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High Prevalence of *vanM* in Vancomycin-Resistant *Enterococcus faecium* Isolates from Shanghai, China

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The *vanM* gene was first found in a vancomycin-resistant *Enterococcus faecium* (VREm) isolate in Shanghai in 2006. In this study, we found that, in 70 VREm strains isolated in nine Shanghai hospitals from 2006 to 2014, *vanM* was more prevalent than the *vanA* gene (64.3% [45/70] versus 35.7% [25/70]). The *vanM*-type isolates showed similar antimicrobial susceptibility patterns with the *vanA* types. The *vanM*-type VREm emerged and disseminated in Shanghai.

The isolation of vancomycin-resistant enterococci (VRE) was first reported in 1988 (1, 2). During the last 2 decades, VRE have become significant nosocomial pathogens worldwide, mainly due to their adaptability in hospital environments and the limited treatment options. Nine types of glycopeptide resistance determinants (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*, and *vanN*) have been reported and well characterized on the basis of phenotypic and genotypic criteria (3). The *vanA and vanB* genotypes predominate worldwide (3, 4).

We first reported the *vanM* gene in a vancomycin-resistant *Enterococcus faecium* (VREm) clinical isolate from a teaching hospital in Shanghai in 2006 (5). Subsequently, only a single study from Singapore has reported *vanM*-type VRE isolates (6). Epidemiology data for strains with *van* determinants other than *vanA* and *vanB* remain rare. In this study, we investigated the prevalence of *van* and virulence genes in VREm strains isolated from 9 hospitals in Shanghai. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence type (MLST) were also performed to elucidate the molecular epidemiology of these strains.

Seventy consecutive and nonduplicate VREm clinical strains were collected from 9 hospitals in Shanghai between 2006 and 2014. MICs of 10 antimicrobial agents (vancomycin, teicoplanin, linezolid, fosfomycin, ampicillin, erythromycin, levofloxacin, gentamicin, minocycline, and rifampin) were determined by agar dilution. Etest (bioMérieux) was used to determine the MICs of tigecycline. Susceptibility to daptomycin was determined by broth microdilution using Mueller-Hinton II broth (cation adjusted) supplemented with calcium 50 μg/ml. Results were interpreted using the 2012 guidelines of the Clinical and Laboratory Standards Institute (www.clsi.org; Wayne, PA, USA). Due to the lack of an acknowledged fosfomycin breakpoint for *E. faecium*, we used the breakpoints of fosfomycin for *Enterococcus faecalis* proposed by the CLSI. *E. faecalis* ATCC 29212 was used as a quality-control strain for MIC determination.

Vancomycin resistance genes were detected by PCR amplification, as previously described (5). The PCR products were sequenced to determine the particular *van* genotype. The presence of five virulence genes (*asa1*, *gelE*, *cylA*, *esp*, and *hyl*) was assayed by multiplex PCR, as described previously (7). PFGE analysis was performed using a contour-clamped homogeneous electric field (CHEF) mapper system (Bio-Rad, USA), as previously described (8). Banding patterns were analyzed with BioNumerics software, version 5.0 (Belgium). Isolates were categorized into the same PFGE pulsotype group if they shared more than 80% similarity. MLST analysis was performed as described previously (9). Alleles and sequence types (STs) were analyzed and determined via the MLST database (http://efaecium.mlst.net/). Clusters of related STs were grouped into clonal complexes (CCs) using the eBURST program, version 3 (http://efaecium.mlst.net/eburst/). Statistical analysis was performed with the chi-square test or Fisher's extract test, as appropriate, using the statistical program SPSS 22.0. A P value of ≤ 0.05 was considered statistically significant.

Among the 70 VREm isolates, 45 strains (64.3%) carried the *vanM* gene, and 25 isolates (35.7%) harbored *vanA*. No other *van* genes were found. The *vanM*-type VREm isolates were detected in 8 hospitals located at the center of Shanghai city. The *vanM* gene has been predominant in VREm strains in Shanghai since 2011 (Fig. 1).

