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Phenotypic and Genotypic Differences of the Vancomycin-Resistant *Enterococcus faecium* Isolates from Humans and Poultry in Korea

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A total of 98 vancomycin-resistant *Enterococcus faecium* (VREF) isolates (58 isolates from patients and 40 isolates from poultry) were compared based on their antimicrobial susceptibility, Tn*1546* element organization, and pulsed-field gel electrophoresis (PFGE) patterns. This comparison aided in determining the relationships between the groups of isolates. All the VREF isolates harbored the *vanA* gene; however, 29 (29.6%) of the isolates exhibited the VanB phenotype-*vanA* genotype. Furthermore, the VREF isolates from humans and poultry exhibited distinct antimicrobial resistance patterns. The PCR mapping of the Tn*1546* elements exhibited 12 different transposon types (A to L). The VREF isolates of poultry were classified into types A to D, whereas the human isolates were classified into types E to L. A PFGE analysis demonstrated a high degree of clonal heterogeneity in both groups of isolates; however, the distinct VREF clones appeared in each group of isolates. The deletion of the *vanX-vanY* genes or insertion of IS*1216V* in the intergenic region from the *vanX-vanY* genes is directly associated with the incongruence of the VanB phenotype-*vanA* genotype in human VREF isolates. These data suggest that the VREF isolates exhibit distinct phenotypic and genotypic traits according to their origins, which suggests that no evidence exists to substantiate the clonal spread or transfer of vancomycin resistance determinants between humans and poultry.

Keywords: *Enetococcus*, vancomycin, Tn*1546*, insertion sequence, clone

Genus *Enterococcus* is a part of the normal intestinal flora in humans and animals; however, enterococci can also be the cause of opportunistic infections, especially among immunocompromised hosts (Murrary, 1998). *Enterococcus faecalis* is generally the predominant species found among human clinical isolates, and accounts for 80 to 90% of enterococci. On the other hand, *Enterococcus faecium* accounts for 5 to 15% of enterococci (Simjee *et al.*, 1999). Since the first emergence of vancomycin-resistant *E. faecalis* in Europe, which occurred in 1988, the frequency of vancomycin-resistant *E. faecalis* and vancomycin-resistant *E. faecium* (VREF) isolates from humans and animals has rapidly increased. Vancomycin resistance has become a major public health concern due to the possible transfer of vancomycin resistance between food-producing animals and humans (Uttley *et al.*, 1998; Hayes *et al.*, 2005; Jung *et al.*, 2006).

Glycopeptides, such as avoparcin and virginiamycin, have been used as growth promoters for livestock or pets, whereas vancomycin and teicoplanin have been used to treat human enterococcal infections (Bager *et al.*, 1997; Hayes *et al.*, 2005). The extensive use of these antimicrobials has caused the emergence of vancomycin-resistant enterococci (VRE) among both groups of isolates. Currently, of the five known *van* genes responsible for vancomycin resistance, the *vanA* gene was the most predominant among the VRE. Based on the susceptibility of teicoplanin, the VRE carrying the *vanA* gene are classified as the VanA phenotype with resistance to both vancomycin and teicoplanin or the VanB phenotype with resistance to vancomycin, but with susceptibility to teicoplanin (Hashimoto *et al.*, 2000; Lee *et al.*, 2004; Kolar *et al.*, 2006). Over the past decade, the majority of VRE observed in humans and animals in Korea exhibited the VanA phenotype, whereas the VanB phenotype-*vanA* genotype enterococci were uncommon (Jung *et al.*, 2006). The mobile genetic Tn*1546* elements mediate the horizontal transfer of the *vanA* gene (Arthur *et al.*, 1993). Moreover, the DNA polymorphisms of the Tn*1546* elements, including the insertion of insertion sequence (IS) elements and the deletion of *van* elements, have been detected and used to trace the origins of vancomycin resistance in enterococci (Lee *et al.*, 2004; Jung *et al.*, 2006).

The animal intestinal tract can act as a reservoir for VRE and food-producing animals can directly transfer VRE to humans via the food chain (Kühn *et al.*, 2005). However, the transmission of VRE or the *van* genes from food-producing animals to humans has been rarely reported (Kühn *et al.*, 2005). With the increasing prevalence of VREF isolates in Korea, we compared the phenotypic and genotypic traits of the VREF isolates from humans and poultry against the antimicrobial agents in order to trace VREF clones and vancomycin resistance determinants.

