Comparison of genetic epidemiology of vancomycin-resistant *Enterococcus faecium* isolates from humans and poultry

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This study was conducted to investigate the molecular characteristics and genetic relatedness of vancomycin-resistant *Enterococcus faecium* (VREF) isolates obtained from humans and poultry in Korea. A total of 147 VREF isolates from humans (71 clinical isolates) and poultry (76 isolates) in Korea were compared with respect to their antibiotic susceptibilities, organization of the Tn*1546* transposon element, detection of virulence genes (*esp* and *hyl*), pulsed-field gel electrophoresis (PFGE) typing and multilocus sequence typing (MLST). All of the human and poultry isolates had the *vanA* gene and 11.3 % (8/71) of the clinical isolates showed the VanB phenotype/*vanA* genotype. PCR mapping of the Tn*1546* elements was different for isolates of the two groups: human isolates were classified into five transposon types, whereas all poultry isolates were identical to Tn*1546* of *E. faecium* strain BM4147. The *esp* gene was detected in both human (93.0 %, 66/71) and poultry (26.3 %, 20/76) isolates, as was the *hyl* gene (human isolates: 80.3 %, 57/71; poultry isolates: 26.3 %, 20/76). Using MLST, the 71 human isolates could be divided into ten sequence types (STs) belonging to clonal complex (CC) 17 (except for one singleton). The 76 poultry isolates were categorized into 14 STs and 88.2 % (67/76) of the poultry isolates belonged to CC26. PFGE typing of the human isolates demonstrated diverse PFGE profiles among the strains. However, the PFGE patterns of the poultry isolates were possibly related to the strains collected from individual farms. These data suggest that epidemic clonal groups of human and poultry VREFs in Korea have evolved through different evolutionary processes.

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INTRODUCTION

Vancomycin-resistant enterococci (VRE) have emerged as an important cause of hospital-acquired infections and outbreaks worldwide. The incidence of VRE ranges from 20 to 40 % in the USA and is >10 % in some European countries (Abele-Horn *et al.*, 2006). Since the use of the glycopeptide antibiotic avoparcin (a vancomycin analogue) as a growth promoter in animals, VRE has also been demonstrated in the faeces of avoparcin-exposed farm animals and animal-derived food products such as raw meat. For this reason, to reduce the exposure of humans to VRE, the use of avoparcin as a therapeutic treatment in animals was banned in all European Union countries starting from 1995 to 1997 (Simonsen *et al.*, 1998).

In Korea, avoparcin was used in the management of poultry and swine from 1983 to 1997 but was banned thereafter to reduce the exposure of humans to vancomycin (Jung *et al.*, 2007). After the occurrence of the first VRE outbreak in 1992, such incidents have increased significantly since 1998 (Lee *et al.*, 2003). After several years of avoparcin discontinuance in Korea, the prevalence of VRE in Korean livestock was investigated, and some studies reported that the VRE incidence rate in chicken samples was higher than that in pig samples (Seo *et al.*, 2005; Lim *et al.*, 2006). According to the Zyvox Annual Appraisal of Potency and Spectrum programme (a surveillance programme of antibiotic resistance in the UK, in 2007), Korea recorded high VRE rates of 22.0 % in 2003 and 47.2 % in 2004 (Ross *et al.*, 2007).

The enterococcal surface protein, encoded by the chromosomal gene *esp*, and the virulence factor hyaluronidase,

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Abbreviations: CC, clonal complex; IS, insertion sequence; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; QD, quinupristin–dalfopristin; ST, sequence type; VRE, vancomycin-resistant enterococci; VREF, vancomycin-resistant *Enterococcus faecium*.

encoded by the *hyl* gene, have recently been identified as markers of VRE clones that are highly prevalent among hospitalized humans (Rice *et al.*, 2003; Vankerckhoven *et al.*, 2004). However, most studies of these enterococcal virulence factors have been limited to human isolates, with few reports of animal sample analysis. Only a few studies have also examined domestic animals, such as pigs (Camargo *et al.*, 2006), or the presence of *E. faecalis* in raw food samples (Templer & Baumgartner 2007).

