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Characterization of Veterinary Hospital-Associated Isolates of *Enterococcus* Species in Korea

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Possible cross-transmission of hospital-associated enterococci between human patients, medical staff, and hospital environments has been extensively studied. However, limited information is available for veterinary hospital-associated *Enterococcus* isolates. This study investigated the possibility of cross-transmission of antibiotic-resistant enterococci between dog patients, their owners, veterinary staff, and hospital environments. Swab samples (n = 465) were obtained from five veterinary hospitals in Seoul, Korea, during 2011. Forty-three *Enterococcus* strains were isolated, representing seven enterococcal species. *E. faecalis* and *E. faecium* were the most dominant species (16 isolates each, 37.2%). Although slight differences in the antibiotic resistance profiles were observed between the phenotypic and the genotypic data, our antibiogram analysis demonstrated high prevalence of the multiple drug-resistant (MDR) isolates of *E. faecalis* (10/16 isolates, 62.5%) and *E. faecium* (12/16 isolates, 75.0%). Pulsed-field gel electrophoretic comparison of the MDR isolates revealed three different clonal sets of *E. faecalis* and a single set of *E. faecium*, which were isolated from different sample groups or dog patients at the same or two separate veterinary hospitals. These results imply a strong possibility of cross-transmission of the antibiotic-resistant enterococcal species between animal patients, owners, veterinary staff, and hospital environments.

Keywords: Antibiotic resistance, enterococci, veterinary hospitals, genetic relatedness, cross-transmission

Introduction

Enterococci are saprophytic, Gram-positive, facultative anaerobes that often occur in pairs or short chains. As part of commensal inhabitants that belong to the normal gastrointestinal microflora of humans and animals [1], the microorganisms are known to be commonly isolated from several food sources, including meats and milk products, as well as from various natural environments [12]. Thus, they were originally considered harmless to humans [10]. However, enterococci have recently emerged and been recognized as one of the leading causative agents of nosocomial infections, especially for species that have multiple drug resistance (MDR) [7, 30].

Human infections caused by enterococci are frequently associated with bacteremia, urinary tract infections, endocarditis, and meningitis [15, 27]. Moreover, the microorganisms can easily acquire antibiotic resistance by either genetic mutation or horizontal gene transfer *via* certain mobile genetic elements such as transposons, bacteriophages, and plasmids [13, 20, 25]. As a result, they possess intrinsic or acquired antibiotic resistance properties against several antibiotics, including glycopeptides, β-lactams, fluoroquinolones, and high levels of aminoglycosides, including gentamicin and streptomycin [6]. Recently, the development of MDR among enterococcal species has become a major public health issue worldwide, partially driven by the overuse or abuse of antibiotics in both human and veterinary practices.

Enterococci thrive well in harsh environments. They can

persist on various *ex vivo* environments, such as medical equipment and/or dry hospital surfaces, aided by their tolerance to heat, chlorine, and alcohol [5, 11, 13]. Thus, it has been hypothesized that enterococci are widely disseminated in hospital environments. Supporting this notion, hospital-acquired enterococcal infections have been extensively reported in human health care units [2, 31]. Indeed, it has been reported that enterococcal isolates are ranked as the second most important pathogens among the intensive care unit-acquired bloodstream infections in Europe [2]. However, much less attention has been given to companion animal patients and their contribution to the cross-transmission of the antibiotic-resistant enterococci within and/or between veterinary hospitals. Only a few studies have reported on the possibility of cross-transmission of antibiotic-resistant bacteria or some pathogenic clones between companion animals and hospital environments in small animal clinics [11].

In this study, veterinary hospital-associated *Enterococcus* species were isolated and identified from samples acquired from dog patients, their owners, veterinary staff, and hospital environments in five veterinary hospitals in Seoul, Korea, during 2011. The antibiotic resistance profiles and molecular fingerprints of the isolates were determined to compare their clonality.

