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Molecular characterization of clinical and environmental isolates of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* from a teaching hospital in Wales

Tomoari Kuriyama,[1] David W. Williams,[1] Mital Patel,[1] Michael A. O. Lewis,[1] Lynda E. Jenkins,[2] Dylan W. Hill[2] and Ian K. Hosein[2]

[1]Department of Oral Surgery, Medicine and Pathology, Dental School, University of Wales College of Medicine, Heath Park, Cardiff CF14 4XY, UK

[2]Department of Infection Prevention and Control, Cardiff and Vale NHS Trust, Heath Park, Cardiff CF14 4XN, UK

Correspondence
David W. Williams
williamsdd@cardiff.ac.uk

The present study describes the first molecular characterization of environmental and clinical isolates of vancomycin-resistant enterococci (VRE) in Wales. Over a 3-month period (May–July 2000), 134 isolates of VRE (89 *Enterococcus faecium* and 45 *Enterococcus faecalis*) were isolated from the patient environment of the University Hospital of Wales (UHW) in Cardiff, Wales, UK. In addition, over the same time-period, 24 clinical isolates of VRE (20 isolates of *E. faecium* and four isolates of *E. faecalis*) were obtained from 14 patients. All study isolates were subjected to PFGE typing and their *van* genotypes were determined by using multiplex PCR. The *vanA* PCR product (231 bp) was evident in 146 (92 %) of 158 VRE isolates; the remaining 12 isolates (8 %) were positive for the *vanB* gene. All isolates of *E. faecalis* were found to be *vanA*-positive. In total, 16 PFGE banding profiles (pulsotypes) were observed for environmental isolates of *E. faecium*, whilst eight pulsotypes were found for isolates of *E. faecalis*. Some of these pulsotypes were isolated from multiple sites, whereas others were more restricted in their distribution. Eleven pulsotypes were evident for clinical isolates and eight of these (representing 11 isolates) were also encountered in environmental isolates. Eleven clinical isolates of *E. faecium* (55 %) shared an identical pulsotype that was not detected in environmental isolates. These results demonstrate a heterogeneous environmental population of VRE and an association of certain strains with clinical isolates. Predominance of a single pulsotype (not detected in the environment) amongst clinical isolates suggests non-environmental transmission between patients.

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INTRODUCTION

Enterococci are important nosocomial pathogens that are often recovered from patients with urinary tract infections, wounds, bacteraemia, endocarditis or meningitis (Murray *et al.*, 1999). Enterococci exhibit intrinsic resistance to several antibiotics and have the ability to acquire antibiotic resistance rapidly (Hayden, 2000). Whilst a treatment regimen with penicillins, alone or in combination with aminoglycosides, is recommended to combat enterococcal infections initially, vancomycin is the antibiotic of choice for infections caused by strains that are resistant to these antibiotics (Murray, 2000). Detection of vancomycin-resistant enterococci (VRE) in the mid-1980s was therefore a major therapeutic concern. VRE were first recovered in France and England (Cetinkaya *et al.*, 2000; Hayden, 2000) but strains have subsequently been reported on a worldwide scale (Goossens, 1998; Cetinkaya *et al.*, 2000; Hayden, 2000). Acquired resistance to vancomycin by enterococci greatly reduces the number of treatment options for disease management (Hayden, 2000; Mundy *et al.*, 2000) and the problem is further compounded by the fact that resistance genes can potentially be transferred to other pathogenic organisms, such as *Staphylococcus aureus* and *Streptococcus* species (Hayden, 2000; Murray, 2000). It is also accepted that prevention of infection and control strategies for VRE have been of limited benefit once a clinical problem has become established (Hayden, 2000; Mundy *et al.*, 2000).

Seven genotypes (*vanA*, *vanB*, *vanC1*, *vanC2/3*, *vanD*, *vanE* and *vanG*) of vancomycin resistance have been reported for

Abbreviations: UHW, University Hospital of Wales; VRE, vancomycin-resistant enterococci.

enterococci to date (McKessar *et al.*, 2000). *vanA* and *vanB* are the principal resistance genotypes reported for *Enterococcus faecium* and *Enterococcus faecalis*, the species isolated most frequently from clinical sites, and are an acquired form of resistance (Murray *et al.*, 1999). Strains with the *vanA* genotype are characterized by high-level vancomycin and teicoplanin resistance, whereas those with the *vanB* genotype exhibit moderate to high resistance to vancomycin only (Cetinkaya *et al.*, 2000; Murray, 2000).

