

## *vanM*, a New Glycopeptide Resistance Gene Cluster Found in *Enterococcus faecium*<sup>▽†</sup>

Xiaogang Xu,<sup>1</sup> Dongfang Lin,<sup>1</sup> Guoquan Yan,<sup>2,3</sup> Xinyu Ye,<sup>1</sup> Shi Wu,<sup>1</sup> Yan Guo,<sup>1</sup> Demei Zhu,<sup>1</sup> Fupin Hu,<sup>1</sup> Yingyuan Zhang,<sup>1</sup> Fu Wang,<sup>1</sup> George A. Jacoby,<sup>4</sup> and Minggui Wang<sup>1,3\*</sup>

Institute of Antibiotics, Huashan Hospital,<sup>1</sup> Department of Chemistry,<sup>2</sup> and Institute of Biomedical Sciences,<sup>3</sup> Fudan University, Shanghai, China, and Lahey Clinic, Burlington, Massachusetts<sup>4</sup>

Received 4 December 2009/Returned for modification 3 March 2010/Accepted 13 August 2010

Since glycopeptide-resistant enterococci (GRE) were reported in 1988, they have appeared in hospitals worldwide. Seven *van* gene cluster types (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, and *vanL*) are currently known. We investigated a clinical strain of *Enterococcus faecium* Efm-HS0661 that was isolated in 2006 from an inpatient with intra-abdominal infection in Shanghai. It was resistant to most antimicrobials, including vancomycin (MIC, >256 µg/ml) and teicoplanin (MIC, 96 µg/ml). Glycopeptide resistance could be transferred to *E. faecium* BM4105RF by conjugation. The donor and its transconjugant were negative by PCR for the known *van* genes. By cloning and primer walk sequencing, we discovered a novel *van* gene cluster, designated *vanM*. The *vanM* ligase gene was 1,032-bp in length and encoded a 343-amino-acid protein that shared 79.9, 70.8, 66.3, and 78.8% amino acid identity with VanA, VanB, VanD, and VanF, respectively. Although the *vanM* DNA sequence was closest to *vanA*, the organization of the *vanM* gene cluster was most similar to that of *vanD*. Upstream from the *vanM* cluster was an IS1216-like element, which may play a role in the dissemination of this resistance determinant. Liquid chromatography-mass spectrometry analysis of peptidoglycan precursors extracted from the VanM-type strain Efm-HS0661 treated with vancomycin or teicoplanin revealed a modified precursor (UDP-*N*-acetylmuramic acid [MurNAc]-tetrapeptide-D-Lac), indicating that VanM, like VanA, confers glycopeptide resistance by the inducible synthesis of precursor ending in D-Ala-D-Lac.

Glycopeptide-resistant enterococci have emerged as important nosocomial pathogens since the late 1980s (18, 25). The glycopeptides vancomycin and teicoplanin act by binding to the D-alanyl-D-alanine (D-Ala-D-Ala) terminus of intermediates in peptidoglycan formation, inhibiting cell wall cross-linking (8). Resistance to glycopeptide antibiotics in enterococci results from the synthesis of peptidoglycan precursors with low affinity for these antibiotics, mediated by different *van* gene clusters (4, 8). Seven types of gene clusters conferring glycopeptide resistance have been described in enterococci based on DNA sequence and organization. They are designated according to the name of the ligase gene, which encodes either a D-Ala:D-Lac (*vanA*, *vanB*, and *vanD*) or a D-Ala:D-Ser (*vanC*, *vanE*, *vanG*, and *vanL*) ligase for the synthesis of peptidoglycan precursors with low affinity for glycopeptides. The *vanA*, *vanB*, and *vanD* gene clusters contain genes for a two-component regulatory system (*vanR* and *vanS*), three resistance genes (*vanH*, encoding dehydrogenase; *vanA*, *vanB*, or *vanD*, encoding ligase; *vanX*, encoding DD-dipeptidase); an accessory gene (*vanY*); and the *vanZ* gene, which is present in the *vanA* gene cluster, whereas the *vanW* gene is found only in the *vanB* operon (8). Another *van* gene cluster, *vanF*, has been described in a biopesticide, *Paenibacillus popilliae* (20), but has not yet been found in enterococci.

Although *vanA*, *vanB*, and *vanD* gene clusters involve genes encoding similar enzymatic functions, they can be distinguished on the basis of the level and inducibility of resistance to glycopeptides and by the location of the genes (8, 11). The VanA type is characterized by acquired resistance to high levels of both vancomycin and teicoplanin, and it is induced by either vancomycin or teicoplanin. The VanB type is characterized by acquired resistance to various concentrations of vancomycin but not to teicoplanin and is induced only by vancomycin (1). VanA- and VanB-type resistances are the most common glycopeptide resistance phenotypes (23). The genes encoding the VanA and VanB phenotypes are carried on transposons, which may be found on plasmids or inserted into the chromosome (2).

