AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EPIDEMIOLOGY AND SURVEILLANCE

Multisite Detection of Tn*1549*-Mediated *vanB* Vancomycin Resistance in Multidrug-Resistant *Enterococcus faecalis* ST6 in Texas and Florida

Shelby R. Simar,[a] Truc T. Tran,[b,c] Kirsten B. Rydell,[b,c] Diana Panesso,[b,c,d] German A. Contreras,[e] Jose M. Munita,[f,g] Renzo O. Cifuentes,[h] Lilian M. Abbo,[h] Pranoti Sahasrabhojane,[i] An Q. Dinh,[b,c] Dierdre B. Axell-House,[b,c] Tor Savidge,[j] Samuel A. Shelburne,[i] Blake M. Hanson,[a] Cesar A. Arias[b,c,k]

[a]Center for Infectious Diseases, University of Texas Health Science Center, School of Public Health, Houston, Texas, USA
[b]Division of Infectious Diseases, Houston Methodist Hospital, Houston, Texas, USA
[c]Center for Infectious Diseases, Houston Methodist Research Institute, Houston, Texas, USA
[d]Molecular Genetics and Antimicrobial Resistance Unit, International Center for Microbial Genomics, Universidad El Bosque, Bogotá, Colombia
[e]Division of Infectious Diseases, Department of Internal Medicine, McGovern Medical School, University of Texas Health Science Center, Houston, Texas, USA
[f]Genomics and Resistant Microbes (GeRM) Group, Facultad de Medicina Clínica Alemana de Santiago, Universidad del Desarrollo, Santiago, Chile
[g]Millennium Initiative for Collaborative Research On Bacterial Resistance (MICROB-R), Santiago, Chile
[h]Jackson Health System, Miami Transplant Institute, Miami, Florida, USA
[i]Department of Infectious Diseases, Division of Internal Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[j]Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, Texas, USA
[k]Department of Medicine, Weill Cornell Medical College, New York, New York, USA

ABSTRACT In the United States, *vanB*-mediated resistance in enterococci is rare. We characterized three sequence type (ST) 6, vancomycin-resistant *Enterococcus faecalis* isolates causing bacteremia in unique patients in spatiotemporally distinct settings. Isolates were recovered between 2018 and 2020 in two cities in the United States (Houston, TX; Miami, FL). The isolates harbored the *vanB* operon on a chromosomally located Tn*1549* transposon, and epidemiological data suggested multiple introductions of the *vanB* gene cluster into ST6 *E. faecalis*.

KEYWORDS *Enterococcus*, VRE, antimicrobial resistance

The prevalence of vancomycin resistance in enterococci in the United States is overwhelmingly higher for *Enterococcus faecium* than *Enterococcus faecalis* (1). This phenotype is primarily mediated by *vanA* in the United States, with *vanB* remaining rare among enterococci. Indeed, the estimated prevalence of *E. faecium* (VREfm) and *E. faecalis* (VREfs) isolates harboring *vanB* in North America was around 3.6% and 1%, respectively (1997 to 2016) (2). In the United States, only sporadic reports of *vanB* resistance have been documented since, usually within a single institution (3, 4). Outside of the United States, *vanB*-mediated vancomycin resistance in enterococci is either endemic (e.g., Australia) (5) or exhibiting a recent sharp increase, as reported in some Western European countries (6, 7). Here, we describe the identification of ST6 *E. faecalis* isolates belonging to three unique patients with bacteremia in two U.S. states, each harboring the *vanB* operon on a Tn*1549* transposon (also knowns as *Tn5382*-like or Tn*1549*/Tn*5382* elements) in differing genomic contexts. When paired with epidemiological data, these findings suggest multiple introductions of the *vanB* gene cluster into ST6 *E. faecalis*.

Patients were identified through the Vancomycin-Resistant Enterococcal Bacteremia Outcomes Study (VENOUS), an ongoing global prospective study investigating the molecular and clinical epidemiology of enterococcal bloodstream infections (8). For inclusion in VENOUS, all eligible patients must have isolates from their first (index) positive blood culture available

Address correspondence to Cesar A. Arias, CAArias@houstonmethodist.org, or Blake M. Hanson, Blake.Hanson@uth.tmc.edu.