Materials and Methods

Bacterial strains and species identification

A total of 98 VREF isolates from humans and poultry

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were collected. Fifty-eight isolates from infected patients were obtained from three university hospitals located in Daegu, Korea between 2001 and 2005 (28 isolates from 2001, 11 from 2004, and 19 from 2005). Forty VREF isolates from poultry were obtained from the intestinal swabs of animals housed in a slaughter facility located in Daegu, Korea between 2001 and 2004 (13 isolates from 2001, 22 from 2003, and 5 from 2005). Initially, we performed biochemical tests using the API20STREP kit (bioMérieux SA, France) to identify the *E. faecium* species. Next, *E. faecium* underwent genotype identification by PCR amplification of the species-specific *ddl* gene (Mac *et al.*, 2003). *E. faecium* ATCC 35667 and *E. faecalis* ATCC 29212 were used as positive controls.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined by agar dilution in Mueller-Hinton agar plates (BD Co. Ltd, USA) using the Steers multiple inoculator, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). *E. faecalis* ATCC 29212 and ATCC 51299 were used as quality control strains. The antimicrobial agents tested included ampicillin, vancomycin, teicoplanin, quinupristin-dalfopristin, linezolid, gentamicin, streptomycin, erythromycin, tetracycline, ciprofloxacin, chloramphenicol, and nitrofurantoin.

Detection of the van genes

The vancomycin-resistant genes, including *vanA*, *vanB*, *vanC1*, and *vanC2/C3*, were detected using a multiplex PCR (Mac

Table 1. List of PCR primers used in this study

Gene	Primer	Sequence (5' to 3')	Size (bp)	Reference
ddl$_{E.\ faecium}$	ddl-F	GCA AGG CTT CTT AGA GA	550	Mac *et al.*, 2003
	ddl-R	CAT CGT GTA AGC TAA CTT C		
vanA	vanA-F	CAT GAA TAG AAT AAA AGT TGC AAT A	1,030	Mac *et al.*, 2003
	vanA-R	CCC CTT TAA CGC TAA TAC GAT CAA		
vanB	vanB-F	GTG ACA AAC CGG AGG CGA GGA	433	Mac *et al.*, 2003
	vanB-R	CCG CCA TCC TCC TGC AAA AAA		
vanC1	vanC1-F	GGT ATC AAG GAA ACC TC	822	Mac *et al.*, 2003
	vanC1-R	CTT CCG CCA TCA TAG CT		
vanC2/C3	vanC23-F	CGG GGA AGA TGG CAG TAT	484	Mac *et al.*, 2003
	vanC23-R	CGC AGG GAC GGT GAT TTT		
Transposase (*orf1*)	Tnp-1	GCT AAT CGC AAA ACA TCC TC	1,515	In this study
	Tnp-2	AAT CCA GAA ACA AAA CGG TAA		
Resolvase (*orf2*)	Res-1	AAC GAG ATC GGA ATG GAT A	390	In this study
	Res-2	TCC TGC GTG ATT TTT ATG ATA		
IS*1542*	IS1542 F2	AAC GTC CTC CTG GGT ATG	variable	Jung *et al.*, 2006
	VanR R1	GGC AAT TTC ATG TTC ATC ATC		
vanS	VanS	AAC GAC TAT TCC AAA CTA GAA C	1,094	Miele *et al.*, 1995
	VanS1	GCT GGA AGC TCT ACC CTA AA		
vanR-vanS	p3967	ATG AGC GAT AAA ATA CTT	1,827	Yu *et al.*, 2003
	p5786	TTA GGA CCT CCT TTT ATC		
vanS-vanH	p4649	TTG GTT ATA AAA TTG AAA AAT	2,337	Yu *et al.*, 2003
	p6969	CTA TTC ATG CTC CTG TCT		
vanH-vanA-vanX	p6081	ATG AAT AAC ATC GGC ATT AC	2,606	Yu *et al.*, 2003
	p8607	TTA TTT AAC GGG GAA ATC		
vanX-vanY	p8061	ATG GAA ATA GGA TTT ACT TT	1,947 or variable	Yu *et al.*, 2003
	p9944	TTA CCT CCT TGA ATT AGT AT		
vanY-vanZ	p9052	ATG AAG AAG TTG TTT TTT TTA	1,550	Yu *et al.*, 2003
	p10585	CTT ACA CGT AAT TTA TTC		
vanZ	vanZ-1	ATC TGG TTA GTG TTA TTC AAA	340	In this study
	vanZ-2	GAT TCA TAT GCT TAT TGC TTA		
IS*1216V*	IS1216-F	ATA CCC CAA TAG CTA TTT TGA	variable	In this study
	IS1216-R	TTC ATT TTC AGT CTC CTC CT		
Tn*1546*	pTn*1546*	GGA AAA TGC GGA TTT ACA ACG CTA AG	10,851	Yu *et al.*, 2003

