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NOTES

Diversity of Tn*1546*-Like Elements in Vancomycin-Resistant Enterococci Isolated from Humans and Poultry in Korea

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To investigate the possible spread of vancomycin resistance among enterococci through horizontal gene transfer between two sources of vancomycin-resistant enterococci (VRE) isolated from farm animals and humans in Korea, molecular characterization of VRE isolated from poultry (20 isolates) and humans (35 isolates) was performed by PCR-restriction fragment length polymorphism typing of the *vanA* gene cluster (Tn*1546*). PCR mapping of Tn*1546* finally distinguished seven different transposon types (types A to G). Type A was observed only in the poultry isolates, while the other six types were present only in the human isolates. An insertion sequence and deleted sequence were detected in most of the human isolates in the *orf2-vanR* and *vanX-vanY* regions in the Tn*1546*-like element, but not in the poultry isolates. Tn*1546*-like elements were found in conjugal plasmids of most human VRE, whereas they were detected in the chromosomes of all poultry VRE. Accordingly, no evidence was found of any recent transmission of vancomycin resistance genes between poultry and humans in Korea.

Vancomycin-resistant enterococci (VRE) were first reported in Europe in 1988 and have since emerged as important nosocomial pathogens (4). Dissemination of vancomycin resistance can occur through horizontal transmission of resistant genes (2). Horizontal transfer of vancomycin resistance can be explained by the fact that the genetic determinant for the VanA type of vancomycin resistance typically resides in a mobile DNA element, such as Tn*1546*, which can be transferred to enterococci by plasmids. Several recent studies have described the heterogeneity of the VanA resistance determinant (9, 10). In these studies, indistinguishable Tn*1546*-like elements were found in VRE isolated from farm animals and humans, suggesting the possible transfer of vancomycin-resistant elements from farm animals to humans. In Korea, there is only limited information on vancomycin resistance dissemination between farm animals and humans. Thus, the aim of this study was to investigate the dissemination of vancomycin resistance by horizontal transfer of resistant genes between farm animals and humans based on molecular characterization of VRE isolated from poultry and humans by PCR typing of the VanA gene cluster (Tn*1546*).

Fifty-five vancomycin-resistant *Enterococcus faecium* isolates were subjected to this study. Thirty-five human clinical isolates of vancomycin-resistant *Enterococcus faecium* were isolated from patients at four university hospitals in Korea from January 2001 to May 2001. During the same period, 20 vancomycin-resistant *E. faecium* strains were isolated from poultry feces. These isolates were then identified by using a Vitek system, and identifications were confirmed by using an APIstrep kit (both from bioMérieux Inc., Hazelwood, Mo.). The MICs of vancomycin (Eli Lilly, Indianapolis, Ind.) and teicoplanin (Aventis Pharmaceutical Co., Somerset, N.J.) for these isolates were evaluated by using the agar dilution method according to NCCLS standards (6). All the isolates were resistant to vancomycin (MICs at which 50% [MIC_{50}] and 90% [MIC_{90}] of the isolates were inhibited, 512 and 1,024 μg/ml, respectively). In contrast, with teicoplanin, there was some difference between the MICs for the human and poultry VRE isolates: the MIC_{50} and MIC_{90} for the human VRE were 32 and 128 μg/ml, respectively, while those for the poultry VRE isolates were 16 and 32 μg/ml, respectively.

Pulsed-field gel electrophoresis (PFGE) analysis and interpretation were performed as previously described (5, 7). The 55 isolates were very heterogeneous, with some discrete clusters of related isolates identified. No VRE isolates from the two groups belonged to the same cluster in the PFGE pattern.