The authors declare a conflict of interest. C.A.A. has received grant support from MeMed Diagnostics, Merck Pharmaceuticals, and Entasis Pharmaceuticals.

Received 21 September 2022
Returned for modification 25 October 2022
Accepted 1 December 2022
Published 21 December 2022

TABLE 1 Patient demographic and hospitalization information[a]

Characteristic	Patient HTX1	Patient MFL1	Patient HTX2
Institution	Houston, TX CC	Miami, FL HS	Houston, TX HS
Year of admission	2018	2020	2020
Length of stay, days	29	83	15
Age, yrs	40	79	51
Location prior to admission	Community/home	LTAC	Community/home
Location upon admission	Non-ICU	ICU	ICU
Antibiotic history 30 days prior to admission	FEP, LZD, MIN	FEP, MEM, VAN	None
History of hospitalization in past year?	Yes	No	Yes
No. of positive blood cultures during hospitalization	3	1	6[b]
Duration of bacteremia, days	3	3	5
Antibiotics given during hospitalization	AMP, CRO, LZD, MEM	LZD	SAM, DAP, GEN, MEM, VAN
Outcome	Death	Discharged	Discharged

[a]CC, cancer center; HS, hospital system; LTAC, long-term acute care; FEP, cefepime; LZD, linezolid; MIN, minocycline; AMP, ampicillin; CRO, ceftriaxone; MEM, meropenem; VAN, vancomycin; DAP, daptomycin; SAM, ampicillin, sulbactam; GEN, gentamicin.
[b]2/6 isolates from cultures were available for sequencing.

for further characterization. MICs to vancomycin for each index isolate were performed through Etest following the Clinical and Laboratory Standards Institute (CLSI) guidelines (9). Isolates were sequenced on Illumina and Oxford Nanopore Technologies platforms (supplementary material; Table S2). To test the transferability of the *vanB* gene cluster on Tn*1549*, a filter mating protocol for conjugative transfer was used for each of the three index *E. faecalis* isolates. Conjugation assay methods are described in the supplementary material. To identify potential extracellular circular intermediates of Tn*1549*, inverse PCR experiments with the three index *E. faecalis* isolates were performed (supplementary material).

We identified six *E. faecalis* isolates harboring *vanB* (Table 1) belonging to three unique patients in our 203-patient VENOUS cohort (1.5%). Patients were hospitalized in a major cancer center (Patient HTX1) or a general hospital (Patient HTX2) in Houston, TX (2018 and 2020, respectively), or in a Miami, FL, general hospital (Patient MFL1) in 2018. Patient HTX1 was undergoing active chemotherapy treatment for acute lymphocytic leukemia, but the remaining two patients were immunocompetent. Of these, two were directly admitted to the intensive care unit (ICU) due to either worsening kidney function or hypotension secondary to septic shock. While patients HTX1 and HTX2 had a history of hospitalization in the year prior to study enrollment, they were not admitted to the same hospitals during this time frame. The index positive blood culture from each patient was obtained on the first day of hospital admission. There was no documented history of recent international travel for any of the patients.

All isolates from patients' follow-up blood cultures exhibited 3 to 12 single nucleotide polymorphisms (SNPs) relative to their index isolate (Table S1; Fig. 1), indicating that each