A patient can acquire VRE from a range of sources (Weber & Rutala, 1997; Falk *et al.*, 2000; Hayden, 2000) and the hospital environment may be an important reservoir (Weber & Rutala, 1997; Hayden, 2000). Epidemiological analysis of VRE has been used to elucidate the movement of VRE between patients and their environment and the extent of clonal transmission within a hospital setting (Cetinkaya *et al.*, 2000). There is still a need for data that clarify the role of the environment in transmission of VRE to patients within a hospital setting.

In 1995, six index patients were found to be culture-positive for VRE at the University Hospital of Wales (UHW), a 920-bed teaching hospital and tertiary care referral centre. Since 1995, there has been an annual increase in isolation of VRE in patients at UHW; 94 patients were found to be positive for VRE in 2001. Between 1995 and 2001, 393 VRE-positive patients have been reported.

PFGE has been used previously with success as a molecular typing technique for VRE (Gordillo *et al.*, 1993; Gambarotto *et al.*, 2000; Kawalec *et al.*, 2000). The aim of the present study was to use PFGE to characterize isolates of *E. faecium* and *E. faecalis* from the environment (wards) and patients in UHW over a specific time-period in 2000. The *van* genotypes of the VRE isolates were also determined, by using multiplex PCR.

METHODS

Isolates of VRE. VRE isolates were obtained between 1 May and 31 July 2000. Clinical isolates were recovered from blood, sputum, urine, bile

Table 1. Sources of isolation and molecular characteristics of VRE isolates

ICU, Intensive Care Unit; SHDU, Surgical High Dependency Unit; TP, transplant section in nephrology unit. Unless stated, all isolates were of the *vanA* genotype. Pulsotype classification was based on Tenover *et al.* (1995).

Ward	Patient or location	Source or site	*van* genotype and pulsotype	
			E. faecium	*E. faecalis*
ICU	Patient 1	Blood	C17, C17*, C17*	
	Patient 2	Blood		A4
		Tap		A5
SHDU	Nine-bedded room	Curtain-rail	C5	
		Shelf	C5	
		Table	C5, C5*	A4
		Work surface	C5, C5*	A4
Haematology	Patient 1	Blood	C17, C17*	
	Patient 3	Blood	C17	
	Patient 4	Blood	C17, C17* C20	
	Patient 5	Blood	C17, C17*, C18	
	Patient 6	Blood	C19	
	Patient 7	Blood	C2	
	Four-bedded room	Toilet	C3, C5, C10	
	Isolation room 1	Chair	C5, C5*	A4
		Fan		A4
		Floor	C2, C3, C5	A2, A4
		Locker	C2, C2*, C5	A2, A4, A4*
		Shelf	C2, C5, C5*, C5*, C5*, C10	A1, A1*, A2, A4
		Table	C2, C5, C5*	A1, A1*, A2, A4
		Television	C5, C5*	A2
		Television table	C5	A4, A4*
		Window sill	C2, C5, C5*, C11	A1
		Walking aid	A4	
	Isolation room 2	Fan	C1, C1*, C3	
		Locker	C2	
		Shelf	C3	
		Table	C1	

Table 1. cont.

