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 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 gene variant. 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 pulsoype 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 exact 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, ≥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 vanA-type VREm isolates. The gentamicin resistance rates were 64.4% and 76% in vanM-type and vanA-type isolates, respectively. No statistically significant differences in susceptibility to the 12 antimicrobial agents were observed between vanM- and vanA-type strains (Table 1).
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/H11005 3), ST 18 (n/H11005 2), ST 78 (n/H11005 46), ST 203 (n = 2), ST 252 (n = 1), ST 262 (n = 1), ST 290 (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/H11005 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/H11005 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.

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

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>vanA-type VREm (n = 25)</th>
<th>vanM-type VREm (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/ml) data:</td>
<td>MIC (µg/ml) data:</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>128 to &gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.5 to &gt;256</td>
<td>32</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1 to 2</td>
<td>1</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2 to 4</td>
<td>4</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.032 to 0.094</td>
<td>0.064</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.5 to &gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32 to &gt;256</td>
<td>64</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.125 to &gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>64 to &gt;512</td>
<td>64</td>
</tr>
<tr>
<td>Rifampin</td>
<td>2 to &gt;256</td>
<td>8</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤0.06 to 32</td>
<td>0.125</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4 to &gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

<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 vanA genotype has predominated in VREm clinical isolates in Shanghai since 2011. Similar to vanA-type VREm strains, vanB-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 vanB might contribute to the increasing VRE prevalence in Shanghai. PFGE analysis indicated that the vanB gene spread among diverse VRE strains in different hospitals instead of as a single clone.

The vanB gene was first found in a VREm clinical isolate from our hospital in Shanghai in 2006 (5). In 2011, Teo et al. reported a vanB-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 vanB-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 vanB 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 vanB, might be missed based on current screening methods.

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

New eBURST sequence type. ST 881 is a new sequence type based on current screening methods.

ACKNOWLEDGMENTS

We thank George A. Jacoby (Lahey Hospital and Medical Center, Burlington, MA, USA) for his critical review of the manuscript.

This study was supported by grants from National Natural Science Foundation of China (81171613 to X.X. and 8110108024 to M.W.) and by the National Major Scientific and Technological Special Project for Significant New Drugs Development (2014ZX09507-009).

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