The VanD type is characterized by resistance to intermediate levels of vancomycin and to low levels of teicoplanin and is expressed constitutively (10, 12, 21). *vanD* genes appear to be located on the chromosome and are not transferable to other enterococci (12). This could explain the scarcity of recognized VanD strains in contrast to the widespread and high prevalence of VanA and VanB strains.

Glycopeptide-resistant enterococci (GRE) were rare in China a few years ago (22), although vancomycin has been used in clinical practice for multidrug-resistant Gram-positive infections in mainland China for decades. In recent years, GRE strains isolated from hospitalized patients in mainland China have been increasing (6, 22, 27). In the course of determining the genotypes of glycopeptide-resistant *Enterococcus faecium* strains isolated from a teaching hospital in Shanghai, we found that several strains were negative by PCR for *vanA*, *vanB*, and *vanD* genes. The responsible glycopeptide resistance

\* Corresponding author. Mailing address: Institute of Antibiotics, Huashan Hospital, Fudan University, 12 M. Wulumuqi Rd., Shanghai 200040, P. R. China. Phone: (86-21)-52888195. Fax: (86-21)-62482859. E-mail: mgwang@fudan.edu.cn.

<sup>▽</sup> Published ahead of print on 23 August 2010.

<sup>†</sup> The authors have paid a fee to allow immediate free access to this article.

TABLE 1. Characteristics of the 10 isolates of glycopeptide-resistant *E. faecium*, recipient BM4105, and transconjugant BM-HS0661<sup>a</sup>

Strain	Source	Date of isolation (yr/mo/day)	Ward	Gene cluster type	MLST sequence type	PFGE type	MIC ( $\mu\text{g/ml}$ )					
							VAN	TEC	LNZ	RIF	NIT	DOX
Efm-HS05417	Urine	2005/12/6	Neurology	<i>vanA</i>	ST17	A	>256	96	1.5	0.25	128	0.19
Efm-HS05446	Urine	2005/12/26	Neurology	<i>vanA</i>	ST17	B	>256	96	1.5	4	16	6
Efm-HS0649	Exudate	2006/1/27	General Surgery	<i>vanA</i>	ST17	B	>256	64	1.5	4	>512	6
Efm-HS06188	Urine	2006/6/1	ICU	<i>vanA</i>	ST78	A	>256	64	1.5	0.19	128	0.125
Efm-HS0661	Exudate	2006/2/4	General Surgery	<i>vanM</i>	ST78	C	>256	96	1.5	4	64	0.125
Efm-HS0761	Urine	2007/3/9	Neurosurgery	<i>vanM</i>	ST78	C	>256	64	1.5	3	64	0.125
Efm-HS07216	Urine	2007/7/9	Neurosurgery	<i>vanM</i>	ST78	D	>256	256	1.5	>32	128	0.19
Efm-HS0847	Urine	2008/2/5	ICU	<i>vanM</i>	ST78	E	>256	>256	1.0	>32	64	0.094
Efm-HS08257	Urine	2008/8/5	Neurosurgery	<i>vanM</i>	ST341	F	>256	0.75	2	8	196	0.19
Efm-HS08369	Urine	2008/10/22	Neurology	<i>vanM</i>	ST18	G	>256	>256	2	16	32	32
BM4105RF	NA	NA	NA	NA	NA	NA	0.75	0.38	1.5	>32	16	0.094
BM-HS0661	NA	NA	NA	<i>vanM</i>	NA	NA	>256	48	1.0	>32	16	0.094

<sup>a</sup> VAN, vancomycin; TEC, teicoplanin; LNZ, linezolid; RIF, rifampin; CIP, ciprofloxacin; NIT, nitrofurantoin; DOX, doxycycline; NA, not applicable; ICU, intensive care unit.

gene was cloned and sequenced from strain Efm-HS0661 and found to be novel. This new glycopeptide resistance gene cluster has been termed the *vanM* cluster. Of 10 unique GRE clinical strains isolated at our teaching hospital from 2005 to 2008, 6 carried *vanM*, which plays a role in the increasing GRE prevalence in China.