To verify the clonal spread of VRE strains and the horizontal transmission of resistance genes from animals to humans, molecular epidemiological studies and *vanA* cluster analysis are used to clarify the genetic relatedness and molecular evolution of human and poultry VRE clones (van den Bogaard *et al.*, 1997). Molecular typing by pulsed-field gel electrophoresis (PFGE) has led to major advancements in our understanding of the epidemiology of VRE, allowing comparisons of isolates from different sources, especially animal and human isolates (Stampone *et al.*, 2005; Willems *et al.*, 2005). Recently, a multilocus sequence typing (MLST) scheme was developed to explore the evolution of *E. faecium* (Bonora *et al.*, 2004). Studies on the diversity of the *vanA* cluster in *E. faecium* isolates have been reported in university hospitals and livestock in Korea (Jung *et al.*, 2006b, 2007).

Here, we studied the prevalence of several enterococcal virulence genes, antibiotic susceptibility and characterization of the *vanA* gene cluster in human and poultry isolates. In addition, the genetic relatedness of these human and poultry VRE isolates from the past 5 years was analysed using PFGE and MLST.

METHODS

Bacterial strains. We analysed VRE isolates from 328 humans (working in the food industry) at five public health centres in 2003, 758 clinical isolates from 13 general hospitals from 2000 to 2005, faecal samples from 431 chickens at nine farms in 2003 and 400 chickens at three farms in 2000, and 100 samples from raw chicken meat in 2003. All VRE were identified as *E. faecium*. A total of 147 vancomycin-resistant *E. faecium* (VREF) isolates were included in this study, comprising 71 clinical samples from 13 general hospitals and 76 poultry samples from six different poultry farms within Chung-Ju, Ham-Yang, Tong-Yung, Yang-Pyung, Yeo-Ju and Yeon-Chen provinces, Korea, and three markets located in Kyung-Gi, Chung-Cheong and Kang-Won provinces, Korea. The poultry isolates were collected from the rectums of chickens and from raw meat, whereas all human isolates were isolated from clinical specimens: blood (two isolates), pus (five isolates), wounds (ten isolates), rectal swabs (three isolates) and urine (51 isolates).

Antimicrobial susceptibility test. The susceptibility of vancomycin-resistant genes was tested using a disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). Ten antibiotics were used for the susceptibility test: ampicillin, chloramphenicol, teicoplanin, ciprofloxacin, erythromycin, gentamicin, quinupristin–dalfopristin (QD), rifampicin, streptomycin and tetracycline. High-level resistance was tested for streptomycin (1000 mg l^{-1}) and gentamicin (500 mg l^{-1}). To verify the phenotype of *vanA*-type VRE, the MICs of vancomycin and teicoplanin for these isolates were determined by Etest (AB Biodisk) using a suspension equivalent to a 0.5 McFarland standard. Resistant and susceptible strains were defined according to CLSI breakpoints (CLSI, 2007). *E. faecalis* ATCC 29212 was used as a quality-control strain.

Detection of vancomycin resistance genes and virulence genes. The resistance genotype of vancomycin-resistant isolates was determined by PCR using primers specific for the *vanA*/*B*/*C* genes as described previously (Clark *et al.*, 1993). PCR amplification of the *esp* and *hyl* genes was performed with previously described primer sets (*esp*: forward, 5′-AGATTTCATCTTTGATTCTTGG-3′, and reverse, 5′-AATTGATTCTTTAGCATCTGG-3′; *hyl*: forward, 5′-CGATGCGC-AAGAATTAGACA-3′, and reverse 5′-CATGATTGGACAACCGA-GTG-3′) using previously described parameters (Camargo *et al.*, 2006).

PFGE. We selected 74 VREF isolates (31 clinical and 43 poultry isolates) according to the regions of the hospitals and farms. The clonal relatedness of these VREF isolates was assessed by PFGE with the following specific modifications. Whole-cell DNA in agarose plugs was prepared as described previously (Miranda *et al.*, 1991) and digested with 20 U *Sma*I for a minimum of 4 h. DNA fragments were separated on a 1.4 % agarose gel using a CHEF DR II apparatus (Bio-Rad) for 20 h with pulse times of 2–8 s. PFGE types and subtypes were defined when PFGE profiles that differed by more than six fragment variations in the same type were observed (Tenover *et al.*, 1995). PFGE patterns were analysed using Gelcompar software (Fingerprinting II Informatix, Bio-Rad) using the Dice coefficient UPMGA method with 1.2 % band tolerance and 0.7 % optimization setting for the entire profile and a dendrogram was constructed.

