



Original Article

Vancomycin-resistant *Enterococcus* sequence type 1478 spread across hospitals participating in the Canadian Nosocomial Infection Surveillance Program from 2013 to 2018

David R. Kleinman MD¹, Robyn Mitchell MHSc², Melissa McCracken MSc³, Susy S. Hota MD MSc⁴ ,
George R. Golding PhD⁵, CNISP VRE Working Group^a and Stephanie W. Smith MD MSc⁶ 

¹Division of Infectious Diseases, Department of Medicine, University of Alberta, Edmonton, AB, Canada, ²Public Health Agency of Canada, Ottawa, Ontario, Canada, ³Antimicrobial Resistance and Nosocomial Infections, Public Health Agency of Canada, Winnipeg, Manitoba, Canada, ⁴Infection Prevention and Control Department, University Health Network, Toronto, Ontario, Canada; Department of Medicine, University of Toronto, Toronto Ontario, Canada, ⁵Antimicrobial Resistance and Nosocomial Infections, One Health Division, Surveillance, Reference and Science Directorate of the National Microbiology Laboratory Branch, Winnipeg, Manitoba, Canada and ⁶Division of Infectious Diseases, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada

Abstract

Objective: To analyze the spread of a novel sequence type (ST1478) of vancomycin-resistant *Enterococcus faecium* across Canadian hospitals.

Design: Retrospective chart review of patients identified as having ST1478 VRE bloodstream infection.

Setting: Canadian hospitals that participate in the Canadian Nosocomial Infection Surveillance Program (CNISP).

Methods: From 2013 to 2018, VRE bloodstream isolates collected from participating CNISP hospitals were sent to the National Microbiology Laboratory (NML). ST1478 isolates were identified using multilocus sequence typing, and whole-genome sequencing was performed. Patient characteristics and location data were collected for patients with ST1478 bloodstream infection (BSI). The sequence and patient location information were used to generate clusters of infections and assess for intrahospital and interhospital spread.

Results: ST1478 VRE BSI occurred predominantly in a small number of hospitals in central and western Canada. Within these hospitals, infections were clustered on certain wards, and isolates often had <20 single-nucleotide variants (SNV) differences from one another, suggesting a large component of intrahospital spread. Furthermore, some patients with bloodstream infections were identified as moving from one hospital to another, potentially having led to interhospital spread. Genomic analysis of all isolates revealed close relatedness between isolates at multiple different hospitals (<20 SNV) not predicted from our epidemiologic data.

Conclusions: Both intrahospital and regional interhospital spread have contributed to the emergence of VRE ST1478 infections across Canada. Whole-genome sequencing provides evidence of spread that might be missed with epidemiologic investigation alone.

(Received 6 October 2021; accepted 30 December 2021; electronically published 10 March 2022)

Vancomycin-resistant *Enterococcus* (VRE) infections cause significant morbidity and mortality in hospitalized patients, particularly in immunocompromised patients and those requiring intensive care.¹ The incidence of VRE bloodstream infection (BSI) is increasing in Canada,² thought to be due in part to the removal of contact precautions for VRE in certain hospitals.³

In Australia, a novel *vanA* containing VRE strain, identified by *pstS* gene loss through multilocus sequence typing (MLST), was identified in

2015.⁴ This strain has since spread throughout the country, both within hospitals and through geographically dispersed regions.⁵ A similar *pstS*-null mutant was identified in Scotland, Denmark, and the United Kingdom but has not been reported to have spread significantly.^{6–8}

In 2013, the Canadian Nosocomial Infection Surveillance Program (CNISP) identified a novel *Enterococcus faecium pstS* null strain in 2 isolates, named sequence type 1478 (ST1478).² ST1478 had spread to 19 acute-care hospitals in 6 provinces by 2018, representing 38.7% of VRE BSIs in that year.² ST1478 isolates had significantly more daptomycin resistance (13%) than non-ST1478 isolates (4%) in Canada,² along with more tetracycline and gentamicin resistance; therefore, infections with this strain may be more difficult to treat.

Whole-genome sequencing has been used in conjunction with epidemiologic investigation to characterize VRE outbreaks.^{9–12} This study aimed to use whole-genome sequencing and enhanced epidemiologic investigation to better understand the intra and

Author for correspondence: Stephanie Smith, E-mail: ss@ualberta.ca

^aOther members of the CNISP VRE working group include Ghada Al-Rawahi, James Brooks, John Conly, Jennifer Ellison, John M. Embil, Jennie Johnstone, Kevin Katz, Kathy N. Suh and Jen Tomlinson.

