Detection of clonally related *vanB2*-containing *Enterococcus faecium* strains in two Spanish hospitals

Carmen Torres,[1] Susanna Escobar,[2] Aránzazu Portillo,[1,3] Luis Torres,[2] Antonio Rezusta,[2] Fernanda Ruiz-Larrea,[1] Maria José Revillo,[2] Carmen Aspiroz[4] and Myriam Zarazaga[1]

Correspondence
Carmen Torres
carmen.torres@daa.unirioja.es

[1]Área Bioquímica y Biología Molecular, Universidad de La Rioja, Madre de Dios 51, Logroño 26006, Spain

[2]Hospital Universitario Miguel Servet, Zaragoza, Spain

[3]Área de Enfermedades Infecciosas, Hospital de La Rioja, Logroño, Spain

[4]Hospital Royo Villanova, Zaragoza, Spain

The aim of this study was to characterize the resistance mechanism in four clinical and five intestinal vancomycin-resistant *Enterococcus faecium* strains with VanB phenotype recovered from unrelated patients confined in two Spanish hospitals and to determine their clonal relationships. MIC values for vancomycin and teicoplanin were 16–32 and 0·5 μg ml^{-1}, respectively. The mechanism of vancomycin resistance, as well as the genetic environment of the implicated gene, was analysed by PCR and sequencing. The *vanB2* gene was detected in all nine *E. faecium* strains and the intergenic $vanS_B$–Y_B region showed the characteristic mutations of the *vanB2* subtype. Two possibly related PFGE patterns, A (seven strains) and B (two strains), were distinguished among these enterococci. The $vanX_B$–*ORFC* intergenic region was amplified in the nine strains and two amino acid changes were detected in the protein encoded by the $vanX_B$ gene in strains of pattern A with respect to those of pattern B. The *vanB2* gene cluster was integrated into Tn*5382* in all nine strains, being *pbp5* gene-linked to this transposon. The *ant*(6′)-*Ia*, *aph*(3′)-*IIIa* and *erm*(B) genes were also detected in all of the strains. Both isolates with PFGE pattern B contained the *esp* gene. In summary, *vanB2*-containing *E. faecium* strains with indistinguishable PFGE patterns were recovered from seven patients from two Spanish hospitals.

Received 8 February 2006
Accepted 17 May 2006

INTRODUCTION

Different mechanisms of vancomycin resistance have been detected in enterococci (*vanA*, *vanB*, *vanC*, *vanD*, *vanE* and *vanG*) (Cetinkaya *et al.*, 2000; Gholizadeh & Courvalin, 2000; Murray, 1997; Perichon *et al.*, 1997; Woodford, 2001). The *vanB* genotype is characterized by acquired inducible resistance to various levels of vancomycin and susceptibility to teicoplanin (Quintiliani *et al.*, 1993). It is determined by a cluster of genes, $vanR_B$, $vanS_B$, $vanY_B$, *vanW*, $vanH_B$, *vanB* and $vanX_B$. Three different subtypes of the *vanB* gene have been documented, *vanB1*, *vanB2* and *vanB3*, based on sequence variability in the *vanB* ligase gene (Dahl *et al.*, 1999). The *vanB2* subtype has been found in human enterococcal strains from different countries (Dahl *et al.*, 1999, 2000; Gold *et al.*, 1993; Granlund *et al.*, 2006; Kawalec *et al.*, 2001; Lee *et al.*, 2001; Lu *et al.*, 2001, 2005; Mato *et al.*, 1996; McGregor & Young, 2000; McGregor *et al.*, 2001; Simonsen *et al.*, 1998). In Spain, there has been one previous report on the detection of a *vanB2*-containing *Enterococcus faecium* clinical strain (Lorenzo-Díaz *et al.*, 2004) and one on the detection of a *vanB2*-containing *Enterococcus hirae* strain from a faecal sample of a healthy pig (Torres *et al.*, 2003). Recently, the *vanB2* gene cluster associated with a Tn*5382*-like transposon has been found in a *Clostridium* strain from human faeces in Canada (Domingo *et al.*, 2005).

Over a 1 year period, a series of clinical and intestinal vancomycin-resistant *E. faecium* strains with VanB phenotype (VREF-VBP) were recovered from two Spanish hospitals. The aim of this study was to characterize the resistance mechanisms of these strains and to determine their clonal relationships.