Ward	Patient or location	Source or site	*van* genotype and pulsotype	
			E. faecium	*E. faecalis*
	Sluice room	Sluice	C2, C5, C10	
Nephrology	Patient 8	Sputum		A3
	Patient 9	Urine	C12	
	Patient 10	Bile specimen	C12	
	Patient 11	Urine	C13	
	Patient 12	Blood	C4 (*vanB*)	
	Patient 13	Dialysis fluid	C4 (*vanB*), C9	A6
	Patient 14	Urine		A6
	High-care unit	Chair	C9	
		Curtain	C7, C9	A6
		Fan	C9, C5*, C7*	
		Locker	C9, C7	A1, A2
		Shelf 1	C4 (*vanB*)	
		Shelf 2	C7	
		Shelf 3	C9	
		Table	C7	
		Window sill	C8	
	Nine-bedded room	Bed-rail		A3
		Chair 1	C4 (*vanB*)	
		Chair 2	C9	
		Chair 3		A3
		Curtain 1	C4 (*vanB*)	
		Curtain 2	C4 (*vanB*)	
		Fan	C7	
		Locker 1	C9	
		Locker 2	C4 (*vanB*), C6	A3
		Locker 3	C9	
		Shelf 1	C9	
		Shelf 2	C4 (*vanB*), C4* (*vanB*), C7, C8	
		Shelf 3	C3, C7, C15	A3
		Shelf 4		A3
		Table 1	C9	
		Table 2	C4 (*vanB*), C13	
		Table 3		A3
		Table 4	C6	
		Television	C14	
		Window sill		A3
	Isolation room	Bed-rail		A3
		Fan	C9	A3
		Floor		A3
		Locker		A3
		Shelf		A3
		Table		A3
		Tap 1		A3, A3*, A3*, A3*
		Window sill		A3
	Sluice room	Sluice	C4 (*vanB*), C7, C7, C8, C9	
	TP four-bedded room	Toilet	C4 (*vanB*)	A4
	TP treatment room	Refrigerator handle	C12	
		Work surface	C9	
	TP sluice room	Sluice	C8, C9, C16	

*Strain obtained at separate time-point.

and dialysis fluids from patients in UHW over this time-period. Environmental isolates were obtained from selected sites within the Intensive Care Unit, Surgical High-Dependency Unit, haematology ward and nephrology unit at UHW. A saline-moistened sterile swab was applied to a variety of surfaces to collect environmental isolates (Table 1). Each swab was rolled five times over the selected sampling site. Swabs were inoculated on to Slanetz and Bartley agar and *Staphylococcus*/*Streptococcus* agar (both from Oxoid). All plates were incubated aerobically at 35 °C for 48 h.

Identification of VRE. Enterococci were identified in accordance with American Society for Microbiology (ASM) recommendations (Murray *et al.*, 1999). Resistance of each isolate to vancomycin was determined by the disc-diffusion method recommended by the National Committee for Clinical Laboratory Standards (2000).

Molecular typing of VRE by using PFGE. Test isolates were subcultured on blood agar for 4 h at 37 °C. DNA was prepared by using a previously described method (Bartie *et al.*, 2000). Restriction enzyme *Sma*I (New England BioLabs) was used as described previously (Bartie *et al.*, 2000). PFGE parameters comprised ramp pulse times of 1–35 s for 30 h at 6 V cm^{-1}. Comparison of banding patterns was done by visual analysis (Tenover *et al.*, 1995) and through the use of GelCompar software (Applied Maths; Bartie *et al.*, 2000).

Characterization of vancomycin-resistance genotype. Identification of *van* genotypes (*vanA* or *vanB*) for each isolate of VRE was performed by using multiplex PCR as described by Bell *et al.* (1998).

RESULTS

Isolates of VRE

In total, 24 clinical isolates of VRE (20 isolates of *E. faecium* and four of *E. faecalis*), obtained from 14 patients, were studied (Table 1). All patients were from the Intensive Care Unit, haematology ward or nephrology unit of UHW. A total of 134 isolates of VRE (89 isolates of *E. faecium* and 45 isolates of *E. faecalis*) was recovered from targeted environmental sampling sites (Table 1).

PFGE

All study isolates were amenable to typing by using the PFGE methodology described (Fig. 1). Sixteen banding profiles (pulsotypes) were identifiable by visual analysis of environmental *E. faecium* isolates ($n = 89$; Table 1). In the case of the 45 isolates of *E. faecalis*, eight pulsotypes were evident (Table 1). Certain pulsotypes (e.g. *E. faecium* C5 and *E. faecalis* A4) were noted for isolates recovered from multiple environmental sites within the hospital, whereas others were more limited in their distribution (e.g. *E. faecium* C11 and *E. faecalis* A5) and were often restricted to individual wards.