Cite this article: Kleinman DR, et al. (2023). Vancomycin-resistant *Enterococcus* sequence type 1478 spread across hospitals participating in the Canadian Nosocomial Infection Surveillance Program from 2013 to 2018. *Infection Control & Hospital Epidemiology*, 44: 17–23, <https://doi.org/10.1017/ice.2022.7>

© The Author(s), 2022. Published by Cambridge University Press on behalf of The Society for Healthcare Epidemiology of America. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

interhospital transmission of this novel *Enterococcus* strain in a network of Canadian acute-care hospitals to inform infection control practices.

Methods

Study design and sources of data

CNISP is a collaboration between the Public Health Agency of Canada, the Association of Medical Microbiology and Infection Disease Canada, the National Microbiology Laboratory (NML) of Canada and sentinel acute-care hospitals across Canada. CNISP has conducted prospective surveillance of VRE BSI among inpatients in Canadian acute-care hospitals since 1999. Medical records from patients with ST1478 bloodstream infections across 19 acute-care hospitals were further interrogated for this study.

Case definitions

Hospitalized patients with enterococcal bacteremia characterized as having vancomycin MICs >8 mg/L, using local laboratory methods, were eligible. Patients were included more than once if a positive VRE blood isolate was identified >14 days after completion of therapy for previous infection and the isolate was believed to be unrelated to previous infection in accordance with best clinical judgment.¹³

Data collection

Through CNISP routine surveillance, the medical records of each patient identified with VRE BSI were reviewed for demographic, clinical, risk factor and outcome data by trained infection prevention and control professionals (Appendix 1 online). For this study, further data were extracted from patient charts, including hospitalizations in the 12 months preceding ST1478 BSI, patient location at CNISP hospital(s), patient comorbidities and additional risk factor data (Appendix 2 online). Patient data from were linked using a unique patient identifier.

Molecular methods

VRE bloodstream isolates were sent to the NML for molecular typing (*van* gene polymerase chain reaction [PCR], speciation, and MLST), and whole-genome sequencing (WGS). WGS data were generated using the MiSeq platform (Illumina, San Diego, CA). Assembled reads (contigs) were analysed with an in-house MLST tool based on one from the Centre for Genomic Epidemiology website (<https://cge.cbs.dtu.dk/services>). Phylogenetic relationships were determined by single nucleotide variant (SNV) analysis using the SNVPhyl pipeline.¹⁴ The reference genomes used in the SNV analysis was the *Enterococcus faecium* 07B18012 and 26G17009 pseudogenomes.

Data compilation

Patient characteristics, including comorbidities, and 30-day all-cause mortality were calculated using Excel. For reporting purposes, and to ensure confidentiality, we grouped provinces into 3 regions: west (British Columbia, Alberta, Saskatchewan and Manitoba), central (Ontario and Quebec) and east (Nova Scotia, New Brunswick, Prince Edward Island, and Newfoundland and Labrador). To identify potential interhospital transmission, patient movement data at the hospital level were plotted for each patient for central and western Canada.

Hospital-based cluster analysis

Clusters were defined as ≥ 5 BSIs in one hospital or within a network of regional hospitals with regular interhospital transfers within a 12-month period. Outbreaks were defined as an unexpected increase in the prevalence of a pathogen; VRE outbreaks have been defined by as few as 2 in the same period in an individual hospital.¹⁵ To allow for patient movement analysis, a minimum of 5 infections was chosen. VRE has been reported to have variable mutation rates, with estimates between 5 and 147 SNVs per genome per year^{16,17}; therefore, periods <1 year were used such that SNV differences could be used to assess genetic relatedness between isolates. Furthermore, utilizing WGS data, the SNVPhyl pipeline was used to generate a SNV matrix for each cluster.

Genome-based cluster analysis

Using an SNV matrix containing all sequenced ST1478 isolates, clusters of isolates with <20 SNVs were identified. The threshold of 10 SNV has been suggested to identify related isolates within hospitals¹⁸; to allow sufficient variation to detect relationships between hospitals, the threshold of 20 SNV was used. Phylogenetic trees of these clusters of interest were generated using the SNVPhyl pipeline.

