Infection, Genetics and Evolution 68 (2019) 145–152

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Characterization of *vanM* carrying clinical *Enterococcus* isolates and diversity of the suppressed *vanM* gene cluster

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ARTICLE INFO

Keywords:
Suppressed *vanM* gene cluster
Enterococci
IS*1216E*
Vancomycin resistance

ABSTRACT

Here we report the prevalence of the suppressed *vanM* gene cluster as a reservoir of vancomycin resistance genes. Among 1284 clinical isolates of enterococci from four hospitals in Hangzhou, China, 55 isolates of *Enterococcus faecium* and one isolate of *Enterococcus faecalis* were screened positive for the *vanM* genotype. Antimicrobial susceptibility testing showed that 55 of the 56 *vanM*-positive isolates were susceptible to vancomycin and teicoplanin. Most of them (54/56) belonged to the main epidemic lineage CC17, mostly the ST78 type. The *vanM* gene clusters in the 55 vancomycin-susceptible isolates showed sequence diversity owing to different insertion locations of IS*1216E*. The *vanM* transposons could be classified into five types and they all carried two or more IS*1216E* elements, leading to complete or partial deletions of *vanR*, *vanS*, or *vanX*. Quantitative reverse transcription polymerase chain reaction showed that the expression level of *vanM* was significantly lower in the vancomycin-susceptible isolates than in the vancomycin-resistant isolate. Considering the prevalence of the *vanM* genotype and the potential for conversion to a resistant phenotype, *vanM* might act as an important determinant of glycopeptide resistance in the future. It is essential to strengthen the surveillance of *vanM*-containing enterococci to control the dissemination of vancomycin resistance.

1. Introduction

Vancomycin-resistant enterococci (VRE) are of global importance as nosocomial pathogens (Ranotkar et al., 2014). Eight gene clusters (*vanA*, *vanB*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*) have been associated with acquired vancomycin resistance, among which *vanA* and *vanB* are the most common (Ranotkar et al., 2014; Xu et al., 2010). VanA type is characterized by acquired resistance to high levels of both vancomycin and teicoplanin, whereas the VanB positive enterococci showed a wide range of MICs to vancomycin while retaining susceptibility to teicoplanin (Cetinkaya et al., 2000). In China, VRE strains have increasingly been isolated from hospitalized patients in recent years and they have mainly carried the *vanA* gene cluster (Kang et al., 2014; Yang et al., 2015). In 2010, the *vanM* gene cluster was first reported in a clinical *Enterococcus faecium* (*E. faecium*) strain isolated in 2006 from an inpatient with intra-abdominal infection in Shanghai, China (Xu et al., 2010). The *vanM*-type isolates showed similar antimicrobial susceptibility patterns to the *vanA* types, with resistance to both vancomycin and teicoplanin. Subsequently, *vanM*-type VRE isolates were also reported in Singapore (Teo et al., 2011). In 2015, the prevalence of *van* gene clusters was investigated in 70 vancomycin-resistant *Enterococcus faecium* (VREm) strains isolated from nine Shanghai hospitals in China between 2006–2014 and *vanM* was found to be more prevalent than *vanA* (64.3% vs. 35.7%) (Chen et al., 2015). These observations suggested that *vanM*-type VREm had disseminated in Shanghai, China. Recently, *vanM*-carrying VREm strains were reported to cause the emergence of infections and environmental contamination in an intensive care unit in Hangzhou (Zhang et al., 2018). The *vanM* transposons in two isolates from a patient and other environmental samples were similar to that of *E. faecium* Efm-HS0661 from Shanghai, suggesting that *vanM* gene might spread between Shanghai and Hangzhou in China. Enhanced hospital infection controls and active surveillance of *vanM*-carrying enterococci become essential to prevent the dissemination of the *vanM* genes in clinical settings.

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https://doi.org/10.1016/j.meegid.2018.12.015
Received 6 June 2018; Received in revised form 24 November 2018; Accepted 11 December 2018
Available online 12 December 2018
1567-1348/

It is known that insertion sequence (IS) elements are highly mobile, which could cause structural alterations in the resistance gene, and even alteration of resistance phenotype (Nzabarushimana and Tang, 2018; Partridge et al., 2018). Recent reports have revealed that the *vanA* gene cluster is prone to insertion sequence element-mediated alterations that occasionally influence the vancomycin resistance phenotype, leading to glycopeptide susceptibility (Darini et al., 2000; Gagnon et al., 2011). An outbreak of vancomycin-susceptible enterococci containing *vanA* that are capable of undergoing conversion into a glycopeptide-resistant phenotype was recently reported in Canada (Szakacs et al., 2014). This clone was termed vancomycin-variable enterococci (VVE) due to its ability to revert to a glycopeptide-resistant phenotype, which could potentially cause serious clinical problems such as potentially escaping detection and surveillance, facilitating the horizontal spread of vancomycin resistance, and presenting a risk of treatment failure.

We were concerned about whether silencing of the vancomycin resistance gene can occur in *vanM*-carrying clinical enterococcus isolates. Therefore, in this study, we investigated the prevalence of *vanM* in clinical *Enterococcus* isolates from four hospitals in Hangzhou, China, between January 2016 and July 2017. We characterized these *vanM*-containing *Enterococcus* isolates and their *vanM* gene clusters.

2. Materials and methods

2.1. Bacterial isolates

From January 2016 until July 2017, 1284 non-duplicate *Enterococcus* isolates were collected from four different hospitals in Hangzhou, Zhejiang Province, China (585 isolates from Sir Run Run Shaw Hospital, Zhejiang University; 457 isolates from the first affiliated hospital, Zhejiang University; 131 isolates from Hangzhou First People's Hospital; and 111 isolates from Zhejiang Provincial People's Hospital).

