

## High Prevalence of VanB2 Vancomycin-Resistant *Enterococcus faecium* in Taiwan

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Thirty-six VanB glycopeptide-resistant *Enterococcus faecium* isolates were collected from patients in five different hospitals in Taiwan. The vancomycin resistance genes were amplified by the long *vanB* PCR, which amplifies the 6,373-bp *vanB* gene cluster including the *vanR<sub>B2</sub>*, *vanS<sub>B2</sub>*, *vanY<sub>B2</sub>*, *vanW<sub>B2</sub>*, *vanH<sub>B2</sub>*, *vanB2*, and *vanX<sub>B2</sub>* genes. The deduced amino acid sequences were found to be 95 to 98% homologous to those of the *vanB1* gene cluster: VanR<sub>B1</sub>, 97%; VanS<sub>B1</sub>, 97%; VanY<sub>B1</sub>, 96%; VanH<sub>B1</sub>, 95%; VanB1, 96%; and VanX<sub>B1</sub>, 98%. Restriction enzyme analysis of the long *vanB* PCR products revealed that all 36 isolates had the same *vanB2*-specific pattern. DNA sequence analysis of the *vanB2* gene, which is a D-Ala–D-Lac ligase gene, revealed that none of the 36 sequences were identical to the previously published *vanB2* sequence. Thirty-one isolates had 1 nucleotide different from the published *vanB2* sequence. The sequences of the other five isolates differed from the published *vanB2* sequence by 2 or 3 nucleotides. Four isolates with a low or moderate resistance to vancomycin (MIC = 4 to 32 µg/ml) were found to have the same leucine-to-methionine change at amino acid position 308 of the *vanB2* gene. The genomic DNAs of all 36 isolates were digested with *Sma*I and then typed by pulsed-field gel electrophoresis (PFGE). Eight different PFGE types (I to VIII) were observed, and type I was found to be prevalent in all hospitals examined in this study. This result suggests that intra- and interhospital dissemination of this *E. faecium* strain has occurred in Taiwan.

Glycopeptide resistance in enterococci is genotypically and phenotypically diverse. The VanA and VanB types are the two most common classes of acquired glycopeptide resistance in enterococci. VanA enterococci are resistant to high levels of vancomycin and teicoplanin due to the presence of the *vanA* gene cluster that includes the *vanR*, *vanS*, *vanH*, *vanA*, *vanX*, *vanY*, and *vanZ* genes (1). The *vanA* gene cluster is usually located on transposon Tn1546 or related elements (2). Strains with the VanB phenotype have various levels of resistance to vancomycin but are susceptible to teicoplanin (25). The VanB-type resistance is mediated by the *vanB* gene cluster that includes the *vanR<sub>B</sub>*, *vanS<sub>B</sub>*, *vanY<sub>B</sub>*, *vanW*, *vanH<sub>B</sub>*, *vanB*, and *vanX<sub>B</sub>* genes (8). The *vanB* gene cluster may reside on Tn1547, which is flanked by two distantly related insertion sequences, an IS256-like element and IS16, in direct-repeat orientation (24). A novel *vanB*-containing transposon, Tn5382, was recently described in a vancomycin-resistant *Enterococcus faecium* (4).

The *vanB* gene encodes a D-Ala–D-Lac ligase and is classified into three different types, *vanB1*, *vanB2*, and *vanB3*, based on DNA sequence heterogeneity (11, 22). The *vanB1* gene was previously referred to as *vanB* (6, 10). The *vanB2* gene was first found in *Enterococcus faecalis* SF300 (11). The *vanB3* gene was found recently in two *E. faecalis* isolates (22). Its nucleotide sequence differs from that of *vanB1* by 5% (22) and from the published *vanB2* sequence by 3.6% (22).

We have recently used PCR primers specific for the *vanB1*

gene (6) to amplify the vancomycin resistance gene from VanB enterococci in Taiwan. The resulting PCR products were found to be 1,100 bp, not the expected 433 bp. The PCR product of one isolate, *E. faecium* TSGH1, was sequenced and found to contain a portion of the *vanH<sub>B2</sub>* gene, the entire *vanB2* gene, and a portion of the *vanX<sub>B2</sub>* gene (GenBank accession no. Z83305). The *vanB2* sequence of TSGH1 was found to differ by only 1 nucleotide from that of the published 801-bp *vanB2* gene (22). This result suggests that *E. faecium* TSGH1 harbors a *vanB2* gene cluster. We therefore examined all VanB *E. faecium* isolates that we have collected. The entire *vanB* gene cluster of each isolate was amplified, and the *vanB* ligase gene of each isolate was sequenced. Pulsed-field gel electrophoresis (PFGE) was performed to identify the predominant strain of *E. faecium* in Taiwan.