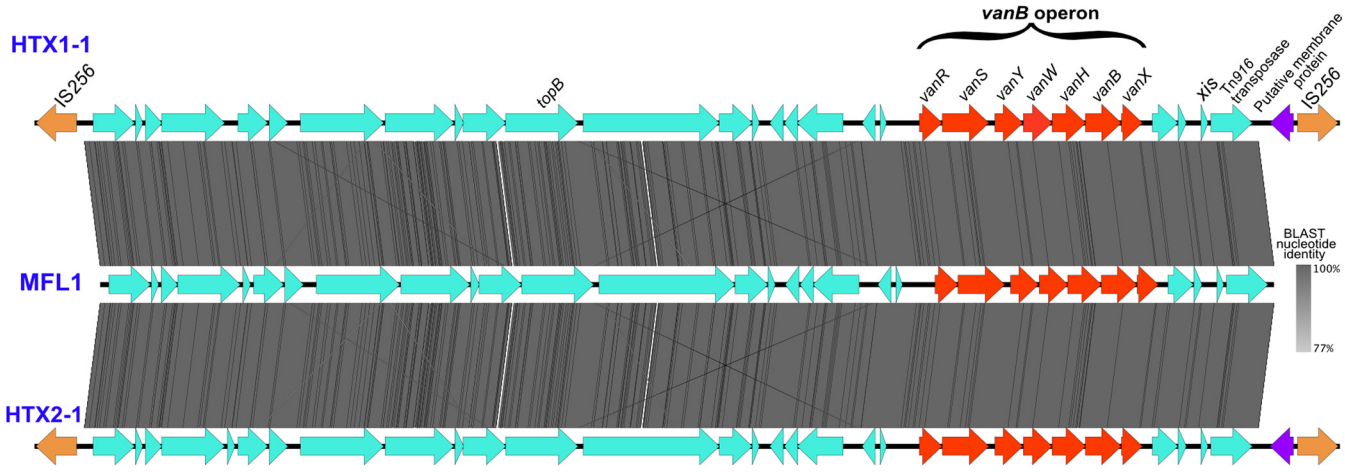

FIG 1 Characterization of Tn*1549* transposons harboring *vanB*. Characterization of transposon Tn*1549* carrying the *vanB* operon in each index isolate ($n = 3$). Genes belonging to Tn*1549* are depicted in aqua, genes comprising the *vanB* operon are shown in red, and mobile genetic elements are shown in orange. All other genes are depicted in purple.

patient was infected with the same strain throughout the course of bacteremia. Thus, only isolates from each patient's index blood culture ($n = 3$; isolates HTX1-1, HTX2-1, and MFL1) were further analyzed. The two Houston, TX, index isolates were more similar to each other than to MFL1, the latter differing by 181 whole-genome SNPs (Fig. S1). Comparison to *E. faecalis* V583, the first ST6 vancomycin-resistant *E. faecalis* reported in the United States, revealed wgSNP differences of >2000 SNPs (Fig. S2). All isolates harbored a chromosomally located Tn*1549* conjugative transposon carrying the *vanB* operon (Fig. 1). All identified Tn*1549* were ca. 33.8 kb and displayed 99% identity relative to a reference Tn*1549* (10). In isolates HTX1-1 and HTX2-1, Tn*1549* had nearly identical chromosomal insertion sites within genes encoding a bacterial sugar uptake phosphotransferase system (PTS) (10). In contrast, in *E. faecalis* MLF1, Tn*1549* was inserted closer to the bacterial replication initiator gene *dnaA*. Interestingly, although the left and right ends and inverted right and left repeat regions of Tn*1549* were identical in all three strains, in both *E. faecalis* HTX1-1 and HTX2-1, Tn*1549* was flanked by additional mobile genetic elements (MGEs), specifically, insertion sequence IS*256*. However, these additional MGEs were not detected in MFL1. We did not detect conjugative transfer of Tn*1549* from any of the three strains to the laboratory strain *E. faecalis* OG1RF under the chosen experimental conditions, nor were circular intermediates of Tn*1549* observed with inverse PCR.

Given the heterogeneous contexts of Tn*1549*, we next investigated structural variations in the chromosomes using long-read sequencing to further explore genetic relatedness among the strains. We confirmed that HTX1-1 and HTX2-1 possessed nearly identical chromosomal gene content and synteny (Fig. S3a), with a total size of roughly 3.11 Mb. However, the chromosome of MFL1 was significantly larger (ca. 3.28-Mb), and a 2.9-Mb inversion was noted relative to the Houston, TX isolates (Fig. S3b) within tRNA regions (Fig. S3c). Indeed, tRNA regions are known sites of genomic recombination and integration (11). All isolates were multidrug-resistant, possessing similar antimicrobial resistance (AMR) gene content, including the presence of *aac(6′)-Ie-aph(2″)-Ia* conferring high-level aminoglycoside resistance (Table S4). Isolates also possessed unique plasmid repertoires, ranging from 1 to 3 plasmids, and at least one plasmid associated with each index isolate harbored one or more AMR genes. (Fig. S4).