All the isolates were screened for the presence of *vanM* by polymerase chain reaction (PCR) with the primers *vanM*-F (5′-CAGAGATT GCCAACAACATTGA-3′) and *vanM*-R (5′-TCGGGAATTGTTATACCTG CTG-3′), and the amplicons were sequenced. We retrospectively collected clinical data for the *vanM*-positive isolates, including the age and sex of the patient, the date of isolation, the involved hospitals/wards, and the infection sites.

2.2. Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) of ten antimicrobial agents (vancomycin, teicoplanin, levofloxacin, chloramphenicol, fosfomycin, ampicillin, erythromycin, tetracycline, rifampicin, and linezolid) were measured by the agar dilution method and high-level gentamicin resistance was determined by the Kirby–Bauer method for the *vanM*-positive enterococci. *E. faecalis* ATCC 29212 was used as a control. The results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute (CLSI 2017).

2.3. Molecular typing

*Sma*I-pulsed-field gel electrophoresis (PFGE) was performed to classify all *vanM*-positive isolates (Saeedi et al., 2002). Comparison of PFGE patterns was performed using BioNumerics® version 7.6 (Applied Maths, Ghent, Belgium) with the Dice coefficient, applying a band position tolerance of 1.7% and an optimization of 1.5%. Isolates were classified into the same PFGE group if their Dice similarity coefficient was over 81% (Carrico et al., 2005).

Sequence types (STs) were assigned using https://cge.cbs.dtu.dk/services/MLST/ according to their Illumina whole-genome sequences.

2.4. Whole-genome sequencing (WGS) and analysis

Single-molecule real-time sequencing using an RSII sequencer (Pacific Biosciences, Menlo Park, CA) was used to perform WGS for *E. faecium* SRR6. Genome annotation was managed using the RAST server and supplemented by the NCBI Prokaryotic Genome Annotation Pipeline. The circular map of the pEMASRR6 plasmid was generated using the CGview server.

The other *vanM*-positive isolates were sequenced using a HiSeq2000™ platform (Illumina, San Diego, CA) with 2 × 100 bp paired-end reads. The raw data were mapped to a reference sequence using CLC Genomics Workbench 8.0 (CLCbio, Aarhus, Denmark).

2.5. S1 nuclease-based plasmid analysis and Southern blot hybridization

To confirm whether the *vanM* gene was present on plasmids, S1-PFGE was performed referring to a previously published protocol (Barton et al., 1995).

2.6. Confirmation of vanM gene cluster sequences

The *vanM* transposon elements of all *vanM*-positive isolates were characterized by Sanger sequencing using three overlapping primers (M1, M2, and M3) based on the complete sequence of the plasmid pEMSRR6 (Fig. 2) and supplemented by their Illumina WGS data. The sequences of the primers M1, M2, and M3 are provided in Table S1.

2.7. Quantitative reverse transcription PCR (qRT-PCR)

qRT-PCR was performed to measure the transcriptional level of *vanM* in the clinical isolates in the absence and presence of vancomycin. The chromosomal housekeeping gene *purK* was chosen as an internal control to quantify the normalized fold expression of *vanM* in different strains. The sequences of the *Q-vanM* and *Q-purK* primers used are listed in Table S1. RNA was extracted with RNAprotect Bacteria reagent and an RNeasy® mini-kit (Qiagen, Valencia, CA, USA). Reverse transcription of the RNA was performed using random hexamers transcriptase (Takara Bio, Ōtsu, Japan), according to the manufacturer's instructions. Quantitative PCR reactions were performed using the SYBR® Premix Ex Taq™ PCR kit (Takara Bio, Japan) on a LightCycler® 480 instrument (Roche Molecular Diagnostics, Rotkreuz, Switzerland). The Ct value of each sample was measured under appropriate PCR conditions (pre-heated at 95 °C for 5 min; 40 amplification cycles at 95 °C for 5 s, 52 °C for 30 s, and 72 °C for 30 s). Data were calculated based on the ΔΔCt method.