Abbreviations: ICU, intensive care unit; VREF-VBP, vancomycin-resistant *Enterococcus faecium* strains with VanB phenotype.

METHODS

Strains and patients. Three VREF-VBP clinical strains (*E. faecium* C393, C395 and C461) were recovered from blood and urine

samples from three patients from the Miguel Servet Hospital in Zaragoza, Spain (Table 1). MIC values of vancomycin and teicoplanin for these strains (16 and 0·5 μg ml^{-1}, respectively) were indicative of the VanB-resistant phenotype. They were detected among a series of 1036 clinical enterococcal isolates recovered over a 1 year period (February 2002 to January 2003) in that hospital (0·3 %). An additional VREF-VBP clinical strain (*E. faecium* C396) was recovered in March 2002 from the abdominal abscess of a patient from Royo Villanova Hospital, located 4 km away from Miguel Servet Hospital. Relationships among the patients from the two hospitals were not known.

In order to detect intestinal colonization by VREF-VBP strains, a surveillance programme was carried out with all faecal samples (n=632) collected in Miguel Servet Hospital from February to June 2002. Faecal samples were diluted in sterile saline solution and seeded in bile aesculin agar plates supplemented with vancomycin (6 μg ml^{-1}) and aztreonam (75 μg ml^{-1}). Colonies with a typical enterococcal morphology were identified by classical biochemical methods and putative enterococci were verified using specific PCR assays (Table 2). Four of these 632 faecal samples (0·6 %) were shown to be colonized by VREF-VBP strains (*E. faecium* C397, C402, C404 and C406). In addition, from July 2002 to January 2003, sporadic faecal samples from particular patients [confined in the intensive care unit (ICU) and oncology wards, among others] were also analysed for this purpose in Miguel Servet Hospital and one additional VREF-VBP strain was collected (*E. faecium* C460) (Table 1).

The nine VREF-VBP strains included in this study, implicated in infection processes (four strains) or in intestinal colonization (five strains), were recovered from nine different patients from these two Spanish hospitals (Table 1). All patients had severe diseases and six were or had been admitted to an ICU ward of the hospital. Five had been treated previously with glycopeptides, whilst two others had received broad-spectrum antibiotics, such as imipenem or ceftazidime. No information about previous antibiotic consumption was available for the remaining two patients.

Antibiotic susceptibility testing. Determination of the MICs of different antibiotics (vancomycin, teicoplanin, streptomycin, gentamicin, kanamycin, ampicillin, erythromycin, ciprofloxacin, tetracycline and chloramphenicol) was carried out using the NCCLS agar dilution method (NCCLS, 2002). *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 were used as control strains.

Characterization of the vancomycin resistance mechanism. Vancomycin resistance mechanisms were analysed in VREF-VBP strains using specific primers for amplification of the *vanA*, *vanC-1*, *vanC-2/3* and *vanD* genes (Table 2). *E. faecium* AR1 and *E. faecalis* SF300 (kindly supplied by E. Cercenado, Hosp. Gregorio Marañón, Madrid, Spain) were used as positive controls for the *vanA* and *vanB* PCR assays, respectively (Gold *et al.*, 1993; Torres *et al.*, 1994). *Enterococcus gallinarum* Z380 and *Enterococcus casseliflavus* Z406, from the collection of strains of the University of La Rioja, were used as positive controls for PCR detection of the *vanC-1* and *vanC-2/3* genes, respectively. *vanB* ligase genes, as well as the intergenic $vanS_B$–$vanY_B$ region, were amplified by PCR. In order to determine whether the *vanB2* operon of the *vanB2*-positive strains was associated with a Tn*5382*-like element, specific primers for the non-integrase (left) end of Tn*5382* and for the $vanX_B$–*ORFC* intergenic region and flanking coding sequences in Tn*5382* were used for PCR assays. The same strains were examined for *pbp5*–Tn*5382* linkage by PCR amplifying a 1079 bp region between *pbp5* and Tn*5382*. Subsequent sequence analysis of PCR products was performed for all PCR assays mentioned above. PCR primers and conditions, as well as the relevant references, are given in Table 2.