The PCR amplicons of *vanA* and *vanB* were produced by methods described in previous reports (3). The PCR amplicons from the internal region of the Tn*1546*-like elements were produced by an initial cycle at 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 3.5 min, and a final extension step at 72°C for 10 min. Primers are listed in Table 1. For a Long PCR of the Tn*1546*-like elements, an Expand Long Template PCR System (Roche, Mannheim, Germany) was used according to the manufacturer's instructions. The *vanA* gene was detected in all the isolates, while the *vanB* gene was not detected in any (data not shown). The polymorphic regions of Tn*1546*-like elements in the 55 VREs were analyzed by a Tn*1546* internal-region PCR method. The PCR mapping finally distinguished seven different Tn*1546*-like element types (Fig. 1). The PCR product pattern for type A was identical to the predicted pattern for the published sequence of

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TABLE 1. PCR primers for amplification of the Tn*1546*-like element internal region

Primer	Amplicon	Nucleotide sequence (5′–3′)	Expected size (bp)	Positions[a]
pTn*1546*	Tn*1546*	GGAAAATGCGGATTTACAACGCTAAG	10,851	13–38 10839–10814
p72	*orf1*-5′	ACGTTAAGAAAGTTTTAGTGG	1,120	72–92
p1175	*orf1*-5′	GCCCTTTTAGGAATGG		1190–1175
p1085	*orf1*-3′	CATACATGCGCCATTGAGATA	1,696	1085–1110
p2760	*orf1*-3′	GTTAGTCCATCCTCGCTTGAT		2780–2760
p2524	*orf2-vanR*	CTTGCTTCCCACACCATT	1,497	2541–2524
p4000	*orf2-vanR*	GGCAATTTCATGTTCATCATC		4020–4000
p3976	*vanRS*	ATGAGCGATAAAATACTT	1,827	3976–3993
p5786	*vanRS*	TTAGGACCTCCTTTTATC		5803–5786
p4649	*vanSH*	TTGGTTATAAAATTGAAAAAT	2,337	4649–4669
p6969	*vanSH*	CTATTCATGCTCCTGTCT		6986–6969
p6018	*vanHAX*	ATGAATAACATCGGCATTAC	2,606	6018–6037
p8607	*vanHAX*	TTATTTAACGGGGAAATC		8624–8607
p8016	*vanXY*	ATGGAAATAGGATTTACTTT	1,947	8016–8035
p9944	*vanXY*	TTACCTCCTTGAATTAGTAT		9963–9944
p9052	*vanYZ*	ATGAAGAAGTTGTTTTTTTTA	1,550	9052–9072
p10585	*vanYZ*	CTTACACGTAATTTATTC		10602–10585

[a] Based on the published Tn*1546* sequence (GenBank accession number M97297).

Tn*1546* (1), and all the elements were conserved and indistinguishable from those of Tn*1546*. The PCR-restriction fragment length polymorphism (RFLP) patterns for isolates of this type were also identical to each other. All 20 VRE isolates from poultry were included in this type, yet none of the human VRE isolates belonged to it. Types B, C, and D exhibited larger PCR products for the *orf2-vanR* region, whereas types C and F exhibited larger PCR products for the *vanX-vanY* region than

