

Molecular epidemiology of vancomycin-resistant enterococcal bacteraemia: results from the Canadian Nosocomial Infection Surveillance Program, 1999–2009

M. McCracken¹, A. Wong², R. Mitchell³, D. Gravel³, J. Conly⁴, J. Embil⁵, L. Johnston⁶, A. Matlow⁷, D. Ormiston⁵, A. E. Simor⁸, S. Smith⁹, T. Du¹, R. Hizon¹ and M. R. Mulvey^{1*} on behalf of the members of the Canadian Nosocomial Infection Surveillance Program†

¹Public Health Agency of Canada, Winnipeg, MB, Canada; ²Royal University Hospital, Saskatoon, SK, Canada; ³Public Health Agency of Canada, Ottawa, ON, Canada; ⁴Foothills Medical Centre, Alberta Health Services—Calgary and area and University of Calgary, Calgary, AB, Canada; ⁵Health Sciences Centre, Winnipeg, MB, Canada; ⁶QEII Health Sciences Centre, Halifax, NS, Canada; ⁷Hospital for Sick Children, Toronto, ON, Canada; ⁸Sunnybrook Health Sciences Centre, Toronto, ON, Canada; ⁹University of Alberta, Edmonton, AB, Canada

*Corresponding author. Tel: +1-204-789-2133; Fax: +1-204-789-5020; E-mail: Michael_mulvey@phac-aspc.gc.ca

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Objectives: Vancomycin-resistant enterococci (VRE) can be associated with serious bacteraemia. The focus of this study was to characterize the molecular epidemiology of VRE from bacteraemia cases that were isolated from 1999 to 2009 as part of Canadian Nosocomial Infection Surveillance Program (CNISP) surveillance activities.

Methods: From 1999 to 2009, enterococci were collected from across Canada in accordance with the CNISP VRE surveillance protocol. MICs were determined using broth microdilution. PCR was used to identify *vanA*, *B*, *C*, *D*, *E*, *G* and *L* genes. Genetic relatedness was examined using multilocus sequence typing (MLST).

Results: A total of 128 cases of bacteraemia were reported to CNISP from 1999 to 2009. In 2007, a significant increase in bacteraemia rates was observed in western and central Canada. Eighty-one of the 128 bacteraemia isolates were received for further characterization and were identified as *Enterococcus faecium*. The majority of isolates were from western Canada (60.5%), followed by central (37.0%) and eastern (2.5%) Canada. Susceptibilities were as follows: daptomycin, linezolid, tigecycline and chloramphenicol, 100%; quinupristin/dalfopristin, 96.3%; high-level gentamicin, 71.6%; tetracycline, 50.6%; high-level streptomycin, 44.4%; rifampicin, 21.0%; nitrofurantoin, 11.1%; clindamycin, 8.6%; ciprofloxacin, levofloxacin and moxifloxacin, 1.2%; and ampicillin, 0.0%. *vanA* contributed to vancomycin resistance in 90.1% of isolates and *vanB* in 9.9%. A total of 17 sequence types (STs) were observed. Beginning in 2006 there was a shift in ST from ST16, ST17, ST154 and ST80 to ST18, ST412, ST203 and ST584.

Conclusions: The increase in bacteraemia observed since 2007 in western and central Canada appears to coincide with the shift of MLST STs. All VRE isolates remained susceptible to daptomycin, linezolid, chloramphenicol and tigecycline.

Keywords: *E. faecium*, CC17, *vanA*, MLST, vancomycin-resistant *Enterococcus*

Introduction

Since their first appearance in 1986, vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens and have been observed worldwide.^{1,2} The occurrence of VRE has been increasing globally, particularly in countries such as the USA, Europe and Taiwan.^{3,4} In the USA, a 12 year

surveillance study conducted at a single hospital reported 6% VRE in 1998 and 25% in 2009 among elderly patients.⁵ Reports of VRE from the USA and Europe have also been as high as 65% in some hospitals⁶ and 25% in intensive care units (ICUs).^{3,7}

In Canada, VRE were first identified in 1993⁸ and rates of infection have remained relatively low since then. In 1996, a national VRE prevalence study conducted by the Canadian

Nosocomial Infection Surveillance Program (CNISP) found a VRE infection rate of 0.1% in non-endemic hospitals and 3.7% in endemic hospitals.⁹ Between 1999 and 2005, CNISP surveillance reported a slight increase in the rate of infection from 0.02 to 0.05 cases per 1000 patients admitted.¹⁰ Furthermore, rates of VRE infections have been shown to increase from 1.8% in 2007 to 4.6% in 2009 in another Canadian study¹¹ and have even been reported at 6.7% in Canadian ICUs.¹² More recently, in 2010 a Canadian prevalence study reported a prevalence of VRE infection and colonization of 2% and a VRE infection prevalence of 0.06%.¹³ Bacteraemia caused by VRE is of particular concern as these patients are generally debilitated and there is an association with increased morbidity, mortality, length of stay and healthcare costs.³ The focus of this study was to characterize the molecular epidemiology of VRE from bacteraemia cases that were isolated from 1999 to 2009 as part of CNISP surveillance activities.