In total, 11 pulsotypes were encountered from the 24 clinical VRE isolates. Eight of these pulsotypes, representing 11 isolates (P3, K28, K95, I95, K30, G75, H3, K29, Q48, L94 and J24) were identical to some of the environmental pulsotypes (C2, C9, C13, C4, C12, A6, A3 and A4; Fig. 2). Of the remaining 13 clinical *E. faecium* isolates, 11 were found to share an identical pulsotype (C17) that was not evident for any of the environmental isolates. Two clinical isolates (*E. faecium* P35 and *E. faecium* N95) had unique pulsotypes.

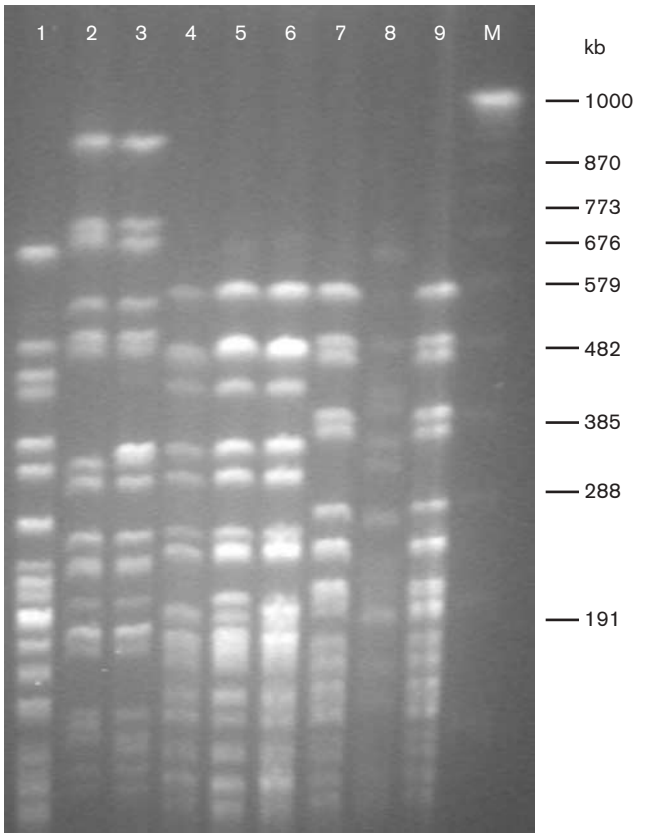

Fig. 1. PFGE of *Sma*I-restricted genomic DNA of VRE. Lanes contain DNA from the following strains (origin, pulsotype): 1, Q41 (patient, C17); 2, Q48 (patient, A6); 3, e30 (environment, A6); 4, e14 (environment, C12); 5, G75 (patient, C12); 6, H3 (patient, C12); 7, e55 (environment, C4); 8, P35 (patient, C19); 9, e117 (environment, C4); M, λ DNA marker.

van genotype analysis

Multiplex PCR detected either the *vanA* or *vanB* genotype for all VRE isolates studied. The *vanA* PCR product was represented by a 231 bp fragment, which was clearly distinct from the 330 bp *vanB* product. The *vanA* genotype predominated (146 of 158 isolates; 92 %) and was the only *van* genotype encountered for isolates of *E. faecalis*.

DISCUSSION

It has been suggested that hospitalized patients can become colonized by VRE from the local environment (Weber & Rutala, 1997; Hayden, 2000). However, there have not been any studies to assess the molecular relationship between environmental and clinical isolates from hospitalized patients in Wales. Clarification of the patterns of VRE dis-