### MATERIALS AND METHODS

**Bacterial isolates.** Thirty-six clinical isolates of VanB *E. faecium* collected from five hospitals (13 isolates from hospital A, 4 from hospital B, 5 from hospital C, 2 from hospital D, and 12 from hospital E) in Taiwan were studied. They were not consecutive vancomycin-resistant enterococcus (VRE) isolates. They were selected because their stock cultures were available and they were previously determined to carry the *vanB* gene (20). *E. faecalis* V583 CDC containing *vanB1* (26), *E. faecium* TUH2-18 containing *vanB2*(7), and *E. faecium* VRE45 containing *vanB3* (7) were used as controls in PCR, PFGE, and DNA sequencing.

**Antimicrobial susceptibility assay.** VanB *E. faecium* isolates were assayed for susceptibility to vancomycin and teicoplanin by the E test (AB Biodisk, Piscataway, N.J.) according to the manufacturer's instructions. *E. faecalis* ATCC 29212 was used as the control strain for the susceptibility test.

**PCR amplification.** Bacterial DNA was isolated as previously described (19). Briefly, the DNA from a loop of cultured bacteria was extracted by boiling the bacteria with lysis buffer (1% Triton X-100, 10 mM Tris-HCl [pH 8.0], 1 mM EDTA) for 30 min. After centrifugation, the DNA in the supernatant was analyzed for the presence of the *van* gene cluster by PCR. PCRs were performed in the GenAmp PCR system (model 2400; Perkin-Elmer, Norwalk, Conn.).

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TABLE 1. Primers used to characterize the *vanB2* gene cluster in this study

Name	Sequence 5'→3'	Position <sup>a</sup>
For long <i>vanB</i> PCR		
VB211F	TCTACTTGTGCGAGGATGATG	191–211
VB6545R	AACTTATAGTGCCACCATTG	6564–6545
For sequencing of <i>vanB2</i> gene cluster		
VB498F	CAAAGCCATTCAAGATGCGG	479–498
VB893F	CCTATACGATCATTGTCCCTG	874–893
VB1043R	TATTGTAGTGAAAACAGCCCT	1062–1043
VB1655R	CGGAAATGGTTTTGCCTGC	1674–1655
VB2028R	TGCTTCGCGCTGATCAATG	2047–2028
VB2047F	CATTGATCAGGCGCGAAGCA	2028–2047
VB2248R	TCTGTAGTTTTCTGTAGC	2256–2248
VB2556F	CTCATAACAGGAGCAGCACT	2537–2556
VB2596R	TCATCTCCCTGTTCTTTGGC	2615–2596
VB2944F	CTTGGTCTTGCTGTGGATA	2926–2944
VB3478F	TTCTTTCTGGTGGCTGGT	3461–3478
VB3959R	CGGGAGGGGATAACAGATT	3977–3959
VB4322R	CATAATCCCGCAACGCTGTC	4340–4322
VB4871R	CCGTATGGGGTGTGATGAT	4889–4871
VB5367R	AAGAATGTAGGCCAGTGA	5382–5367
VB5382F	CAAATCACTGGCCATTC	5363–5382
VB5454F <sup>b</sup>	GTGACAAACCGAGGCGAGGA	5434–5454
VB5745F	AGAAAATGCGATGATTACAG	5726–5745
VB5808R	TCTGCATCCAAGCACCCG	5825–5808
VB5846R <sup>b</sup>	CCGCCATCTCTGCAAAAAA	5866–5846
VB6115F	GGTGGATGGGTATGAGGTG	6097–6115

<sup>a</sup> Nucleotide positions are those of the V583 *vanB* gene cluster (9).

<sup>b</sup> These primers are described by Clark et al. (6) for the amplification of the *vanB1* gene.

