Journal of Global Antimicrobial Resistance 2 (2014) 115–118

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Short Communication

Diversity of vancomycin-resistant enterococci in a low endemicity area

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ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form 26 December 2013
Accepted 10 February 2014

Keywords:
Vancomycin-resistant enterococci
VRE
Multilocus sequence typing
MLST
vanB gene

ABSTRACT

Although vancomycin-resistant enterococci (VRE) are frequent nosocomial pathogens worldwide, in Japan their prevalence is low and their molecular epidemiology remains unclear. In The University of Tokyo Hospital (Tokyo, Japan), only five isolates of VRE (*vanA*-type *Enterococcus faecium*) were identified in the 20 years before 2010; however, nine isolates of *vanB*-type *E. faecium* were identified from 2011 to 2012. Multilocus sequence typing (MLST) revealed that they belonged to five sequence types (ST18, ST78, ST203, ST412 and ST612). Despite the limited isolation, including one from a medical tourist, there was significant diversity in genotypes. Increasing medical travel might enhance the diversity of VRE.

1. Introduction

Vancomycin-resistant enterococci (VRE) are one of the most frequent nosocomial pathogens worldwide. Vancomycin resistance is reported in 15–25% of *Enterococcus faecium* and 2–3% of *Enterococcus faecalis* in the UK and in 80% of *E. faecium* and 7% of *E. faecalis* in the USA. This vancomycin resistance is mediated by *vanA* or *vanB* genes. *vanA*-type VRE is predominant in Europe, the USA and South Korea, whereas *vanB* type is predominant in Singapore and Australia [1]. Multilocus sequence typing (MLST) is used for genotyping of VRE, and several clonal complexes (CCs) have been identified. *Enterococcus faecium* CC17 (now subdivided into sequence type ST17, ST18 and ST78 lineages) is the most prevalent genotype in nosocomial settings [2]. Most *E. faecium* strains belonging to CC17 are ampicillin-resistant, which may facilitate their nosocomial transmission. Several sequence types are regionally predominant, for example ST203 in Australia and ST17 in Singapore, although predominant sequence types may change over time [3,4].

The number of VRE infections in Japan is as low as ca. 100 per year (http://idsc.nih.go.jp/idwr/ydata/report-Ea.html). Despite several outbreak reports of VRE in Japan, regional spread was reported in only one area [5–8]. Moreover, only one study has reported on the epidemiology of VRE using MLST [7], which indicated that ST78 *vanA*-type *E. faecium* was predominant in a region of Japan, and no studies have been reported on virulence genes. In The University of Tokyo Hospital (Tokyo, Japan), only one isolate of VRE was identified before the year 2000, and only four from 2001 to 2010. However, in the 10 months from July 2011 to April 2012, nine isolates of *vanB*-type vancomycin-resistant *E. faecium* were identified from clinical samples, although one of them was recovered from a medical tourist from Singapore. The aim of this study was to determine the molecular characteristics of these isolates and to clarify the molecular epidemiology in a hospital in a low endemicity area.

2. Materials and methods

2.1. Bacterial and epidemiological data and antibiotic susceptibility testing

The University of Tokyo Hospital is a 1217-bed, tertiary care teaching hospital in Tokyo, Japan.

A vancomycin-resistant *E. faecium* was isolated from a urine sample of an inpatient in the internal medicine ward at the end of July 2011. Immediately after detection of the isolate, active surveillance culturing for VRE was conducted in the ward and seven isolates of vancomycin-resistant *E. faecium* were detected

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http://dx.doi.org/10.1016/j.jgar.2014.02.001
2213-7165/

from stool, anal swab or urine samples from 42 patients. All cases were colonisation. In response to the outbreak, the following infection control measures were enhanced: (i) isolation or cohorting of VRE-colonised patients; (ii) restriction of new admissions to the ward; (iii) cessation of the use of the shower room in the ward because VRE-colonised patients had used the same shower room; (iv) enhanced hand hygiene initiatives; and (v) promotion of judicious antibiotic use, e.g. restriction of vancomycin usage, because one of the VRE-colonised patients had been administrated oral vancomycin for recurrent *Clostridium difficile* colitis. Active surveillance culturing for VRE was conducted six times every 1 or 2 weeks until November 2011. No additional isolate of VRE was identified in the ward and the transmission pathways remained unclear. In April 2012 another vancomycin-resistant *E. faecium* was detected from the stool of a medical tourist from Singapore in another ward. Active surveillance culturing for VRE was conducted in this ward, however no further VRE was identified. After that, no VRE have been identified in this hospital so far. In addition to these strains, five strains isolated before 2010 were reviewed and the molecular characteristics of four of the five strains that were stored were analysed. The first VRE in this hospital was a *vanA*-type *E. faecium* isolated in 1999 from a Japanese patient admitted into the hospital after undergoing a liver transplantation in the USA. It was isolated from stool samples and was considered to be colonisation. The second strain was isolated from stool and blood culture in 2007. This case was considered to be infection. The third strain was isolated from the stool of a patient in the same ward and was considered to be colonisation. The fourth and fifth strains were isolated in another ward. These cases were considered to be colonisation and sporadic isolations, since the interval between isolations was ca. 1 year (Table 1).

