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NOTE

VanB-*vanA* Incongruent VRE Isolated from Animals and Humans in 1999

Enjoo Shin, Hyun-gin Hong, Yasuyoshi Ike[1], Kyungwon Lee[2], Yong Ho Park[3], Dong Taek Lee[4] and Yeonhee Lee*

[1]*Department of Bacteriology and Bacterial Infection Control, Laboratory of Bacterial Drug Resistance, Graduate School of Medicine, Gunma University, Maebashi, Gunma 371-8511, Japan*
[2]*Department of Clinical Laboratory, Severance Hospital, Yonsei University, Seoul 120-749, Republic of Korea*
[3]*Department of Veterinary Medicine, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea*
[4]*Laboratory of Microbiology, College of Medicine, Kyungpook National University, Daegu 700-422, Republic of Korea*
**Culture Collection of Antimicrobial Resistant Microbes, Department of Biology, Seoul Women's University, Seoul 139-774, Republic of Korea*

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16 chicken isolates and four clinical isolates of VanB-*vanA* incongruent vancomycin-resistant *Enterococcus faecium* strains without *vanS* were isolated in 1999. Pulsed-field gel electrophoresis revealed only a peripheral relationship between the chicken isolates and clinical isolates, but suggested clonal spread in the chicken isolates.

Keywords: enterococcus, *Enterococcus faecium*, incongruence, vancomycin-resistance, VanA, VanB

Enterococci are gram-positive cocci which are responsible for severe human infections, including endocarditis, meningitis, and septicemia. The enterococci constitute an increasingly frequently observed cause of nosocomial infections, and have become the second or third most commonly isolated organisms in cases of nosocomial infections (Coque *et al.*, 1996; Yeh *et al.*, 2002). Since its first detection in 1986, enterococcal resistance to glycopeptides has become widespread, and has been recognized as an increasingly salient problem in clinical environments (McDonald *et al.*, 1997).

The assumption that the use of the glycopeptide, avoparcin, as a feed additive resulted in a reservoir of glycopeptide-resistant *E. faecium* (GREF) in the animal husbandry field was verified by the demonstration of GREF in animal feces (pigs and chickens) in farms in which avoparcin was utilized (Bates *et al.*, 1994; Devriese *et al.*, 1996). The presence of GREF in the intestinal flora of meat animals also indicated their presence in meat products, and this was demonstrated in both poultry carcasses and raw minced pork (Chadwick *et al.*, 1996). The presence of GREF in meat products makes its spread to healthy, non-hospitalized humans quite likely, and this has, in fact, been confirmed (Descheemaeker *et al.*, 1999; Lu *et al.*, 2002). Since the discontinuation of clinical avoparcin usage, a decline has been recorded in the rates of GREF in animals and humans within the community (Klare *et al.*, 1999). This supports the notion that the ban of avoparcin for use as an antibacterial growth promoter might hinder human chemotherapy regimens in many countries, including Korea. In Korea, the prevalence of vancomycin-resistant enterococcus (VRE) in hospitalized patients has evidenced a significant increase (Lee *et al.*, 2001; Shin *et al.*, 2003) since the first isolation of vancomycin- resistant *E. durans*, in 1992 (Park *et al.*, 1992). Recently, VanB-*vanA* incongruent VRE has been detected in Japan (Hashimoto *et al.*, 2000), Taiwan (Lauderdale *et al.*, 2002), and Korea (Eom *et al.*, 2004).

In this work, animal and clinical isolates of VRE, all of which were collected in 1999 and stored in the Culture Collection of Antimicrobial Resistant Microbes (http://www.ccarm.or.kr) were screened for the presence of VanB-*vanA* incongruent VRE, and the relationships between the animal and human isolates were determined via pulsed-field gel electrophoresis (PFGE).

Five hundreds and ninty four chicken isolates and 16 clinical isolates of *E. faecium* obtained in 1999

* To whom correspondence should be addressed.
(Tel) 82-2-970-5664; (Fax) 82-2-970-5901
(E-mail) yhlee@swu.ac.kr