2.8. Nucleotide sequence accession number

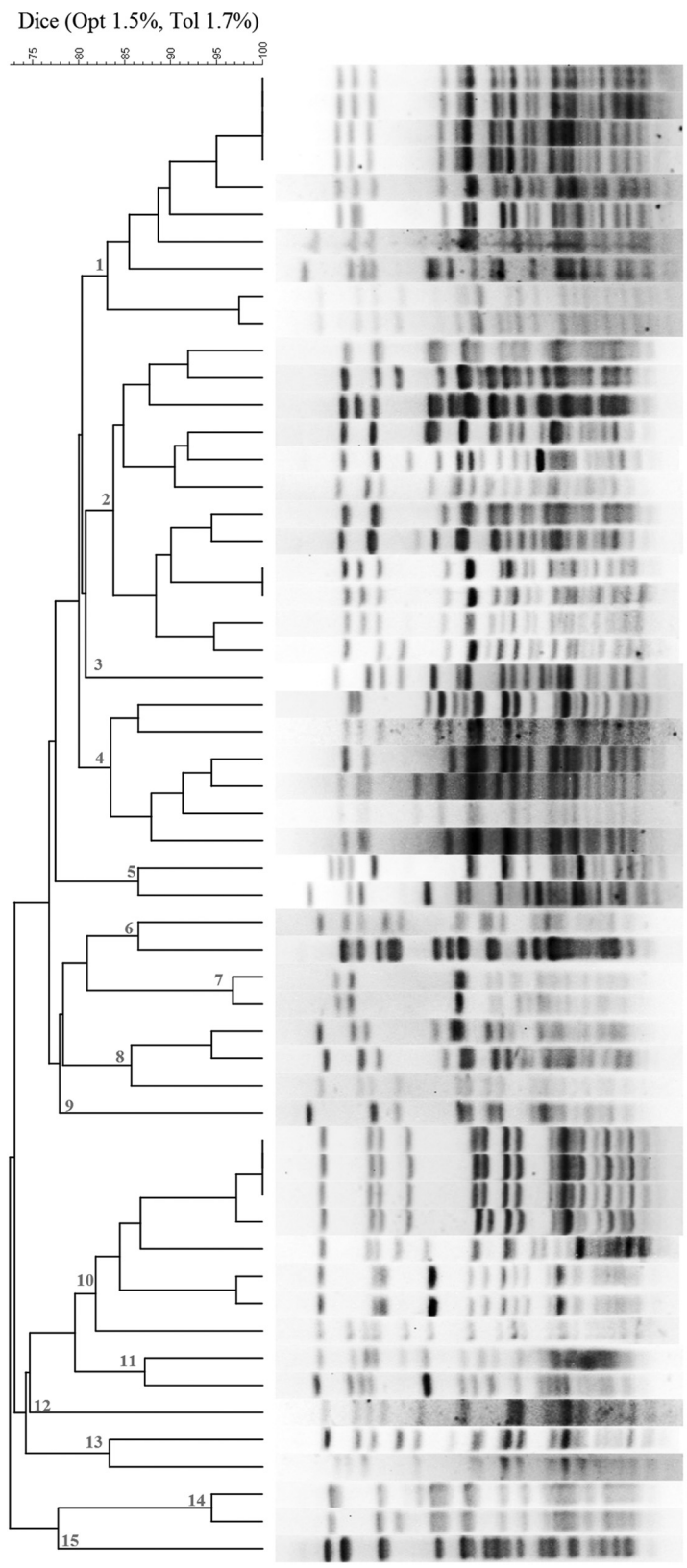
The nucleotide sequence of *E. faecium* plasmid pEMSRR6 has been submitted to the EMBL/GenBank database under the following accession number:MG640601.

3. Results

3.1. Prevalence of vanM-positive Enterococcus isolates

Among the 1284 clinical *Enterococcus* isolates from four hospitals in Hangzhou, 56 isolates including 55 *E. faecium* isolates and one *E. faecalis* isolate were positive for *vanM* genes. The origins of these 56 *vanM*-positive enterococci were relatively diverse, including urine (26/56), bile (8/56), blood (6/56), and other infected sites (Table S3).

Of the 56 *vanM*-positive isolates, 55 isolates were susceptible to vancomycin and teicoplanin. Only one *E. faecium* isolate, SRR22, was found to express high resistances to vancomycin with MIC ≥ 256 μg/ml and to teicoplanin with an MIC of 256 μg/ml. All the isolates were susceptible to linezolid. The resistance rates of the *vanM*-positive



Strain no.	Hospital	Date of Isolation	Origin	ST	VSE/VRE
ZY3	ZY	2017-01	Blood	78	VSE
ZY4	ZY	2017-01	Bile	78	VSE
ZY7	ZY	2017-03	Drainage	78	VSE
ZY10	ZY	2017-03	Bile	78	VSE
SY4	SY	2017-07	Bile	78	VSE
ZY2	ZY	2017-01	Urine	78	VSE
SRM1	SRM	2017-07	Urine	78	VSE
SY5	SY	2017-08	Bile	78	VSE
SY1	SY	2017-06	Urine	78	VSE
SY2	SY	2017-06	Urine	78	VSE
SRR2	SRR	2016-02	Urine	78	VSE
SRR7	SRR	2016-07	Ascites	78	VSE
SRR9	SRR	2016-07	Drainage	78	VSE
SRR11	SRR	2016-08	Secretion	78	VSE
SRR12	SRR	2016-08	Urine	78	VSE
SRR30	SRR	2017-06	Urine	78	VSE
SRR4	SRR	2016-03	Drainage	78	VSE
SRR10	SRR	2016-08	Urine	78	VSE
SRR13	SRR	2016-09	Urine	78	VSE
SRR18	SRR	2016-12	Secretion	78	VSE
SRR17	SRR	2016-12	Urine	78	VSE
SRR20	SRR	2017-01	Drainage	78	VSE
ZY11	ZY	2017-03	Urine	78	VSE
ZY9	ZY	2017-03	Blood	192	VSE
ZY17	ZY	2017-06	Urine	78	VSE
ZY13	ZY	2017-04	Urine	78	VSE
ZY15	ZY	2017-04	Urine	78	VSE
ZY20	ZY	2017-05	Blood	78	VSE
ZY16	ZY	2017-05	Drainage	78	VSE
ZY1	ZY	2017-01	Bile	78	VSE
ZY8	ZY	2017-03	Blood	78	VSE
SRR29	SRR	2017-06	Bile	78	VSE
SRR8	SRR	2016-07	Urine	78	VSE
SRR27	SRR	2017-05	Urine	78	VSE
SRR28	SRR	2017-05	Urine	78	VSE
SRR26	SRR	2017-05	Incision	78	VSE
SRR6	SRR	2016-03	Urine	78	VSE
SRR25	SRR	2017-04	Drainage	78	VSE
SRR24	SRR	2017-04	Drainage	78	VSE
ZY5	ZY	2017-01	Drainage	78	VSE
ZY6	ZY	2017-02	Blood	78	VSE
ZY12	ZY	2017-04	Urine	78	VSE
ZY14	ZY	2017-04	Ascites	78	VSE
SRR19	SRR	2017-01	Bile	555	VSE
SRR14	SRR	2016-10	Urine	78	VSE
SRR15	SRR	2016-10	Urine	78	VSE
SRR16	SRR	2016-11	Urine	78	VSE
SRR22	SRR	2017-02	Pus	78	VRE
SRR23	SRR	2017-03	Drainage	78	VSE
ZY19	ZY	2017-05	Ascites	78	VSE
SRR21	SRR	2017-02	Bile	78	VSE
SY3	SY	2017-06	Blood	78	VSE
SRR1	SRR	2016-01	Urine	78	VSE
SRR3	SRR	2016-02	Urine	78	VSE
SRR5	SRR	2016-03	Urine	761	VSE