et al., 2003). The primers used in this study are listed in Table 1. PCR amplification was performed using a PTC 200 (MJ-Research Co., USA) DNA thermal cycler. The PCR cycle was as follows: 1 cycle of 5 min at 94°C, 30 cycles at 94°C for 30 sec, 54°C for 45 sec, and 72°C for 45 sec, followed by a final 10 min extension at 72°C.

Molecular analysis of the Tn1546 elements

The PCR mapping for the overlapping internal region of the Tn*1546* elements was performed as described in Yu *et al.* (2003) and Jung *et al.* (2006). The detection of the IS*1216V* internal region, between the downstream of *vanX* and the upstream of *vanY* from the published sequence of Tn*1546*, was designed to be amplified (GenBank accession no. M97297) (Table 1). The PCR products were purified using a PCR purification kit (Qiagen Co., Germany) and sequenced with an automatic sequencer (ABI 3100; Applied Biosystems, USA).

Pulsed-field gel electrophoresis (PFGE)

The preparation of plugs followed the method described in Van Den Braak *et al.* (2000) and the genomic DNA was digested with *Sma*I (Roche Diagnostics, Germany) and separated on a 1.0% agarose gel using a contour-clamped homogeneous-field apparatus (CHEF DRIII systems; Bio-Rad Laboratories, USA). A lambda ladder comprising 48.5 kb concatemers (Bio-Rad Laboratories) was used as a size marker. The agarose gels were run in a 0.5× TBE buffer at 14°C and 6 V/cm for 19 h, with a pulse time of 5 to 40 sec with a linear ramp. The gel images were stored electronically as TIFF files and analyzed using the GelCompar II computer program (Applied Maths, Belgium).

Statistical analysis

Statistical analyses were performed using the χ^2 or Fisher's exact test. A *P*-value <0.05 was considered to be statistically significant.

Results and Discussion

Antimicrobial susceptibility of VREF isolates

All VREF isolates showed a high level of resistance to vancomycin (MIC_{90}, >256 μg/ml); however, 29 (29.6%) of these isolates were susceptible to teicoplanin (Table 2), suggesting an incongruence of the VanB phenotype-*vanA* genotype. Overall, the VREF isolates were highly resistant to ampicillin

Table 2. Antimicrobial susceptibility of vancomycin-resistant *E. faecium* isolates from humans (n=58) and poultry (n=40)

Antibiotic	Origin	MIC (μg/ml)			No. (%) of resistant isolates
		Range	MIC_{50}	MIC_{90}	
Ampicillin	Poultry	8 → 256	64	256	40 (100)
	Human		> 256	> 256	58 (100)
Gentamicin	Poultry	64 → 2,048	< 64	< 64	0 (0)
	Human		> 2,048	> 2,048	53 (91)
Streptomycin	Poultry	64 → 2,048	> 2,048	> 2,048	36 (90)
	Human		512	> 2,048	39 (67)
Vancomycin	Poultry	2 → 256	> 256	> 256	40 (100)
	Human		> 256	> 256	58 (100)
Teicoplanin	Poultry	4 - 512	16	64	26 (65)
	Human		32	256	43 (74)
Quinupristin-dalfopristin	Poultry	0.25 - 8	1	4	10 (25)
	Human		2	2	3 (5)
Erythromycin	Poultry	1 → 256	< 1	< 1	2 (5)
	Human		> 256	> 256	57 (98)
Chloramphenicol	Poultry	1 - 64	4	8	0 (0)
	Human		8	16	0 (0)
Tetracycline	Poultry	2 - 64	16	32	22 (55)
	Human		2	16	7 (12)
Ciprofloxacin	Poultry	0.5 → 32	8	8	39 (98)
	Human		> 32	> 32	52 (90)
Linezolid	Poultry	0.25 - 8	1	1	0 (0)
	Human		2	2	0 (0)
Nitrofurantoin	Poultry	16 - 512	32	64	0 (0)
	Human		64	256	21 (36)

(100%), ciprofloxacin (92.9%), and streptomycin (76.5%). No isolates were resistant to chloramphenicol or linezolid. We found a significant difference in antimicrobial susceptibility between the human and poultry VREF isolates: resistance to gentamicin ($P<0.001$), erythromycin ($P<0.001$), and nitrofurantoin ($P<0.001$) was significantly higher in the human isolates, whereas resistance to streptomycin ($P<0.01$), quinupristin-dalfopristin ($P<0.05$), and tetracycline ($P<0.001$) was significantly higher in the poultry isolates.