Materials and Methods

Sampling

A total of 465 swab samples were collected from four private small veterinary clinics and one veterinary teaching hospital in Seoul, Korea, throughout 2011. All the individual samples from 171 dog patients (external auditory meatus, 43 samples; medial canthus, 43 samples; interdigital cleft, 42 samples; nasal cavity, 2 samples; skin, 1 sample; anus, 40 samples), 123 pet owners (external auditory meatus, 41 samples; nasal cavity, 41 samples; medial side of arms, 40 samples; medial canthus, 1 sample), 150 veterinary staff members (external auditory meatus, 50 samples; nasal cavity, 50 samples; medial side of arms, 50 samples), and 21 hospital environments (tables, otoscopes, stethoscopes, telephones, computer keyboards, floor, and sinks; 3 samples each) were aseptically obtained, immediately placed into the individual sterile collection tubes containing Amies transport medium (Yu-Han Lab Tech, Korea), and transported on ice to the laboratory within 6 h after collection. Each human sample was routinely taken from the external auditory meatus, nasal cavity, medial side of the arms, and medial canthus. Each animal sample was routinely acquired from the external auditory meatus, medial canthus, interdigital cleft, nasal cavity, skin, and anus. Each environmental sample was routinely taken from tables, otoscopes, stethoscopes, telephones, computer keyboards, floors, and sinks in the veterinary hospitals.

Isolation and Identification of *Enterococcus* Species

All the swab samples were streaked on 5% sheep blood agar plates (Komed, Seongnam, Korea) and incubated at 37ºC for 24 h. Putative *Enterococcus* spp. were isolated according to a standard protocol previously established in our laboratory [24]. For species differentiation, both the genus-specific polymerase chain reaction (PCR) identification method [23] and the VITEK 2 bacterial identification system (BioMerieux, Craponne, France) were carried out based on the manufacturer's instructions. For further confirmation, *E. faecalis* and *E. faecium* were identified by species-specific PCR [14], whereas the other *Enterococcus* spp. were identified by 16S ribosomal RNA sequencing [14, 16]. The PCR primers in this study are shown in Table 1.

Antibiotic Resistance Profiling

Antibiotic susceptibility was determined by a standard disk diffusion test [29] with the following antibiotic disks (Becton Dickinson, Sparks, MD, USA): tetracycline (TE, 30 μg), chloramphenicol (C, 30 μg), erythromycin (E, 15 μg), quinupristin/dalfopristin (SYN, 15 μg), ciprofloxacin (CIP, 5 μg), ampicillin (AM, 10 μg), vancomycin (VA, 30 μg), high-level gentamicin (HLG, 120 μg), high-level streptomycin (HLS, 300 μg), teicoplanin (TEC, 30 μg), and linezolid (LZD, 30 μg). The interpretation of antibiotic resistance, intermediate resistance, or susceptibility was done as described by the Clinical and Laboratory Standards Institute guidelines [29]. *E. faecalis* ATCC 29212 (American Type Culture Collection, Manassas, VA, USA) was used as the reference strain. The MDR isolates were defined as *Enterococcus* isolates resistant to three or more different categories of the evaluated antibiotics [22].

Detection of the Antibiotic Resistance Genes

To determine the mechanisms of antimicrobial resistance among the antibiotic-resistant *Enterococcus* isolates, all the isolates resistant to vancomycin, erythromycin, tetracycline, chloramphenicol, high-level gentamicin, and high-level streptomycin were PCR-screened for the presence of the following six resistance genes; vancomycin (*vanA* and *vanB*), erythromycin (*ermB*), tetracycline (*tetM* and *tetL*) [26], chloramphenicol (*cat*), high-level gentamicin (*aac6'*-Ie-*aph2"*-Ia), and high-level streptomycin (*ant6*-Ia) [21]. The PCR primers specific to the individual target genes are listed in Table 1.

Molecular Fingerprinting

The genetic relatedness among the antibiotic-resistant *Enterococcus* isolates was determined by standard pulsed-field gel electrophoresis (PFGE) using CHEF MAPPER (Bio-Rad, Hercules, CA, USA) as described by the manufacture. In brief, bacterial cells from an overnight culture in 3 ml of Tryptic Soy Broth (Becton Dickinson) were pelleted at 13,000 rpm for 5 min. The pelleted cells were embedded in 1.6% agarose plugs and lysed by lysozyme (Sigma-Aldrich, St. Louis, MO, USA) and proteinase K (Sigma-Aldrich). Lysed plugs were then digested overnight with 40 U of *Sma*I (New England Biolabs, Waltham, MA, USA) at 25ºC. Digested plugs were placed on 1.2% SeaKem Gold agarose (Lonza, Allendale, NJ,

Table 1. Oligonucleotide sequences used in this study.