Ethics approval

This study was either considered exempt from the requirement for ethics approval as a quality assurance study within the mandate of hospital infection prevention and control programs or were approved by the research ethics boards at participating hospitals if required by institution-specific policies.

Results

ST1478 bloodstream infections

From 2013 to 2018, 115 ST1478 BSI VRE isolates among 111 patients were identified in 19 CNISP hospitals (Table 1),² with increasing incidence over the study period. Infections were largely concentrated in central and western Canada (Table 1 and Fig. 1). The most common pre-existing comorbidities were active malignancy (37%), liver disease (27%), kidney disease (26%), and cardiac disease (21%) (Table 1). Surgical procedures, particularly those involving manipulation of the gastrointestinal tract, were frequent among patients with an ST1478 VRE BSI (Table 1). The 30-day all-cause mortality was 32.4%. (Table 1).

Figure 1 displays hospitalizations and the date of positive blood culture for central and western Canada. Central Canada (Fig. 1A) had a large burden of infection concentrated in 2 hospitals, whereas western Canada (Fig. 1B) had infections distributed in multiple hospitals, though with a large concentration at “hospital 1.”

Transmission analysis of hospital clusters

We identified 4 clusters within 2 hospitals in central Canada that had repeated interhospital transfers, 2 of which are shown (Fig. 2A and B), and 3 clusters in 3 separate hospitals in western Canada, 2 of which are shown (Fig. 2C and D).

All epidemiologic defined clusters contained some isolates that appeared genetically related when comparing WGS SNV analysis. The central clusters spanned 2 hospitals (hospital 1 and hospital 2, in Fig. 2A and 2B) and had cases clustered on transplant, leukemia, bone marrow transplant, and the intensive care units. In Fig. 2A and in hospital 2 in Fig. 2B, most isolates were within 20 SNVs

Table 1. Geographic Location and Characteristics of Patients Identified With ST1478 VRE BSI, 2013–2018

Characteristics	No./Total No. (%)
Region	
Western Canada	65/115 (56.5)
Central Canada	49/115 (42.6)
Eastern Canada	1/115 (0.9)
Pre-existing comorbidities	
Active malignancy	40/107 (37.4)
Liver disease	29/107 (27.1)
Kidney disease	28/107 (26.2)
Cardiac disease	22/107 (20.6)
Diabetes	19/107 (17.8)
Solid organ transplant	16/107 (15.0)
Lung disease	14/107 (13.1)
Bone marrow transplant	9/107 (8.4)
Procedures	
All procedures ^a	44/104 (42.3%)
Procedures involving GI tract	28/104 (26.9%)
30-day all-cause mortality	35/108 (32.4)

Note. VRE, vancomycin-resistant *Enterococcus*; BSI, bloodstream infection; GI, gastrointestinal.

^aAll procedures include and surgeries or endoscopic procedures that took place in the 3 months prior to the positive VRE blood culture.

of each other. These suggest a major role for intrahospital transmission in propagating ST1478 within these hospitals.

Interestingly, in Fig. 2A, the first patient with a ST1478 BSI in hospital 1 (labelled B in Fig. 2A) was in hospital 2 two months earlier. This patient's isolate had an SNV difference of 1 from the other ST1478 isolate found in hospital 2 (labelled A) and was within 15 SNV of most ST1478 cases at hospital 1 in the cluster (ie, clusters C–L in Fig. 2A). Given these genetically similar isolates in hospital 1 appeared after the patient labelled B arrived, it is possible that patient B was the source of ST1478 that caused the cluster at hospital 1.

Similarly, a major cluster at hospital 2 may have been initiated by a patient previously hospitalized at hospital 1. In Figure 2B, patient A had a short stay at hospital 1 followed by a longer hospitalization on the leukemia ward of hospital 2, with development of a ST1478 BSI. This BSI was followed by multiple ST1478 BSI (Fig. 2B patients B–D) within 4 SNV of A, along with another BSI in hospital 1 (E in Fig. 2B) within 7 SNV of the BSI at hospital 2. Although patient A's stay at hospital 1 was short, the close relatedness between isolates at both hospitals suggests interhospital transmission; patient A was a plausible ST1478 source of introduction to hospital 2 in our data set.

In the Figure 2C cluster, which represents a single hospital, 3 groups of patients vary by 4 SNV or less on similar wards, suggesting transmission. However, within wards, there was evidence of multiple introductions of ST1478; however, patient C's infection occurred in ICU at a similar time as patient I's and patient K's infections (Fig. 2C), SNV variation suggests 2 unrelated introductions. Figure 2D shows a separate western Canada hospital with high variability between many isolates despite many patients spending on the bone marrow transplant units.