were stored in the Culture Collection of Antimicrobial Resistant Microbes (CCARM), and re-identified via 16S rRNA sequencing, and their MICs were determined via agar dilution, in accordance with the guidelines established by the National Committee for Clinical Laboratory Standards (2004). Genes responsible for vancomycin- resistance were detected using multiplex PCR with primers specific to *vanA*; 5'-GCTATTCA GCTGTACTC-3' and 5'-CAGCGGCCATCATACGG-3', 783 bp, *vanB*; 5'-CATCGCCGTCCCCGAATTTCAAA -3' and 5'-GATGCG GAAGATACCGTGGCT-3', 297 bp, *vanC1*; 5'-GGTATCAAGGAAACCTC-3' and 5'-C TTCCGCATCATAGCT -3', 822 bp, and *vanC2*; 5'-CT CCTACGATTCTCTTG-3' and 5'-CGAGCAAGACCT TTAG-3', 439 bp, as previously described by Dukta-Malen *et al.* (1995). PCR was conducted as follows: 30 cycles of denaturation at 90°C for 3 min, annealing at 52°C for 1 min, and polymerization at 72°C for 1 min. *vanS* was sequenced following PCR amplification using a primer set specific to *vanS*. *vanS*-F; 5'-CGA CAC CAT TGA TAA CCC GA-3' and *vanS*-R; 5'-ACA TCT CTT AGG ACC TCC TT-3' corresponded to nucleotides 4605 to 4623 and 5791 to 5810 of *vanS*. PCR was conducted as follows: 1 cycle at 94°C for 10 min, 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and 1 cycle at 72°C for 10 min. DNA fragments generated by PCR were then electrophoresed and purified with a QIAGEN gel extraction system (Qiagen, USA) and sequenced using an ABI prism 310 Genetic Analyzer (Perkin-Elmer, USA). Pulse field gel electrophoresis (PFGE) was conducted as described by Manson *et al.* (2003) in a CHEF-DR-III system (Bio-Rad, USA) with 6 V/cm pulse with increasing pulse time from 5.3 sec to 34.9 sec at 120°C and 19 h at 4°C. Restricted DNA fragments with *Sma*I were stained with ethidium bromide

Table 1. MICs of VanB-*vanA* incongruent VRE isolated from animals and humans

CCARM No.	Source	Species	Genotype	Phenotype	MIC (μg/ml)	
					Vancomycin	Teicoplanin
5046	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5051	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5054	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5061	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5065	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5066	Chicken	*E. faecium*	*vanA*	VanB	≥64	8
5063	Chicken	*E. faecium*	*vanA*	VanB	≥64	4
5073	Chicken	*E. faecium*	*vanA*	VanB	≥64	4
5095	Chicken	*E. faecium*	*vanA*	VanB	≥64	4
5052	Chicken	*E. faecium*	*vanA*	VanB	≥64	4
5062	Chicken	*E. faecium*	*vanA*	VanB	≥64	2
5059	Chicken	*E. faecium*	*vanA*	VanB	≥64	1
5047	Chicken	*E. faecium*	*vanA*	VanB	≥64	1
5067	Chicken	*E. faecium*	*vanA*	VanB	≥64	1
5070	Chicken	*E. faecium*	*vanA*	VanB	≥64	≤0.5
5071	Chicken	*E. faecium*	*vanA*	VanB	8	1
5102	Human	*E. faecium*	*vanA*	VanB	≥64	8
5109	Human	*E. faecium*	*vanA*	VanB	≥64	8
5113	Human	*E. faecium*	*vanA*	VanB	≥64	8
5119	Human	*E. faecium*	*vanA*	VanB	≥64	8
5103	Human	*E. faecalis*	*vanA*	VanB	≥64	4
5105	Human	*E. faecalis*	*vanA*	VanB	≥64	4

and analyzed using a Fingerprinting II Infomatix system (Bio-Rad, USA).

Over a one-year period in 1999, 594 enterococcal isolates were collected from chicken caecum tissues in one of the largest slaughterhouses in Kyung-gi Province, via three separate samplings. 69 of these isolates (11.6%) exhibited growth in the presence of 6 μg/ml of vancomycin. These VRE were identified as *E. faecium* (n=54), *E. gallinarum* (n=16), and *E. casseliflavus* (n=1), but no vancomycin-resistant *E. faecalis* (VRE *faecalis*) was detected. Among the enterococcal isolates obtained from patients hospitalized at the Severance Hospital at Yonsei University (Seoul, Korea) in 1999, 16 of the isolates were identified as vancomycin-resistant *E. faecium* (VRE *faecium*) via disk diffusion test. However, two isolates among these turned out to be *E. faecalis,* according to the results of 16S rRNA sequencing. Among the animal and clinical VRE *faecium* isolates, 38 were found to exhibit a VanA phenotype, characterized by resistance to both vancomycin (MIC≥8 μg/ml) and teicoplanin (MIC≥16 μg/ml). 16 of the animal isolates and four of the clinical isolates manifested a VanB phenotype, characterized by resistance to vancomycin (MIC≥8 μg/ml) but not teicoplanin (MIC≤8 μg/ml) (Table 1). When the multiplex PCR was conducted, *vanA,* but not *vanB,* was found in every animal and clinical VRE *faecium* isolate, including the VRE *faecium* strains which evidenced the VanB phenotype. In a departure from other reports asserting that *vanS* mutation is responsible for VanB-*vanA* incongruence (Hashimoto *et al.*, 2000; Lauderdale *et al.,* 2002), the DNA sequences of *vanS* in every animal and clinical VanB-*vanA* incongruent isolate in our study evidenced no point mutation in *vanS*. PFGE of the *Sma* I-digested genomic DNA of the VanB-*vanA* incongruent VRE *faecium* strains in this study evidenced two major patterns, and the clinical isolates and animal isolates were found to bear little similarity (less than 85%) to one another (Fig. 1). Every isolate with MIC to teicoplanin 8 μg/ml belongs to the same group (group B), thereby indicating the presence of clonal spread among the chickens. This result is contradictory with previous reports (Hashimoto *et al.,* 2000; Laudale *et al.*, 2002; Ko *et al.*, 2005) suggesting that vancomycin-resistance is transferred principally via horizontal, rather than clonal, spread.

