High Prevalence of \textit{vanM} in Vancomycin-Resistant \textit{Enterococcus faecium} Isolates from Shanghai, China

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The \textit{vanM} gene was first found in a vancomycin-resistant \textit{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, \textit{vanM} was more prevalent than the \textit{vanA} gene (64.3\% [45/70] versus 35.7\% [25/70]). The \textit{vanM}-type isolates showed similar antimicrobial susceptibility patterns with the \textit{vanA} types. The \textit{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 (\textit{vanA}, \textit{vanB}, \textit{vanC}, \textit{vanD}, \textit{vanE}, \textit{vanG}, \textit{vanL}, \textit{vanM}, and \textit{vanN}) have been reported and well characterized on the basis of phenotypic and genotypic criteria (3). The \textit{vanA} and \textit{vanB} genotypes predominate worldwide (3, 4).

We first reported the \textit{vanM} gene in a vancomycin-resistant \textit{Enterococcus faecium} (VREM) clinical isolate from a teaching hospital in Shanghai in 2006 (5). Subsequently, only a single study from Singapore has reported \textit{vanM}-type VRE isolates (6). Epidemiology data for strains with \textit{van} determinants other than \textit{vanA} and \textit{vanB} remain rare. In this study, we investigated the prevalence of \textit{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 gentamicin, and all were susceptible to linezolid, daptomycin, and tigecycline. The teicoplanin resistance rates were 71.1\% (32/45) in \textit{vanM}-type and 84.0\% (21/25) in \textit{vanA}-type VREM isolates. The gentamicin resistance rates were 64.4\% and 76\% in \textit{vanM}-type and \textit{vanA}-type isolates, respectively. No statistically significant differences in susceptibility to the 12 antimicrobial agents were observed between \textit{vanM}- and \textit{vanA}-type strains (Table 1).

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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 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.

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

| Antibacterial agent | vanA-type VREm (n = 25) | vanM-type VREm (n = 45) |
|---------------------|-------------------------|-------------------------|
|                     | MIC (µg/ml) data:       | MIC (µg/ml) data:       |
|                     | MIC range | MIC<sub>50</sub> | MIC<sub>90</sub> | R<sup>a</sup> (%) | MIC range | MIC<sub>50</sub> | MIC<sub>90</sub> | R<sup>b</sup> (%) | P    |
| Vancomycin          | 128 to >256 | >256 | >256 | 100 | 128 to >256 | >256 | >256 | 100 | NA<sup>a</sup> |
| 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 | >256 | 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 |

<sup>a</sup> Resistance rate.
<sup>b</sup> 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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