Primers	Nucleotide sequences (5′ to 3′)	Product size (bp)	Reference
Enterococcus spp.	FW: TACTGACAAACCATTCATGATG	112	[20]
	RV: AACTTCGTCACCAACGCGAAC		
E. faecalis	FW: ACTTATGTGACTAACTTAACC	360	[16]
	RV: TAATGGTGAATCTTGGTTTGG		
E. faecium	FW: GAAAAAACAATAGAAGAATTAT	215	[16]
	RV: TGCTTTTTTGAATTCTTCTTTA		
vanA	FW: CATGAATAGAATAAAAGTTGCAATA	1,030	[6]
	RV: CCCCTTTAACGCTAATACGATCAA		
vanB	FW: GTGACAAACCGGAGGCGAGGA	433	[6]
	RV: CCGCCATCCTCCTGCAAAAAA		
ermB	FW: TGGTATTCCAAATGCGTAATG	745	[26]
	RV: CTGTGGTATGGCGGGTAAGT		
tetM	FW: GTGGACAAAGGTACAACGAG	406	[26]
	RV: CGGTAAAGTTCGTCACACAC		
tetL	FW: TGGTGGAATGATAGCCCATT	229	[26]
	RV: CAGGAATGACAGCACGCTAA		
cat	FW: ATGACTTTTAATATTATTRAWTT	648	[14]
	RV: TCATYTACMYTATSAATTATAT		
aac6′-Ie-*aph2′′*-Ia	FW: CCAAGAGCAATAAGGGCATA	220	[21]
	RV: CACTATCATACCACTACCG		
ant6-Ia	FW: ACTGGCTTAATCAATTTGGG	597	[21]
	RV: GCCTTTCCGCCACCTCACCG		

USA) and PFGE was carried out at 6.0 V for 19 h with a ramped pulse time of 1-20 sec in 0.5× Tris-Borate-EDTA (TBE) buffer at 14°C. BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) was used to establish a DNA similarity matrix using the Dice coefficient (0.5% optimization, 1.0% tolerance) and the unweighted pair group method (UPGMA). All statistical comparisons were performed using the chi-square test and the SPSS ver. 12 software (SPSS, Chicago, IL, USA).

Results and Discussion

In this study, 43 veterinary hospital-associated *Enterococcus* strains from five veterinary hospitals in Seoul, Korea, were isolated, speciated, and characterized for their antibiotic resistance profiles as well as molecular fingerprints to determine genetic similarities between those isolates. Our results imply a strong possibility of cross-transmission between dog patients, their owners, veterinary staff, and hospital environments within and/or among veterinary hospitals in Korea.

Prevalence of *Enterococcus* spp. from the Veterinary Hospital-Associated Swab Samples

Among the 465 veterinary hospital-associated swab samples analyzed, 43 *Enterococcus* spp. (9.2%) were isolated and further differentiated into seven different species: *E. faecalis*, *E. faecium*, *E. hirae*, *E. gallinarum*, *E. casseliflavus*, *E. canintestini*, and *E. dispar* (Table 2). Our results showed that both *E. faecalis* (16/43 isolates; 37.2%) and *E. faecium* (16/43; 37.2%) were the most dominant *Enterococcus* spp., collectively accounting for 74.4% of the total *Enterococcus* isolates (Table 2). Interestingly, both *E. faecalis* and *E. faecium* are also known as the predominant species involved in human infections [28]. Other *Enterococcus* spp. were also isolated but seemed to be rare [23], which included *E. hirae* (4/43 isolates; 8.5%), *E. gallinarum* (3/43; 6.4%), *E. canintestini* (2/43; 4.7%), *E. casseliflavus* (1/43; 2.3%), and *E. dispar* (1/43; 2.3%) (Table 2). Notably, a higher prevalence of *Enterococcus* spp. in the samples of dog patients (33/171 isolates; 19.3%) and hospital environment (3/21; 14.3%) was observed than those of pet owners (3/123; 2.4%) and veterinary staff (4/

Table 2. Prevalence of *Enterococcus* spp. from veterinary hospitals in Korea, 2011.

Group of samples	No. of samples	No. of the following *Enterococcus* isolates/No. of the samples tested (%)					
		Total	*E. faecalis*	*E. faecium*	*E. gallinarum*	*E. hirae*	Others[a]
Dog patient	171	33 (19.3)	14 (8.2)	9 (5.3)	3 (1.8)	3 (1.8)	4 (2.3)
Pet owner	123	3 (2.4)	0 (0.0)	2 (1.6)	0 (0.0)	1 (0.8)	0 (0.0)
Veterinary staff	150	4 (2.7)	2 (1.3)	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Environment[b]	21	3 (14.3)	0 (0.0)	3 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)
Total	465	43 (9.3)	16 (3.4)	16 (3.4)	3 (0.6)	4 (0.9)	4 (0.9)

[a]Includes *E. casseliflavus*, *E. canintestini*, and *E. dispar*.