Detection of other antibiotic resistance or virulence genes. The presence of other resistance genes, such as *aac*(6′)-*Ie*-*aph*(2″)-*Ia*, *aph*(3′)-*IIIa*, *ant*(6)-*Ia*, *erm*(A), *erm*(B) and *erm*(C), was analysed by specific PCRs using primers and conditions reported previously (Table 2). In addition, the presence of the *esp* and *hyl* genes (encoding an enterococcal surface protein and hyaluronidase, respectively) was also studied and positive amplicons were sequenced for confirmation. Positive and negative controls from our collection of strains were included in all PCR assays.

Mating experiments. The transferability of vancomycin resistance genetic determinants was tested by conjugation using a filter-mating method (Dunny *et al.*, 1979), with *E. faecalis* strain JH2-2 as recipient (rifampicin- and fusidic acid-resistant, vancomycin-susceptible) and two VREF-VBP strains (C393 and C461) as donors (fusidic acid-susceptible, vancomycin-resistant). Vancomycin-resistant transconjugants were selected on brain heart infusion agar plates supplemented with fusidic acid (25 μg ml^{-1}) and vancomycin (5 μg ml^{-1}).

PFGE analysis of VREF-VBP strains. The clonal relationships of the nine *vanB2*-containing *E. faecium* strains were studied by PFGE by analysing their genomic DNA after digestion with *Sma*I (New England Biolabs), as reported previously (Turabelidze *et al.*, 2000). This technique was performed using clamped homogeneous electric fields (CHEF DRII; Bio-Rad). The running parameters were as follows: voltage, 6 V cm^{-1}; block 1: run time 12 h; initial switch time 3·5 s; final switch time 25 s; block 2: run time 8 h; initial switch time 1 s; final switch time 5 s. A bacteriophage λ ladder (Bio-Rad) was used as a size marker. PFGE banding patterns were analysed and compared visually. Isolates were classified as indistinguishable, closely related, possibly related or unrelated by following previously defined criteria for bacterial strain typing (Tenover *et al.*, 1995).

RESULTS AND DISCUSSION

All nine of the VREF-VBP strains of clinical and intestinal origin included in this study showed low-level vancomycin resistance (MIC 16–32 μg ml^{-1}) and susceptibility to teicoplanin (MIC 0·5 μg ml^{-1}). Positive PCR assays were obtained with the consensus *vanB* primers for the nine strains, showing negative results for the remaining *vanA*, *vanC-1*, *vanC-2/3* and *vanD* PCR tests. Nucleotide sequences of the *vanB* amplicons revealed the presence of the *vanB2* subtype in all nine strains (Table 1) and they were 100 % identical to that previously reported for the *vanB2* gene in human and animal enterococcal isolates (Garnier *et al.*, 2000; Gold *et al.*, 1993; Torres *et al.*, 2003).

According to the PFGE analysis, two possibly related PFGE patterns, A (seven strains) and B (two strains), could be distinguished (Table 1 and Fig. 1). Curiously, strains of PFGE pattern A were recovered at the beginning of the study (from seven patients of the two hospitals from February to May 2002), whilst strains belonging to PFGE pattern B were isolated at the end of the study (from two patients of Miguel Servet Hospital in December 2002 and January 2003).

Sequencing of the $vanS_B$–Y_B intergenic regions for the nine *vanB2*-containing strains showed the typical 11 point mutations and the 5 bp deletion of the *vanB2* subtype (Dahl *et al.*, 2000). All of the strains showed positive PCR results for Tn*5382* and the sequences obtained showed

Table 1. Characteristics of the VREF strains included in this study and of the patients from whom they were recovered

Characteristics of the strains							Characteristics of the patients			
VREF strain	Source and isolation date (month/year)	VAN resistance mechanism	MIC* (μg ml^{-1})		Resistance to other antibiotics*	PFGE pattern	Pathology	Previous exposure in ICU ward	Previous antibiotic treatment*	Hospital
			VAN	TEI						
Clinical infection										
C393	Blood (2/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Kidney failure	No	VAN	MS
C395	Urine (3/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Peritonitis	Yes	Glycopeptides	MS
C461	Urine (1/03)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-TET-CIP	B	Heart surgery	Yes	Unknown	MS
C396	Abdominal abscess (3/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Intestinal surgery	No	Unknown	RV
Intestinal colonization										
C397	Faeces (4/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Heart surgery	Yes	Glycopeptides and others	MS
C402	Faeces (4/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Heart surgery	Yes	CAZ and others	MS
C404	Faeces (5/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Lung disease	Yes	TEI and FEP	MS
C406	Faeces (5/02)	*vanB2*	16	0·5	STR-KAN-AMP-ERY-CIP	A	Heart surgery	Yes	VAN	MS
C460	Faeces (12/02)	*vanB2*	32	0·5	STR-KAN-AMP-ERY-TET-CIP	B	Human immuno-deficiency virus	No	IPM and others	MS