#### MATERIALS AND METHODS

**Bacterial strains and plasmids.** Ten glycopeptide-resistant *E. faecium* clinical isolates and 100 glycopeptide-sensitive clinical strains (36 of *Enterococcus faecalis* and 64 of *E. faecium*) were collected from individual patients at the teaching hospital of Fudan University in Shanghai between 2005 and 2008. *Escherichia coli* DH5 $\alpha$  (Invitrogen), *E. faecium* BM4105RF (22), and plasmid pRB473 (Gram<sup>+</sup>/Gram<sup>-</sup> shuttle vector; Amp<sup>r</sup> Chl<sup>r</sup>) (5) were used in cloning or mating experiments.

**Conjugation and MIC determination.** To determine if the glycopeptide resistance in clinical strains was transferable, filter-mating experiments were carried out with *E. faecium* BM4105RF (Fus<sup>r</sup>, Rif<sup>r</sup>) as the recipient. Transconjugants were selected on brain heart infusion (BHI) agar (Oxoid, Basingstoke, England) plates containing rifampin (100  $\mu\text{g/ml}$ ) or fusidic acid (10  $\mu\text{g/ml}$ ) for counterselection and vancomycin (10  $\mu\text{g/ml}$ ) to select for plasmid-encoded resistance.

The MICs for all antimicrobial agents for the donor, recipient, and transconjugant strains were measured by Etest on Mueller-Hinton agar (Oxoid, Basingstoke, England) according to the manufacturer's instructions (AB Biodisk, Sweden) and interpreted according to recommendations of the Clinical and Laboratory Standards Institute (CLSI).

**PFGE and MLST analysis.** Clinical strains and transconjugants were typed by the pulsed-field gel electrophoresis (PFGE) of genomic DNA using the CHEF mapper system (Bio-Rad). Agarose plugs were prepared with proteinase K (Merck, Germany) at 1 mg/ml and digested with SmaI, and the DNA was subjected to electrophoresis at 6 V/cm, 14°C, in a 1.0% agarose gel (Bio-Rad) with pulse times of 5 to 30 s for 22 h. Banding patterns were interpreted using the criteria devised by Tenover et al. (24).

Multilocus sequence typing (MLST) of *E. faecium* isolates was performed as previously reported (16). The alleles and sequence types (STs) were analyzed and determined through the MLST database (<http://efaecium.mlst.net/>).

***van* gene cluster sequencing.** To determine the *van* genotype present in the GRE clinical isolates, PCR with specific primers for *vanA* and *vanB* was used (4). A set of universal primers was designed in this study to detect D-Ala:D-Lac ligase genes in strains negative for *vanA* and *vanB*, NvanF (5'-GTTTGGGG GTTGCTCAGAGG3') and NvanR (5'-TCACCCCTTTAACGCTAATACGA TC3'), which selected a nucleotide region from position 27 to 1032 of the *vanA* gene (GenBank accession number AM296544) as the target to produce a PCR product of 1,006 bp. Purified PCR products were sequenced on both strands to determine the glycopeptide resistance genotype. The sequence of DNA adjacent to the *vanM* ligase gene was determined with a series of outward-facing primers starting from both sides of the *vanM* gene on plasmid DNA extracted from the transconjugant BM-HS0661 with the plasmid midi kit (Qiagen, Germany).

To confirm that the donor contained the same sequence as that found in the transconjugant, plasmid DNA extracted from Efm-HS0661 was digested with

HindIII and PshAI, and the fragments were ligated into pRB473 digested with HindIII and SmaI (with blunt ends similar to those produced by PshAI). The recombinant plasmid pRB-0661 was transformed into *E. coli* DH5 $\alpha$ , and the transformants were screened by PCR with primers NvanF and NvanR for the *van* gene. The plasmid DNA extracted from the *van* gene-positive transformant was sequenced.

Sequence analyses and comparisons to known sequences were performed with the BLAST programs at the National Center for Biotechnology Information (NCBI).