Based on sequence analysis of the *vanA* and *vanB* gene clusters, primers VB211F and VB6545R were designed (Table 1) to amplify the 6,373-bp *vanB2* gene cluster. This PCR method was named the long *vanB* PCR. The PCR mixture contained 50 ng of template DNA, PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.001% gelatin), 20 pmol of each PCR primer, 0.4 mM each deoxynucleoside triphosphate, and 2.5 U of *TaKaRa Ex Taq* DNA polymerase (Takara Shuzo Co., Ltd., Otsu, Shiga, Japan) in a total volume of 100 µl. After a 10-min denaturation at 94°C, the reaction mixture was run through 40 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 6 min at 72°C and then incubated for 10 min at 72°C. After amplification, 5 µl of PCR products was electrophoresed on a 1% agarose gel in TBE buffer (0.0445 M Tris, 0.0445 M boric acid, 0.001 M EDTA) containing ethidium bromide (0.5 µg/ml). The PCR products were purified with the GFX PCR DNA and Gel Band Purification kits (Amersham Pharmacia Biotech, Inc., Piscataway, N.J.) according to the manufacturer's instructions.

**DNA sequencing.** The long *vanB* PCR products were sequenced by primer walking using primers designed according to the published sequence of the V583 *vanB* gene cluster or new sequences obtained in this study (Table 1). The *vanB2* ligase genes in various isolates were sequenced using primers VB5382F, VB5367R, VB5745F, and VB5808R (Table 1). The BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Inc., Foster City, Calif.) was used for sequencing.

**Restriction fragment length polymorphism (RFLP) analysis.** Ten microliters of each long *vanB* PCR product was digested with different restriction enzymes in a total volume of 20 µl. After incubation for 2 h at 37°C, the digested DNA samples were electrophoresed on a 1% agarose gel in TBE buffer containing ethidium bromide (0.5 µg/ml).

**Typing of VRE by PFGE.** Preparation of bacterial DNA for PFGE was performed as described by Gouby et al. (12). Bacteria were grown overnight on blood agar plates and then suspended in 10 mM Tris–0.1 mM EDTA solution to a concentration of 10 on the McFarland scale. This bacterial suspension was mixed with an equal volume of molten 1.6% low-melting-point agarose and then cast into plugs. The plugs were treated with lysis solution (6 mM Tris-HCl [pH 7.6], 100 mM EDTA [pH 7.5], 1 M NaCl, 0.2% deoxycholate, 0.5% sodium lauroylsarcosine, 1 mg of lysozyme/ml) at 37°C for 24 h. The solution was subsequently replaced with ESP buffer (0.5 M EDTA, 1% sodium lauroylsarcosine, 0.5 mg of proteinase K/ml) and incubated at 37°C for 24 h. The plugs

were then washed once in distilled water and four times in 10 mM Tris–0.1 mM EDTA buffer at 37°C for 30 min each time.

The genomic DNA embedded in the plugs was digested overnight with 50 U of *Sma*I (New England Biolabs, Beverly, Mass.) in a total reaction volume of 0.3 ml in NEB4 buffer (New England Biolabs) at 25°C. The plugs were then washed in 10 mM Tris–0.1 mM EDTA buffer at 37°C for 1 h, loaded into wells of a 1% agarose gel, and then electrophoresed in 0.5× TBE buffer. A plug containing a 50-kb-to-1-Mb lambda DNA ladder (Bio-Rad Laboratories, Hercules, Calif.) was used as the molecular size marker. PFGE was performed with a CHEF Mapper XA system (Bio-Rad Laboratories) at 14°C under the autoalgorithm mode (gradient, 6 V/cm; run time, 27 h 12 min; included angle, 120; initial switch time, 2.91 s; final switch time, 35.38 s; ramping factor, linear). The gel was stained with ethidium bromide and then photographed. The PFGE results were interpreted according to the criteria of Tenover et al. (27).

**Nucleotide sequence accession numbers.** The nucleotide sequences of the whole *vanB2* gene cluster of isolate TSGH1 and the *vanB2* ligase genes of isolates VRE-1, SLH475, and CG4248 have been deposited in GenBank with accession numbers AF310956, AF310953, AF310954, and AF310957, respectively.

## RESULTS

All 36 VanB isolates were amplified by the long *vanB* PCR (Table 2). The sizes of amplified products were approximately 6,400 bp. The PCR products were digested with *Hae*II or *Bcl*II, and all of the products yielded identical *Hae*II or *Bcl*II digestion patterns that were characteristic of *vanB2* but different from those of the *vanB1* or the *vanB3* gene cluster (Fig. 1).