MLST. MLST was performed as described previously (Enright Spratt, 1999) using seven housekeeping genes (*atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS* and *adk*). The allele number for each gene was assigned based on the *E. faecium* MLST database (http://efaecium.mlst.net/). Allelic profiles are presented as a series of seven integers corresponding to the alleles at each of the loci in the order: *atpA*, *ddl*, *gdh*, *purK*, *gyd*, *pstS* and *adk*. A sequence type (ST) was designated for each unique allelic profile. The eBURST version 3 program was used to determine single-locus variants within groups of related isolates [clonal complexes (CCs)] and to obtain a population snapshot. The eBURST algorithm was implemented as a Java applet at http://eburst.mlst.net.

Structural analysis of the *vanA* cluster. Transposon analysis of the *vanA* cluster was performed using overlapping PCR and primer sequences for PCR as described previously (Arthur *et al.*, 1993). *i*-Max II *Taq* polymerase (Intron) was used for the overlapping PCR, and PCR conditions and components of the PCR mixture were set according to the manufacturer's recommendations. We divided the structures of the *vanA* cluster into five types according to the insertion of insertion sequences (ISs) and the deletion of genes at the left and/or right end of the transposon Tn*1546*. Prototype Tn*1546* was classified as type I, insertion of an IS into the *orf2*-*vanR* and *vanX*-*vanY* intergenic region within Tn*1546* was classified as type II, deletion of *orf1* and/or *orf2* at the left end was classified as type III, deletion of *vanY* and/or *vanZ* at the right end was classified as type IV, and the deletion of the left and right ends was classified as type V.

RESULTS

Characteristics of the strains

The resistance rates for the isolates against the ten antimicrobial agents tested are shown in Fig. 1. The resistance rates of the clinical isolates for five of the antibiotics were higher than those of the poultry isolates, namely ampicillin (93.0 vs 72.4 %), ciprofloxacin (87.3 vs

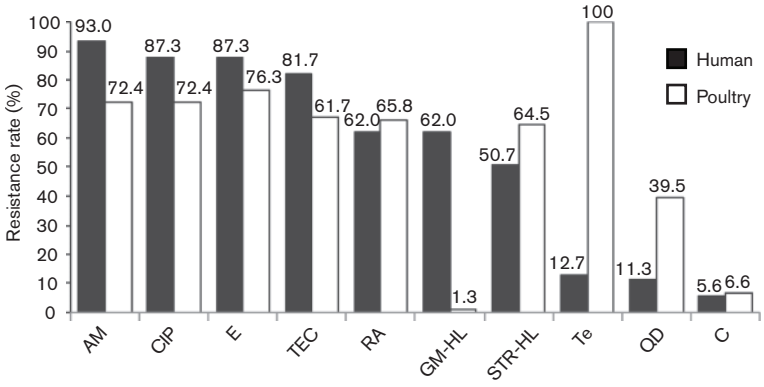


Fig. 1. Antimicrobial resistant rates of 147 VREF isolates from humans and poultry. Ten antimicrobial agents used in this study and their resistance rates were compared. AM, ampicillin; CIP, ciprofloxacin; E, erythromycin; TEC, teicoplanin; RA, rifampicin; GM-HL, high-level gentamicin; STR-HL, high-level streptomycin; Te, tetracycline; QD, quinupristin–dalfopristin; C, chloramphenicol.

72.4 %), erythromycin (87.3 vs 76.3 %), teicoplanin (81.7 vs 67.1 %) and high-level gentamicin (62.0 vs 1.3 %). However, the resistance rates for rifampicin (62.0 vs 65.8 %), high-level streptomycin (50.7 vs 64.5 %), tetracycline (12.7 vs 100 %) and QD (11.3 vs 39.5 %) were lower than those of the poultry isolates. The molecular characteristics of the human and poultry isolates are presented in Table 1. All VREFs were PCR positive for *vanA*. Among 71 clinical isolates, 63 isolates (88.7 %) had the VanA phenotype/*vanA* genotype showing high-level resistance to both vancomycin and teicoplanin, whilst eight isolates (11.3 %) had the VanB phenotype/*vanA* genotype with teicoplanin MICs of 4–24 mg l^{-1} (Hashimoto *et al.*, 2000; Lauderdale *et al.*, 2002; Song *et al.*, 2006). The occurrence of the two virulence genes *esp* and *hyl* differed between the human and poultry isolates; the *esp*-positive isolates made up 93.0 % (66/71) of the clinical isolates and 26.3 % (20/76) of the poultry isolates. The *hyl* gene was detected in 80.3 % (57/71) of the clinical isolates and in 26.3 % (20/76) of the poultry isolates.