In addition to glycopeptide resistance, the VRE strains were resistant to currently used antimicrobial agents, such as aminoglycosides, macrolides, and fluoroquinolones (Zhanel *et al.*, 2001). In particular, a high-level aminoglycoside resistance caused a serious therapeutic problem in a clinical setting, because aminoglycosides have been used in combination therapy with glycopeptides in the treatment of enterococcal infections (Del Campo *et al.*, 2000). In the current study, a high level of gentamicin resistance was detected in the human VREF isolates, whereas a high level of streptomycin resistance was found for both the human and poultry groups. Furthermore, significant differences were observed for the prevalence of resistance to erythromycin, nitrofurantoin, and tetracycline between the VREF isolates from humans and poultry. This may reflect the possible differences in antibiotic selective pressure exerted in humans and poultry. Since susceptible bacteria overcome antibiotic selective pressure through the acquisition of resistance determinants, differences in antimicrobial susceptibility and resistance determinants are useful markers to differentiate the origins of resistant bacteria between humans and animals.

Polymorphism of Tn1546 elements

All VREF isolates were found to carry the *vanA* gene. To analyze the structures of the Tn*1546* elements, the VREF isolates were typed by the PCR mapping of the Tn*1546* elements, including *orf1, orf2,* IS*1542-vanR*, *vanR-vanS*, *vanS-vanH, vanH-vanA-vanX, vanX-vanY,* and *vanY-vanZ*. This analysis revealed that the 98 VREF isolates could be classified into 12 different transposon types (Table 3). Overall, transposon type A was the most predominant (n=37), followed by type F (n=20), type H (n=14), and type I (n=9). However, the prevalence of transposon types was different between the two groups of isolates. The VREF isolates from poultry belonged to types A through D, whereas the human isolates belonged to types E through L. Transposon type A (n=37) was the most predominant in poultry isolates, whereas type F (n=20) and H (n=14) were the most predominant in human isolates. All the human VREF isolates harbored IS*1542* in the intergenic region of *orf2-vanR* genes. Forty-seven (81.0%) of the 58 VREF isolates from humans harbored IS*1216V* in the intergenic region of *vanX-vanY* genes, whereas the insertion of IS*1216V* was not found in the VREF isolates from poultry.

We observed a noticeable difference in the structures of the Tn*1546* elements between the human and poultry VREF isolates. The transposon types were more diverse in the human VREF isolates, because the genetic mutations, including the insertion of IS elements or the deletion of Tn*1546* elements, were more common in human isolates. The majority of the poultry VREF isolates belonged to the prototype of Tn*1546* elements (type A), whereas the insertion of IS*1542* and IS*1216V* was most typical in the human isolates. The insertion of IS*1542* in the *orf2-vanR* intergenic region and IS*1216V* in the intergenic region of *vanX-vanY* has already been detected in the VREF isolates from Korea in previous studies (Lee *et al.*, 2004; Jung *et al.*, 2006; Park *et al.*, 2006). The variability in transposon types may be explained by the different antibiotic selective pressures against glycopeptides, as well as the origins of the strains and the geographical differences in the transposon distributions. The presence of identical transposon types among both groups may indicate a possible exchange of glycopeptide resistance determinants

Table 3. DNA Polymorphism of the Tn*1546* elements in vancomycin-resistant *E. faecium* isolates