Methods

CNISP is administered by the Public Health Agency of Canada (PHAC) and has conducted prospective surveillance for VRE infection and colonization since 1999.¹⁰ CNISP is a partnership between the Centre for Communicable Disease and Infection Control and the National Microbiology Laboratory (NML) at PHAC and the Canadian Hospital Epidemiology Committee (CHEC), a sub-committee of the Association of Medical Microbiology and Infectious Diseases Canada. Currently CNISP comprises 53 hospital facilities in nine Canadian provinces. Inpatients with enterococcal bacteraemia characterized by having MICs of vancomycin of ≥ 8 mg/L were included only once in the surveillance.¹⁰ Once a VRE bacteraemia was determined, epidemiological data were collected as previously described and the isolate was forwarded to the NML for further characterization.¹⁰

At the NML, antimicrobial susceptibilities were determined by broth microdilution using the Sensititre GPALL1F panels (Trek Diagnostics, Cleveland, OH, USA) and MICs were interpreted using breakpoints described by the CLSI.¹⁴ A total of 15 antimicrobial agents were tested: ampicillin, chloramphenicol, ciprofloxacin, clindamycin, daptomycin, high-level gentamicin (500 mg/L), levofloxacin, linezolid, moxifloxacin, nitrofurantoin, quinupristin/dalfopristin, rifampicin, high-level streptomycin (1000 mg/L), tetracycline and tigecycline. Sensititre breakpoints for *Enterococcus* species were used to interpret tigecycline MICs (susceptible, ≤ 0.25 mg/L) (Trek Diagnostics).

PCR was used to determine the presence of vancomycin resistance genes *vanA*, *B*, *C*, *D*, *E*, *G* and *L* as well as the *ddl* gene to identify species, using a previously published protocol.¹⁵ *Enterococcus faecium* multilocus sequence typing (MLST) was conducted using previously published methods.¹⁶ Sequence types (STs) were determined using the MLST database (<http://efaecium.mlst.net>) and genetic relatedness was explored using goeBURST.¹⁷

Results and discussion

A total of 128 epidemiological cases of VRE bacteraemia were reported to CNISP from 1999 to 2009. Figure 1 illustrates that from 1999 to 2006 VRE bacteraemia rates remained consistent with minor fluctuations. In 2007, a significant increase in VRE bacteraemia rates was observed in western Canada (from 0.016 per 1000 patient admissions in 2006 to 0.022 per 1000 patient admissions in 2007) as well as in central Canada (from 0.005 per 1000 patient admissions in 2006 to 0.027 per 1000 patient admissions in 2007). VRE bacteraemia rates in the eastern provinces have remained consistently low from 1999 to 2009. Previous Canadian studies have also shown an increase from 1.8% in 2007 to 4.6% in 2009 in rates of VRE causing nosocomial infection.¹¹ Similarly, a recent study in the USA showed that in a 2.5 year period beginning in 2005 the rate of VRE bacteraemia increased significantly from 0.06 infections per 1000 patient-days to 0.17 infections per 1000 patient-days ($P=0.03$).¹⁸ In contrast, reports from Taiwan have indicated a more significant increase in VRE bacteraemia, from 3.9% in 2003 to 18.9% in 2010.⁴

Of the 128 epidemiological bacteraemia cases, 81 isolates were obtained and submitted to the NML for further characterization. These 81 isolates were the focus for the remainder of the study. All isolates were confirmed as *E. faecium* using PCR. The majority of bloodstream isolates were from western Canada (British Columbia, Alberta, Saskatchewan and Manitoba; 60.5%, $n=49$), followed by central Canada (Ontario and Quebec; 37.0%, $n=30$) and eastern Canada (Nova Scotia, New Brunswick, Newfoundland and Labrador; 2.5%, $n=2$). Patient age ranged from 3 to 95 years of age with 1.6% of cases <18 years, 53.1% 18–64 years and 45.3% >65 years old. Males represented 60.5% ($n=49$) of isolates. All isolates were susceptible to daptomycin, linezolid, tigecycline and chloramphenicol. Similar