PFGE profiles and genetic relatedness

PFGE analysis of 31 human and 43 poultry isolates from different regions revealed that the human and poultry isolates were genetically unrelated (Fig. 2). The PFGE patterns of poultry isolates indicated that these isolates could be assigned to seven groups (A–G) and that the isolates collected from individual farms were possibly related, except for three groups (E–G). By contrast, the PFGE patterns of the clinical isolates had various types and revealed heterogeneity among hospitals. The isolates having PFGE type C1 (four poultry isolates with ST10) and PFGE type K (four clinical isolates with ST203) were each distributed over two different regions.

Analysis of STs and CCs

The results of MLST typing are shown in Table 1. The 71 clinical isolates were divided into ten STs: ST17, ST78, ST80, ST192, ST203, ST204, ST205, ST233, ST234 and ST333. ST78 (35.2 %, 25/71) was the most prevalent type. ST203, ST17, and ST192 were identified in 21.1 % (15/71), 12.7 % (9/71) and 9.8 % (7/71) of isolates, respectively. Three additional STs were newly registered in this study and were confirmed via the MLST website: ST233 (three isolates), ST234 and ST333 (each identified in a single isolate). The 76 poultry samples were identified as 14 STs: ST8, ST9, ST10, ST12, ST26, ST195, ST231, ST232, ST236, ST237, ST238, ST250, ST251 and ST297. Of these isolates, ST10 (15/76, 19.4 %), ST12 (13/76, 17.1 %) and ST26 (14/76 isolates, 18.2 %) were the most frequently identified. Two STs, ST236 (11/76, 14.3 %) and ST232 (8/76, 10.4 %), were also frequently observed in poultry strains. Eight STs were newly registered in this study: ST231, ST232, ST236, ST237, ST238, ST250, ST251 and ST297.

Population analysis performed using the eBURST algorithm found that all of the clinical isolates were members of CC17, except for the singleton ST333. ST17 is the predicted founder of CC17, which is composed of four STs (ST78, ST205, ST233 and ST234) that are single-locus variants of ST17. Four STs (ST80, ST192, ST203 and ST204) are double-locus variants of ST17. Within CC17, ST78 represents a secondary founder of a distinct branch, which is designated complex-17. ST192 is also a secondary founder of another branch, complex-78, which also descends from ST333. Of the poultry isolates, 88.2 % (67/76) were members of CC26, excluding five STs (ST9, ST231, ST237, ST238 and ST250), which were singletons. CC26 comprises four single-locus variants (ST10, ST195, ST236 and ST251) and four double-locus variants (ST8, ST12, ST232 and ST297).