*VAN, vancomycin; TEI, teicoplanin; STR, streptomycin; KAN, kanamycin; AMP, ampicillin; ERY, erythromycin; TET, tetracycline; CIP, ciprofloxacin; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem. High-level resistance was assumed for aminoglycosides.

Table 2. Primers and conditions used in PCR reactions in this study

Gene or fragment amplified	Primer name	Primer sequence (5′→3′)	T_a (°C)*	Product size (bp)	Reference
vanA	*vanA*-1	GGG AAA ACG ACA ATT GC	54	732	Dutka-Malen *et al.* (1995)
	vanA-2	GTA CAA TGC GGC CGT TA			
vanB consensus	*vanB*-1	CAA AGC TCC GCA GCT TGC ATG	58	484	Dahl *et al.* (1999)
	vanB-2	TGC ATC CAA GCA CCC GAT ATA C			
vanC-1	*vanC1*-1	GCT GAA ATA TGA AGT AAT GAC CA	58	811	Miele *et al.* (1995)
	vanC1-2	CGG CAT GGT GTT GAT TTC GTT			
vanC-2/3	*vanC2/3*-1	CTC CTA CGA TTC TCT TG	54	439	Dutka-Malen *et al.* (1995)
	vanC2/3-2	CGA GCA AGA CCT TTA AG			
vanD	*vanD*-1	TAA GGC GCT TGC ATA TAC CG	54	461	Perichon *et al.* (1997)
	vanD-2	TGC AGC CAA GTA TCC GGT AA			
$vanS_B$–$vanY_B$	$vanS_BY_B$-1	ATA TGC GCT GGA AAA CAC CTC	60	309	Dahl *et al.* (1999)
	$vanS_BY_B$-2	CCC CAG ATT GTT TCA TAT GCC			
Tn*5382*-like	Tn*5382*-1	GTT CTT ATT CCG CAG GTG GTG ATT	60	311	Carias *et al.* (1998)
	Tn*5382*-2	ACG CCA TGC TAT TTA CTT CCG GC			Dahl *et al.* (2000)
$vanX_B$–*ORFC*	$vanX_B$-*ORFC*-1	GAT GCC AAG TAC GCT ACA TGG GA	57	873	Carias *et al.* (1998)
	$vanX_B$-*ORFC*-2	TGA GTT GTG GAA GTC GAT TAG AG			Dahl *et al.* (2000)
pbp5–Tn*5382*	*pbp5*-Tn*5382*-1	TCA GCC GAT TTG CGA CAG GTT ATG	55	1079	Carias *et al.* (1998)
	pbp5-Tn*5382*-2	TGG GGT GGC GGG TAT TAG CAG TAT			
ant(6)-*Ia*	*ant*(6)-*Ia*-1	ACT GGC TTA ATC AAT TTG GG	58	597	Clark *et al.* (1999)
	ant(6)-*Ia*-2	GCC TTT CCG CCA CCT CAC CG			
aac(6′)-*Ie-aph*(2″)-*Ia*	*aac*(6′)-*Ie-aph*(2″)-*Ia*-1	CCA AGA GCA ATA AGG GCA TA	60	220	Van de Klundert & Vliegenthart (1993)
	aac(6′)-*Ie-aph*(2″)-*Ia*-2	CAC TAT CAT AAC CAC TAC CG			
aph(3′)-*IIIa*	*aph*(3′)-*IIIa*-1	GCC GAT GTG GAT TGC GAA AA	60	292	Van de Klundert & Vliegenthart (1993)
	aph(3′)-*IIIa*-2	GCT TGA TCC CCA GTA AGT CA			
erm(A)	*erm*(A)-1	TCT AAA AAG CAT GTA AAA GAA	52	645	Sutcliffe *et al.* (1996)
	erm(A)-2	CTT CGA TAG TTT ATT AAT ATT AGT			
erm(B)	*erm*(B)-1	GAA AAG GTA CTC AAC CAA ATA	52	639	Sutcliffe *et al.* (1996)
	erm(B)-2	AGT AAC GGT ACT TAA ATT GTT TAC			
erm(C)	*erm*(C)-1	TCA AAA CAT AAT ATA GAT AAA	52	642	Sutcliffe *et al.* (1996)
	erm(C)-2	GCT AAT ATT GTT TAA ATC GTC AAT			
tet(M)	*tet*(M)-1	GTT AAA TAG TGT TCT TGG AG	55	696	Aarestrup *et al.* (2000)
	tet(M)-2	CTA AGA TAT GGC TCT AAC AA			
esp	*esp*-1	CTT TGA TTC TTG GTT GTC GGA TAC	55	475	Klare *et al.* (2005)
	esp-2	TTC AAC TAC CAC GGT TTG TTT ATC			
hyl	*hyl-1*	GAG TAG AGG AAT ATC TTA GC	50	661	Klare *et al.* (2005)
	hyl-2	AGG CTC CAA TTC TGT			