The entire 6,400-bp *vanB2* gene cluster of isolate TSGH1 was sequenced. This gene cluster was found to contain the 663-bp *vanR*<sub>B2</sub>, 1,344-bp *vanS*<sub>B2</sub>, 807-bp *vanY*<sub>B2</sub>, 828-bp *vanW*<sub>B2</sub>, 972-bp *vanH*<sub>B2</sub>, 1,029-bp *vanB2*, and 609-bp *vanX*<sub>B2</sub> genes in addition to the 175-bp *vanS*<sub>B</sub>-*vanY*<sub>B</sub> intergenic region. The nucleotide sequences of the *vanB2* gene cluster were found to

TABLE 2. General information for VanB *E. faecium* strains and isolates

Origin or hospital	Strain	MIC ( $\mu\text{g/ml}$ )		Long <i>vanB</i> PCR-RFLP profile	<i>vanB</i> gene type	PFGE type
		Vancomycin	Teicoplanin			
United States	V583 CDC	96	0.38	B1	B1	IX
Norway	TUH2-18	12	0.38	B2	B2	X
United States	VRE45	$\geq 256$	0.5	B3	B3	XI
A	TSGH1	>256	1	B2	B2 <sup>a</sup>	I
A	TSGH2	>256	1	B2	B2 <sup>a</sup>	I
A	F901	>256	0.25	B2	B2 <sup>a</sup>	I
A	F909	>256	0.5	B2	B2 <sup>a</sup>	I
A	F910	>256	0.5	B2	B2 <sup>a</sup>	I
A	1716	>256	0.5	B2	B2 <sup>a</sup>	II
A	1741	>256	1.5	B2	B2 <sup>a</sup>	II
A	1742	>256	2	B2	B2 <sup>a</sup>	II
A	1746	>256	1	B2	B2 <sup>a</sup>	I
A	P6-31	>256	0.75	B2	B2 <sup>a</sup>	I
A	M908	>256	1	B2	B2 <sup>a</sup>	I
A	M909	>256	1	B2	B2 <sup>a</sup>	I
A	M941	>256	1	B2	B2 <sup>a</sup>	I
B	16-43	>256	2	B2	B2 <sup>a</sup>	I
B	24-24	>256	1.5	B2	B2 <sup>a</sup>	I
B	Y1	>256	1.5	B2	B2 <sup>a</sup>	I
B	2-50	>256	1	B2	B2 <sup>a</sup>	I
C	VRE-1	4	1	B2	B2 <sup>b</sup>	III
C	VRE-3	8	1	B2	B2 <sup>b</sup>	III
C	VRE-8	32	1	B2	B2 <sup>b</sup>	IV
C	VRE-20	>256	4	B2	B2 <sup>a</sup>	I
C	CG4248	32	1	B2	B2 <sup>c</sup>	I
D	SLH475	>256	1	B2	B2 <sup>d</sup>	I
D	SLH476	3	1	B2	B2 <sup>a</sup>	V
E	CKU-2	48	0.5	B2	B2 <sup>a</sup>	I
E	CKU-4	>256	0.5	B2	B2 <sup>a</sup>	I
E	CKU-6	>256	1	B2	B2 <sup>a</sup>	VI
E	CKU-8	128	1	B2	B2 <sup>a</sup>	I
E	CKU-10	>256	1.5	B2	B2 <sup>a</sup>	I
E	CKU-11	>256	2	B2	B2 <sup>a</sup>	VII
E	CKU-12	>256	1	B2	B2 <sup>a</sup>	VIII
E	CKU-13	>256	1	B2	B2 <sup>a</sup>	I
E	CKU-14	>256	4	B2	B2 <sup>a</sup>	VIII
E	CKU-16	>256	4	B2	B2 <sup>a</sup>	I
E	CKU-20	>256	0.5	B2	B2 <sup>a</sup>	I
E	CKU-21	>256	4	B2	B2 <sup>a</sup>	I

<sup>a</sup> One base different from the published *vanB2* gene sequence of Patel et al. (22).

<sup>b</sup> Two bases different from the published *vanB2* gene sequence of Patel et al. (22).

<sup>c</sup> Three bases different from the published *vanB2* gene sequence of Patel et al. (22).