Clusters predicted from sequence data

In addition to using WGS to better understand epidemiologic clusters, we sought to use WGS to analyze transmission of ST1478 across Canada. We used WGS data from the whole data set to generate an SNV matrix containing all isolates, and we identified clusters of isolates containing <20. Most clusters were in individual hospitals, some of which are explored in the previous section. However, 2 clusters spanned multiple hospitals, and they highlight the clonal spread of VRE ST1478 across Canada particularly well. The first cluster (not shown) identified 33 closely related isolates in 5 hospitals across 3 provinces; most occurred at a single hospital in central Canada. The second cluster, shown in Figure 3, spans 7 hospitals in multiple cities in western Canada.

The cluster in Figure 3 highlights that there can be dispersion of this pathogen across hospitals regionally and that seemingly unrelated isolates epidemiologically can be very closely related through sequence data. However, as the precise patient movement that transmitted these strains between hospitals cannot be identified from our patient movement data, the exact mechanism by which this pathogen spread is difficult to elucidate.

Discussion

In this study, we explored the spread of a previously reported *pstS* null VRE strain with increased daptomycin resistance, ST1478,² across and within Canadian hospitals through whole-genome sequencing and epidemiologic data. All hospitals with a high incidence of this strain had clusters of low SNV variation, suggesting the role of intrahospital transmission. This finding may reflect high outbreak potential of *psTs* null VRE isolates, with Australia having had a similar *pstS*-null VRE strain establish itself in hospitals around the country.⁵ Furthermore, we identified regional clustering of isolates with low SNV variation, suggesting interhospital transmission.

In our data set, this pathogen appears to have spread differently in central Canada than in western Canada. In central Canada, we identified significant intrahospital transmission. In western Canada, there appears to have been significant interhospital transmission and more variation within individual hospitals. This may be due to healthcare systems in western Canada, where patients living outside major centers often frequent multiple tertiary-care hospitals in different provinces, potentially leading to spread between hospitals.

The SNV variation between isolates was used to estimate relatedness in our study. *Enterococcus faecium* has been found to have a variable mutation rate.^{5,16,17} SNV variance in VRE can be further complicated by recombination events¹² and by rapid evolution in certain hosts; a VRE isolate accumulated 25 SNV in 133 days in a colonized mouse.¹⁹ Other studies demonstrated low SNV variability of in VRE outbreaks, with isolates differing by 0–3 SNV in one analysis.¹¹

ST1478 showed increased resistance to multiple antimicrobials, including daptomycin^{2,2} which is the first-line treatment for serious VRE infections. This pathogen's ability to spread rapidly within and between hospitals represents a potential threat to patients and highlights the need for effective infection control practices. The 30-day all-cause mortality for VRE BSI ST1478 was 32.4%, similar to the mortality rate of non-ST1478 VRE BSI Canada during the same time period, 30.8%.² VRE BSI has consistently been associated with higher mortality than vancomycin-sensitive enterococcus BSI.^{20,21}