*T_a, Annealing temperature.

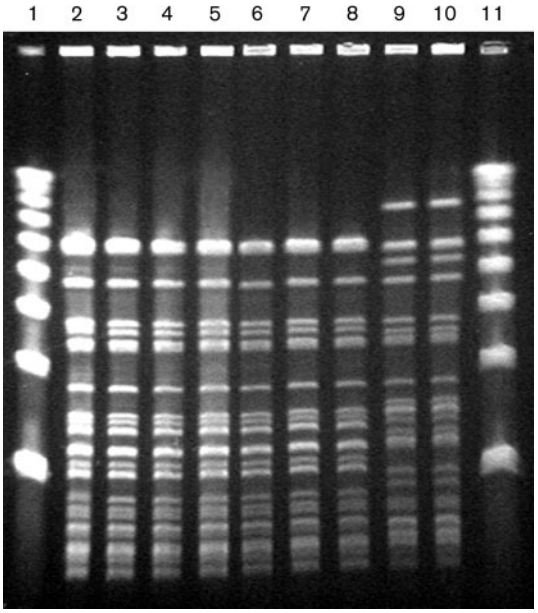

Fig. 1. PFGE of *Sma*I-digested genomic DNA from the nine *vanB2 E. faecium* strains. Lanes: 1 and 11, PFGE DNA marker; 2, *E. faecium* C393; 3, *E. faecium* C395; 4, *E. faecium* C396, 5, *E. faecium* C397; 6, *E. faecium* C402; 7, *E. faecium* C404; 8, *E. faecium* C406; 9, *E. faecium* C460; 10, *E. faecium* C461.

100 % identity with a partial sequence of the previously reported Tn*5382* and Tn*1549* transposons (GenBank accession nos AF175739 and AF192329, respectively). These sequences were also 100 % identical to that previously found by our group in a *vanB2*-containing *E. hirae* strain of animal origin (Torres *et al.*, 2003). However, slight differences for the *vanX*$_B$–*ORFC* amplicons of the nine strains were observed. The seven strains belonging to PFGE pattern A showed a sequence 100 % identical to that reported previously for *vanB2*-containing *E. faecalis* and *E. faecium* strains with the Tn*5382* transposon (GenBank accession nos AF203411 and AF203417, respectively). Nevertheless, a different sequence with a deduced valine residue (codon GTG) at position 46 of the VanX$_B$ protein and an asparagine residue (codon AAT) at position 89 was obtained for the remaining two strains with the PFGE pattern B (*E. faecium* C460 and C461). This VanX$_B$ sequence was 100 % identical to that previously reported for other *vanB2*-containing *E. faecium* strains with Tn*5382* (GenBank accession no. AF203412 and others) and also identical to that found in the *vanB2 E. hirae* strain of animal origin previously published (Torres *et al.*, 2003). The impact of these amino acid changes on the activity of the VanX$_B$ protein remains to be investigated. According to our results, the *vanB2* gene cluster could be included in a Tn*5382*-like element in our strains. It should be noted that Tn*5382* and Tn*1549* show a strong similarity at both ends of the transposons (Carias *et al.*, 1998; Garnier *et al.*, 2000; Umeda *et al.*, 2002) and it has been proposed that both could be integrated in the Tn*5382*–Tn*1549* family (Umeda *et al.*, 2002).