Fig. 1. Strain particulars and UPGMA dendrogram of patterns generated by *Sma*I-PFGE of *vanM*-carrying *E. faecium* with the Dice coefficient with a band position tolerance of 1.7% and an optimization of 1.5%. The numbers 1-15 represented 15 groups classified by a cut-off at 81% of the similarity values. SRR: Sir Run Run Shaw hospital, Zhejiang university, Hangzhou, China; ZY: The first affiliated hospital, Zhejiang university, Hangzhou, China; SY: Hangzhou First People's Hospital, Hangzhou, China; SRM: Zhejiang Provincial People's Hospital, Hangzhou, China.

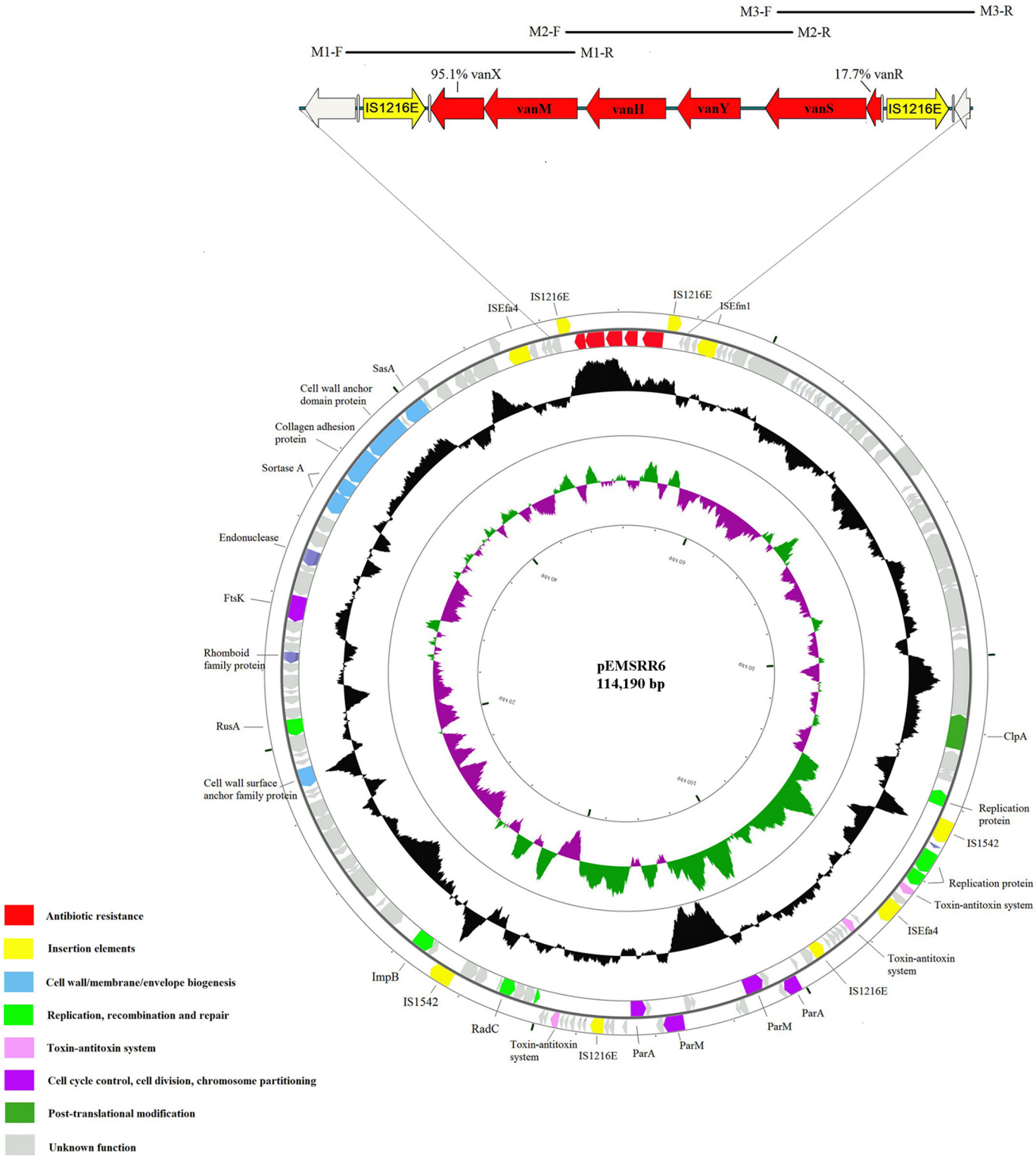


Fig. 2. Genetic map of the full-length 114,190 bp plasmid, pEMSRR6 and the partial enlarged view of the *vanM* transposon. The two outer circles represent ORFs in the plus (outside) and minus (inside) orientations, respectively. The two inner circles represent the G + C content plotted against the average G + C content of 33.8% (black circle) and GC skew information (green and purple circles). The overlapping primers of M1, M2, and M3 are indicated by bold lines above *vanM*-transposon. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isolates to ampicillin, levofloxacin, rifampicin, erythromycin, high-level gentamicin, tetracycline, fosfomycin, and chloramphenicol, were 98.2, 98.2, 98.2, 96.4, 44.6, 12.5, 1.8, and 1.8%, respectively (Table S2).