The *vanM*-type *E. faecium* isolates showed similar antimicrobial susceptibility patterns to the *vanA*-type isolates. All 70 VREm isolates were resistant to vancomycin (MICs, 128 to >256 μg/ml) and levofloxacin, and all were susceptible to linezolid, daptomycin, and tigecycline. The teicoplanin resistance rates were 71.1% (32/45) in *vanM*-type and 84.0% (21/25) in *van*A-type VREm isolates. The gentamicin resistance rates were 64.4% and 76% in *vanM*-type and *van*A-type isolates, respectively. No statistically significant differences in susceptibility to the 12 antimicrobial agents were observed between *vanM*- and *vanA*-type strains (Table 1).

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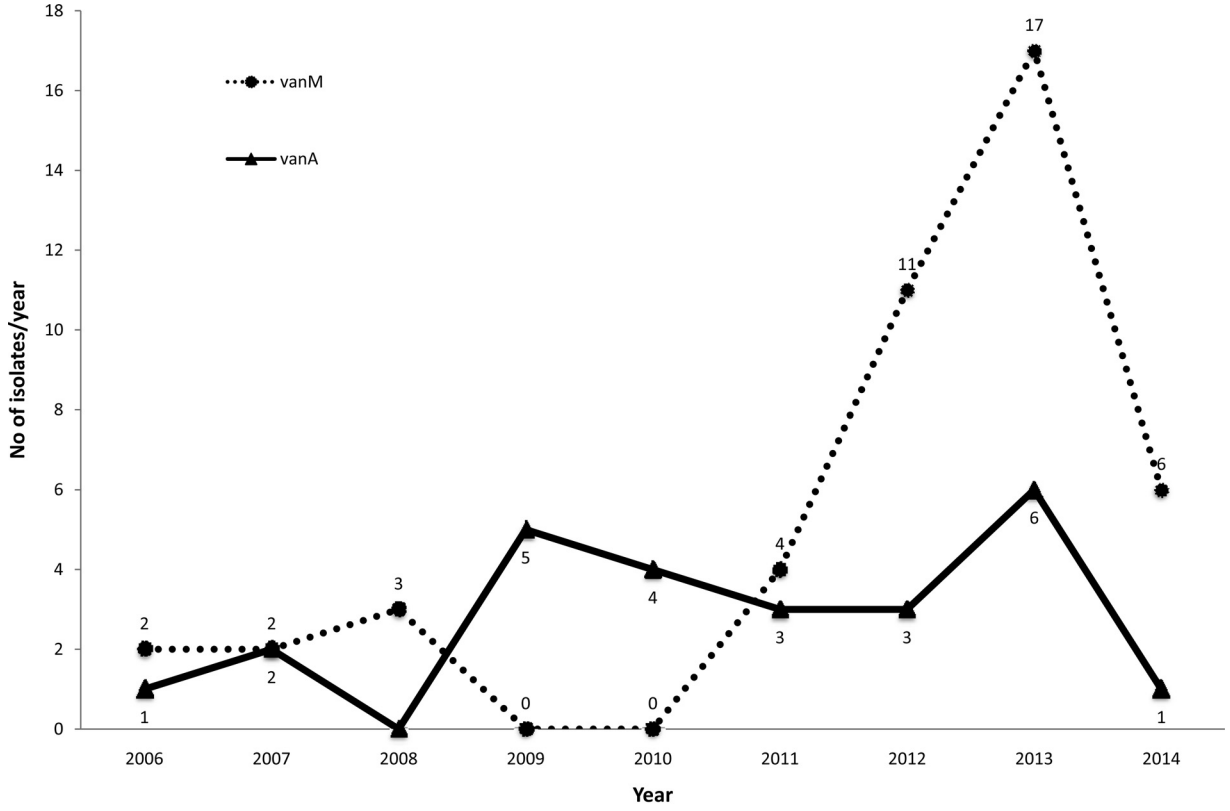

FIG 1 Distribution of vancomycin-resistant genes in 70 VREm strains isolated from nine hospitals, Shanghai, China, 2006 to 2014.

Five different pulsotypes were found among the 70 VREm strains, and each pulsotype included strains from at least 2 different hospitals (Fig. 2). By MLST analysis, 12 sequence types (STs) were identified, including ST 17 ($n = 3$), ST 18 ($n = 2$), ST 78 ($n = 46$), ST 203 ($n = 2$), ST 252 ($n = 1$), ST 262 ($n = 2$), ST 290 ($n = 1$), ST 341 ($n = 1$), ST 389 ($n = 1$), ST 555 ($n = 7$), ST 564 ($n = 3$), and ST 881 ($n = 1$). ST 881 is a new sequence type found in this study, and the data were uploaded to the eBURST database. eBURST analysis showed that all of the 70 VREm isolates belonged to clonal complex (CC) 17.