Transposon type	PCR amplification of Tn*1546* elements								No. of isolates		
	ORF1	*ORF2*	IS*1542-vanR*	*vanR-VanS*	*vanS-vanH*	*vanH-vanA-vanX*	*vanX-vanY*	*vanY-vanZ*	Humans (n=58)	Poultry (n=40)	Total (n=98)
A	+	+	-	+	+	+	+	+	0	37	37
B	+	-	-	+	+	+	+	+	0	1	1
C	+	+	-	+	+	+	-	-	0	1	1
D	-	+	-	+	+	+	-	-	0	1	1
E	+	+	+ (IS*1542*)[a]	+	+	+	+	+	2	0	2
F	+	+	+ (IS*1542*)	+	+	+	+ (IS*1216V*)[b]	+	20	0	20
G	+	-	+ (IS*1542*)	+	+	+	+ (IS*1216V*)	+	3	0	3
H	-	+	+ (IS*1542*)	+	+	+	+ (IS*1216V*)	+	14	0	14
I	-	-	+ (IS*1542*)	+	+	+	+ (IS*1216V*)	+	9	0	9
J	-	+	+ (IS*1542*)	+	+	+	+ (IS*1216V*)	-	1	0	1
K	-	-	+ (IS*1542*)	+	+	+	-	+	4	0	4
L	-	-	+ (IS*1542*)	+	+	+	-	-	5	0	5

[a]Insertion of IS*1542* within the *orf2-vanR* intergenic region
[b]Insertion of IS*1216V* within the *vanX-vanY* intergenic region

between human and animal isolates or the spread of a specific clone from common reservoirs (Willems *et al.*, 1999). Nevertheless, our data suggests no existence of common transposon types between the VREF isolates from humans and poultry.

Clonal distribution of VREF isolates

The PFGE was performed to compare the genomic similarities of the VREF isolates, which were classified into 58 clones with a similarity value of 0.8 (Fig. 1). The VREF isolates showed a high degree of genomic heterogeneity with