**Extraction and analysis of peptidoglycan precursors.** *E. faecium* Efm-HS0661 was grown in BHI broth (Oxoid, Basingstoke, England) to an optical density at 650 nm (OD<sub>650</sub>) of 0.4 and was treated with vancomycin (100  $\mu\text{g/ml}$ ) or teicoplanin (50  $\mu\text{g/ml}$ ) for an additional 120 min. Peptidoglycan precursors were extracted as previously described (3). The peptidoglycan precursors were analyzed by liquid chromatography-mass spectrometry (LC-MS). The experiments were performed on an LC-20AD system (Shimadzu, Japan) connected to an LTQ Orbitrap XL mass spectrometer (Thermo, Bremen, Germany) equipped with an online electrospray ion source (Michrom Bioresources, Auburn, GA). The samples were separated with a Zorbax 300SB-C<sub>18</sub> column (1.0 by 250 mm) from Agilent. The peptides were eluted with a linear gradient, starting from 0 to 100% B in 18 min (eluent A, 10 mM ammonium acetate, pH 5; eluent B, 2% acetonitrile in 10 mM ammonium acetate, pH 5). The column flow rate was maintained at 30  $\mu\text{l/min}$ . The electrospray voltage of 4.0 kV versus the inlet of the mass spectrometer was used. Survey full-scan MS spectra ( $m/z$  400 to 1,800) were acquired in the orbitrap with a mass resolution of 100,000 at  $m/z$  400. MS/MS spectra were acquired in the LTQ. The UDP-*N*-acetylmuramic acid (MurNAc) peptide structures were deduced from their molecular mass determined by MS/MS performed on singly charged protonated molecules. The quantification of the molecules under study was carried out by extracting selected ions (extracted ion chromatograms; XIC) from the respective full-scan MS chromatograms. The Xcalibur 2.0.7 software (Thermo Fisher Scientific) was used for acquiring and handling the data.

**Nucleotide sequence accession numbers.** The sequences of the *vanM* gene cluster of vancomycin-resistant *E. faecium* Efm-HS0661 have been submitted to the GenBank database and assigned accession number FJ349556.

#### RESULTS AND DISCUSSION

**Characterization of glycopeptide-resistant *E. faecium*.** The characteristics of the 10 isolates of glycopeptide-resistant *E. faecium* are listed in Table 1. All 10 patients had been administered glycopeptide antibiotics and/or broad-spectrum cephalosporins. All 10 *E. faecium* isolates were resistant to vancomycin, erythromycin (MIC > 256  $\mu\text{g/ml}$ ), ciprofloxacin (MIC > 32  $\mu\text{g/ml}$ ), ampicillin (MIC > 32  $\mu\text{g/ml}$ ), and gentamicin (MIC > 1,024  $\mu\text{g/ml}$ ), and 9 of them were resistant to teicoplanin, but none was resistant to linezolid (Table 1). The glycopeptide resistance of Efm-HS0661 could

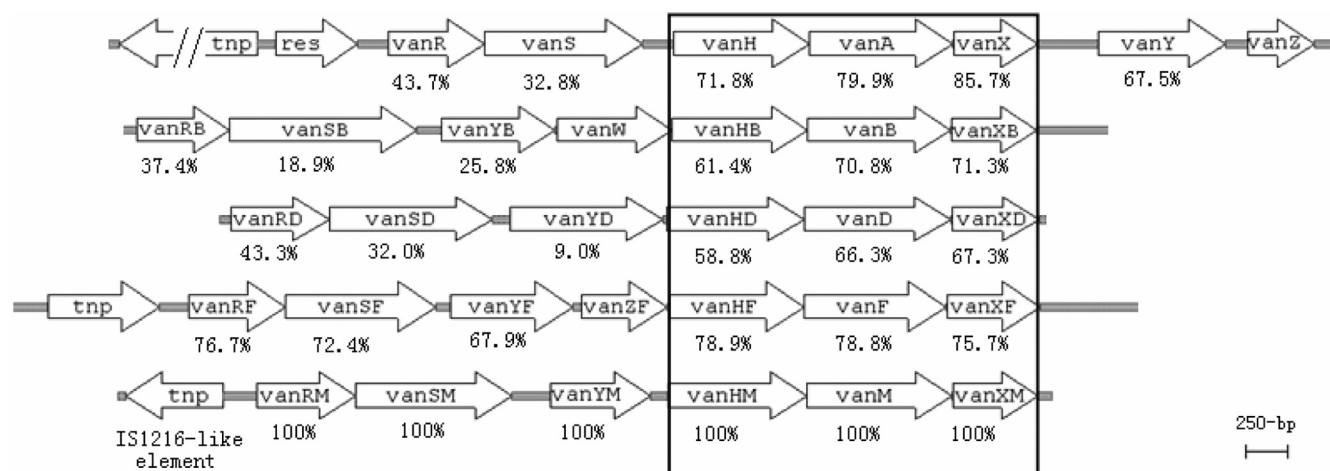


FIG. 1. Alignment of vancomycin resistance gene clusters of *vanA* (M97297), *vanB* (EFU35369), *vanD* (AF130997), *vanF* (AF155139), and *vanM* (FJ349556). The percent amino acid identities to *vanRM*, *vanSM*, *vanYM*, *vanHM*, *vanM*, and *vanXM* products are shown below the respective genes.

be transferred by conjugation to *E. faecium* BM4105 RF, but the resistance to other drugs could not.