3.2. Molecular characteristics

The PFGE clustering showed that the 55 *vanM*-positive *E. faecium* isolates were visually classified into 15 pulsotypes using the cut-off of 81% similarity. From multilocus sequence typing analysis, the 55 *vanM*-

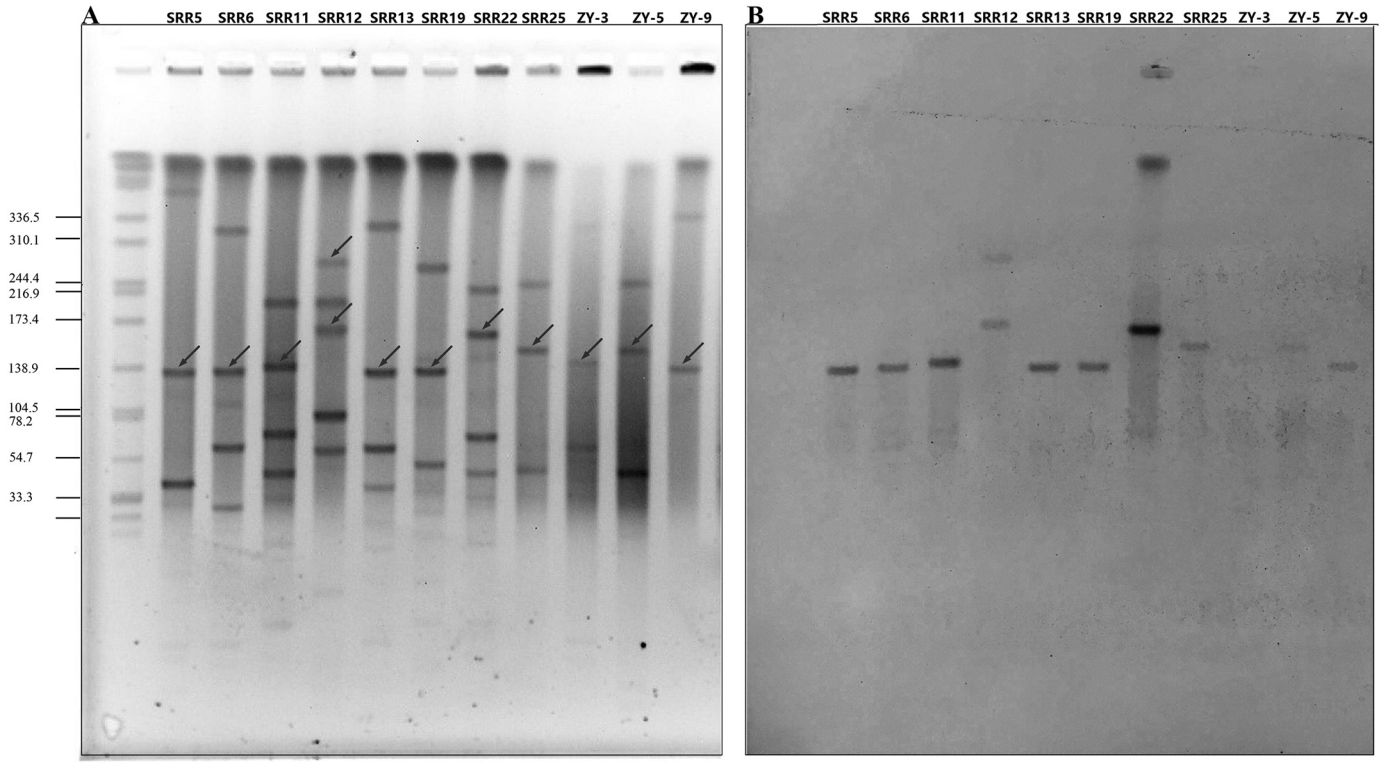


Fig. 3. Plasmid profiles of 11 representative *vanM*-carrying *E. faecium* isolates as shown by S1-PFGE (A) and Southern blotting hybridization with *vanM* probes (B). The sizes of the molecular marker (M) are indicated. The ST type, *vanM*-transposon structure type and origin of the selected strains are as follows: SRR5: ST761, typeI, urine; SRR6: ST78, typeI, urine; SRR11: ST78, typeI, anastomotic secretion; SRR12: ST78, type V, urine; SRR13: ST78, typeI, urine; SRR19: ST555, type IV, bile; **The only VRE SRR22**: ST78, the pus of lips; SRR25: ST78, type II, liver surgery drainage; ZY3: ST78, typeI, blood; ZY5: ST78, typeI, drainage; ZY9: ST192, undetermined type, blood.

positive *E. faecium* isolates were grouped into 4 distinct STs including ST78 (52 isolates), ST555 (1 isolate), ST192 (1 isolate), and ST761 (1 isolate) (Fig. 1 and Table S3). Most isolates (54/55), except the ST761-type isolate SRR5, belonged to the clonal complex CC17. One isolate of *E. faecalis*, ZY18, belonged to ST32.