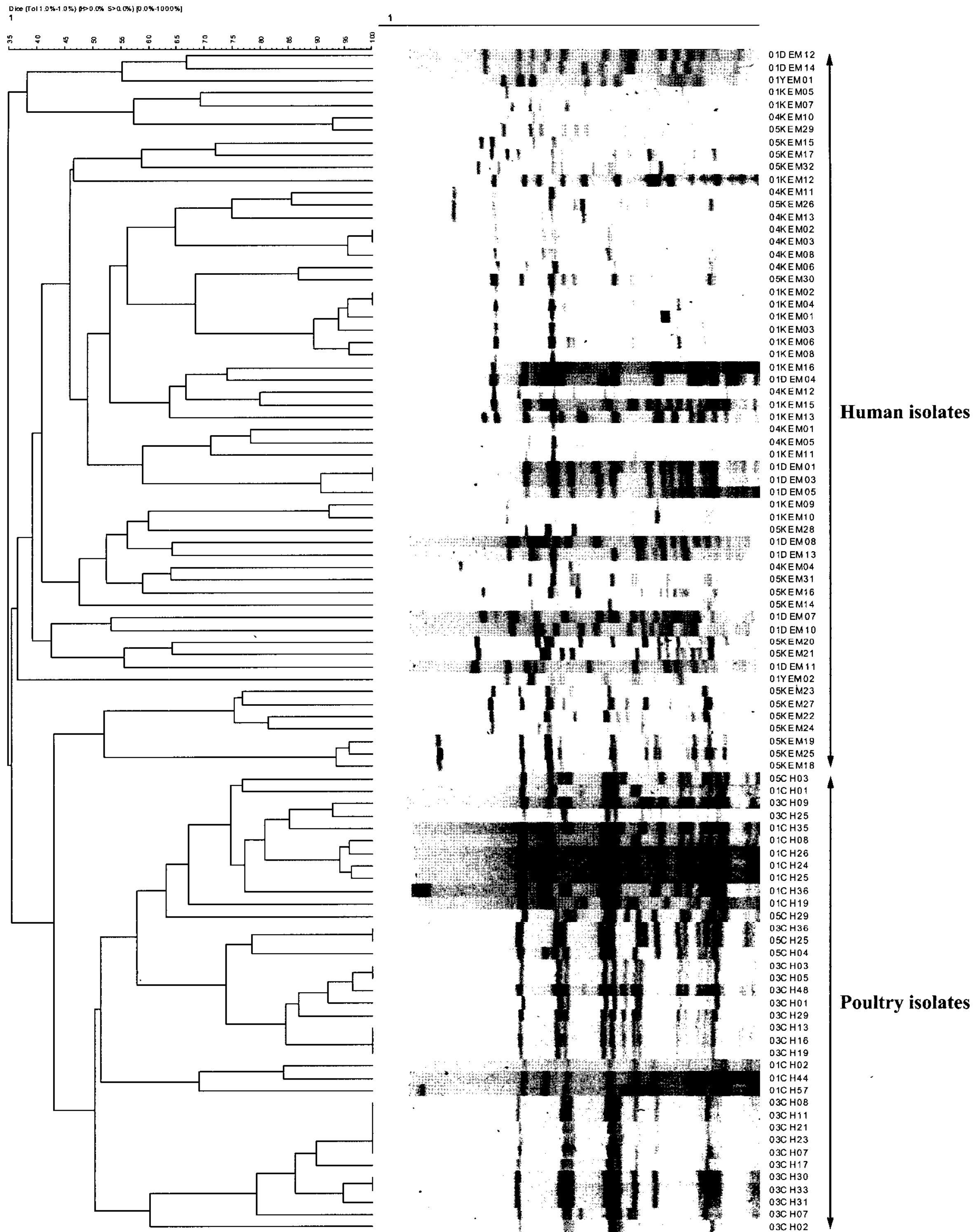

Fig. 1. PFGE analysis of vancomycin-resistant *E. faecium* isolates from humans and poultry. The dendrogram is based on a cluster analysis of the unweighted-pair group method with average linkages.

41 clones from 58 human isolates and 17 clones from 40 poultry isolates.

Nonetheless, the VREF strains can still be potentially transferred from food-producing animals to humans via the food chain. Despite this, the PFGE analysis suggests that no evidence of this horizontal transfer of the VREF strains exist from poultry to humans. The high polyclonal nature of the VREF isolates from both groups suggests a limited spread of the VREF strains, even though they were found in the same hospital or farm. However, Ko *et al.* (2005) reported that the clonal complex 78, which was a globally epidemic VREF clone, was widely distributed among the human clinical VREF isolates in Korea. We did not determine the multilocus sequence types of the VREF isolates in the current study; however, a PFGE analysis demonstrated that diverse VREF clones circulated among both groups.

Incongruence of VanB phenotype-vanA genotype

Sixty-nine (70.4%) of 98 VREF isolates showed the VanA phenotype, whereas 29 (29.6%) of 98 isolates (14 from poultry and 15 from humans) showed the VanB phenotype. These results are consistent with other studies, which demonstrate that the VRE isolates with the VanA phenotype were the most predominant in the human and animal isolates in Korea (Ko *et al.,* 2005; Jung *et al.*, 2006). Nevertheless, the prevalence of the VREF isolates with the VanB phenotype-*vanA* genotype was higher in comparison with the two previous Korean studies (Ko *et al.,* 2005; Jung *et al.*, 2006). Hashimoto *et al.* (2000) demonstrated that the point mutations of the *vanS* gene for the glycopeptide sensor protein, including L50V, E54Q, and Q69H, could be responsible for the incongruence of the VanB phenotype-*vanA* genotype in the VRE strains from Japan. These point mutations of the *vanS* gene were also detected in the VRE isolates from Taiwan and Korea (Lauderdale *et al.*, 2002; Eom *et al.*, 2004). To determine whether the incongruence of the VanB phenotype-*vanA* genotype was due to the point mutations in the *vanS* gene, PCR products with the *vanS*-specific primers (VanS and VanS1) were sequenced. The results revealed that the nucleotide sequences and the deduced amino acid residues from the *vanS* gene in 29 VREF isolates showing the VanB phenotype-*vanA* genotype were identical to the VRE isolates showing the VanA phenotype. The impairment of the accessory proteins from VanY and VanZ or rearrangements of the *vanX*, *vanY*, and *vanZ* genes are also responsible for incongruence of the VanB phenotype-*vanA* genotype (Arthur *et al.*, 1995; Simonsen *et al.*, 2000; Park *et al.*, 2006). Based on the PCR mapping of Tn*1546* elements, the VREF isolates showing the VanB phenotype-*vanA* genotype were classified into 9 transposon types. Fourteen poultry VREF isolates showing the VanB phenotype-*vanA* genotype belonged to transposon type A (n=12), B (n=1), and C (n=1). Fifteen human VREF isolates showing VanB phenotype-*vanA* genotype belonged to transposon type F (n=2), G (n=1), H (n=1), I (n=3), K (n=4), and L (n=4). All the human VREF isolates showing the VanB phenotype-*vanA* genotype exhibited either the insertion of IS*1216V* in the intergenic region of *vanX-vanY* genes (n=7) or the deletion of *vanX-vanY* genes (n=8). On the other hand, one poultry isolate with the VanB phenotype-*vanA* genotype showed the deletion of the *vanX-vanY* genes (transposon type C). Of the 11 VREF isolates carrying the Tn*1546* elements with the deleted form of *vanX-vanY* genes, 9 were shown to belong to the VanB phenotype-*vanA* genotype. This result suggests that the deletion of *vanX-vanY* genes is directly associated with the incongruence of the VanB phenotype-*vanA* genotype in Korean VREF isolates. We could not determine the mechanisms of incongruence of the VanB phenotype-*vanA* genotype in 13 poultry VREF isolates carrying the prototype of the Tn*1546* elements by PCR mapping. The nucleotide sequence analysis of the *vanX-vanY* genes is necessary to characterize VanB phenotype-*vanA* genotype in VREF isolates carrying transposon type A.