**Sequencing and characterization of *vanM*.** Efm-HS0661, a GRE clinical isolate, was negative for *vanA* and *vanB* by PCR amplification. The strain amplified with primers NvanF and NvanR yielded a 1,006-bp product, which was sequenced on both strands. Using outward-facing primers that matched both ends of this sequence, primer walk sequencing was carried out on the original unmodified plasmid DNA extracted from a transconjugant, BM-HS0661. The analysis of the sequence and its putative protein indicated the presence of a novel *van* gene, designated *vanM*. *vanM* was 1,032 bp in length and encoded a 343-amino-acid protein. *vanM* had 81.8, 72.5, 67.7, and 78.2% nucleotide identity with *vanA*, *vanB*, *vanD*, and *vanF*, respectively. The VanM protein had the highest identity (79.9%) to VanA and the lowest similarity (66.3%) to VanD (Fig. 1). The PEKG motif specifically found in the  $\omega$  loop of D-Ala:D-Lac ligases in VanA, VanB, and VanD also was present in VanM between positions 249 and 252 (7, 13).

**Adjacent sequence analysis.** By sequencing with a series of outward-facing primers from both sides of the *vanM* gene, a 6,592-bp DNA fragment was obtained. Cleavage site analysis showed that there were two unique restriction sites, HindIII and PshAI, at the ends of the fragment. Plasmid DNA extracted from clinical isolate Efm-HS0661 was digested with HindIII and PshAI, and the 6,280-bp fragment was ligated into pRB473 digested with HindIII and SmaI. The *vanM*-positive recombinant plasmid was sequenced. The sequences from Efm-HS0661 and BM-HS0661 were identical, confirming that the *vanM*-bearing plasmid had been transferred from donor to transconjugant.

The analysis of the 6,592-bp fragment revealed seven open reading frames (ORFs). Based on the identity of the deduced amino acid sequences to those of the proteins encoded by the *vanA*, *vanB*, and *vanD* gene clusters, six ORFs were assigned to *vanRM*, *vanSM*, *vanYM*, *vanHM*, *vanM*, and *vanXM*, respectively. VanRM and VanSM were similar to VanR and VanS (Fig. 1), which are part of a two-component regulatory system in the *vanA* gene cluster. VanYM was similar to VanY, displaying DD-carboxypeptidase activity (26). The identities

among the VanHM, VanH, VanHB, and VanHD dehydrogenases; the VanM, VanA, VanB, and VanD ligases; and the VanXM, VanXA, VanXB, and VanXD DD-dipeptidases were from 58.8 to 85.7% (Fig. 1). The three conserved residues, Arg, Glu, and His, which were predicted to participate in substrate binding and catalysis of D-Lac dehydrogenases (7), were present in VanHM at positions 235, 264, and 296, respectively.

The organization of the *vanM* gene cluster was most similar to that of the *vanD* gene cluster, although proteins encoded by genes in the *vanM* gene cluster shared higher deduced amino acid identity with those encoded by the *vanA*, *vanB*, and *vanF* gene clusters than with those of the *vanD* gene cluster (Fig. 1). No genes similar to *vanZ* or *vanW* present in the *vanA* and *vanB* gene clusters were found. Therefore, the *vanM* gene cluster may have a common ancestral origin with the *vanA*, *vanB*, and *vanD* gene clusters, but each has evolved independently. Meanwhile, VanRM and VanSM have more identity with VanRF and VanSF, which indicate that the genes in *Paenibacillus popilliae* are a precursor to the vancomycin resistance genes in VanM-type enterococci. The use of *P. popilliae* biopesticidal preparations in agricultural practice may have an impact on bacterial resistance in human pathogens (20).

Upstream from the *vanM* gene cluster was an insertion sequence that was identical to an IS1216-like element (Fig. 1). A similar IS element, IS1216V, is widespread among VanA-type GRE and may play an important role in the dissemination of resistance determinants by the transposon-mediated fusion of *vanA* plasmids with other plasmids (9, 15, 19). In this study, we observed that the plasmids bearing *vanM* in donor and transconjugant were of different sizes (data not shown), which may have resulted from an IS1216-mediated transposition event (17). Another interesting phenomenon is that a *vanM*-type strain, Efm-HS08257, had a VanB phenotype (vancomycin MIC, >256  $\mu\text{g/ml}$ ; teicoplanin MIC, 0.75  $\mu\text{g/ml}$ ). Sequence analysis showed that there was a 173-base insertion between the IS1216-like element and *vanRM* of the *vanM* gene cluster in this strain (data not shown). This insertion might induce the change of phenotype, and we are carrying out a further study to confirm the relationship.