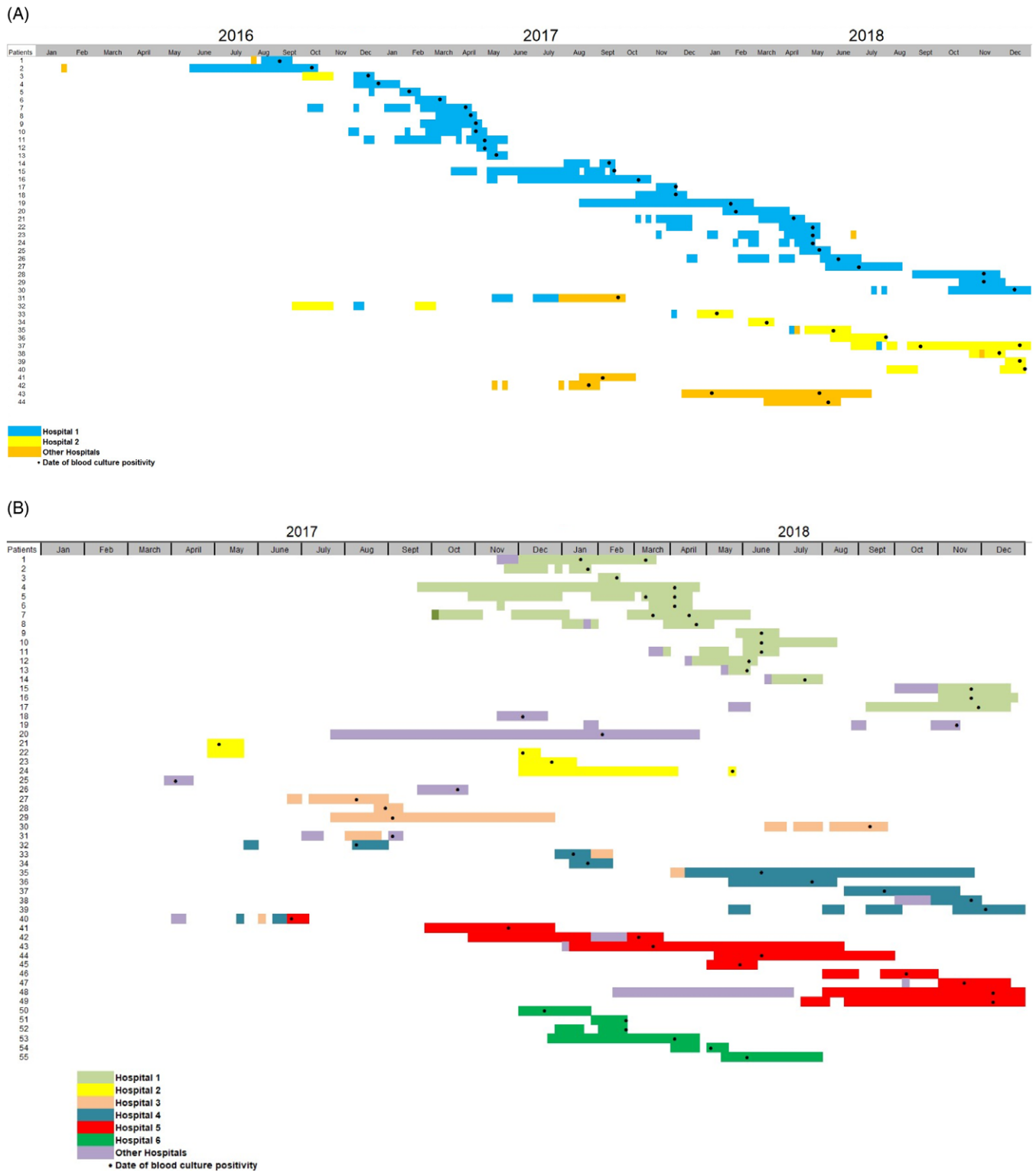


Fig. 1. Both figures plot the hospital location of patients who developed ST1478 bloodstream infections. Colors represent hospitals, dots represent date of blood culture positivity, x-axis represents time of hospitalization and positive blood culture, and the left-hand column contains isolate or patient identifiers. (A) Hospitalizations of ST1478 patients along with date of blood culture positivity in central Canada. (B) Hospitalizations of ST1478 patients along with date of blood culture positivity in western Canada. Hospital 1 only began sending VRE bloodstream isolates to the National Microbiology Laboratory in 2018.