<sup>d</sup> One base different from the published *vanB2* gene sequence of Patel et al. (22).

be more similar to those of the *vanB1* gene cluster (94 to 96% homology) than to those of the *vanA* gene cluster (48 to 81% homology) and the *vanD* gene cluster (33 to 71% homology). The deduced amino acid sequences of the *vanB2* gene cluster also had a higher degree of homology with those of the *vanB1* gene cluster (95 to 97% homology) than to those of the *vanA* and *vanD* gene clusters (14 to 76% homology) (Table 3).

The *vanB2* ligase genes of all 36 isolates were then sequenced. Four types of *vanB2* ligase gene sequences, designated TSGH-1, V4248, VRE-1, and SLH475, were observed. Thirty-one isolates belonged to sequence TSGH-1, three belonged to VRE-1, and one each belonged to CG4248 and SLH475 (Table 2). These four sequences differed from each other by only 5 nucleotides at positions 520, 538, 556, 922, and 967. The nucleotides of TSGH-1 at these positions were T, A, C, C, and A, respectively. Those of V4248 were T, G, C, A, and G. Sequence VRE-1 had T, C, A, A, and A at these positions, and SLH475 had G, G, A, C, and A. These sequences differed

from that of the *vanB1* ligase gene at 44 to 46 (4.3 to 4.5%) nucleotide positions, resulting in 14 or 15 amino acid changes.

The 1,029-bp *vanB2* sequences from the 36 isolates were also compared with the previously published 801-bp *vanB2* ligase gene sequence (22), which is missing the first 102 and the last 126 bp. Only 1 to 3 nucleotides were found to be different from the published *vanB2* ligase gene sequence. The G-to-C change at nucleotide position 538 of the 31 sequences represented by TSGH-1 resulted in a valine-to-leucine change. The A-to-C and C-to-A changes at positions 556 and 922 of the sequence CG4248 resulted in threonine-to-proline and leucine-to-methionine changes at these positions. The VRE-1 sequence had G-to-C and C-to-A changes at positions 538 and 922, causing valine-to-leucine and leucine-to-methionine changes at these positions. The T-to-G change at position 520 of the sequence SLH475 caused a serine-to-alanine change at that position. Four isolates (CG4248, VRE-1, VRE-3, and VRE-8) were found to have a low or moderate resistance to vancomycin

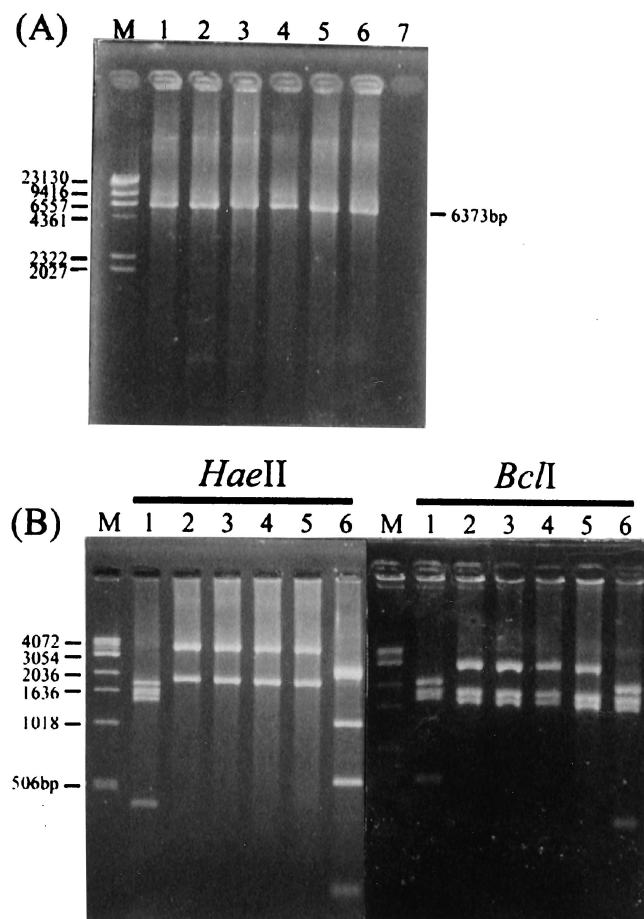


FIG. 1. Analysis of long *vanB* PCR products. (A) PCR products of VRE harboring the *vanB* gene cluster amplified with primers VB211 and VB6545R. The sizes of PCR products are indicated on the right-hand side of the gel. Lane M, lambda phage DNA digested with *Hind*III; lane 1, VanB1 V583; lanes 2 to 5, VanB2 TUH2-18, TSGH1, CG4248, and VRE-1; lane 6, VanB3 VRE45. (B) RFLP analysis of long *vanB* PCR products by *Hae*II or *Bcl*I digestion. Lane M, 1-kb DNA ladder (Life Technologies, Grand Island, N.Y.); lane 1, VanB1 V583; lanes 2 to 5, VanB2 TUH2-18, TSGH1, CG4248, and VRE-1; lane 6, VanB3 VRE45. The numbers on the left are in base pairs.