Tn*1549* carrying *vanB* has been extensively observed in *E. faecium* clinical strains worldwide (12, 13) but far less in *E. faecalis* (14, 15). To our knowledge, only one other instance of Tn*1549*-mediated *vanB* vancomycin resistance in *E. faecalis* has been described in the United States, and the observation of additional *IS256* MGEs flanking Tn*1549* in the two Houston, TX isolates has not before been described in this context. We also identified two distinct chromosomal insertion points of Tn*1549* in HTX1-1/HTX2-1 and MFL1, suggesting independent acquisitions of the *vanB*-carrying transposon in these isolates that is supported by strain geographical and temporal separation. Given that the chromosomal gene structure and synteny—including the context of insertion for Tn*1549*—in the two Houston, TX patients were nearly identical, these isolates may have originated from a common ancestor some time ago. However, the considerable SNP distance and the lack of a spatiotemporal link between patients provide evidence against a more recent direct transmission event between the two patients.

All *vanB*-carrying isolates belonged to ST6, a sequence type highly prevalent in clinical settings in the United States and known for enhanced virulence and multidrug-resistant phenotypes (8, 16). Our findings suggest that *vanB*-carrying Tn*1549* might be circulating in U.S. enterococci and that a dearth of comprehensive U.S. enterococcal genomic surveillance has precluded further discovery of this resistance phenotype in *E. faecalis*.

In summary, we found three epidemiologically distinct instances of multidrug-resistant ST6 *E. faecalis* bacteremia isolates harboring a *vanB*-carrying Tn*1549* transposon in the United States. This observation, along with the evolving emergence of multi-drug resistant *E. faecalis*, support the need for more extensive surveillance for these nosocomial pathogens.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 1.3 MB.

ACKNOWLEDGMENTS

C.A.A. has received grant support from MeMed Diagnostics, Merck Pharmaceuticals, and Entasis Pharmaceuticals.

This work was supported by the National Institute of Allergy and Infectious Diseases (NIAID) K24AI121296, R01AI134637, R01AI148342-01, P01AI152999-01 to C.A.A. S.R.S. was partially funded under National Institutes of Health (NIH) pre-doctoral training grant (5T32AI055449-15 to Theresa M. Koehler/Michael Lorenz). B.M.H. was supported by NIAID K01AI148593-01 and P01AI152999-01.