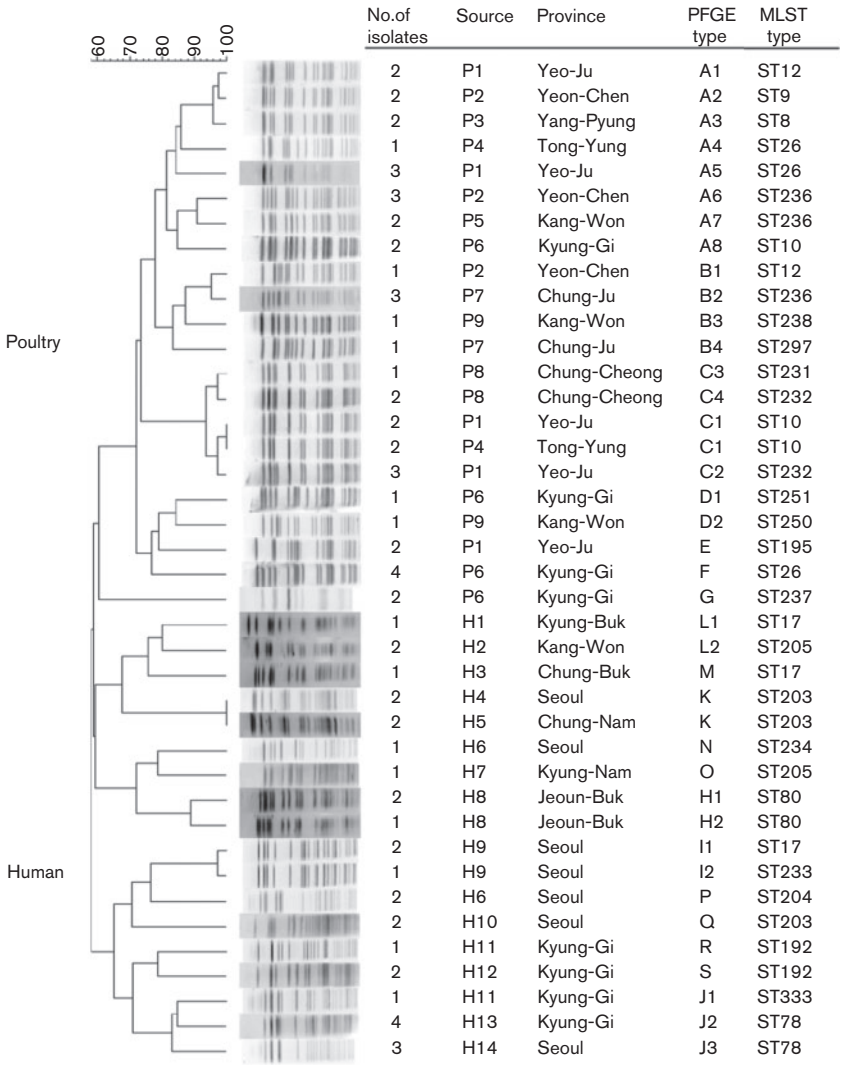
Polymorphism of Tn*1546*-like elements

The distribution of the Tn*1546* cluster in 86 VRE (47 clinical and 39 poultry isolates) is shown in Table 2. All isolates were divided into five types (I–V) on the basis of insertion of ISs and deletion and/or insertion of genes in the *vanA* cluster. The human and poultry isolates belonged to different types. The transposons of all poultry isolates belonged to type I, which is identical to the prototype Tn*1546*. Those of the human isolates were diverse, but most of the transposons were classified into types II and III: four isolates belonged to type I, 18 isolates to type II, 17 isolates to type III, two isolates to type IV and six isolates to type V.

