JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1999, p. 1229–1230 Vol. 37, No. 4
0095-1137/99/$04.00+0

Letters to the Editor

Should Genotypic Testing Be Done on All Phenotypically Vancomycin-Resistant Enterococci Detected in Hospitals?

We wish to report the detection of *vanA* and *vanC* genes in *Enterococcus gallinarum*, because the detection of *vanA* genes in a motile enterococcus has potentially important implications for infection control practice in hospitals.

Since 1986, vancomycin-resistant *E. faecium* and *E. faecalis* have become major nosocomial pathogens in the United States and in several European countries (1, 7). Two principal phenotypes of acquired vancomycin resistance have been described, VanA and VanB, encoded by two distinct gene clusters, the *vanA* and *vanB* clusters (5). Both genes are mobile on either plasmids or transposons. The *vanA* genes typically confer high-level resistance to vancomycin (MIC ≥ 128 mg/liter) and teicoplanin (MIC ≥ 16 mg/liter), while *vanB* genes typically result in moderate to high-level resistance to vancomycin (MIC = 16 to 64 mg/liter). However, *vanA* and *vanB* genotypes associated with different resistance phenotypes have been reported. The motile enterococci, *E. gallinarum*, *E. casseliflavus*, and *E. flavescens*, have low-level intrinsic vancomycin resistance (MIC = 4 to 16 mg/liter) due to the *vanC-1*, *vanC-2*, and *vanC-3* genes, respectively. VanA- and VanB-type resistance in the motile enterococci has been reported on only a few occasions, and as far as we are aware not in Australia (2, 8).

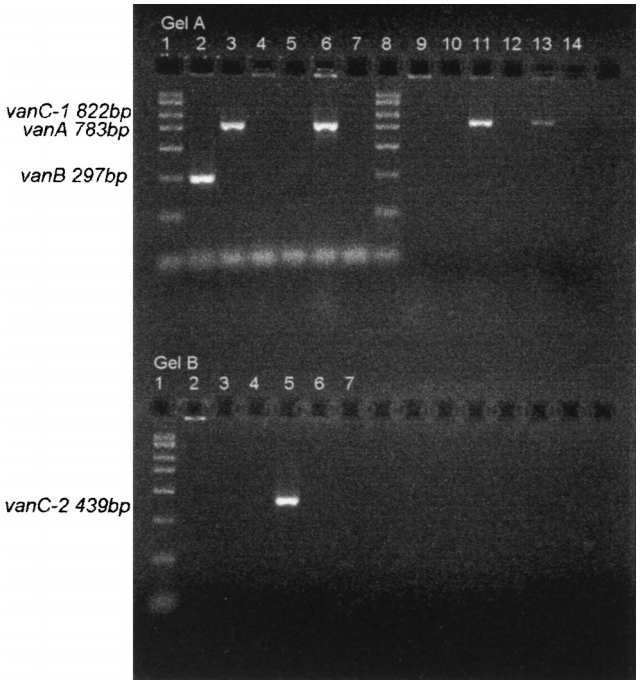

FIG. 1. Agarose gel electrophoresis of *vanA*, *vanB*, *vanC-1*, and *vanC-2* PCR products. (A) Multiplex *vanA* and *vanB* gene PCR (lanes 2 to 7) and *vanC-1* gene PCR (lanes 9 to 14). Lanes: 1 and 8, Amresco PCR DNA marker; 2, *E. faecalis* ATCC 51299 *vanB*, 297 bp (product size, 297 bp); 3, *E. faecium* wild strain *vanA*, 783 bp (product size, 783 bp); 4, *E. gallinarum* NCTC 11428 *vanC-1*, 822 bp (no product detected); 5, *E. casseliflavus* ATCC 25788 *vanC-2*, 439 bp (no product detected); 6, WBG 9213 (product size, 783 bp); 7, water control; 9, *E. faecalis* ATCC 51299 *vanB*, 297 bp (no product detected); 10, *E. faecium* wild strain *vanA*, 783 bp (no product detected); 11, *E. gallinarum* NCTC 11428 *vanC-1*, 822 bp (product size, 822 bp); 12, *E. casseliflavus* ATCC 25788 *vanC-2*, 439 bp (no product detected); 13, WBG 9213 (product size, 822 bp); 14, water control. (B) *vanC-2* gene PCR. Lanes: 1, Amresco PCR DNA marker; 2, *E. faecalis* ATCC 51299 *vanB*, 297 bp (no product detected); 3, *E. faecium* wild strain *vanA*, 783 bp (no product detected); 4, *E. gallinarum* NCTC 11428 *vanC-1*, 822 bp (no product detected); 5, *E. casseliflavus* ATCC 25788 *vanC-2*, 439 bp (product size, 439 bp); 6, WBG 9213 (no product detected); 7, water control.

Recently, an *E. gallinarum* strain (WBG 9213) isolated from a chicken-processing plant was found to have both *vanA* and *vanC* genes. This isolate was identified by the Facklam and Collins identification scheme (3). Differentiation between *E. gallinarum* and *E. casseliflavus* was based on the lack of pigment production on 5% sheep blood agar after 24 h of incubation. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion and E test (AB Biodisk, Sweden) methods by using Mueller-Hinton agar supplemented with 5% horse blood, and by using the Vitek GPS-TB card (bioMérieux-Vitek). The interpretative criteria of the National Committee for Clinical Laboratory Standards (NCCLS) were used for determining susceptibility of the isolates (6). WBG 9213 grew on the NCCLS vancomycin resistance screening test plate, brain heart infusion agar supplemented with 6 mg of vancomycin per liter.

The *van* genes were detected by PCR using the oligonucleotide primers for *vanA*, *vanB*, *vanC-1*, and *vanC-2* genes as reported by Free and Sahm (4). The target gene and product size for each of the control strains are described in Fig. 1. *vanA* and *vanB* amplifications were performed as a multiplex reaction. *vanC-1* and *vanC-2* amplifications were performed separately.

Both the 822-bp (*vanC-1*) and 783-bp (*vanA*) PCR products were detected in WBG 9213 (Fig. 1). This isolate had a VanA phenotype. MICs of vancomycin and teicoplanin as determined by the E test were ≥256 and 32 mg/liter, respectively. Vancomycin resistance was confirmed by Kirby-Bauer disk diffusion (no zone detected) and the Vitek GPS-TB card (≥32 mg/liter).

The emergence of acquired and transferable high-level vancomycin resistance in the motile enterococci is of great potential clinical and infection control significance. Enterococci with VanA- or VanB-type vancomycin resistance encoded by *vanA* and *vanB* gene clusters, respectively, are a major cause of nosocomial infections in some hospitals in the United States and Europe. Whenever such a strain is detected in a hospital, strict infection control procedures are applied to prevent transmission to other patients. However, motile enterococci with their intrinsic VanC-type low-level vancomycin resistance have not required special infection control measures. This policy may no longer be adequate. When vancomycin resistance is detected by phenotypic methods in enterococci other than *E. faecium* and *E. faecalis*, it can no longer be assumed that such isolates are not carrying mobile plasmids or transposons encoding high-level vancomycin resistance. PCR assays need to be done to ensure that these enterococci are not carrying *vanA* or *vanB* genes in addition to *vanC* genes.

We now recommend that genotypic testing be routinely performed on all clinical isolates of vancomycin-resistant enterococci.

VanA phenotype resistance is often mediated by self-transferable plasmids that have acquired Tn*516*-related transposons that carry the *vanA* gene cluster. Plasmid DNA studies are being performed on the isolate described here and will be the subject of a further report.

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