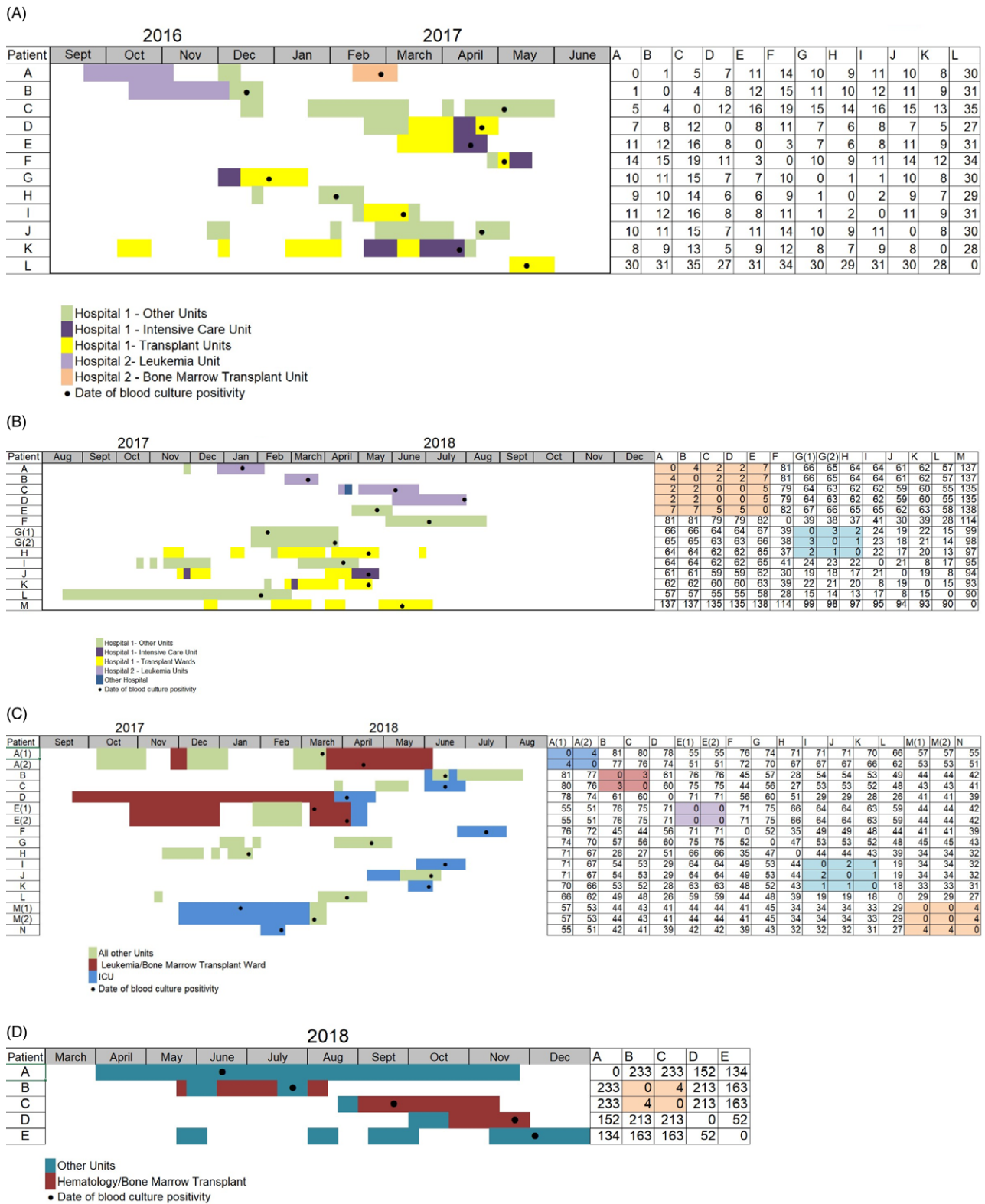
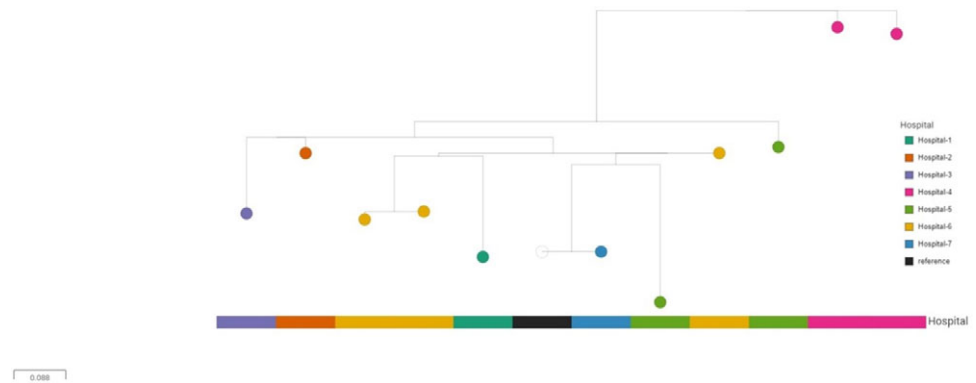