3.3. vanM-containing plasmid structure

E. faecium SRR6 was the first isolate that screened positive for the *vanM*-genotype but showed a vancomycin-susceptible phenotype. The WGS analysis of *E. faecium* SRR6 showed that *vanM* was located on the plasmid pEMSRR6, which is 114,190 bp in size with an average G + C content of 33.8% (Fig. 2). Sequence alignment showed that the plasmid pEMSRR6 shared similarity with the *vanA*-carrying *E. faecium* plasmid p63-1 (accession no. CP019989.1) with 75% coverage and 99% identity. The regions with ≥97% similarity included the positions 15,421–33,785, 33,788–45,744, 80,198–88,668, 89,993–98,863, and 99,234–110,211 bp, which had homologies of 99, 97, 97, 99, and 99%, respectively. The region 80,198–98,863 bp interrupted by IS*1542* contains replication protein-related genes. As shown in Fig. 2, three genes encoding replication proteins were found in the plasmid pEMSRR6. However, no plasmid replicons were found via the PlasmidFinder server (identity threshold 60%) and the plasmid did not belong to any of 19 rep families (< 80% identity by both DNA and protein sequence comparisons) according to the previously reported classification system for plasmids from enterococci (Jensen et al. 2010).

As regards the antibiotic resistance regions, only one resistance determinant was found, which was an imperfect *vanM*-type transposon. Two IS*1216E* elements were inserted into *vanR* and *vanX*, respectively, leading to a 575-bp deletion in *vanR* and a 30-bp deletion in *vanX*. Besides these two IS*1216E* elements, there were two more IS*1216E* elements within the plasmid genome surrounding the region 98,790–110,656 bp. Additionally, other types of insertion sequence elements including IS*Efa4*, IS*1542*, and IS*Efm1* were found.

3.4. vanM location

Eleven representative strains were selected to include as much diversity in ST type, *vanM*-transposon structure type and isolate origin as possible. As shown in Fig. 3, each *vanM*-positive *E. faecium* isolate contained several plasmids and the plasmid profiles were diverse. In the isolates SRR5(ST761), SRR6(ST78), SRR11(ST78), SRR13(ST78), SRR19(ST555), ZY3(ST78), and ZY9(ST192), the *vanM* genes were located on plasmids of similar size (ca. 110 kb), regardless of their diverse ST types and plasmid profiles. In isolate SRR12, two *vanM* signals of hybridization of different sizes (ca. 160 kb and ca. 280 kb) were detected. However, only one sequence type of the *vanM* cluster was detected by PCR, indicating that these two operons are identical. In addition, the Southern blotting band for the VRE isolate SRR22 was obviously stronger than the bands for the other VSE isolates, indicating probably more copy numbers of the same *vanM* carrying plasmid (ca. 155 kb).

3.5. Diversity of the vanM gene cluster

The *vanM* transposon structures of 56 *vanM*-positive isolates were analyzed from the results of the WGS and overlapping PCR analyses. In the one *vanM*-positive VRE isolate, SRR22, no deletion or insertion was found in the *vanM* transposon including the *vanR*, *vanS*, *vanY*, *vanH*, *vanM*, and *vanX* genes (Fig. 4). This result was similar to the *vanM* transposon structure reported by Xu et al. (2010) (GenBank no. FJ349556), except that IS*1216E* was inserted not only upstream of the *vanR* gene but also downstream of the *vanX* gene.