(MIC = 4 to 32 µg/ml) (Table 2). Analysis of the sequences revealed that all four of these isolates had a C-to-A change at position 922, resulting in a leucine-to-methionine change at codon 308 of the *vanB2* ligase gene.

Eight different PFGE types, designated types I to VIII, were observed (Fig. 2). Type I was found to be predominant (Table 2). Twenty-five (69.4%) isolates belonged to type I, three isolates were type II, and two isolates each belonged to types III and VIII. One isolate each belonged to types IV, V, VI, and VII. Type I was also found to be the predominant type in all of the hospitals. Types II and III were found only in hospitals A and C. None of the control strains, one each VanB1 and VanB3 strain from the United States and one VanB2 strain from Norway, belonged to these eight PFGE types (Table 2).

**DISCUSSION**

In this study, we found that all 36 VanB *E. faecium* isolates examined harbored the *vanB2* gene cluster. No VanB1 or

TABLE 3. Homology between the deduced amino acid sequences of proteins encoded by *vanB2*, *vanB*, *vanA*, and *vanD* gene clusters

Sequence compared	% Identity					
	VanR <sub>B2</sub>	VanS <sub>B2</sub>	VanY <sub>B2</sub>	VanH <sub>B2</sub>	VanB <sub>2</sub>	VanX <sub>B2</sub>
VanR <sub>B1</sub>	97					
VanS <sub>B1</sub>		97				
VanY <sub>B1</sub>			96			
VanH <sub>B1</sub>				95		
VanB <sub>1</sub>					96	
VanX <sub>B1</sub>						98
VanR	44					
VanS		17				
VanY			22			
VanH				67		
VanA					76	
VanX						75
VanR <sub>D</sub>	34					
VanS <sub>D</sub>		15				
VanY <sub>D</sub>			14			
VanH <sub>D</sub>				62		
VanD					68	
VanX <sub>D</sub>						71

VanB3 *E. faecium* were found. These 36 VanB *E. faecium* isolates were collected from five different hospitals in Taiwan. To our knowledge, this is the first report that all VanB *E. faecium* isolates from a certain geographical location harbor the *vanB2* gene. Previous studies have shown that VanB2 VRE are uncommon in the United States but are quite common in Europe (6, 7, 21). Dahl et al. (7) have recently examined 17 VanB VRE isolates from hospitalized patients in Europe and the United States and showed that only 2 of 9 U.S. isolates but 7 of 8 European isolates harbored the *vanB2* gene. McGregor and Young (21) found that 28 of 32 VanB VRE from hospitalized patients in Scotland harbored *vanB2*. Clark et al. (6) examined 105 clinical isolates of VRE collected from 31 U.S. hospitals in 14 states and found that all 26 VanB VRE isolates harbored the *vanB1* gene.

The results of this study also revealed sequence heterogeneity of the *vanB* ligase genes in different VRE isolates. The *vanB2* sequences obtained in this study had a marked difference from those of *vanB1* (4.3 to 4.5% difference) and *vanB3* (2.8 to 3% difference). In addition, none of the 36 *vanB2* ligase gene sequences determined in this study were identical to that of the previously described *vanB2* ligase gene (22). There were 1- to 3-nucleotide differences between that sequence and our *vanB2* ligase gene sequences. However, the *Hae*II and *Bcl*I digestion patterns of the long *vanB* PCR products of all 36 isolates are identical. This observation could provide an alternative means for identification of VanB2 VRE for epidemiological or surveillance studies.

Although there are several types of *vanB*, no correlation has been observed between *vanB* subtypes and levels of vancomycin resistance (7). A unique finding in this study is that all four VanB2 VRE isolates with a low or moderate resistance to vancomycin (MIC = 4 to 32 µg/ml) had a leucine-to-methionine change at codon 308 of the *vanB2* gene. Whether this change actually results in a low or moderate vancomycin resistance in VanB2 VRE remains to be investigated.