The *esp* gene was present 97.8% (44/45) and 84% (21/25) of *vanM*-type and *vanA*-type isolates, respectively ($P = 0.033$). The *hyl* gene was detected in 17.8% (6/45) and 32% (8/25) of *vanM*-type and *vanA*-type isolates, respectively ($P = 0.063$). All strains were negative for the presence of *cylA*, *gelE*, and *asa1* virulence genes.

TABLE 1 Comparison of the MICs of 12 antimicrobial agents between *vanA*- and *vanM*-type VREm isolates

	vanA-type VREm ($n = 25$)				*vanM*-type VREm ($n = 45$)				
	MIC (μg/ml) data:				MIC (μg/ml) data:				
Antibacterial agent	Range	MIC_{50}	MIC_{90}	R[a] (%)	MIC range	MIC_{50}	MIC_{90}	R[a] (%)	*P*
Vancomycin	128 to >256	>256	>256	100	128 to >256	>256	>256	100	NA[b]
Teicoplanin	0.5 to >256	32	128	71.1	0.5->256	64	128	84	0.232
Linezolid	1 to 2	1	2	0	1 to 2	1	2	0	NA
Daptomycin	2 to 4	4	4	0	0.5 to 4	4	4	0	NA
Tigecycline	0.032 to 0.094	0.064	0.064	0	0.032 to 0.125	0.064	0.094	0	NA
Ampicillin	0.5 to >256	>256	>256	97.8	0.5 to >256	>256	>256	96	0.671
Levofloxacin	32 to >256	64	>256	100	32 to >256	64	128	100	NA
Erythromycin	0.125 to >256	>256	>256	91.1	0.125 to >256	>256	>256	92	0.899
Fosfomycin	64 to >512	64	>512	26.7	16 to >512	64	>512	24	0.808
Rifampin	2 to >256	8	16	82.2	≤0.06 to 16	8	16	80	0.820
Minocycline	≤0.06 to 32	0.125	16	15.6	≤0.06 to 32	0.125	16	16	0.961
Gentamicin	4 to >256	>256	>256	64.4	2 to >256	>256	>256	76	0.322

[a] Resistance rate.
[b] Not applicable.