The *vanM* transposon structures of the other *vanM*-positive VSE

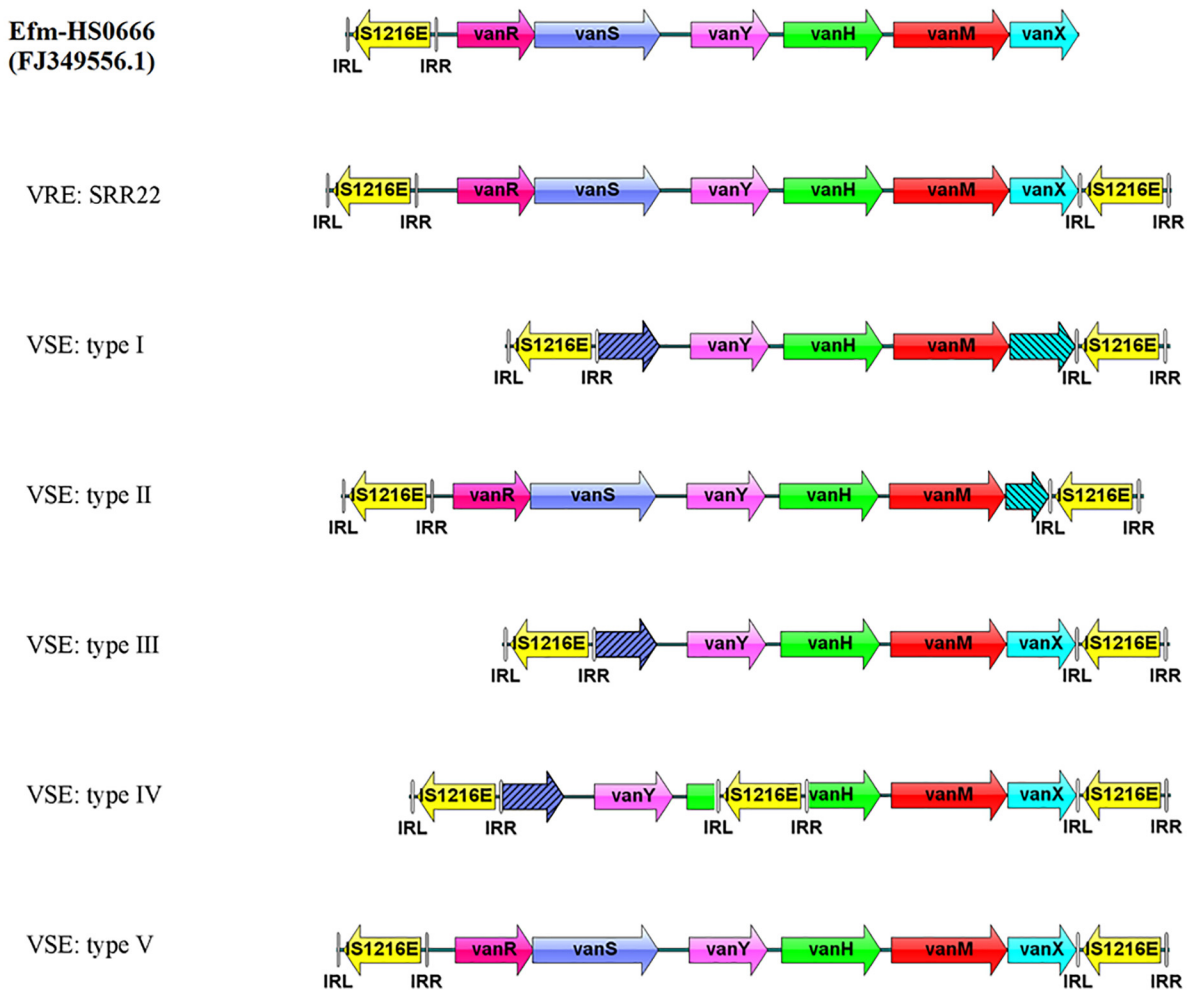


Fig. 4. Schematic map of *vanM* gene clusters in 56 *vanM*-carrying enterococci. The *vanM* gene cluster of vancomycin-resistant *E. faecium* Efm-HS0661 (GenBank accession no. FJ349556) are shown at the top. Arrows in yellow represent IS*1216E* elements. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

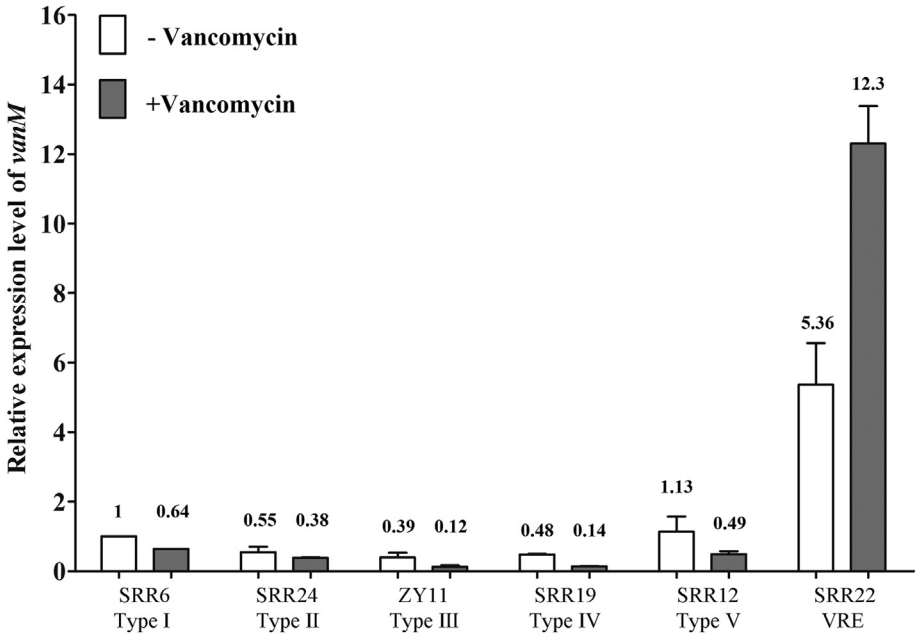


Fig. 5. Expression levels of *vanM* after bacterial growth in the absence (□) or presence of vancomycin at concentration 0.5 μg/ml (■) assessed by RT-qPCR. All measurements were normalized against the housekeeping gene *purK*. The results were represented as relative fold expression to the *vanM* expression of SRR6 as a benchmark. SRR22 was the only VRE isolate. Other five VSE isolates were representative of different kinds of *vanM* transposon structure types.

isolates showed different IS*1216E* insertion positions in the *vanM* transposons, as shown in Fig. 4. The type I *vanM* transposon structure was the dominant type at the rate of 70.9% (39/55), with two IS*1216E* elements inserted into the *vanRS* and *vanX* genes leading to the partial deletions of both these genes. Seven isolates had the type II *vanM* transposon structure with *vanX* broken by IS*1216E*. The type III *vanM* transposon structure showed only deletions in the two-component regulatory system. The type IV *vanM* transposon structure of isolate SRR19 showed three IS*1216E* elements, leading to a complete loss of *vanR,* partial deletions of *vanS*, and an interruption of *vanH*. Most surprisingly, in the isolate SRR12, no deletion or interruption was detected in the *vanR*, *vanS*, *vanY*, *vanH*, *vanM,* and *vanX* genes of the *vanM* gene cluster, and this was classified as a type V transposon structure.