In conclusion, the differences in antimicrobial susceptibility, Tn*1546* element organization, and *Sma*I-digested PFGE patterns were observed in the VREF isolates from humans and poultry in Daegu, Korea. These results suggest no evidence supporting the transfer of VREF strains between poultry and humans via the food chain nor a possible exchange of glycopeptide resistance determinants among either group of VREF isolates. However, surveillance studies should be continued in order to monitor the possible future exchange of VREF clones or *van* genes from food-producing animals to humans.

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References

Arthur, M., C. Molinas, F. Depardieu, and P. Courvalin. 1993. Characterization of Tn*1546*, a Tn*3*-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in *Enterococcus faecium* BM4147. *J. Bacteriol.* 175, 117-127.

Arthur, M., F. Depardieu, C. Molinas, P. Reynolds, and P. Courvalin. 1995. The *vanZ* gene of Tn*1546* from *Enterococcus faecium* BM4147 confers resistance to teicoplanin. *Gene.* 154, 87-92.

Bager, F., M. Madsen, J. Christensen, and F.M. Aarestrup. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. *Prev. Vet. Med.* 31, 95-112.

Clinical and Laboratory Standards Institute. 2006. Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M100-S16. Clinical and Laboratory Standards Institute, Wayne, Pa, USA.

Del Campo, R., C. Tenorio, C. Rubio, J. Castillo, C. Torres, and R. Gomez-Lus. 2000. Aminoglycoside-modifying enzymes in high-level streptomycin and gentamicin resistant *Enterococcus* spp. in Spain. *Int. J. Antimicrob. Agents* 15, 221-226.

Eom, J.S., I.S. Hwang, B.Y. Hwang, J.G. Lee, Y.J. Lee, H.J. Cheong, Y.H. Park, S.C. Park, and W.J. Kim. 2004. Emergence of *vanA* genotype vancomycin-resistant enterococci with low or moderate levels of teicoplanin resistance in Korea. *J. Clin. Microbiol.* 42, 1785-1786.

Hashimoto, Y., K. Tanimoto, Y. Ozawa, T. Murata, and Y. Ike. 2000. Amino acid substitutions in the VanS sensor of the VanA-type vancomycin-resistant *Enterococcus* strains result in high-level vancomycin resistance and low-level teicoplanin resistance. *FEMS Microbiol. Lett.* 185, 247-254.

Hayes, J.R., D.D. Wagner, L.L. English, L.E. Carr, and S.W. Joseph. 2005. Distribution of streptogramin resistance determinants among *Enterococcus faecium* from a poultry production environment of the USA. *J. Antimicrob. Chemother.* 55, 123-126.

Jung, W.K., S.K. Hong, J.Y. Lim, S.K. Lim, N.H. Kwon, J.M. Kim, H.C. Koo, S.H. Kim, K.S. Seo, Y. Ike, K. Tanimoto, and Y.H. Park. 2006. Phenotypic and genetic characterization of vancomycin-resistant enterococci from hospitalized humans and from poultry in Korea. *FEMS Microbiol. Lett.* 260, 193-200.

Ko, K.S., J.Y. Baek, J.Y. Lee, W.S. Oh, K.R. Peck, N. Lee, W.G. Lee, K. Lee, and J.H. Song. 2005. Molecular characterization of vancomycin-resistant *Enterococcus faecium* isolates from Korea. *J. Clin. Microbiol.* 43, 2303-2306.