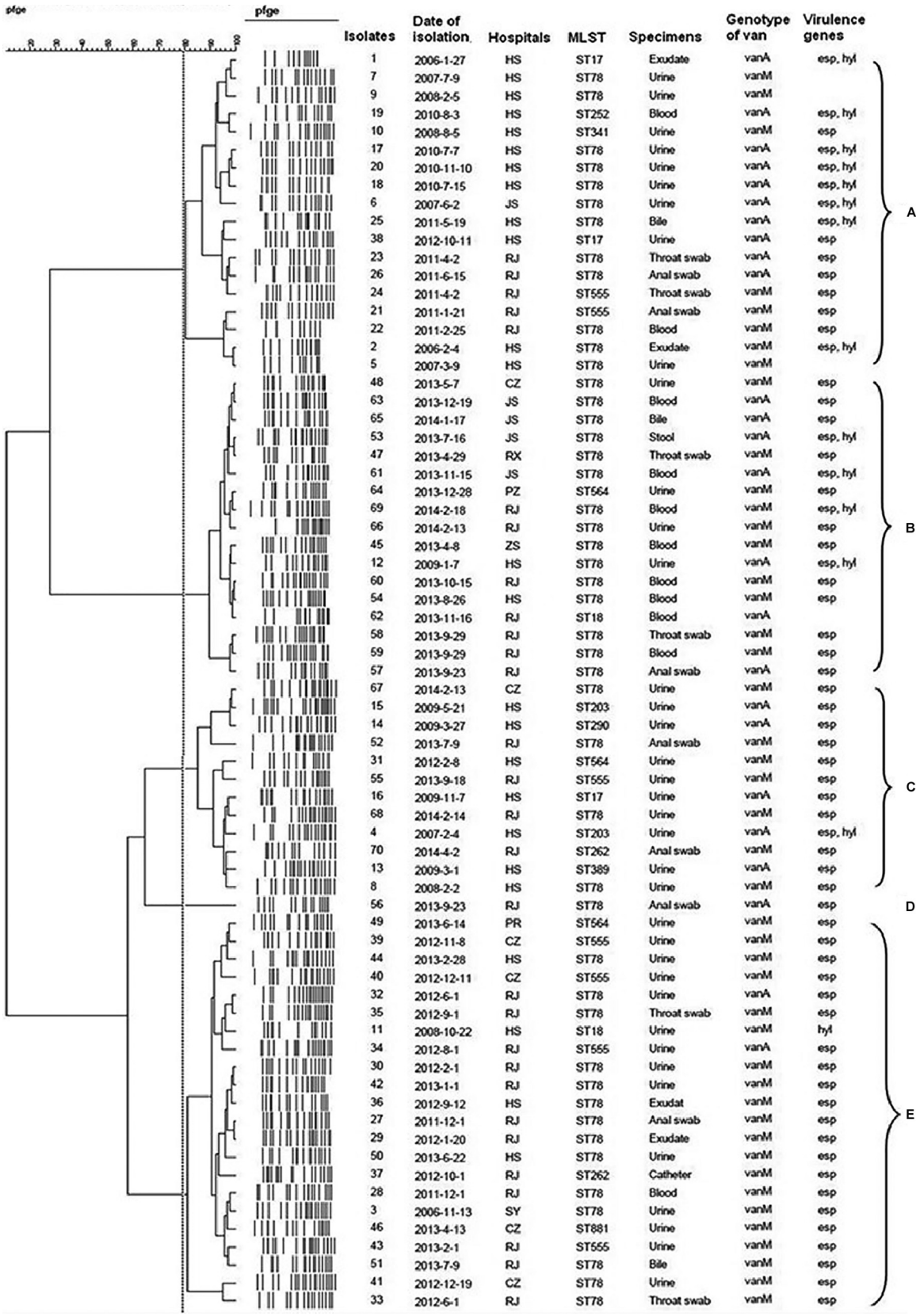

FIG 2 Strains particulars and PFGE dendrogram of the 70 VREm isolates from nine hospitals in Shanghai. Detailed information of the isolated dates, hospitals, specimen sources, MLST, *van* genotypes, and virulence genes are listed for each isolate. Pulsotypes A through E are clustered based on 80% similarity of the PFGE pattern.

Previous studies found that *vanA* is the most frequently encountered genotype of VREm in Asia, as in other countries worldwide (10–12). This study, however, showed that the *vanM* genotype has predominated in VREm clinical isolates in Shanghai since 2011. Similar to *vanA*-type VREm strains, *vanM*-type VREm strains are multidrug resistant, belong to CC17, and carry virulence genes *esp* and *hyl*, which provide these VREm strains more advantages to adapt to the hospital environment. Data from annual bacterial resistance surveillance program in Shanghai, China, showed that vancomycin resistance strains in *E. faecium* (VREm) increased from 0.33% in 2006 to 1.62% in 2011 and to 1.95% in 2014 (unpublished data). Thus, the high prevalence of *vanM* might contribute to the increasing VRE prevalence in Shanghai. PFGE analysis indicated that the *vanM* gene spread among diverse VRE strains in different hospitals instead of as a single clone.

The *vanM* gene was first found in a VREm clinical isolate from our hospital in Shanghai in 2006 (5). In 2011, Teo et al. reported a *vanM*-type *E. faecium* clinical strain in Singapore (6), thus indicating that this new vancomycin resistance gene might spread to other countries.

One of the reasons for the rarity of *vanM*-type VREm strains might be that most clinical laboratories and commercial molecular detection kits (Cepheid, Bouwel, Belgium; BD Diagnostics-GeneOhm, San Diego, CA) mainly focus on *vanA* and *vanB* genes and do not include the *vanM* gene (13, 14). In a study conducted in Mexico, one isolate of *E. faecium* demonstrated high-level resistance to vancomycin and teicoplanin, but it was classified as non-*vanA*, non-*vanB* isolate (15), which suggests that detection of new vancomycin resistance genes, such as *vanM*, might be missed based on current screening methods.

Overall, the results presented here suggest that *vanM* gene plays an important role in vancomycin resistance and dissemination in *E. faecium* strains in Shanghai. Therefore, it is necessary to screen for *vanM* in *E. faecium* strains to better control *vanM*-type VREm infection and dissemination.

New eBURST sequence type. ST 881 is a new sequence type found in this study, and the data were uploaded to the eBURST database.

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