “Difference” is the CFSAN SNP distance minus the SKA SNP distance.

TABLE IN SEPARATE FILE: Table\_s3.xlsx

**Figure s1. Vancomycin-resistant *Enterococcus faecium* isolate pairs meeting the threshold of ≤ 15 single nucleotide polymorphisms (SNPs) by split k-mer analysis (SKA), reference-based alignment (CFSAN), or both.** A) Isolates with and without epidemiologic (epi) links in each group. The percentage in parenthesis is the percent of the total number of isolates in the row. B) and C) Comparison of SNP distances calculated using each method for isolates with SNP distance ≤ 15 by SKA (B) or CFSAN (C). Isolates in the orange area (including those on the boundary) are the 82 with SNP distance ≤ 15 by both methods. Isolates in the red or yellow areas are those with SNP distance ≤ 15 by SKA (red) or CFSAN (yellow) only. Numbers inside dots indicate the number of isolate pairs with that combination of CFSAN and SKA SNP distance. Dots with no numbers represent one isolate pair. Dark blue dots represent pairs for which an epidemiologic link was identified, while light blue dots represent pairs for which an epidemiologic link was not identified. For dark blue dots that represent multiple isolate pairs, the number of pairs with an epidemiologic link is indicated in dark blue text to the right of the dot.

A screenshot of a graph

Description automatically generated

**Figure s2. Additional transmission clusters identified via surveillance whole-genome sequencing of vancomycin-resistant *Enterococcus faecium*.** Refer to Figure 3A for key to transmission cluster maps. AH, Brigham and Women’s Hospital (BWH)-affiliated partner hospital (units numbered separately from BWH units). ED, emergency department. GI surg, gastrointestinal surgery. Int, intensive care. Med, medicine. Neuro, neurology. Onc, oncology. PACU, post-anesthesia care unit. Thorac surg, thoracic surgery.