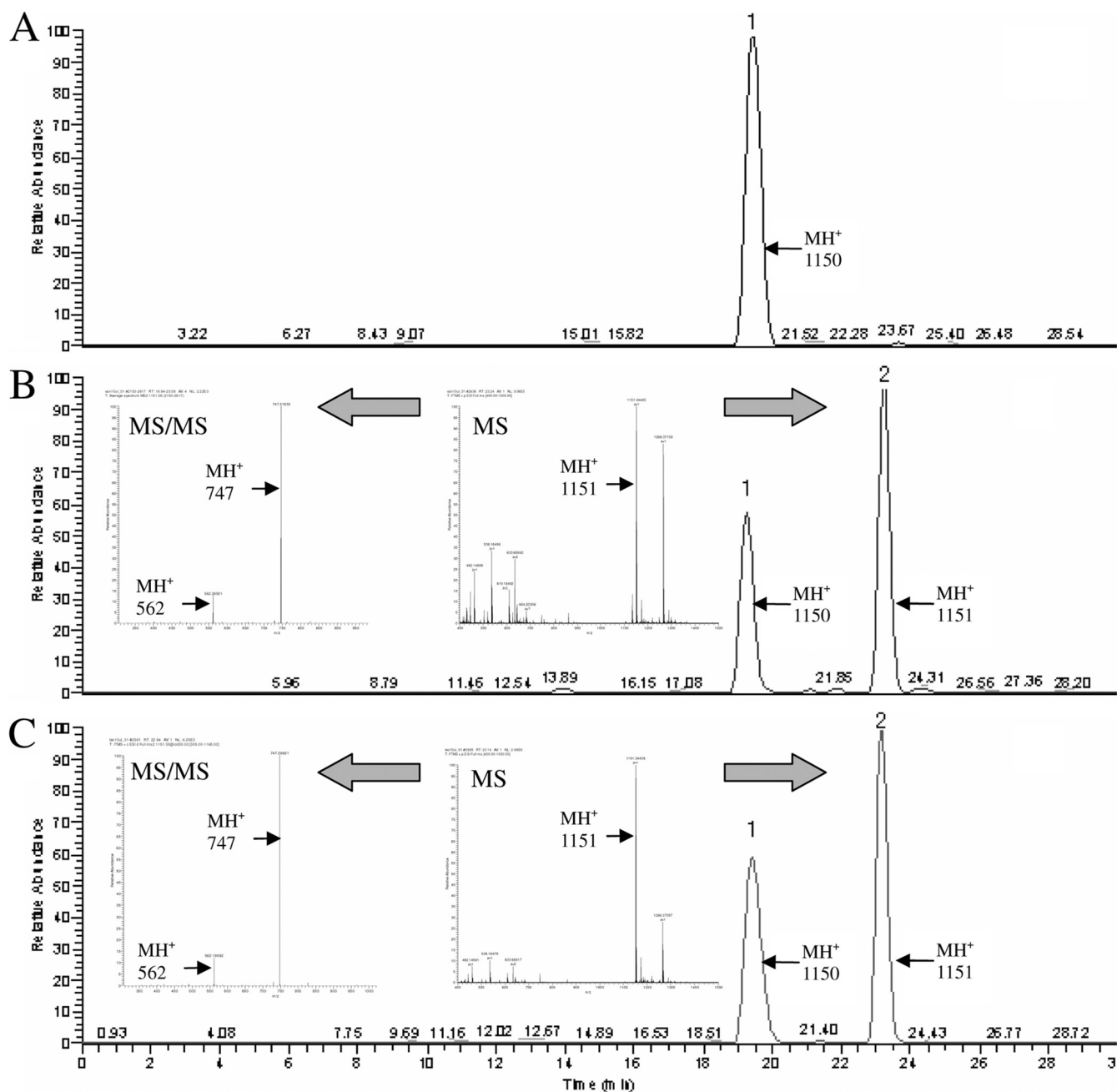


FIG. 2. Analysis of peptidoglycan precursors by liquid chromatography-mass spectrometry. Shown is the XIC of protonated molecules at  $m/z$  1,150 ( $MH^+$ ) and 1,151 ( $MH^+$ ), which correspond to UDP-MurNAc-tetrapeptide-D-alanine (peak 1) and UDP-MurNAc-tetrapeptide-D-lactate (peak 2), in the peptidoglycan precursors (A to C). The  $m/z$  value of the selected precursor ions for the MS/MS analysis is 1,151 ( $MH^+$ ). The protonated molecule could produce two protonated molecules at  $m/z$  562 and 747, which is consistent with the UDP-MurNAc-tetrapeptide-D-lactate fragments tetrapeptide-D-lactate and MurNAc-tetrapeptide-D-lactate, respectively. The precursors were extracted from untreated *E. faecium* Efm-HS0661 (A), Efm-HS0661 treated with vancomycin (B), and Efm-HS0661 treated with teicoplanin (C).