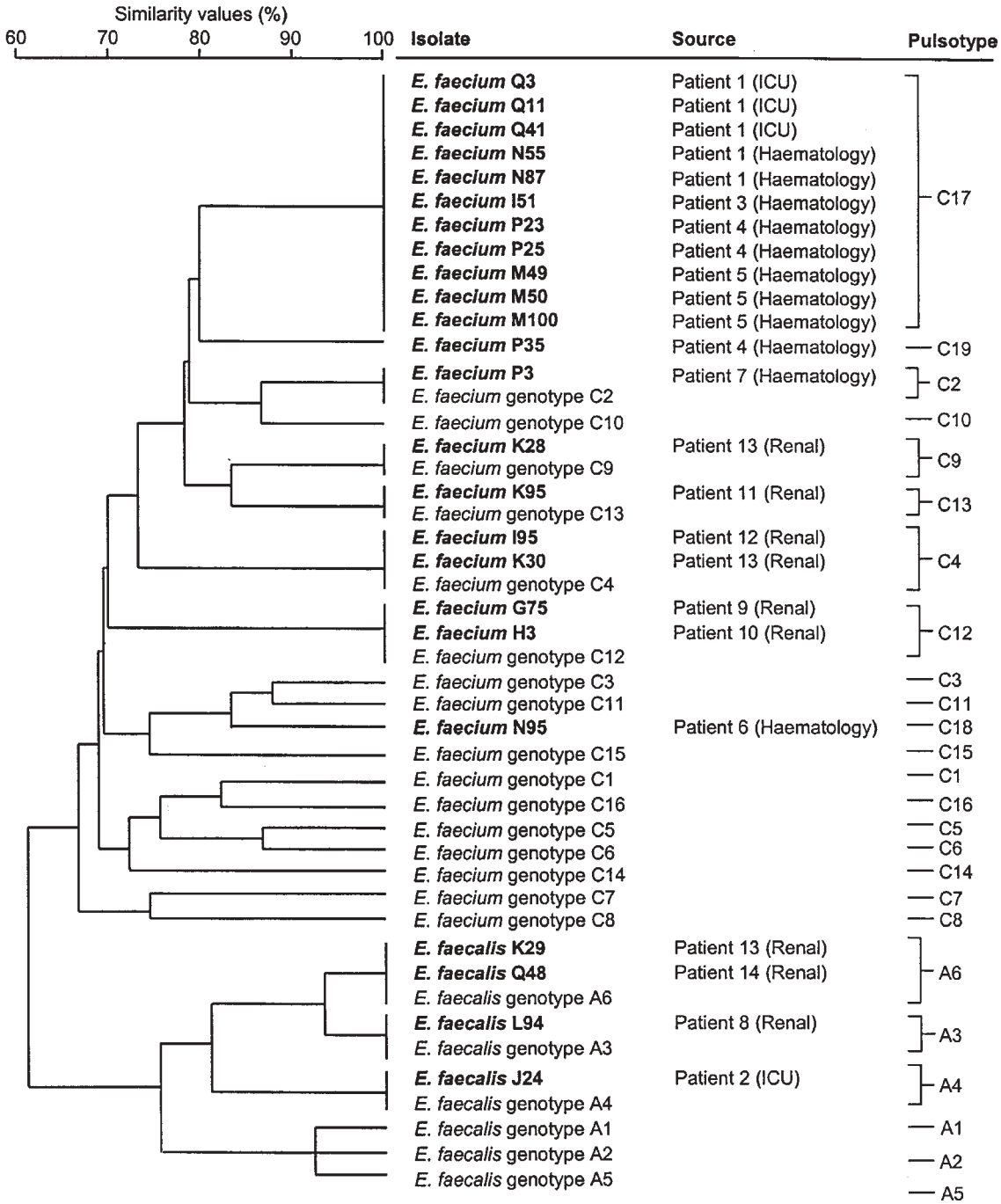

Fig. 2. Correlation of pulsotypes from 24 clinical (bold) and 22 environmental VRE isolates. Arithmetic mean dendrograms were generated from similarity values determined by the Dice coefficient. ICU, Intensive Cure Unit. Environmental VRE pulsotypes represent all previous visually distinct pulsotypes (C1–C16, A1–A6).

semination in the hospital environment and determination of genetic similarity between isolates could provide information on possible routes of transmission.

Although amplified fragment-length polymorphism analysis and multilocus sequence typing schemes have recently been developed for typing enterococci (Willems *et al.*, 2001; Homan *et al.*, 2002), PFGE remains the most widely used tool for enterococcal typing (Tenover *et al.*, 1995). Several methodologies for PFGE analysis of enterococci have been reported (Gordillo *et al.*, 1993; Gambarotto *et al.*, 2000; Kawalec *et al.*, 2000), although experience gained during the

present study revealed that suggested procedures were inconsistent with regard to lysis of some VRE strains (data not shown). Previous studies have recommended that test cultures should be incubated on blood agar at 37 °C for 16–24 h to generate plugs for PFGE. However, this incubation time was reduced to 4 h in the present study, which led to a noticeable improvement in DNA extraction and subsequent band intensity. Furthermore, initial evaluation of the band profiles obtained by using electrophoresis times of either 24 or 30 h revealed that 30 h yielded optimal resolution of banding patterns.