According to a recent report, *vanB2*-containing *E. faecium* strains from Taiwan were found to harbour Tn*5382* and to be associated with the *pbp5* gene (Lu *et al.*, 2005). In our study, the nine VREF-VBP strains showed ampicillin resistance and using PCR it was possible to detect the presence of *pbp5*–Tn*5382* amplicons of the expected size in all of them. Two of these amplicons were sequenced (*E. faecium* C395 and C393), confirming that sequences of Tn*5382* were located downstream from a *pbp5* gene. The sequence of the C-terminal part of the protein encoded by *pbp5* in these two isolates presented nine amino acid changes with respect to the deduced sequence of the protein encoded by *pbp5* (GenBank accession no. X84860) (Jureen *et al.*, 2003). The amino acid changes detected in the protein encoded by the *pbp5* gene in both isolates were: 461Q→K, 470H→Q, 485M→A, 496N→K, 499A→T, 525E→D, 586V→L, 629E→V and 667P→S. Most of these amino acid changes have been detected previously in the protein encoded by the *pbp5* gene of ampicillin-resistant *E. faecium* isolates (Jureen *et al.*, 2003; Rice *et al.*, 2004).

The transferability of genetic determinants implicated in vancomycin resistance was tested by conjugation using *E. faecium* C393 (pattern A) or C461 (pattern B) as donor and *E. faecalis* strain JH2-2 as recipient. Results were negative in both cases and no transconjugants were detected.

The presence of the *esp* and *hyl* genes encoding virulence factors (enterococcal surface protein and hyaluronidase, respectively) was investigated by PCR and sequencing. The *esp* gene was detected in the two *vanB2*-containing *E. faecium* strains belonging to PFGE pattern B, but not in those of pattern A. The *hyl* gene was not identified in our VREF-VRP strains. The clonal spread of ampicillin/vancomycin-resistant *E. faecium* carrying the *esp* and *hyl* genes in German hospitals has been reported recently (Klare *et al.*, 2005).

All of the strains in this study showed high-level streptomycin and kanamycin resistance, as well as resistance to erythromycin and ciprofloxacin. The two strains included in the PFGE pattern B also showed tetracycline resistance. The *ant*(6′)-*Ia*, *aph*(3′)-*IIIa* and *erm*(B) genes were detected in all nine strains.

In conclusion, this study reports for the first time the persistence of clonally related *vanB2*-containing *E. faecium* strains affecting patients in two hospitals in the same city in Spain. A previous clinical *vanB2*-containing *E. faecium* strain was detected in a Spanish hospital by other authors (Lorenzo-Díaz *et al.*, 2004) and very recently a nosocomial outbreak of *vanB2* vancomycin-resistant *E. faecium* was reported in Sweden (Granlund *et al.*, 2006). In most cases, our vancomycin-resistant enterococci were recovered from patients with severe diseases (the majority had been hospitalized in ICU wards) who had previously received

glycopeptides and other broad-spectrum antibiotics. Special care should be taken in the future to ensure the rapid detection and control of this type of strain in order to avoid its dissemination within a hospital.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Fondo de Investigaciones Sanitarias of Spain (FIS 01/973).

REFERENCES

Aarestrup, F. M., Agerso, Y., Gerner-Smidt, P., Madsen, M. & Jensen, L. B. (2000). Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis* **37**, 127–137.

Carias, L. L., Rudin, S. D., Donskey, C. J. & Rice, L. B. (1998). Genetic linkage and cotransfer of a novel *vanB*-containing transposon (Tn*5382*) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant *Enterococcus faecium* isolate. *J Bacteriol* **180**, 4426–4434.

Cetinkaya, Y., Falk, P. & Mayhall, C. G. (2000). Vancomycin-resistant enterococci. *Clin Microbiol Rev* **13**, 686–707.

Clark, N. C., Olsvik, Ø., Swenson, J. M., Spiegel, C. A. & Tenover, F. C. (1999). Detection of a streptomycin/spectinomycin adenylyltransferase gene (*aadA*) in *Enterococcus faecalis*. *Antimicrob Agents Chemother* **43**, 157–160.