**Distribution of *vanM*.** By PCR with primers NvanF and NvanR and sequencing, *vanM* was found in six glycopeptide-resistant *E. faecium* strains, and *vanA* was found in the other four strains (Table 1). No *van* gene (*vanA*, *vanB*, *vanD*, *vanF*, or *vanM*) was found in the 100 glycopeptide-sensitive *Enterococcus* spp. clinical isolates.

Of the 10 vancomycin-resistant GRE strains, the PFGE profiles of SmaI-digested chromosomal DNA divided them into

seven types (A to G), while MLST revealed four different sequence types. One ST78 isolate and three ST17 isolates harbored *vanA* genes. Four ST78 isolates, one ST18 isolate, and one ST341 isolate harbored *vanM* genes (Table 1).

**Analysis of peptidoglycan precursors.** The peptidoglycan precursors in *E. faecium* Efm-HS0661 and vancomycin- or teicoplanin-treated Efm-HS0661 were analyzed by LC-MS. The results are presented in Fig. 2. The full-scan mass spec-

trum of the peptidoglycan precursors extracted from vancomycin or teicoplanin treated Efm-HS0661 showed a protonated molecule at  $m/z$  1,151 ( $MH^+$ ), which indicates a molecular weight of 1,150, which is consistent with UDP-MurNAc-tetrapeptide-D-lactate (1,150 Da). This protonated molecule was not detected in the peptidoglycan precursors extracted from Efm-HS0661 not treated with glycopeptide. MS-MS analysis showed that the protonated molecule at  $m/z$  1,151 ( $MH^+$ ) could produce two protonated molecules at  $m/z$  562 and 747, which also were consistent with the UDP-MurNAc-tetrapeptide-D-lactate fragments tetrapeptide-D-lactate and MurNAc-tetrapeptide-D-lactate, respectively (14). These data indicate that the *vanM* gene cluster confers glycopeptide resistance by the production of peptidoglycan precursors ending in D-Ala-D-Lac, which could be induced by either vancomycin or teicoplanin.

In conclusion, a new glycopeptide resistance determinant, the *vanM* gene cluster, was characterized from a glycopeptide-resistant clinical isolate of *E. faecium*. The *vanM* gene encoded a D-Ala:D-Lac ligase related to VanA, VanB, and VanD and could be transferred by conjugation.

#### ACKNOWLEDGMENTS

This work was supported by grant 2005CB0523101 (to M.W.) from the National Basic Research Program of China from the Ministry of Science and Technology, China, grant LJ06052 (to M.W.) from the Shanghai Municipal Health Bureau, and grant 09411967900 (to X.X.) from the Shanghai Municipal Science and Technology Commission.