Molecular typing of clinical VRE strains has shown that VRE isolates obtained from hospitalized patients in Europe tend to be heterogeneous with respect to their PFGE fingerprints (Goossens, 1998; Willems *et al.*, 2001). This observation is in contrast to some reports from the USA, where greater homogeneity was found (Goossens, 1998; Falk *et al.*, 2000). The current study represents the first report of characterization and epidemiology of VRE in a Welsh hospital.

Over the sampling period, 134 VRE isolates (*E. faecium*, $n = 89$; and *E. faecalis*, $n = 45$) were obtained from a range of inanimate environmental surfaces. The predominance of *E. faecium* over *E. faecalis* is in agreement with other epidemiological analyses of VRE populations (Gambarotto *et al.*, 2000). As VRE have been shown to survive on environmental surfaces for time-periods in excess of 5 weeks (Falk *et al.*, 2000), there may be significant potential for their transmission to patients.

PFGE analysis of the environmental VRE isolates identified 16 pulsotypes for *E. faecium* and five for *E. faecalis*. These results indicate that a variety of VRE strain types are present within the UHW environment; this is consistent with other European hospitals. Several VRE types (*E. faecium* C5; *E. faecalis* A1, A2 and A4) were found at two or more locations in the hospital and could be indicative of local transmission within UHW. It is interesting that the outlying of patients has increased in UHW over recent years; this could play a contributory role in environmental transmission of VRE. In contrast, other VRE types (C1–4, C6–16, A3, A5 and A6) were found only at specific sites in the hospital, although localized spread of these strains was evident (e.g. strain A3 was located at several sites in a nine-bedded room in nephrology).

During the sampling, 24 clinical strains of VRE were isolated from 14 patients. PFGE analysis revealed that 11 of these clinical isolates (P3, K28, K95, I95, K30, G75, H3, K29, Q48, L94 and J24) were genetically similar to environmental isolates (C2, C9, C13, C4, C12, A6, A3 and A4). Therefore, it is likely that spread of VRE occurs between patients and the environment, although it is difficult to ascertain whether or not the environment was responsible for infecting these patients or vice versa. It was interesting to note that nine of 11 clinical VRE isolates that were similar to environmental isolates were from patients within the nephrology unit. The remaining two clinical isolates that shared profiles with environmental strains were from one patient who was attending the haematology ward and one patient in the Intensive Care Unit. The reason for the high concordance between clinical and environmental isolates in the nephrology unit is unclear. It is possible that that as there is a tendency for patients with chronic renal disease to spend longer and more frequent time-periods within the hospital setting, a greater likelihood of exposure to environmental VRE exists.

The remaining 13 clinical isolates (recovered from five patients) had pulsotypes that were distinct from those of environmental isolates. The origin of these VRE isolates is uncertain, but they may represent strains that were acquired previously from the community setting (Bonten *et al.*, 2001). However, 11 of the clinical isolates (from four of five patients) had an identical pulsotype (C17). All strains with this pulsotype were isolated from patients on the haematology ward, although one of these patients also spent time in the Intensive Care Unit. This suggests probable patient-to-patient transfer, possibly via contact with health-care workers or patient visitors. It was also noted over this sampling period that visitors caring for relatives had frequent contact with other patients on the haematology ward. Further studies are required to confirm these possible routes of transmission.

VRE isolates were found to possess either the *vanA* or *vanB* genotype. There was a predominance of the *vanA* genotype (92 % of all isolates), which is consistent with the majority of findings from other countries (Bell *et al.*, 1998; Goossens, 1998; Kawalec *et al.*, 2000; Scagnelli *et al.*, 2001) with the exception of Australia, where a higher incidence of *vanB* isolates has been reported (Bell *et al.*, 1998). Although *vanB* VRE were encountered rarely in the present investigation, it was interesting to note that all *vanB*-positive isolates encountered were of pulsotype C4. This finding would not only support the reliability of the PFGE typing procedure, but would also further substantiate the dissemination of this particular strain and the 'environment-to-patient' link.

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