Kolar, M., R. Pantucek, I. Vagnerova, P. Sauer, M. Kesselova, L. Cekanova, D. Koukalova, J. Doskar, and V. Ruzickova. 2006. Prevalence of vancomycin-resistant enterococci in hospitalized patients and those living in the community in the Czech Republic. *New Microbiol.* 29, 121-125.

Kühn, I., A. Iversen, M. Finn, C. Greko, L.G. Burman, A.R. Blanch, X. Vilanova, A. Manero, H. Taylor, J. Caplin, L. Domínguez, I.A. Herrero, M.A. Moreno, and R. Möllby. 2005. Occurrence and relatedness of vancomycin-resistant enterococci in animals, humans, and the environment in different European regions. *Appl. Environ. Microbiol.* 71, 5383-5390.

Lauderdale, T.L., L.C. McDonald, Y.R. Shiau, P.C. Chen, H.Y. Wang, J.F. Lai, and M. Ho. 2002. Vancomycin-resistant enterococci from humans and retail chickens in Taiwan with unique VanB phenotype-*vanA* genotype incongruence. *Antimicrob. Agents Chemother.* 46, 525-527.

Lee, W.G., J.Y. Huh, S.R. Cho, and Y.A. Lim. 2004. Reduction in glycopeptide resistance in vancomycin-resistant enterococci as a result of *vanA* cluster rearrangements. *Antimicrob. Agents Chemother.* 48, 1379-1381.

Mac, K., H. Wichmann-Schauer, J. Peters, and L. Ellerbroek. 2003. Species identification and detection of vancomycin resistance genes in enterococci of animal origin by multiplex PCR. *Int. J. Food Microbiol.* 88, 305-309.

Miele, A., M. Bandera, and B.P. Goldstein. 1995. Use of primers selective for vancomycin resistance genes to determine *van* genotype in enterococci and to study gene organization in VanA isolates. *Antimicrob. Agents Chemother.* 39, 1772-1778.

Murrary, B.E. 1998. Diversity among multidrug-resistant enterococci. *Emerg. Infect. Dis.* 4, 37-47.

Park, I.J., W.G. Lee, H. Lee, D. Yong, K. Lee, E-C, Lim, S. H. Jeong, Y.J. Park, T.Y. Choi, Y. Uh, J.H. Shin, J. Lee, J.Y. Ahn, S-H. Lee, and G-J. Woo. 2006. Mechanism of VanB phenotype in vancomycin-resistant enterococci carrying *vanA* gene. *Korean J. Lab. Med.* 26, 412-417.

Simjee, S., A.P. Fraise, and M.J. Gill. 1999. Plasmid heterogeneity and identification of a Tn*5281*-like element in clinical isolates of high-level gentamicin-resistant *Enterococcus faecium* isolated in the UK. *J. Antimicrob. Chemother.* 43, 625-635.

Simonsen, G.S., M.R. Myhre, K.H. Dahl, Ø. Olsvik, and A. Sundsfjord. 2000. Typeability of Tn*1546*-like elements in vancomycin-resistant enterococci using long-range PCRs and specific analysis of polymorphic regions. *Microb. Drug Resist.* 6, 49-57.

Uttley, A.H., C.H. Collins, J. Naidoo, and R.C. George. 1998. Vancomycin-resistant enterococci. *Lancet* 1, 57-58.

Van Den Braak, N., E. Power, R. Anthony, H.P. Endtz, H.A. Verbrugh, and A. van Belkum. 2000. Random amplification of polymorphic DNA versus pulsed field gel electrophoresis of *Sma*I DNA macrorestriction fragments for typing strains of vancomycin-resistant enterococci. *FEMS Microbiol. Lett.* 192, 45-52.

Willems, R.J., J. Top, N. van den Braak, A. van Belkum, D.J. Mevius, G. Hendriks, M. van Santen-Verheuvel, J.D. van Embden. 1999. Molecular diversity and evolutionary relationships of Tn*1546*-like elements in enterococci from humans and animals. *Antimicrob. Agents Chemother.* 43, 483-491.

Yu, H.S., S.Y. Seol, and D.T. Cho. 2003. Diversity of Tn*1546*-like elements in vancomycin-resistant enterococci isolated from humans and poultry in Korea. *J. Clin. Microbiol.* 41, 2641-2643.

Zhanel, G.G., D.J. Hoban, and J.A. Karlowsky. 2001. Nitrofurantoin is active against vancomycin-resistant enterococci. *Antimicrob. Agents Chemother.* 45, 324-326.