VanB-*vanA* incongruent VRE has been detected in chickens in Japan (Hashimoto *et al.,* 2000), chickens and humans in Taiwan (Lauderdale *et al.,* 2002), and in humans in Korea (Eom *et al.,* 2004). All of these were determined to be highly vancomycin resistant, whereas their MICs to teicoplanin varied quite significantly. The MIC values were 0.75-12 μg/ml in the chicken and human isolates from Taiwan, 1-2 μg/ml in the chicken isolates from Japan, and 4-8 μg/ml in the human isolates from Korea. In this study, MICs to teicoplanin were 0.5-8 μg/ml in the chicken isolates, 8 μg/ml in human isolates of VRE *faecium,* and 4 μg/ml in human VRE *faecalis* isolates. These results indicated that teicoplanin resistance in VRE *faecium* is more pronounced in human isolates than in chicken isolates. This may be attributable to the use of higher concentrations of antimicrobial agents for therapeutic use in humans, as compared to the low concentrations for prophylactic use in animals. Unlike the isolates with mutations in *vanS* in Japan and Taiwan, the VanB-*vanA* incongruent VRE isolates analyzed in this study harbored no *vanS* mutations, which suggests the operation of another mechanism,

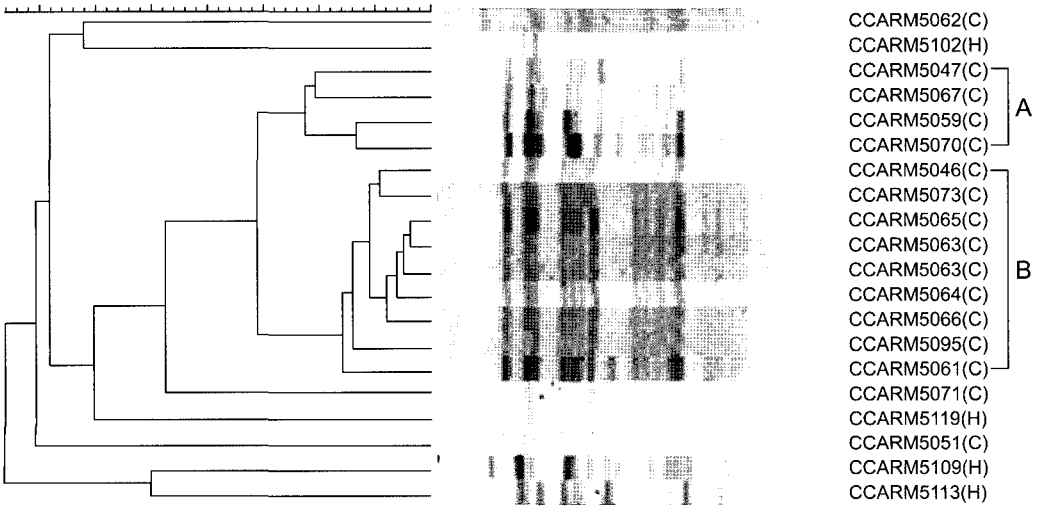

Fig. 1. PFGE of VanB-*vanA* incongruent VRE *faecium* isolated from animals and humans. *Sma*I-digested genomic DNA fragments were separated via pulse field gel electrophoresis. C, chicken isolate; H, human isolate.

such as the gene arrangement hypothesis of Lee *et al.* (2004). This, in turn, appears to indicate the operation of diverse mechanisms with regional specificity in VanB-*vanA* incongruent VRE. The PFGE profiles of VanB-*vanA* incongruent VRE in this study revealed only a peripheral (if any) relationship between the chicken and human isolates obtained in 1999. As clonal spread was detected in chickens in 1999, continuous monitoring of VRE occurrence in animals is necessary, most notably for the prevention of the possible spread of VRE into humans.

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