Fig. 2. These figures show the inpatient location of patients who developed ST1478 infection, date of blood culture positivity, and the SNV difference between different isolates within each cluster. Patients are identified by letters; patients who developed 2 infections are identified by A(1) and A(2). Colors represent different wards dots represent date of blood culture positivity, and the x-axis represents time. The right contains an SNV matrix containing SNV differences between isolates, with groups of isolates with few SNV differences highlighted. (A–B) Two clusters within 2 hospitals in central Canada, hospital 1 and hospital 2, with repeated interhospital transfers. Most isolates within hospitals vary by <20 SNV, suggesting intrahospital transmission. (A) First cluster in central Canada hospitals. Patient B was hospitalized in hospital 2 shortly before hospitalization in hospital 1; their isolate varied from another in hospital 2 (isolate A) by 1 SNV and was within 16 SNVs of isolates C through K at hospital 1. (B) Third cluster in central Canada. Patient A had a short previous hospitalization in Hospital prior to a longer one in Hospital 2 with ST1478 BSI. (2C–D) Two clusters identified in separate hospitals in western Canada. (C) Cluster in a western Canada hospital. Highlighted isolates in the SNV matrix show closely related isolates, suggesting intrahospital transmission of these isolates. (D) Cluster in another western Canada hospital. Although all patients developed infections at similar times and spent time on the bone marrow transplant ward, there is significant SNV variation between most isolates, suggesting repeated introduction of this strain.

Fig. 3. Phylogeny of a cluster of isolates, with 35 SNV between, spanning 7 western Canada hospitals. Phylogeny was determined using 88.44% of the core genome. The reference genome used was the *E. faecium* 26G17009 pseudogenome. SNV variation is more than the <20 SNV used to identify isolates within the larger SNV matrix that contains all isolates, with the smaller sample sizes allowing more core genome to be compared.



This study had several limitations. Because this study involved only hospitals that participate in the CNISP, we may have missed important VRE ST1478 bloodstream isolates, which might have prevented us from fully elucidating the transmission dynamics of this pathogen. Furthermore, certain hospitals did not consistently send isolates to the NML, which limited our ability to detect ST1478 in these hospitals. Additionally, CNISP only collects isolates from inpatients with bloodstream infections, meaning that ST1478 colonized patients or healthcare workers are not represented. Given that colonized patients are likely contributing to spread of this pathogen, our bloodstream isolate approach will miss transmission events and limit our ability to identify routes of transmission. In addition, we did not collect data on post-acute-care facilities, such as rehabilitation hospitals; spread that may have taken place at these institutions was not included in our study. Furthermore, the difficulty in using SNV variance in estimating genetic relatedness may limit the findings of this study.

In this study, we analyzed the spread of a VRE strain, ST1478, across Canadian hospitals. Using both SNV analysis and patient movement data, we found evidence of intrahospital spread along with regional interhospital spread, pronounced in western Canada. Additionally, as demonstrated by the insights we gained from our phylogenetic data, we show the ability of whole-genome sequencing to supplement epidemiologic data to better elucidate the transmission of pathogens within and between hospitals. With movement between hospitals appearing to be an important mediator of spread of this strain of VRE between hospitals, targeted screening for VRE among previously hospitalized patients could limit the spread between hospitals. Furthermore, the apparent spread of this strain within hospitals underscores the need for compliance with hand hygiene and isolation precautions for VRE positive patients. To better understand the spread of VRE, hospital networks with more VRE could be further investigated, through the collection of VRE colonization, bloodstream isolates, and nonbloodstream isolates, which could be integrated with patient movement data to further explore the spread of this pathogen.

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/ice.2022.7>

Acknowledgments. We thank the physicians, epidemiologists, infection control practitioners, and laboratory staff at each participating hospital for their contributions to this study.

Financial support. No financial support was received for this study.

Conflicts of interest. All authors report no conflicts of interest relevant to this article.