Table 1. Molecular characteristics of human and poultry VREF isolates in Korea

Origin of isolates (*n*)	CC (no. isolates)	ST (*atpA-ddl-gdh-purK-gyd-pstS-adk*)*	Total isolates	Source (no. isolates)†	Specimens (no. isolates)	No. positive isolates (%)		MIC (mg l^{-1})		Year of isolation
						esp	*hyl*	VA	TEC	
Human (71)	CC17 (70)	17 (1-1-1-1-1-1-1)	9	GH (9)	Urine (5), wound (4)	8/9 (88.8)	6/9 (66.6)	⩾1024	12 to ⩾1024	2000–2004
		78 (15-1-1-1-1-1-1)	25	GH (25)	Urine (20), pus (5)	24/25 (96.0)	24/25 (96.0)	⩾1024	4 to ⩾1024	2003–2005
		80 (9-1-1-1-12-1-1)	4	GH (4)	Urine (3), wound (1)	4/4 (100)	4/4 (100)	⩾1024	>1024	2003
		192 (15-1-1-1-1-7-1)	7	GH (7)	Urine (4), blood (2), rectal swab (1)	7/7 (100)	7/7 (100)	⩾1024	16 to >1024	2004, 2005
		203 (15-1-1-1-1-20-1)	15	GH (15)	Urine (11), wound (4)	15/15 (100)	12/15 (80.0)	⩾1024	16 to >1024	2000–2004
		204 (15-1-6-1-1-1-1)	3	GH (3)	Urine (3)	2/3 (66.6)	0/3 (0.0)	⩾1024	>1024	2000
		205 (3-1-1-1-1-1-1)	3	GH (3)	Urine (3)	1/3 (33.3)	3/3 (100)	⩾1024	24–512	2003
		233 (1-1-1-1-1-20-1)	3	GH (3)	Urine (2), rectal swab (1)	3/3 (100)	0/3 (0.0)	⩾1024	>1024	2000
		234 (1-1-6-1-1-1-1)	1	GH (1)	Wound (1)	1/1 (100)	0/1 (0.0)	>1024	>1024	2000
	Singleton (1)	**333** (15-2-1-1-1-7-1)	1	GH (1)	Rectal swab (1)	1/1 (100)	1/1 (100)	⩾1024	>1024	2004
Poultry (76)	CC26 (67)	8 (5-2-1-6-1-7-1)	2	F (2)	Faeces (2)	0/2 (0.0)	0/2 (0.0)	⩾512	64, 256	2003
		10 (5-2-1-6-1-1-1)	15	F (12), M (3)	Faeces (12), meat (3)	2/15 (13.3)	4/15 (26.6)	⩾256	⩾256	2000–2003
		12 (5-2-6-6-1-1-1)	13	F (13)	Faeces (13)	7/13 (53.8)	7/13 (53.8)	⩾256	⩾64	2003
		26 (9-2-1-6-1-1-1)	14	F (8), M (6)	Faeces (8), meat (6)	6/14 (42.8)	6/14 (42.8)	⩾1024	⩾256	2000–2003
		195 (9-2-1-6-1-7-1)	2	F (1), M (1)	Faeces (1), meat (1)	0/2 (0.0)	0/2 (0.0)	⩾256	128	2002, 2003
		232 (15-2-24-6-1-1-1)	8	F (4), M (4)	Faeces (4), meat (4)	2/8 (25.0)	1/8 (12.5)	⩾1024	⩾512	2000–2003
		236 (9-2-6-6-1-1-1)	11	F (7), M (4)	Faeces (7), meat (4)	0/11 (0.0)	0/11 (0.0)	⩾1024	⩾256	2000–2003
		251 (9-2-1-34-1-1-1)	1	M (1)	Meat (1)	1/1 (100)	1/1 (100)	⩾1024	512	2000
		297 (9-2-6-36-1-1-1)	1	F (1)	Faeces (1)	0/1 (0.0)	0/1 (0.0)	⩾1024	256	2003
	Singleton (9)	9 (5-2-6-6-1-7-1)	2	F (2)	Faeces (2)	0/2 (0.0)	1/2 (50.0)	⩾512	256	2003
		231 (15-1-24-6-1-1-1)	1	M (1)	Meat (1)	1/1 (100)	0/1 (0.0)	⩾1024	⩾256	2000
		237 (5-2-24-14-1-7-1)	4	M (4)	Meat (4)	0/4 (0.0)	0/4 (0.0)	⩾256	⩾64	2000–2003
		238 (2-2-6-6-1-7-1)	1	M (1)	Meat (1)	0/1 (0.0)	0/1 (0.0)	⩾1024	⩾512	2000
		250 (3-2-7-34-1-1-1)	1	M (1)	Meat (1)	1/1 (100)	0/1 (0.0)	⩾512	64	2000

*Bold numbers indicate new STs identified in this study.
†GH, General hospital; F, poultry farm; M, market.



No.of isolates	Source	Province	PFGE type	MLST type
2	P1	Yeo-Ju	A1	ST12
2	P2	Yeon-Chen	A2	ST9
2	P3	Yang-Pyung	A3	ST8
1	P4	Tong-Yung	A4	ST26
3	P1	Yeo-Ju	A5	ST26
3	P2	Yeon-Chen	A6	ST236
2	P5	Kang-Won	A7	ST236
2	P6	Kyung-Gi	A8	ST10
1	P2	Yeon-Chen	B1	ST12
3	P7	Chung-Ju	B2	ST236
1	P9	Kang-Won	B3	ST238
1	P7	Chung-Ju	B4	ST297
1	P8	Chung-Cheong	C3	ST231
2	P8	Chung-Cheong	C4	ST232
2	P1	Yeo-Ju	C1	ST10
2	P4	Tong-Yung	C1	ST10
3	P1	Yeo-Ju	C2	ST232
1	P6	Kyung-Gi	D1	ST251
1	P9	Kang-Won	D2	ST250
2	P1	Yeo-Ju	E	ST195
4	P6	Kyung-Gi	F	ST26
2	P6	Kyung-Gi	G	ST237
1	H1	Kyung-Buk	L1	ST17
2	H2	Kang-Won	L2	ST205
1	H3	Chung-Buk	M	ST17
2	H4	Seoul	K	ST203
2	H5	Chung-Nam	K	ST203
1	H6	Seoul	N	ST234
1	H7	Kyung-Nam	O	ST205
2	H8	Jeoun-Buk	H1	ST80
1	H8	Jeoun-Buk	H2	ST80
2	H9	Seoul	I1	ST17
1	H9	Seoul	I2	ST233
2	H6	Seoul	P	ST204
2	H10	Seoul	Q	ST203
1	H11	Kyung-Gi	R	ST192
2	H12	Kyung-Gi	S	ST192
1	H11	Kyung-Gi	J1	ST333
4	H13	Kyung-Gi	J2	ST78
3	H14	Seoul	J3	ST78