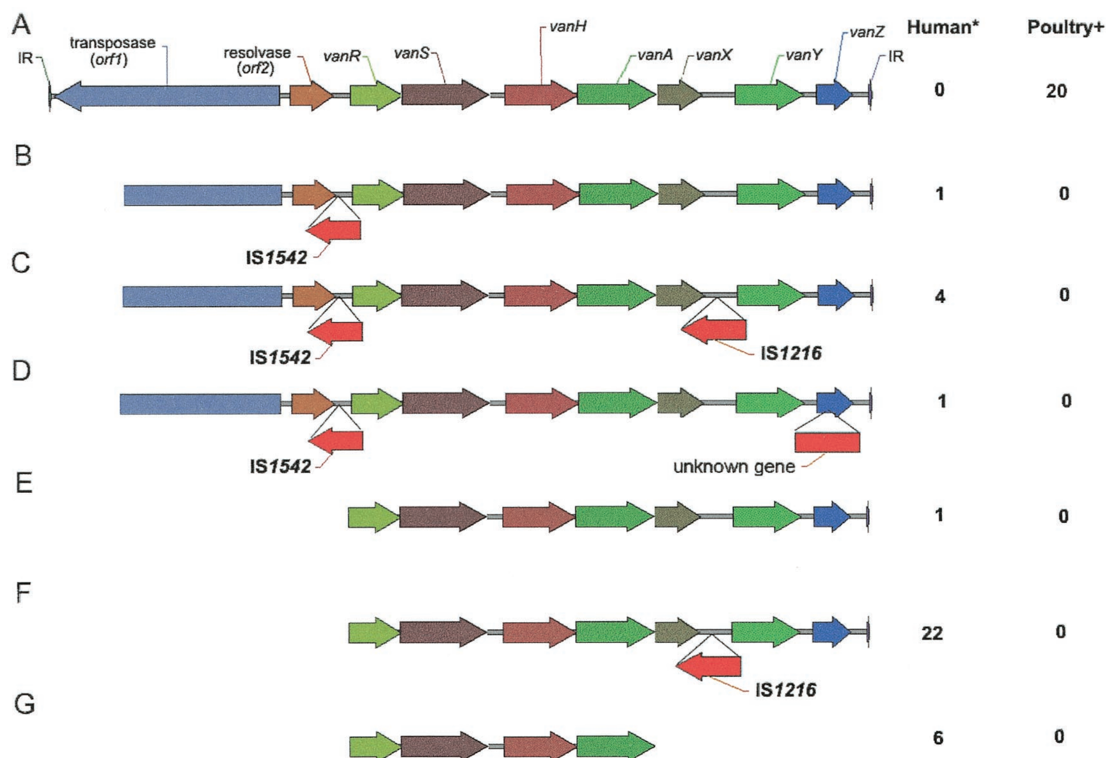

FIG. 1. Genetic maps for classification of VRE isolates by molecular type of Tn*1546*. IR, inverted repeat sequence. Human*, number of isolates isolated from humans. Poultry+, number of isolates isolated from poultry.

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for those regions of type A, the Tn*1546* element prototype. The PCR products that were larger than those of the prototype *vanA* elements were digested with *Alu*I and *Dde*I (Roche), respectively, and separated by agarose gel electrophoresis (1.5% agarose gels). The isolates included in type F were further divided into four subtypes (subtypes F1 through F4) based on PCR-RFLP analysis. These subtypes differed in only one fragment, the left end region of the insertion (IS) elements. Types C and F2 produced identical PCR-RFLP results for the *vanX-vanY* region. The *orf2-vanR*, *vanX-vanY*, and *vanY-vanZ* region PCR products that were larger than expected were purified with a PCR purification kit (Qiagen Inc., Hilden, Germany) according to the manufacturer's instructions. The purified PCR products were sequenced using a T7 sequencing kit (Amersham Biosciences, Piscataway, N.J.). The results of the RFLP and sequencing analysis revealed that the IS elements in the *orf2-vanR* and *vanX-vanY* regions were IS*1542* and IS*1216V*, respectively, in all the isolates bearing IS elements. IS*1542* elements were detected in 6 (17%) of the 35 human isolates, while IS*1216V* elements were detected in 26 (74%). Type D included an IS element in the *vanY-vanZ* region of the Tn*1546*-like element. This IS element sequence did not match any previously reported sequence.

To determine the locations of Tn*1546*-like elements in plasmids or chromosomes, Southern hybridization analysis using a digoxigenin DNA labeling and detection kit (Roche) was performed according to the manufacturer's instructions. According to the results, most of the *vanA* gene clusters in the human VRE isolates (33 of 35) were detected on a conjugal plasmid, whereas all the *vanA* gene clusters in the poultry VRE isolates were detected on a chromosome. Previously, a difference in the rate of *vanA* gene transfer was also found between human and poultry VRE isolates in a conjugal transfer test using filter-mating with *E. faecalis* JH2-2 as the recipient (8). The *vanA* gene transfer rates of the human VRE isolates (about 10^{-4} to 10^{-5} per donor) were higher than those of the poultry VRE isolates (about 10^{-8} to 10^{-9} per donor).

In conclusion, we found that *vanA* gene clusters and DNA fingerprint patterns by PFGE were quite different for the two groups of VRE, those from humans and poultry, in Korea, suggesting that the VRE from the two sources were clonally unrelated. No evidence was found of any recent transmission of vancomycin-resistant genes between farm animals and humans in Korea.

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