Dahl, K. H., Simonsen, G. S., Olsvik, Ø. & Sundsfjord, A. (1999). Heterogeneity in the *vanB* gene cluster of genomically diverse clinical strains of vancomycin-resistant enterococci. *Antimicrob Agents Chemother* **43**, 1105–1110.

Dahl, K. H., Lundblad, E. W., Rokenes, T. P., Olsvik, Ø. & Sundsfjord, A. (2000). Genetic linkage of the *vanB2* gene cluster to Tn*5382* in vancomycin-resistant enterococci and characterization of two novel insertion sequences. *Microbiology* **146**, 1469–1479.

Domingo, M.-C., Huletsky, A., Bernal, A., Giroux, R., Boudreau, D. K., Picard, F. J. & Bergeron, M. G. (2005). Characterization of a Tn*5382*-like transposon containing the *vanB2* gene cluster in a *Clostridium* strain isolated from human faeces. *J Antimicrob Chemother* **55**, 466–474.

Dunny, G. M., Craig, R. A., Carron, R. L. & Clewell, D. B. (1979). Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. *Plasmid* **2**, 454–465.

Dutka-Malen, S., Evers, S. & Courvalin, P. (1995). Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* **33**, 24–27.

Garnier, F., Taourit, S., Glaser, P., Courvalin, P. & Galimand, M. (2000). Characterization of transposon Tn*1549*, conferring VanB-type resistance in *Enterococcus* spp. *Microbiology* **146**, 1481–1489.

Gholizadeh, Y. & Courvalin, P. (2000). Acquired and intrinsic glycopeptide resistance in enterococci. *Int J Antimicrob Agents* **16**, S11–S17.

Gold, H. S., Ünal, S., Cercenado, E., Thauvin-Eliopoulos, C., Eliopoulos, G. M., Wennersten, C. B. & Moellering, R. C., Jr (1993). A gene conferring resistance to vancomycin but not teicoplanin in isolates of *Enterococcus faecalis* and *Enterococcus faecium* demonstrates homology with *vanB, vanA*, and *vanC* genes of enterococci. *Antimicrob Agents Chemother* **37**, 1604–1609.

Granlund, M., Carlsson, C., Edebro, H., Emanuelsson, K. & Lundholm, R. (2006). Nosocomial outbreak of *vanB2* vancomycin-resistant *Enterococcus faecium* in Sweden. *J Hosp Infect* **62**, 254–256.

Jureen, R., Top, J., Mohn, S. C., Harthug, S., Langeland, N. & Willens, R. J. L. (2003). Molecular characterization of ampicillin-resistant *Enterococcus faecium* isolates from hospitalized patients in Norway. *J Clin Microbiol* **41**, 2330–2336.

Kawalec, M., Gniadkowski, M., Zielinska, U., Klos, W. & Hryniewicz, W. (2001). Vancomycin-resistant *Enterococcus faecium* strain carrying the *vanB2* gene variant in a Polish hospital. *J Clin Microbiol* **39**, 811–815.

Klare, I., Konstabel, C., Mueller-Bertling, S. & 10 other authors (2005). Spread of ampicillin/vancomycin-resistant *Enterococcus faecium* of the epidemic-virulent clonal complex-17 carrying the genes *esp* and *hyl* in German hospitals. *Eur J Clin Microbiol Infect Dis* **24**, 815–825.

Lee, W. G., Jernigan, J. A., Rashed, J. K., Anderson, G. J. & Tenover, F. C. (2001). Possible horizontal transfer of the *vanB2* gene among genetically diverse strains of vancomycin-resistant *Enterococcus faecium* in a Korean hospital. *J Clin Microbiol* **39**, 1165–1168.

Lorenzo-Díaz, F., Delgado, T., Reyes-Darias, J. A., Flores, C., Méndez-Álvarez, S., Villar, J., Sierra, A. & Claverie-Martín, F. (2004). Characterization of the first VanB vancomycin-resistant *Enterococcus faecium* isolated in a Spanish hospital. *Curr Microbiol* **48**, 199–203.

Lu, J.-J., Perng, C.-L., Ho, M.-F., Chiueh, T.-S. & Lee, W.-H. (2001). High prevalence of VanB2 vancomycin-resistant *Enterococcus faecium* in Taiwan. *J Clin Microbiol* **39**, 2140–2145.