Fig. 2. Dendrogram obtained using PFGE profiles for the clinical and poultry VREF isolates analysed in this study. Samples were obtained from nine different farms (P1–P9) and 14 hospitals (H1–H14). PFGE types were allocated according to the criteria of Tenover *et al.* (1995).

DISCUSSION

Avoparcin has been used as a feed additive since the 1980s but was prohibited in 1997 in Korea. Since then, some research on this subject, including that done by us, has been performed. Previously, we reported that VRE rates in poultry in 2003 were 28.1 %, and that >60 % of them were multidrug resistant (Jung *et al.*, 2006b). In addition to these outcomes, in this study, we compared the prevalence and genetic relatedness of VRE between human isolates from hospitals and poultry isolates from farms.

The VRE isolates from humans and poultry showed different resistance rates to three antibiotics: high-level gentamicin (62.0 vs 1.3 %), tetracycline (12.7 vs 100 %) and QD (11.3 vs 39.5 %). The high resistance rates to QD and tetracycline in poultry were expected due to the addition of virginiamycin as a feed additive from 1970 to 1999 and tetracycline as a feed additive in 2008. The QD resistance rate in our clinical isolates appeared not to be associated with the recent use of QD in the hospital for the treatment of VRE, as was reported previously (Oh *et al.*, 2005). In the USA and Greece, QD resistance emerged even before its commercial use was reported (Oh *et al.*, 2005; Karanika *et al.*, 2008). These results suggest that the use of QD may not be the sole reason for the high rate of resistance and that there may be other reasons.

With regard to VREF, the frequency of the virulence factors in hospitals and in poultry was 93.0 and 26.3 % in the *esp*-positive isolates, and 80.3 and 26.3 % in the *hyl*-positive isolates. Over 90 % of the VRE isolates in hospitals in Korea were found to be resistant to ampicillin and ciprofloxacin (Yoo *et al.*, 2006) and carried the *esp* gene (Ko *et al.*, 2005). A 3 % *hyl*-positive incidence rate has been observed in European clinical isolates (Vankerckhoven *et al.*, 2004). High rates of *esp*- or *hyl*-positive isolates in humans have also been documented in the USA (Rice *et al.*, 2003). However, the isolation rates of virulence factors were higher in poultry in Korea than in other countries, and the cause of this should be investigated further.

Table 2. Diversity of Tn*1546* in 86 *E. faecium* isolates carrying the *vanA* gene from humans and poultry

+, Positive; −, negative; ++, larger than the size of the prototype Tn*1546* by insertion of IS elements.

Tn type	No. (%) of samples from:		PCR products of *vanA* cluster*											
	Hospital	Poultry	orf1	orf2	orf2-vanR	vanR	vanS	vanH	vanA	vanX	vanX-vanY	vanY	vanY-vanZ	vanZ
I	4 (8.5)	39 (100)	+	+	+	+	+	+	+	+	+	+	+	+
II	1 (2.1)	0 (0)	+	+	+	+	+	+	+	+	++	+	+	+
	17 (36.2)	0 (0)	+	+	++	+	+	+	+	+	++	+	+	+
III	5 (10.6)	0 (0)	−	+	++	+	+	+	+	+	++	+	+	+
	10 (21.3)	0 (0)	−	−	++	+	+	+	+	+	++	+	+	+
	2 (4.3)	0 (0)	−	−	−	+	+	+	+	+	++	+	+	+
IV	2 (4.3)	0 (0)	+	+	++	+	+	+	+	+	−	−	−	−
V	2 (4.3)	0 (0)	−	−	++	+	+	+	+	+	−	−	+	+
	1 (2.1)	0 (0)	−	−	++	+	+	+	+	+	++	+	−	−
	3 (6.3)	0 (0)	−	−	−	+	+	+	+	+	−	−	−	−
Total	47 (100)	39 (100)												

*PCR was performed using overlapping PCR (Arthur *et al.*, 1993).