Susceptibility of the isolates to antibiotics (ampicillin, erythromycin, minocycline, levofloxacin, vancomycin, teicoplanin, linezolid and gentamicin) was tested by MicroScan® WalkAway® 96 SI (Siemens Healthcare Diagnostics, Erlangen, Germany) and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Exact minimum inhibitory concentrations were not determined because standard protocols of the manufacturers were used.

2.2. Analysis of clonality using pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing

Genomic DNA for PFGE was digested with *Sma*I (New England Biolabs, Ipswich, MA). Separation of DNA fragments was carried out using a GenePatch System (Bio-Rad, Hercules, CA) and 1% agarose gel prepared with 0.5× TBE (Tris–borate–ethylene diamine tetra-acetic acid) buffer. The pulse time was increased from 5.3 s to 34.9 s, with a voltage gradient of 6 V/cm.

Seven housekeeping genes (*adk*, *atpA*, *ddl*, *gdh*, *gyd*, *purK* and *pstS*) were amplified and sequenced with forward and reverse primers as described on the MLST website (http://efaecium.mlst.net/). Sequence types were determined by submitting these data to the database on the MLST website. Cluster analysis was performed using the MLST database and the eBURST algorithm on this website.

2.3. Molecular methods

Types of *van* gene were determined by multiplex PCR with four primer pairs to identify *vanA* and *vanB* [10]. Subtypes of the *vanB* gene were determined as described elsewhere [11]. Briefly, *vanB* long 5959 bp PCR products were amplified using *vanB* long primers. These PCR products were subsequently digested with *Bsp*HI and *Dra*I (New England Biolabs) and were analysed by agarose gel electrophoresis. The *vanB2* gene was identified from *vanB2*-specific patterns. Putative virulence genes (*esp* and *hyl*) and transposon Tn*5382* were detected by PCR with specific primers as described previously [12].

3. Results

MLST analysis revealed that all of the isolates belonged to CC17 (ST18 and ST78 lineages). All but one isolate obtained from July to August 2011 were identified as ST78, the exception corresponding to its single locus variant (SLV) ST203. The isolate obtained in April 2012 in the other ward was identified as ST612, which is a SLV of ST18. Older isolates corresponded to ST412 (a SLV of ST203) (n = 2; 2007), ST78 (n = 1; 2008) and ST18 (n = 1; 2009) (Table 1).

Table 1
Characteristics of vancomycin-resistant *Enterococcus faecium* isolates.

Patient	Ward	Date	Material	*van* type	PFGE	ST	*esp*	*hyl*	Tn*5382*	MIC (μg/mL) (susceptibility category[a])							
										AMP	ERY	MIN	LVX	VAN	TEIC	LZD	GEN [b]
1	A	November 1999	Stool	A	N/A	N/A	N/A	N/A	N/T	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	>16 (R)	N/A	N/A
2	B	July 2007	Stool, blood	A	A	412	+	–	N/T	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	>16 (R)	≤2 (S)	8
3	B	September 2007	Stool	A	B	412	+	–	N/T	>8 (R)	≤2 (S)	≤2 (S)	>4 (R)	>16 (R)	>16 (R)	≤2 (S)	8
4	C	February 2008	Urine	A	C	78	+	+	N/T	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	>16 (R)	≤2 (S)	>500 (R)
5	C	January 2009	Sputum, stool	A	D	18	+	+	N/T	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	16 (I)	≤2 (S)	4
6	D	July 2011	Urine	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
7	D	August 2011	Urine	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
8	D	July 2011	Anal swab	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
9	D	August 2011	Anal swab	B2	F	203	+	–	+	>8 (R)	>4 (R)	>8 (R)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
10	D	August 2011	Stool	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
11	D	August 2011	Anal swab	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
12	D	August 2011	Anal swab	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
13	D	August 2011	Stool	B2	E	78	–	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)
14	E	April 2012	Stool	B2	G	612	+	–	+	>8 (R)	>4 (R)	≤2 (S)	>4 (R)	>16 (R)	≤2 (S)	≤2 (S)	>500 (R)

PFGE: pulsed-field gel electrophoresis; ST: sequence type; MIC: minimum inhibitory concentration; AMP: ampicillin; ERY: erythromycin; MIN: minocycline; LVX: levofloxacin; VAN: vancomycin; TEIC: teicoplanin; LZD: linezolid; GEN: gentamicin; N/A: not available; N/T: not tested; S: susceptible; I: intermediate; R: resistant.

[a] Interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9].

[b] R in gentamicin stands for high-level resistance to gentamicin (>500 μg/mL).