VanB phenotype-vanA genotype Enterococcus faecium with heterogeneous expression of teicoplanin resistance

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Abstract

Six isolates of VanB phenotype-vanA genotype E. faecium with heterogeneous expression of teicoplanin resistance, which gave rise to an outbreak at a Korean tertiary-care teaching hospital, represent IS1216V in the coding region of vanS. This could be the underlying cause of VanB phenotype-vanA genotype with heterogeneous expression of teicoplanin resistance.
VanA glycopeptide resistance is characterized by acquired inducible resistance to both vancomycin and teicoplanin, whereas VanB phenotype is characterized by variable levels of resistance to vancomycin but with susceptibility to teicoplanin (1, 5). During the past 5 years, vancomycin-resistant enterococci (VRE) with the \textit{vanA} genotype that are susceptible to teicoplanin, hence having the VanB phenotype and the \textit{vanA} genotype, have become increasingly prevalent in Asia (8, 11, 12). In 2005, we experienced an outbreak of VanB phenotype-\textit{vanA} genotype \textit{E. faecium} with heterogeneous expression of teicoplanin resistance isolated from six patients at a tertiary-care teaching hospital. To our knowledge, this is the first description of \textit{IS1216V} inserted in the coding region of the \textit{vanS} gene that might be involved in heterogeneous expression of teicoplanin susceptibility.

From November to December 2005, six isolates of VanB phenotype-\textit{vanA} genotype \textit{E. faecium} (AJJ2, AJJ3, AJJ4, AJJ5, AJJ8 and AJJ10) were collected from a tertiary-care teaching hospital. Organisms were identified using conventional biochemical reactions and the Vitek identification system (bioMérieux, Hazelwood, MO). To evaluate genetic relatedness of the isolates, PFGE was performed with \textit{SmaI} digested genomic DNA (Gibco BRL, Gaithersburg, Md.) as described by Murray et al. (13), with pulse times beginning with 1 s and ending with 20 s at 6 V/cm for 24 h. Dendrograms based on Dice coefficients
and clustered using an unweighted pair group method using an arithmetic averages
algorithm were generated with Bio-Gene software (Vilber Lourmat, Mame Lavallec,
France). All six isolates of VanB phenotype-vanA genotype E. faecium revealed identical or
closely related PFGE patterns according to Tenover et al. (14). The MICs of vancomycin
and teicoplanin among the isolates were determined by the E-test (AB Biodisk North
America, Inc., Culver City, Calif.). Previously characterized VRE strains, E. faecium
BM4147 (3) and VanB phenotype-vanA genotype E. faecium JC03 (12) without
heterogeneous expression, served as controls.

All isolates displayed variable levels of vancomycin resistance (MICs, 64-128 µg/mL) and
teicoplanin susceptibility (MICs, 4-12 µg/mL) with heterogeneous expression of
teicoplanin resistance. The heterogeneous expression of resistance was characterized by
growth of colonies in the elliptic inhibition zone when performing E-test susceptibility
testing (Fig. 1A). Colonies in the elliptic inhibition zone displayed a homogeneous
phenotype of resistance to teicoplanin, when retested by E-test (Fig. 1B). VanB phenotype-
vanA genotype E. faecium JC03 (12) without heterogeneous expression represented
teicoplanin susceptibility, and displayed no growth of colonies in the inhibition zone (Fig.
1C), and E. faecium BM4147 (3) displayed a homogeneous resistance to teicoplanin (Fig.
Bacterial DNA was extracted with a Qiagen DNeasy Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer’s instructions. For structural analysis of Tn1546-like elements, an overlapping PCR amplification of internal regions of Tn1546 was performed as described previously (9). PCR amplicons larger than that of the prototype vanA gene cluster were purified with GENECLEAN Kits (Qbiogene Inc., Carlsbad, CA). The purified PCR products were sequenced directly using ABI Prism 3100 DNA SEQUENCER (Applied Biosystem, Foster city, CA). DNA fragments amplified with a combination of Tn1546 primer and IS1216V primer were also purified and subsequently sequenced to determine the exact integration site and orientation of the IS1216V insertion. DNASIS for windows v.2.6 (Hitachi Software Engineering, South San Francisco, CA) was used for sequence analysis. Nucleotide sequences were compared to the reference sequence of transposon Tn1546 (GenBank accession number M97287) (3).