3.6. vanM expression analysis

To confirm the expression of the *vanM* gene cluster, we measured the levels of *vanM* transcripts from six representative isolates (including the VRE isolate SRR22 and five VSE isolates of different *vanM* transposon structure types) after bacterial growth in the absence or presence of sub-MIC vancomycin. As shown in Fig. 5, in the absence of vancomycin, the expression of *vanM* in the VRE isolate SRR22 was > 5 folds higher than that in any of the VSE strains. Additionally, in the presence of a sublethal concentration of vancomycin, the difference in the expression levels of *vanM* between the VRE and VSE isolates became greater - > 12 folds. It seemed that vancomycin could activate higher expression of the *vanM* cluster in VRE isolates but not in VSE isolates.

4. Discussion

In recent years, outbreaks of vancomycin-susceptible *Enterococcus* isolates have been reported involving *vanA*-containing *Enterococcus* isolates, but such outbreaks have not been reported involving *vanM*-containing *Enterococcus* isolates (Sivertsen et al., 2016; Szakacs et al., 2014; Kohler, Eshaghi et al., 2018). In our study, *vanM*-carrying isolates, which were mostly vancomycin-susceptible, were found at a high prevalence among clinical isolates of enterococci from four hospitals in Hangzhou. Our PFGE analysis indicated that the *vanM* gene had spread among diverse *Enterococcus* strains in different hospitals instead of spreading as a single clone. However, most of the *vanM*-positive *E. faecium* isolates belonged to the ST78 (CC17) type, which is the most commonly reported hospital-associated genetic lineage worldwide. ST78 was also reported to be the predominant ST among *vanA*- and *vanM*-type VREm strains in China (Chen et al., 2015). The detection of *vanM*-carrying plasmids in genetically unrelated *E. faecium* isolates, as well as in one *E. faecalis* isolate, strongly points to in vivo horizontal transfer events.

We demonstrated that the insertion of an IS*1216*-like element mediated the silencing of the *vanM* phenotype. IS*1216,* belonging to the IS6 family, has been reported as a widespread mobile genetic element in enterococci (Clewell et al., 2014; Darini et al., 2000). IS*1216* elements play a key role in the structural variation of Tn*1546*-type transposons among VanA type VRE (Willems et al., 1999; Cha et al., 2013; Wardal et al., 2017). In our study, IS*1216E* was found to insert into the *vanM* gene cluster at various positions, leading to the deletions or partial deletions of *vanR*, *vanS*, or *vanX*. Our analysis of the levels of *vanM* transcripts proved that the *vanM* gene clusters in these VSE isolates were suppressed. The *vanR* and *vanS* genes jointly comprising the two-component regulatory system are essential for the expression of the *vanH*, *vanA*, and *vanX* genes (Arthur et al., 1992). The loss of *vanRS* could lead to inactivation of the transcription of vancomycin resistance genes (Gagnon et al., 2011), and might account for a vancomycin-susceptible phenotype in most *vanM*-positive VSE isolates in our study. The impairment of *vanX*, which is crucial for vancomycin resistance (Reynolds et al., 1994), might be responsible for the silencing of the vancomycin resistance phenotype in VSE isolates with the type II *vanM* transposon structure. However, *E. faecium* SRR12 carried an intact *vanM* gene cluster but expressed a negative vancomycin resistance phenotype, suggesting that vancomycin resistance might not be completely dependent on the *van* transposon element. A similar case was previously reported in *vanA*-type *E. faecium* isolates, where two strains with the same *vanA* gene cluster showed distinct resistance phenotypes (Choi et al., 2011).

Enterococci containing remnants of the *vanA* cluster able to revert to a resistant phenotype have been reported in Europe and were termed vancomycin-variable enterococci (VVE) (Szakacs et al., 2014). The susceptible VVE strains could convert to a vancomycin-resistant phenotype in vivo during vancomycin therapy or in vitro exposure to vancomycin (Sivertsen et al., 2016; Coburn et al., 2014). The movement of IS elements could disrupt the expression of the *vanHAX* genes through the excision of some transcription inhibitory factors or the introduction of an accessory promoter, leading to the reversion of resistance phenotype (Sivertsen et al., 2016; Thaker et al., 2015). In our study, no case of in vivo switching from vancomycin-susceptible to vancomycin-resistant was found among the patients before and after treatment with vancomycin. However, we observed the development of resistance during in vitro exposure to vancomycin in preliminary trials (data not shown). It was supposed that these *vanM*-containing vancomycin-susceptible *E. faecium* strains might switch to a vancomycin-resistant phenotype during prolonged vancomycin treatment in a hospital setting, which might lead to a failure of vancomycin treatment.

The detection of VRE in the clinic usually depends on phenotypic screening ahead of genotypic analysis, in which VVE would be overlooked. Therefore, the molecular screening of vancomycin resistance genes including *vanA*, *vanB*, and *vanM* is indispensable. WGS could be a better tool owing to its ability to unveil all the *van* gene sequences, which are correlated with the vancomycin phenotype. *vanM*-type VREm strains have disseminated and even become more prevalent than *vanA*-type VREm strains in Shanghai. Our study also showed a high prevalence of *vanM*-containing vancomycin-susceptible enterococci in Hangzhou. The previous studies on *vanA*-positive enterococci have suggested the potential of VVE strains to switch to a resistant phenotype. Therefore, *vanM* can be expected to play an important role in glycopeptide resistance in the future and it will be necessary to strengthen the surveillance for *vanM* among clinical *Enterococcus* isolates. In addition, it is critical to study the conversion mechanism of VVE strains further to better prevent the emergence and dissemination of vancomycin resistance.

Fundings

This work was supported by research grants from the National Natural Science Foundation of China [Nos. 81371859 and 81871689].

Competing interests

None declared.

Ethical approval

Not required.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2018.12.015.

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