We performed PFGE and MLST as complementary typing methods in order to trace the evolutionary origins of VREF strains in both hospitals and poultry. Most of the clinical isolates isolated in 2000–2005 had unrelated PFGE patterns (Fig. 2). In contrast, the PFGE profiles among the poultry isolates revealed genetically related patterns within farms and within regions (Fig. 2). Therefore, human and poultry clonal groups may have evolved independently over time. Three STs (ST17, ST78 and ST203) were widely distributed in the human population, and the ST78 isolates were isolated predominantly in 2003–2005. ST17, ST78 and ST80 have been isolated previously in Europe and the USA and are distributed worldwide (Stampone *et al.*, 2005; Willems *et al.*, 2005). ST192, ST203, ST204 and ST205, which were first reported by Ko *et al.* (2005), were also observed in this study. According to the MLST analysis, most of the VREF clinical isolates were members of CC17, which is related to a globally epidemic *E. faecium* clone. CC17 (designated the 'C1 lineage'), as described by Homan *et al.* (2002), is a documented hospital-derived VRE that contains the virulence gene *esp*. CC17 is characterized by ampicillin or quinolone resistance and the presence of *esp* or *hyl* genes (Leavis *et al.*, 2006). This high-risk enterococcal CC has been reported worldwide, including in Korean hospitals (Ko *et al.*, 2005; Tenover & McDonald, 2005; Willems *et al.*, 2005; Camargo *et al.*, 2006). ST17 and its descendent ST80 have emerged in hospitalized patients, first in the USA and now in other countries, including Korea (Ko *et al.*, 2005; Stampone *et al.*, 2005).

The structure of the *vanA* cluster for human and poultry isolates in Korea has been reported recently, and the transposons of the *vanA* cluster in human isolates are very different from those in poultry. In this study, the structure of the *vanA* cluster of the poultry isolates from 2000 and 2003 was that of the prototype Tn*1546*. Our clinical isolates were characterized by IS*1216V* insertion in the *orf2-vanR* region and IS*1542* insertion in the *vanX-vanY* region (Table 1). These results are similar to the results of other researchers (Handwerger *et al.*, 1995; MacKinnon *et al.*, 1997; Willems *et al.*, 1999; Yu *et al.*, 2003; Huh *et al.*, 2004; Camargo *et al.*, 2005; Jung *et al.*, 2006a; Park *et al.*, 2007). Changes in the phenotype of VRE carrying the *vanA* gene have been reported in many countries (Jensen *et al.*, 1999; Hashimoto *et al.*, 2000; Lauderdale *et al.*, 2002; Park *et al.*, 2007, 2011; Gu *et al.*, 2009). Of these phenotypic changes, the VanB phenotype/*vanA* genotype isolates harboured point mutations in three *vanS* sites (nt 4796, 4808 and 4855) in the early 2000s, but recently it has been reported that they do not carry point mutations in *vanS*, but carry *vanZ*, which shows resistance to teicoplanin (Park *et al.*, 2007; Gu *et al.*, 2009). Our eight clinical isolates with the VanB phenotype (teicoplanin MICs of 4–24 mg l^{-1}) had the same *vanS* sequence of BM4147 and seven harboured the *vanZ* gene. Therefore, these changes in the *vanA* cluster indicate that the transposons of clinical isolates are expected to be more diverse.

In conclusion, we found that Korean VREFs of the CC17 clonal group had high rates of *esp* and *hyl* genes in clinical isolates and that poultry strains of the CC26 clonal group had relatively lower rates of these genes compared with the clinical isolates. Furthermore, we demonstrated that clonal spread of VREF strains between human and poultry in Korea appears to be absent. Since 2005, in Korea, the use of some antibiotics as feed additives in poultry has been banned. Therefore, continued investigation of the spread and transmission of multidrug-resistant *E. faecium* strains between humans and animals (including poultry) will provide useful strategies for the use of antimicrobials.

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