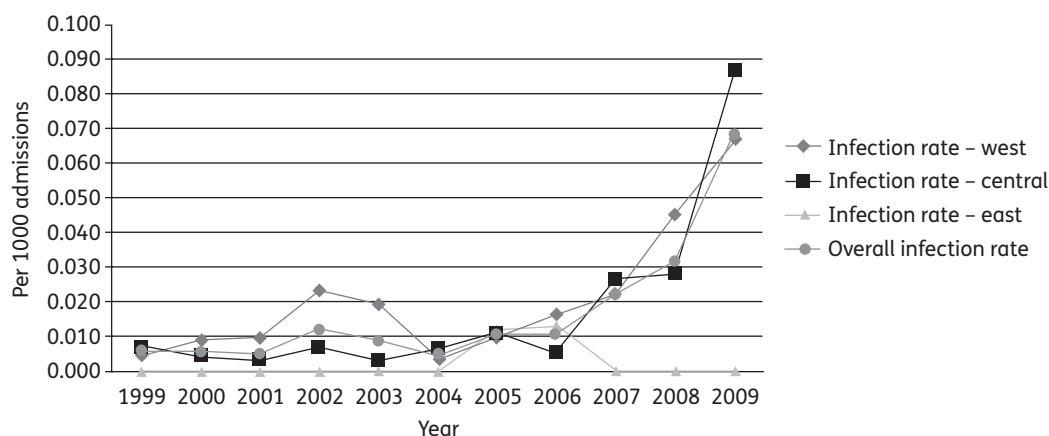


Figure 1. VRE bacteraemia rates per 1000 patient admissions by region, 1999–2009 ($n=128$).

antibiotic susceptibility profiles have also been observed in previous Canadian studies.^{11,12} Susceptibilities to all other isolates were as follows: quinupristin/dalfopristin, 96.3%; high-level gentamicin, 71.6%; tetracycline, 50.6%; high-level streptomycin, 44.4%; rifampicin, 21.0%; nitrofurantoin, 11.1%; clindamycin, 8.6%; ciprofloxacin, levofloxacin and moxifloxacin, 1.2%; and ampicillin, 0.0%. Similar antibiotic susceptibility profiles have been observed in previous Canadian studies.^{11,12} Nearly all isolates were multi-drug resistant (MDR) (resistant to three or more antimicrobial classes). There were no significant changes in susceptibilities over the 11 year time span (data not shown).

Over the 11 years of surveillance, 90.1% of the isolates harboured *vanA*, while the remainder of the isolates carried *vanB*. In this study, it was discovered that *vanB* first appeared in 2005 and the number of isolates harbouring this gene remained low in Canada, in contrast to *vanA*. The widespread occurrence of the *vanA* gene in *E. faecium* is a phenomenon that has been observed in previous Canadian and European studies.^{3,11,12}

MLST was completed on all 81 isolates and 17 STs were identified. The distribution of STs was as follows: ST16, *n*=13; ST17, *n*=11; ST18, *n*=8; ST203, *n*=9; ST412, *n*=9; ST584, *n*=8; ST154, *n*=7; ST117, *n*=6; ST280, *n*=2; ST78, *n*=1; ST80, *n*=1; ST282, *n*=1; ST375, *n*=1; ST662, *n*=1; ST663, *n*=1; ST664, *n*=1; and ST665 (*n*=1). There was no major difference in ST distribution between isolates that were *vanB* positive and *vanA* positive. Clustering analysis by the goeBURST algorithm showed that all CNISP VRE isolates belonged to the previously designated CC17 (where CC stands for clonal complex).¹⁷ CC17 represents a lineage of a virulent VRE hospital clone that has been observed worldwide.^{3,4,19} Characteristics of the CC17 clone often include ampicillin and glycopeptide resistance phenotypes, virulence genes such as *esp* and *hyl*, and colonization determinants, all of which may allow CC17 to thrive and disseminate in the hospital environment.^{3,20} The majority of STs were distributed throughout western (*n*=13) and central (*n*=10) Canada followed by eastern Canada (*n*=2) (Figure 2a). Most STs were observed in both central and western Canada. In the west, ST117 and ST584 were the only STs with multiple isolates identified from a single region. These STs were restricted to a single site within western Canada and were MDR (resistant to three or more antimicrobial classes).

