High prevalence of vanM in vancomycin-resistant Enterococcus faecium isolates
from Shanghai, China

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Running Head: High prevalence of vanM in VREm

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Abstract

vanM gene was first found in a vancomycin-resistant *E. faecium* (VREm) isolated in Shanghai in 2006. In this study, we found that vanM was more prevalent than vanA gene, 64.3% (45/70) vs 35.7% (25/70), in 70 VREm strains isolated in nine Shanghai hospitals, 2006-2014. VanM-type isolates showed similar antimicrobial susceptibility patterns with VanA-type. 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 two decades, VRE have become significant nosocomial pathogens worldwide, mainly due to their adaptability in hospital environments and 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). *vanA* and *vanB* genotypes predominate worldwide (3, 4).

We first reported the vanM gene in a vancomycin-resistant *E. 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* remains rare. In this study, we investigated the prevalence of *van* and virulence genes in VREm strains isolated from nine hospitals in Shanghai. PFGE and MLST were also performed to elucidate the molecular epidemiology of these strains.

Seventy consecutive and non-duplicate VREm clinical strains were collected from nine hospitals in Shanghai between 2006 and 2014. Minimal inhibitory concentrations (MICs) of ten antimicrobial agents (vancomycin, teicoplanin, linezolid, fosfomycin,
ampicillin, erythromycin, levofloxacin, gentamicin, minocycline, and rifampicin) were
determined by agar dilution. Etest (bioMérieux) was used to determine the MICs of
tigecycline. Susceptibility to daptomycin was determined by microbroth dilution using
Mueller Hinton II Broth (Cation-Adjusted) supplemented with calcium at 50 μg/ml.

Results were interpreted using guidelines of the 2012 Clinical and Laboratory
Standards Institute (Wayne, PA, USA; www.clsi.org). Due to the lack of
acknowledged fosfomycin breakpoint for *E. faecium*, we used the breakpoints of
fosfomycin for *E. faecalis* proposed by the CLSI. *E. faecalis* ATCC29212 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 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 (9). Alleles and STs were
analyzed and determined via the MLST database (http://efaecium.mLst.net/). Clusters
of related sequence types (STs) were grouped into clonal complexes (CCs) using the
eBURST program v3 (http://efaecium.mLst.net/eburst/). Statistical analysis was
performed by the Chi-square test or Fisher’s exact test as appropriate using
A p value ≤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. vanM-type VREm isolates were detected in eight hospitals located at the center of Shanghai city. vanM gene was predominant in VREm strains in Shanghai since 2011 (Figure 1).

VanM-type *E. faecium* isolates showed similar antimicrobial susceptibility patterns with 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 vanA-type VREm isolates. The gentamicin-resistant 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 two different hospitals (Figure 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 was uploaded to eBURST database. eBURST analysis showed that all of the 70 VREm isolates belonged to clonal
The esp gene was present 97.8% (44/45) and 84% (21/25) in vanM-type and vanA-type isolates, respectively (P=0.033). The hyl gene was detected in 17.8% (6/45) and 32% (8/25) in vanM-type and vanA-type isolates, respectively (P=0.063). All strains were negative for the presence of cylA, gelE and asa1 virulence genes.

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 predominated in VREm clinical isolates in Shanghai since 2011. Similar to vanA-type VREm, vanM-type VREm strains are multidrug resistant, belonged 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 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 vanM gene spread among diverse VRE strains in different hospitals instead of as a single clone.

The vanM gene was firstly found in a VREm clinical isolate from our hospital in Shanghai in 2006 (5). In 2011, Teo JW 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 focused on \textit{vanA} and \textit{vanB} genes and do not include the \textit{vanM} gene (13, 14). In a study conducted in Mexico, one isolate of \textit{E. faecium} demonstrated high-level resistance to vancomycin and teicoplanin, but it was classified as non-\textit{vanA}, non-\textit{vanB} (15), which suggested that detection for new vancomycin-resistant genes such as \textit{vanM} might be missed based on current screening methods.

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

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\textbf{Figure legends}
Figure 1. Distribution of vancomycin-resistant genes in 70 VREm strains isolated from nine hospitals, Shanghai, China, 2006-2014.

Figure 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 and van genotypes, virulence genes are listed, respectively, for each isolate. Pulsotypes A-E are clustered based on 80% similarity of the PFGE pattern

References


Regional spread and control of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* in Kyoto, Japan. Eur J Clin Microbiol Infect Dis. 31:1095-1100.


Table 1. Comparison of the MICs (μg/ml) of 12 antimicrobial agents between VanA- and VanM-type vancomycin-resistant *E. faecium* (VREm) isolates

<table>
<thead>
<tr>
<th>Antibacterial Agents</th>
<th>VanA-type VREm (n=25)</th>
<th>VanM-type VREm (n=45)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>128-&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.5-&gt;256</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1-2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2-4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.032-0.094</td>
<td>0.064</td>
<td>0.064</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.5-&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32-&gt;256</td>
<td>64</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.125-&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>64-&gt;512</td>
<td>64</td>
<td>&gt;512</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>2-&gt;256</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Minocycline</td>
<td>≤0.06-32</td>
<td>0.125</td>
<td>16</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4-&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

a: resistance rate;  b: not applicable