References

1. Johnstone J, Chen C, Rosella L, *et al*. Patient- and hospital-level predictors of vancomycin-resistant *Enterococcus* (VRE) bacteremia in Ontario, Canada. *Am J Infect Control* 2018;46:1266–1271.
2. McCracken M, Mitchell R, Smith S, *et al*. Emergence of *pstS*-null vancomycin-resistant *Enterococcus faecium* clone ST1478, Canada, 2013–2018. *Emerg Infect Dis* 2020;26:2247–2250.
3. Johnstone J, Policarpio ME, Lam F, *et al*. Rates of blood cultures positive for vancomycin-resistant *Enterococcus* in Ontario: a quasi-experimental study. *CMAJ Open* 2017;5:E273–E280.
4. Carter GP, Buultjens AH, Ballard SA, *et al*. Emergence of endemic MLST nontypeable vancomycin-resistant *Enterococcus faecium*. *J Antimicrob Chemother* 2016;71:3367–3371.
5. van Hal SJ, Beukers AG, Timms VJ, *et al*. Relentless spread and adaptation of nontypeable *vanA* vancomycin-resistant *Enterococcus faecium*: a genome-wide investigation. *J Antimicrob Chemother* 2018;73:1487–1491.
6. Lemonidis K, Salih TS, Dancer SJ, Hunter IS, Tucker NP. Emergence of an Australian-like *pstS*-null vancomycin-resistant *Enterococcus faecium* clone in Scotland. *PLoS One* 2019;14:e0218185.
7. Hansen TA, Pedersen MS, Nielsen LG, *et al*. Emergence of a vancomycin-variable *Enterococcus faecium* ST1421 strain containing a deletion in *vanX*. *J Antimicrob Chemother* 2018;73:2936–2940.
8. Raven KE, Reuter S, Reynolds R, *et al*. A decade of genomic history for healthcare-associated *Enterococcus faecium* in the United Kingdom and Ireland. *Genome Res* 2016;26:1388–1396.
9. Eichel V, Klein S, Bootsvelde C, *et al*. Challenges in interpretation of WGS and epidemiological data to investigate nosocomial transmission of vancomycin-resistant *Enterococcus faecium* in an endemic region: incorporation of patient movement network and admission screening. *J Antimicrob Chemother* 2020;75:1716–1721.
10. Abdelbary MHH, Senn L, Greub G, Chaillou G, Moulin E, Blanc DS. Whole-genome sequencing revealed independent emergence of vancomycin-resistant *Enterococcus faecium* causing sequential outbreaks over 3 years in a tertiary-care hospital. *Eur J Clin Microbiol Infect Dis* 2019;38:1163–1170.
11. Rangberg A, Larsen AL, Kacelnik O, *et al*. Molecular analysis and epidemiological typing of vancomycin-resistant *Enterococcus* outbreak strains. *Sci Rep* 2019;9:11917.
12. Pinholt M, Larner-Svensson H, Littauer P, *et al*. Multiple hospital outbreaks of *vanA* *Enterococcus faecium* in Denmark, 2012–13, investigated by WGS, MLST, and PFGE. *J Antimicrob Chemother* 2015;70:2474–2482.
13. Smith S, Conly J, Embil J, *et al*. Canadian Nosocomial Infection Surveillance Program (CNISP) Surveillance of Vancomycin Resistant *Enterococci* Bloodstream Infections in CNISP Hospitals. Ottawa (ON): Public Health Agency of Canada; 2018.
14. Petkau A, Mabon P, Sieffert C, *et al*. SNVPhyl: a single nucleotide variant phylogenomics pipeline for microbial genomic epidemiology. *Microb Genom* 2017;3:e000116.
15. Buetti N, Wassilew N, Rion V, *et al*. Emergence of vancomycin-resistant enterococci in Switzerland: a nationwide survey. *Antimicrob Resist Infect Control* 2019;8:16.

16. Howden BP, Holt KE, Lam MMC, *et al.* Genomic insights to control the emergence of vancomycin-resistant enterococci. *mBio* 2013;4:e00412–13.
17. Lebreton F, van Schaik W, McGuire AM, *et al.* Emergence of epidemic multidrug-resistant *Enterococcus faecium* from animal and commensal strains. *mBio* 2013;4:e00534–13.
18. van Hal SJ, Ip CLC, Ansari MA, *et al.* Evolutionary dynamics of *Enterococcus faecium* reveals complex genomic relationships between isolates with independent emergence of vancomycin resistance. *Microb Genom* 2016;2(1):e000048.
19. Dubin KA, Mathur D, McKenney PT, *et al.* Diversification and evolution of vancomycin-resistant *Enterococcus faecium* during intestinal domination. *Infect Immun* 2019;87:e00102–19.
20. Prematunge C, MacDougall C, Johnstone J, *et al.* VRE and VSE bacteremia outcomes in the era of effective VRE therapy: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol* 2016;37:26–35.
21. Rottier WC, Pinholt M, van der Bij AK, *et al.* Attributable mortality of vancomycin resistance in ampicillin-resistant *Enterococcus faecium* bacteremia in Denmark and the Netherlands: A matched cohort study. *Infect Control Hosp Epidemiol* 2021. doi: 10.1016/j.cmi.2020.07.014.