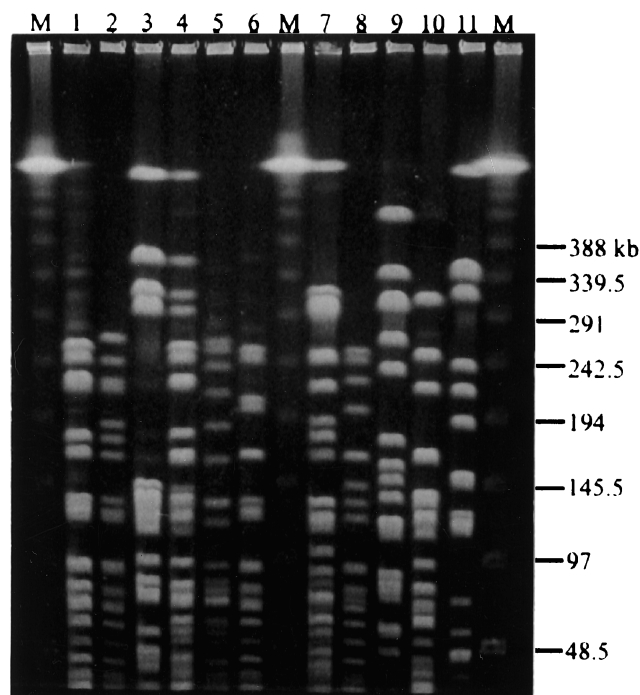


FIG. 2. PFGE patterns of *Sma*I digests of chromosomal DNA from VRE isolates. Lanes M, Lambda DNA-PFGE marker ladder; lanes 1 to 6, TSGH1 (type I), 1716 (type II), VRE-1 (type III), VRE-8 (type IV), SLH476 (type V), and CKU-6 (type VI); lanes 7 to 11, CKU-11 (type VII), CKU-12 (type VIII), VanB1 V583 (type IX), VanB2 TUH2-18 (type X), and VanB3 VRE45 (type XI). The numbers on the right are in base pairs.

The sequence heterogeneity in the *vanB* ligase genes is in contrast to the reported DNA sequence homogeneity in the *vanA* ligase gene (13, 14, 28). The sequences of the *vanA* ligase genes in different VRE isolates appeared to be identical. However, the Tn1546 or Tn1546-like elements that carry the *vanA* gene clusters are structurally different (13, 14, 28). The structural diversity in Tn1546 elements is caused by deletions or insertions which may have occurred during recombination events (13, 22, 28). VanA VRE have been isolated from a variety of sources, including animals, animal foodstuffs, and humans (14–17, 28). The food chain has been suggested to be a source of VanA VRE (5, 18). To our knowledge, VanB VRE have only been detected in hospitalized patients. The finding that type I *E. faecium* harboring the *vanB2* gene was prevalent (69.4%) suggests that intra- and interhospital dissemination of this VRE has occurred in Taiwan. Because of the frequent occurrence of oxacillin-resistant *Staphylococcus aureus*, the use of vancomycin has increased tremendously in Taiwan during the last decade. It is possible that the spread of the type I VanB2 *E. faecium* in Taiwan is the result of unregulated use of vancomycin in humans.

The *vanB* gene cluster has been found to be associated with various DNA elements ranging from 60 to 250 kb (3, 4, 23, 29). A 64-kb composite transposon (Tn1547), bounded by IS256-like and IS16 elements, has been shown to transpose the *vanB* gene cluster from the chromosome to a plasmid (23). A 55-MDa transferable plasmid was found to contain both *vanB* and a gentamicin resistance gene (29). A 27-kb conjugative trans-

poson (Tn5382) which may be part of a 160-kb plasmid containing both the *vanB* gene and the *pbp5* gene, which encodes high-level ampicillin resistance, has also been described (4). The observation that *Hae*II and *Bcl*II restriction maps of the *vanB* gene cluster in various *E. faecium* isolates are identical suggests that the entire *vanB2* gene cluster is transferred from one isolate to another. In addition to horizontal transfer of the vancomycin resistance gene due to DNA transposition or plasmid transfer, clonal dissemination of a certain strain is another mode of transmission. The finding that the majority (65%) of VanB isolates examined in this study belong to the same PFGE type (type I) is one example of this type of transmission. Based on the results of this study, it is recommended that both the mobile vancomycin resistance determinants and the PFGE types should be determined for epidemiological studies of VRE infections.

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