Lu, J.-J., Chang, T.-Y., Perng, C.-L. & Lee, S.-Y. (2005). The *vanB2* gene cluster of the majority of vancomycin-resistant *Enterococcus faecium* isolates from Taiwan is associated with the *pbp5* gene and is carried by Tn*5382* containing a novel insertion sequence. *Antimicrob Agents Chemother* **49**, 3937–3939.

Mato, R., de Lencastre, H., Roberts, R. B. & Tomasz, A. (1996). Multiplicity of genetic backgrounds among vancomycin-resistant *Enterococcus faecium* isolates recovered from an outbreak in a New York City hospital. *Microb Drug Resist* **2**, 309–317.

McGregor, K. F. & Young, H.-K. (2000). Identification and characterization of *vanB2* glycopeptide resistance elements in enterococci isolated in Scotland. *Antimicrob Agents Chemother* **44**, 2341–2348.

McGregor, K. F., Nolan, C., Young, H.-K., Palepou, M.-F. I., Tysall, L. & Woodford, N. (2001). Prevalence of the *vanB2* gene cluster in the VanB glycopeptide-resistant enterococci in the United Kingdom and the Republic of Ireland and its association with a Tn*5382*-like element. *Antimicrob Agents Chemother* **45**, 367–368.

Miele, A., Bandera, M. & Goldstein, B. P. (1995). Use of primers selective for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in VanA isolates. *Antimicrob Agents Chemother* **39**, 1772–1778.

Murray, B. (1997). Vancomycin-resistant enterococci. *Am J Med* **102**, 284–293.

NCCLS (2002). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. NCCLS document M7-A5. Wayne, PA: National Committee for Clinical Laboratory Standards.

Perichon, B., Reynolds, P. & Courvalin, P. (1997). VanD-type glycopeptide-resistant *Enterococcus faecium* BM4339. *Antimicrob Agents Chemother* **41**, 2016–2018.

Quintiliani, R., Jr, Evers, S. & Courvalin, P. (1993). The *vanB* gene confers various levels of self-transferable resistance to vancomycin in enterococci. *J Infect Dis* **167**, 1220–1223.

Rice, L. B., Bellais, S., Carias, L. L., Hutton-Thomas, R., Bonomo, R. A., Caspers, P., Page, M. G. P. & Gutmann, L. (2004). Impact of

specific *pbp5* mutations on expression of β-lactam resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* **48**, 3028–3032.

Simonsen, G. S., Andersen, B. M., Digranes, A. & 9 other authors (1998). Low faecal carrier rate of vancomycin resistant enterococci in Norwegian hospital patients. *Scand J Infect Dis* **30**, 465–468.

Sutcliffe, J., Grebe, T., Tait-Kamradt, A. & Wondrack, L. (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemother* **40**, 2562–2566.

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H. & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **33**, 2233–2239.

Torres, C., Reguera, J. A., Sanmartín, M. J., Pérez-Díaz, J. C. & Baquero, F. (1994). *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. *J Antimicrob Chemother* **33**, 553–561.

Torres, C., Tenorio, C., Portillo, A., García, M., Martínez, C., del Campo, R., Ruiz-Larrea, F. & Zarazaga, M. (2003). Intestinal colonization by *vanA*- or *vanB2*-containing enterococcal isolates of healthy animals in Spain. *Microb Drug Resist* **9** (Suppl. 1), S47–S52.

Turabelidze, D., Kotetishvili, M., Krefer, A., Morris, J. G. & Sulakvelidze, A. (2000). Improved pulsed-field gel electrophoresis for typing vancomycin-resistant enterococci. *J Clin Microbiol* **38**, 4242–4245.

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.

Van de Klundert, J. A. M. & Vliegenthart, J. S. (1993). PCR detection of genes for aminoglycoside-modifying enzymes. In *Diagnostic Molecular Microbiology: Principles and Applications*, pp. 547–552. Edited by D. H. Persing, T. F. Smith, F. C. Tenover & T. J. White. Washington, DC: American Society for Microbiology.

Woodford, N. (2001). Epidemiology of the genetic elements responsible for acquired glycopeptide resistance in enterococci. *Microb Drug Resist* **7**, 229–236.