When the ST types were analysed by year, a shift in ST was observed beginning in 2006 (Figure 2b and c). Prior to 2006, predominant types included ST154, ST16, ST17 and ST80 (Figure 2b). After 2006, ST18, ST203, ST412 and ST584 became predominant (Figure 2c). This shift in ST occurred around the same time bacteraemia rates began to rise in central and western Canada, suggesting there may be a correlation. However, it should be noted that a limitation to this study is the fact that we only examined 81 of a possible 128 cases of bacteraemia. A similar shift in ST types was observed in Taiwan that primarily involved ST17 being replaced by ST18 and ST78.⁴ The shift in that study also correlated with an increase in VRE bacteraemia. It has been previously suggested that the clonal spread of certain STs such as ST18 may have played a role in the increase in VRE.⁴

Conclusions

In Canada during the study period described here, increases in rates of VRE bacteraemia have been observed in the western

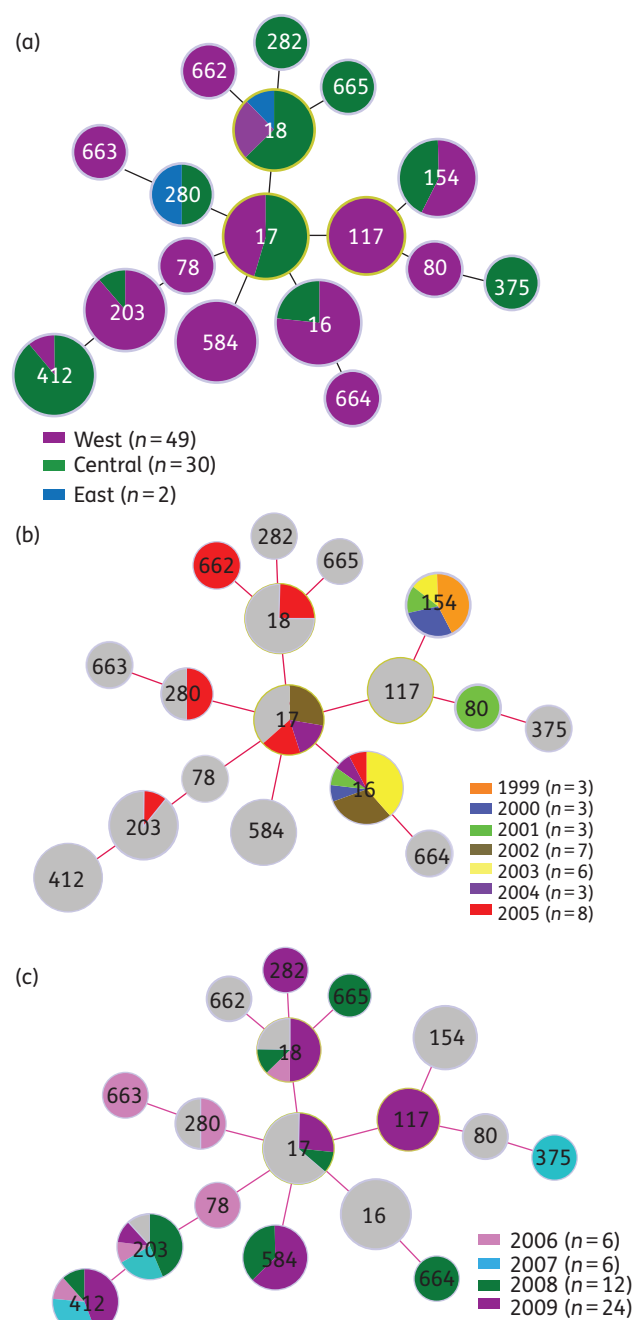


Figure 2. (a) goeBURST diagram showing distribution of all STs subdivided by region (*n*=81). Most STs were observed in western (*n*=49) and central (*n*=30) Canada. ST117 and ST584 are the only STs with multiple isolates from a single region. (b) goeBURST diagram showing distribution of all STs subdivided by year, 1999–2005. STs that were not seen from 1999 to 2005 are shown in grey. (c) goeBURST diagram showing distribution of all STs subdivided by year, 2006–2009. A shift in ST beginning around 2006 was observed. STs that were not seen from 2006 to 2009 are shown in grey.

and central regions. A shift in ST occurred around the same time bloodstream infections began to rise in western and central Canada. Perhaps the clonal spread of certain STs may be partially responsible for the increase in VRE bacteraemia in

Canada, but at this point it is not well understood if there is a link between these two phenomena. It is clear that the global spread of CC17 has also occurred in Canada. The acquisition of resistance to numerous antimicrobials and the increase in bacteraemia cases warrants continued surveillance of VRE in the Canadian setting.

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Transparency declarations

None to declare.

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