#### REFERENCES

- Arthur, M., F. Depardieu, P. Reynolds, and P. Courvalin. 1996. Quantitative analysis of the metabolism of soluble cytoplasmic peptidoglycan precursors of glycopeptide-resistant enterococci. *Mol. Microbiol.* **21**:33–44.
- Arthur, M., P. Reynolds, and P. Courvalin. 1996. Glycopeptide resistance in enterococci. *Trends Microbiol.* **4**:401–407.
- Billot-Klein, D., L. Gutmann, E. Collatz, and J. Van Hejenoort. 1992. Analysis of peptidoglycan precursors in vancomycin resistant enterococci. *Antimicrob. Agents Chemother.* **36**:1487–1490.
- Boyd, D. A., B. M. Willey, D. Fawcett, N. Gillani, and M. R. Mulvey. 2008. Molecular characterization of *Enterococcus faecalis* N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, *vanL*. *Antimicrob. Agents Chemother.* **52**:2667–2672.
- Brückner, R. 1997. Gene replacement in *Staphylococcus carnosus* and *Staphylococcus xylosum*. *FEMS Microbiol. Lett.* **151**:1–8.
- Cao, B., Y. Liu, S. Song, R. Li, H. Wang, and C. Wang. 2008. First report of clinical and epidemiological characterisation of vancomycin-resistant enterococci from mainland China. *Int. J. Antimicrob. Agents.* **32**:279–281.
- Casadewall, B., and P. Courvalin. 1999. Characterization of the *vanD* glycopeptide resistance gene cluster from *Enterococcus faecium* BM4339. *J. Bacteriol.* **181**:3644–3648.
- Courvalin, P. 2006. Vancomycin resistance in gram-positive cocci. *Clin. Infect. Dis.* **42**(Suppl. 1):S25–S34.
- Darini, A. L., M. F. Palepou, and N. Woodford. 2000. Effects of the movement of insertion sequences on the structure of VanA glycopeptide resistance elements in *Enterococcus faecium*. *Antimicrob. Agents Chemother.* **44**:1362–1364.
- Depardieu, F., M. Kolbert, H. Prүүл, J. Bell, and P. Courvalin. 2004. VanD-type vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob. Agents Chemother.* **48**:3892–3904.
- Depardieu, F., I. Podglajen, R. Leclercq, E. Collatz, and P. Courvalin. 2007. Modes and modulations of antibiotic resistance gene expression. *Clin. Microbiol. Rev.* **20**:79–114.
- Depardieu, F., P. E. Reynolds, and P. Courvalin. 2003. VanD-type vancomycin-resistant *Enterococcus faecium* 10/96A. *Antimicrob. Agents Chemother.* **47**:7–18.
- Evers, S., B. Casadewall, M. Charles, S. Dutka-Malen, M. Galimand, and P. Courvalin. 1996. Evolution of structure and substrate specificity in D-alanine:D-alanine ligases and related enzymes. *J. Mol. Evol.* **42**:706–712.
- Handwerger, S., M. J. Pucci, K. J. Volk, J. Liu, and M. S. Lee. 1992. The cytoplasmic peptidoglycan precursor of vancomycin-resistant *Enterococcus faecalis* terminates in lactate. *J. Bacteriol.* **174**:5982–5984.
- Heaton, M. P., L. F. Discotto, M. J. Pucci, and S. Handwerger. 1996. Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an *Enterococcus faecium* sex pheromone-response plasmid. *Gene* **171**:9–17.
- Homan, W. L., D. Tribe, S. Poznanski, M. Li, G. Hogg, E. Spalburg, J. D. Van Embden, and R. J. Willems. 2002. Multilocus sequence typing scheme for *Enterococcus faecium*. *J. Clin. Microbiol.* **40**:1963–1971.
- Jensen, L. B. 1998. Internal size variations in Tn1546-like elements due to the presence of IS1216V. *FEMS Microbiol. Lett.* **169**:349–354.
- Leclercq, R., E. Derlot, J. Duval, and P. Courvalin. 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N. Engl. J. Med.* **319**:157–161.
- Palepou, M. F., A. M. Adebisi, C. H. Tremlett, L. B. Jensen, and N. Woodford. 1998. Molecular analysis of diverse elements mediating VanA glycopeptide resistance in enterococci. *J. Antimicrob. Chemother.* **42**:605–612.
- Patel, R., K. Piper, F. R. Cockerill III, J. M. Steckelberg, and A. A. Yousten. 2000. The biopesticide *Paenibacillus popilliae* has a vancomycin resistance gene cluster homologous to the enterococcal VanA vancomycin resistance gene cluster. *Antimicrob. Agents Chemother.* **44**:705–709.
- Perichon, B., P. Reynolds, and P. Courvalin. 1997. VanD-type glycopeptide-resistant *Enterococcus faecium* BM4339. *Antimicrob. Agents Chemother.* **41**:2016–2018.
- Qu, T. T., Y. G. Chen, Y. S. Yu, H. X. Lv, X. Q. Dong, Z. Xiao, H. Q. Gu, and L. J. Li. 2007. Molecular characterization of vancomycin-resistant enterococci in Hangzhou, China. *J. Antimicrob. Chemother.* **60**:1403–1405.
- Ribeiro, T., M. Abrantes, M. F. Lopes, and M. T. Crespo. 2007. Vancomycin-susceptible dairy and clinical enterococcal isolates carry *vanA* and *vanB* genes. *Int. J. Food Microbiol.* **113**:289–295.
- Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
- Uttley, A. H., C. H. Collins, J. Naidoo, and R. C. George. 1988. Vancomycin-resistant enterococci. *Lancet* **i**:57–58.
- Wright, G. D., C. Molinas, M. Arthur, P. Courvalin, and C. T. Walsh. 1992. Characterization of VanY: a DD-carboxypeptidase from vancomycin resistant *Enterococcus faecium* BM4147. *Antimicrob. Agents Chemother.* **36**:1514–1518.
- Zheng, B., H. Tomita, Y. H. Xiao, S. Wang, Y. Li, and Y. Ike. 2007. Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolates from mainland China. *J. Clin. Microbiol.* **45**:2813–2818.