Filter matings were performed with E. faecalis JH2-2 (10) as the recipient and all six isolates as the donors, as previously described (15). Transconjugants were selected on BHI agar plates containing 50 µg/ml of rifampin, 20 µg/ml of fusidic acid, and 10 µg/ml of vancomycin. The transconjugants were examined for the structural analysis of Tn1546.
Induction of resistance by teicoplanin in VanB phenotype-vanA genotype *E. faecium* AJJ5 with heterogeneous expression was studied by determination of growth rates after overnight incubation with or without teicoplanin. The isolate was grown overnight at 37°C in BHI broth with or without teicoplanin (6 µg/ml). The cultures were diluted 1:20 into 20 ml of BHI with or without teicoplanin (6 µg/ml), grown at 37°C with shaking, and optical density at 600 nm was monitored. Previously characterized VRE strains, *E. faecium* BM4147 (3) and VanB phenotype-vanA genotype *E. faecium* JC03 (12) without heterogeneous expression, served as controls.

A total of twelve isolates (6 original isolates and 6 resistant derivatives obtained from the elliptic inhibition zone on an E-test strip, respectively) were characterized by one copy of IS1216V in the left end of Tn1546, a second in the coding region of vanS, and a third in the vanX-vanY intergenic region as well as IS1542 in the orf2-vanR intergenic region. IS1216V in the vanS was integrated at nucleotide 4900 with an 8-bp duplication of the target sequence (GTCATTAG) in the forward orientation. Although the VanS sensor peptide was reported earlier to have no functional effect due to the disruption of vanS by IS1216V (6), IS1216V in the coding region of vanS in our cases might affect the expression of teicoplanin resistance. The VanRS_A system activates transcription of the resistance genes in
response to vancomycin and teicoplanin, whereas VanB-type enterococci remain susceptible to teicoplanin which is not an inducer (2, 7). Amino acid substitutions due to the three point mutations of \( \textit{vanS} \) are responsible for impaired teicoplanin resistance among \( \textit{vanA} \)-genotype VRE strains (8). On the other hand, the modification of signal transduction due to substitutions in the putative linker of VanS\(_B\) has been shown to be the most common mechanism of acquisition of inducible resistance to teicoplanin among \( \textit{vanB} \)-genotype VRE strains (4). Similarly, the genetic alteration of \( \textit{vanS} \) due to the IS\(_{1216V}\) insertion in our isolates might be associated with heterogeneous teicoplanin resistance.

All isolates transferred vancomycin resistance at a frequency of \( 6 \times 10^{-9} \) to \( 2 \times 10^{-8} \) transconjugants per donor. The transconjugants revealed the same heterogeneous resistance to teicoplanin.

In growth rate studies, after overnight incubation of AJJ5 with teicoplanin, growth was delayed at the beginning of the subculture in the presence of teicoplanin, with growth resuming after a lag phase of 4h (Fig. 2A). The growth of VanB phenotype-\( \textit{vanA} \) genotype \( \textit{E. faecium} \) JC03 without heterogeneous expression was suppressed throughout the subculture in the presence of teicoplanin, regardless of prior overnight incubation (Fig. 2B). The growth of BM4147 did not show and lag phase throughout the subcultures neither in
the absence nor presence of teicoplanin, regardless of prior overnight incubation (Fig. 2C).

The resumption of growth of AJJ5, which characterized by IS1216V insertion in the coding region of the vanS gene, in the presence of teicoplanin after overnight incubation with teicoplanin and the homogeneous expression of teicoplanin resistance in a colony within the inhibition zone suggest that a portion of bacteria that retained teicoplanin resistance was fully induced after incubation with teicoplanin.

The heterogeneous teicoplanin resistance of our isolates was clinically important because the conventional test can not detect the heterogeneous teicoplanin resistance. These isolates might potentially give rise to homogeneous resistance during teicoplanin therapy. PFGE showed a genetically identical or closely related pattern for all isolates, indicating clonal distribution (14). When finding VanB phenotype-vanA genotype E. faecium, molecular typing and MIC determination by E-test are recommended during teicoplanin therapy to prevent a nosocomial outbreak.

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REFERENCES


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FIG. 1. Teicoplanin (TP) MIC determination by E-test of *E. faecium* AJJ5 with heterogeneous expression (A), a colony of *E. faecium* isolate AJJ5 grown in the inhibition zone next to the E-test strip (B), VanB phenotype-vanA genotype *E. faecium* JC03 without heterogeneous expression (C) and *E. faecium* BM4147 (D).

Short title: Teicoplanin (TP) MIC determination by E-test.

FIG. 2. Effect of overnight growth in the presence of teicoplanin (6 μg/ml) on growth of *E. faecium* AJJ5 with heterogeneous expression (A), VanB phenotype-vanA genotype *E. faecium* JC03 without heterogeneous expression (B) and *E. faecium* BM4147 (C) in the absence or presence (6 μg/ml) of teicoplanin. Teicoplanin (TP) concentration in the overnight culture/in the culture medium. O.D., optical density.

Short title: Effect of overnight growth in the presence of teicoplanin.