REFERENCES

1. Weiner-Lastinger LM, Abner S, Edwards JR, Kallen AJ, Karlsson M, Magill SS, Pollock D, See I, Soe MM, Walters MS, Dudeck MA. 2020. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. Infect Control Hosp Epidemiol 41:1–18. https://doi.org/10.1017/ice.2019.296.
2. Pfaller MA, Cormican M, Flamm RK, Mendes RE, Jones RN. 2019. Temporal and geographic variation in antimicrobial susceptibility and resistance patterns of Enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997–2016. Open Forum Infect Dis 6:S54–S62. https://doi.org/10.1093/ofid/ofy344.
3. Ping S, Mayorga-Reyes N, Price VJ, Onuoha M, Bhardwaj P, Rodrigues M, Owen J, Palacios Araya D, Akins RL, Palmer KL. 2021. Characterization of presumptive vancomycin-resistant enterococci recovered during infection control surveillance in Dallas, Texas, USA. Access Microbiol 3:e000214. https://doi.org/10.1099/acmi.0.000214.
4. Erickson KE, Madinger NE, Chatterjee A. 2016. Draft genome sequence for a clinical isolate of vancomycin-resistant *Enterococcus faecalis*. Genome Announc 4:e00584-16. https://doi.org/10.1128/genomeA.00584-16.
5. Lee RS, Gonçalves da Silva A, Baines SL, Strachan J, Ballard S, Carter GP, Kwong JC, Schultz MB, Bulach DM, Seemann T, Stinear TP, Howden BP. 2018. The changing landscape of vancomycin-resistant *Enterococcus faecium* in Australia: a population-level genomic study. J Antimicrob Chemother 73:3268–3278.
6. Werner G, Neumann B, Weber RE, Kresken M, Wendt C, Bender JK, Becker K, Borgmann S, Diefenbach A, Hamprecht A, Hogardt M, Wichelhaus T, Kemp V, Huebner N-O, Kaasch A, Geginat G, Kohnen W, Menzer A, Krause T, Miethke T, Pranada F, Radojn F, Tobisch S, Jansen V, Regnath T, Bührlen U, Schneider-Brachert W, Schwarz R, Luemen M, Skov R, Thuermer A, von Baum H, Weig M, Uwe G, Zabel L, von Wulffen H, Döring S, VRE study group. 2020. Thirty years of VRE in Germany – "expect the unexpected": the view from the National Reference Centre for Staphylococci and Enterococci. Drug Resist Updat 53:100732. https://doi.org/10.1016/j.drup.2020.100732.
7. Sadowy E, Gawryszewska I, Kuch A, Żabicka D, Hryniewicz W. 2018. The changing epidemiology of VanB *Enterococcus faecium* in Poland. Eur J Clin Microbiol Infect Dis 37:927–936. https://doi.org/10.1007/s10096-018-3209-7.
8. Contreras GA, Munita JM, Simar S, Luterbach C, Dinh AQ, Rydell K, Sahasrabhojane PV, Rios R, Diaz L, Reyes K, Zervos M, Misikir HM, Sanchez-Petitto G, Liu C, Doi Y, Abbo LM, Shimose L, Seifert H, Gudiol C, Barberis F, Pedroza C, Aitken SL, Shelburne SA, van Duin D, Tran TT, Hanson BM, Arias CA. 2022. Contemporary clinical and molecular epidemiology of vancomycin-resistant Enterococcal bacteremia: a prospective multicenter cohort study (VENOUS I). Open Forum Infect Dis 9:ofab616. https://doi.org/10.1093/ofid/ofab616.
9. Clinical and Laboratory Standards Institute (CLSI). 2022. CLSI M100-ED32:2022 performance standards for antimicrobial susceptibility testing, 32nd edition. Clinical and Laboratory Standards Institute.
10. Saier MH. 2015. The Bacterial Phosphotransferase System: New frontiers 50 years after its discovery. J Mol Microbiol Biotechnol 25:73–78. https://doi.org/10.1159/000381215.
11. Williams KP. 2002. Integration sites for genetic elements in prokaryotic tRNA and tmRNA genes: sublocation preference of integrase subfamilies. Nucleic Acids Res 30:866–875. https://doi.org/10.1093/nar/30.4.866.
12. Howden BP, Holt KE, Lam MMC, Seemann T, Ballard S, Coombs GW, Tong SYC, Grayson ML, Johnson PDR, Stinear TP. 2013. Genomic insights to control the emergence of vancomycin-resistant Enterococci. mBio 4. https://doi.org/10.1128/mBio.00412-13.
13. Lam MM, Seemann T, Tobias NJ, Chen H, Haring V, Moore RJ, Ballard S, Grayson LM, Johnson PD, Howden BP, Stinear TP. 2013. Comparative analysis of the complete genome of an epidemic hospital sequence type 203 clone of vancomycin-resistant *Enterococcus faecium*. BMC Genomics 14:595. https://doi.org/10.1186/1471-2164-14-595.
14. Garnier F, Taourit S, Glaser P, Courvalin P, Galimand M. 2000. Characterization of transposon Tn1549, conferring VanB-type resistance in *Enterococcus* spp. Microbiol Read Engl 146:1481–1489. https://doi.org/10.1099/00221287-146-6-1481.
15. Umeda A, Garnier F, Courvalin P, Galimand M. 2002. Association between the *vanB2* glycopeptide resistance operon and Tn*1549* in enterococci from France. J Antimicrob Chemother 50:253–256. https://doi.org/10.1093/jac/dkf105.
16. McBride SM, Fischetti VA, LeBlanc DJ, Jr, RCM Gilmore MS. 2007. Genetic diversity among *Enterococcus faecalis*. PLoS One 2:e582. https://doi.org/10.1371/journal.pone.0000582.