Detection of an unusual van genotype in a vancomycin resistant Enterococcus faecium hospital isolate

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ABSTRACT

We highlight the detection of a rare vanM genotype in *Enterococcus faecium*. This isolate exhibited a VanB phenotype, with high levels of resistance to vancomycin (MIC>256mg/L) and susceptibility to teicoplanin (MIC=1mg/L). It was however vanB negative by PCR. Further screening for other van loci revealed the presence of a complete vanM operon.
Glycopeptide resistance in Enterococci is mediated by van gene clusters and to date eight operons (vanA, vanB, vanC, vanD, vanE, vanG, vanL and vanM), each with distinguishing resistance characteristics have been described (1,13,14). Isolates harbouring vanA, vanB and vanM are resistant to high levels of vancomycin (>128mg/L). Additionally, vanA and vanM isolates are typically resistant to high levels of teicoplanin although exceptions have been noted (9,13). The vanB gene cluster on the other hand, produces little or no resistance to teicoplanin (MIC <1mg/L). vanD strains are resistant to moderate levels of vancomycin (MIC 16-128mg/L) and susceptible to teicoplanin whilst vanC, vanE, vanL and vanG strains exhibit low-level resistance to vancomycin (1,13,14). Clinically, the vanA and vanB operons are the two most dominant and relevant vancomycin resistance factors (14).

Locally, infrequent outbreaks of vancomycin-resistant enterococci (VRE) have been traced to a dominant vanB E. faecium clone (8). This is in contrast to the other Asian countries like South Korea (10) and Taiwan (2,7) where vanA is the predominant genotype. We report the detection of an uncommon van genotype, vanM, which has thus far only been reported from China with a handful (n=6) of characterized isolates (13).

A vancomycin resistant E. faecium was isolated from a screening rectal swab of a 64 year-old female who had been a contact of another patient carrying VRE of vanA genotype. She had no travel history. The patient was admitted to the orthopedic unit with a fractured femur with poorly controlled diabetes mellitus and hypertension. She had been on vancomycin for a long period of time for the treatment of methicillin-resistant Staphylococcus aureus (MRSA) osteomyelitis of the right foot but was not treated for the VRE.

The isolate was nonmotile and nonpigmented. Biochemically, it was unable to ferment sorbitol, arabinose, and methyl-α-D-glucopyranoside. It tested positive with raffinose. VITEK 2 gram-positive (GP) identification card #21342 (bioMérieux, Marcy l'Etoile, France), matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF MS) (Bruker Daltonik GmbH, Leipzig, Germany) and species-specific PCR targeting D-alanine–D-alanine ligase
(ddIE. faecium) (3) all confirmed that it was an E. faecium isolate. Susceptibility testing was performed using the Etest (bioMérieux) method with breakpoints defined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The isolate was highly resistant to vancomycin (MIC>256mg/L) and sensitive to teicoplanin (MIC=1mg/L) indicative of a VanB phenotype. The isolate was also sensitive to daptomycin, linezolid and quinupristin/dalfopristin with MICs of 1, 1.5 and 0.75mg/L, respectively, but resistant to ampicillin, ciprofloxacin and rifampicin with MICs >32mg/L. Inducibility of teicoplanin resistance was tested by inoculating cultures onto Muller-Hinton agar plates without vancomycin or containing a subinhibitory concentration of vancomycin (5 mg/L) (6) and a teicoplanin Etest strip was overlaid on the plate. The Etest MIC, determined after a 48hr incubation at 37°C, was unchanged in either plate suggesting that vancomycin was not an inducer of teicoplanin resistance.

As part of the hospital’s infection control policy, all VRE isolates are PCR screened for vanA and vanB, for which the isolate tested negative. Further screening for vanC, vanD, vanE, vanL and vanG ligases (1,4,5) was performed. The isolate was positive for a 377bp fragment of the vanHM gene. This amplification was performed using primers vanHMF (5’-CAGCGTGGGGCACAAGTCTGA-3’) and vanHMR (5’-TGCCGTACGCCAACACGTGA-3’). PCR amplification followed by direct sequencing of the ORFs spanning the vanM locus from IS1216-like element to vanXM (nucleotide positions 55 to 6503 of the vanM operon [GenBank:FJ349556.1] revealed a 100% identity to the GenBank sequence except for a 173bp insertion 171bp upstream of the vanRM ORF. The insertion event has been similarly reported by Xu et al. (13) in one of their isolates exhibiting a high level of vancomycin resistance (>256mg/L) and teicoplanin sensitivity (0.75mg/L).

Since their remaining isolates had both high levels of vancomycin and teicoplanin resistance, it was speculated that the insertion might have caused the loss of teicoplanin resistance (13).
PCRs for the detection of five virulence genes *asa1, gelE, cylA, esp* and *hyl* (12), coding for aggregation substance, gelatinase, cytolysin, enterococcal surface protein and hyaluronidase, respectively were performed. The isolate was positive only for the *esp* gene.

Mutilocus sequence typing (MLST) using the protocol available at [http://efaecium.mlst.net/misc/info.asp](http://efaecium.mlst.net/misc/info.asp) indicated that the isolate belonged to ST 78. This was also the dominant strain type detected amongst four out of the six (67%) Chinese *vanM* isolates (13). Multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA) was performed (http://www.umcutrecht.nl/subsite/MLVA/) and revealed the isolate was MLVA type (MT)-12. Collectively, genotyping results, resistance to ampicillin and quinolones and the presence of an enterococcal surface protein indicates the isolate belongs to clonal complex 17 (CC17). *E. faecium* CC17 has been associated with epidemic nosocomial outbreaks and infections (2,11).

Southern blot hybridization analysis of plasmid and genomic DNA was performed using DIG DNA Labeling and Detection Kit (Roche Diagnostics, Mannheim, Germany). Probing with digoxigenin (DIG)-labeled amplicon derived from nucleotide positions 5513 to 5823 of the *vanM* operon (FJ349556.1) suggested that *vanM* was chromosomally encoded. *vanM* has been previously found to be plasmid localized with the demonstration of conjugal transfer of the *vanM* cluster to an antibiotic susceptible *E. faecium* recipient (13). Xu *et al.* (13) speculate that the transfer may have been facilitated by an IS1216 transposition event. The association of *vanM* with the IS element may also similarly mediate dissemination in our isolate. However, due to the unavailability of a suitable *E. faecium* recipient we were not able to demonstrate transferability of *vanM*.

VRE isolated at the hospital during the study period and from the previous year were of either *vanA* or *vanB* genotype and this is the first instance of *vanM* detection. This report serves to highlight the existence of unusual *van* genotypes in hospitals whose detection may be missed. Commercial molecular assays such as Cepheid GeneXpert vanA/vanB assay (Cepheid, Sunnyvale, CA) or BD GeneOhm VanR assay (BD GeneOhm, San Diego, CA) specifically target the...
vanA and/or vanB genes which have no more than 83% nucleotide identity with vanM. Hence, we believe that it is unlikely that the vanM genotype would be picked up by such assays. Given the rare occurrence of vanM, we are currently uncertain about the clinical significance of vanM in Singapore.

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Reference


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