[b]Samples from veterinary hospital environments.

150; 2.7%). Taken together, our results demonstrate that *E. faecalis* and *E. faecium* were most prevalent among the veterinary hospital-associated *Enterococcus* spp. in Korea, which is consistent with previous studies in Portugal and the United States [18, 30].

Phenotypic Characterization of Antibiotic Resistance Among *E. faecalis* and *E. faecium* Isolates

The antibiotic resistance profiles were examined for all the *E. faecalis* and *E. faecium* isolates because they were the most predominant *Enterococcus* spp. related with veterinary hospitals in Korea (Table 2). None of the 32 isolates displayed vancomycin resistance using a standard disk diffusion test (Table 3). Similar to our observation, previous studies have reported very rare detection of vancomycin-resistant *Enterococcus* (VRE) isolates from veterinary medical equipment or domestic animals, such as dogs and cats, in small animal clinics [19, 23]. Therefore, it appears that VRE strains are not yet prevalent in veterinary hospitals or environments, unlike in human hospitals or environments. The glycopeptide antibiotic avoparcin can induce cross-resistance with vancomycin [6]. In Korea, however, the use of avoparcin in feed and feed additives has been banned since 1998. The absence of VRE in the veterinary hospitals examined in this study might be directly or indirectly related with the governmental ban on the use of avoparcin.

As shown in Table 3, however, our antibiogram analyses revealed that the antibiotic resistance rates of the *E. faecalis* isolates were 68.8% and 56.3% for tetracycline (11/16 isolates) and erythromycin (9/16), respectively, which were followed by 37.6% for chloramphenicol (6/16) and 6.25% for both high-level gentamicin and high-level streptomycin (1/16 each). Although limited information has been available, a recent study in the United States revealed that resistance to enrofloxacin (73.0%), erythromycin (53.9%), ampicillin (51.0%), and doxycycline (42.9%) was detected among 115 *E. faecium* isolates from small animal clinics [23], suggesting that the resistance profiles are similar to those in Korea. Since their intrinsic resistance against quinupristin/dalfopristin has been well established [3, 9], we originally decided to exclude evaluation of quinupristin/dalfopristin resistance among the *E. faecalis* isolates. However, a recent study demonstrated that some clinical strains of *E. faecalis* carry premature stop codons in the *lsa* gene responsible for quinupristin/dalfopristin resistance [8]. In support of the latter observation, four *E. faecalis* isolates were susceptible to quinupristin/dalfopristin, implying that such a nonsense mutation might be increased among strains of *E. faecalis*. In comparison, the antibiotic resistance rates of the *E. faecium* isolates were 81.3% for tetracycline and ampicillin (13/16 isolates each), followed by 68.8% for erythromycin and ciprofloxacin (11/16 each), 56.3% for high-level gentamicin (9/16), and 37.5% for high-level streptomycin (6/16) (Table 3). It is noteworthy that most of the *E. faecalis* and *E. faecium* isolates were resistant to tetracycline (24/32 isolates; 75.0%) and erythromycin (20/32; 62.5%) (Table 3).

It is known that enterococci are intrinsically resistant to several antibiotics and can readily accumulate certain genetic mutations and exogenous genes that confer additional resistance [1, 4]. In support of this observation, 68.8% of the *E. faecalis* and *E. faecium* isolates displayed the MDR phenotypes; 10/16 isolates (62.5%) for *E. faecalis* and 12/16 (75.5%) for *E. faecium*. The 10 MDR isolates of *E. faecalis* were resistant to three (5 isolates; 50%) or four (5 isolates; 50%) different antibiotics evaluated, whereas the 12 MDR isolates of *E. faecium* were resistant to four (3 isolates; 25.0%) or five (9 isolates; 75.0%) different antibiotics. All the *E. faecalis* and *E. faecium* isolates were susceptible to linezolid and teicoplanin (data not shown).

Detection of the Antibiotic Resistance Genes Among the Resistant *E. faecalis* and *E. faecium* Isolates

To evaluate the presence of appropriate antibiotic resistance genes in the resistant *E. faecalis* and *E. faecium*

Table 3. Antibiotic resistance profiling of the *E. faecalis* and *E. faecium* isolates.

Sample group	Species	No. (%) of isolates resistant to the following antibiotics[a]											
		VA	E	TE	C	HLG	HLS	AM	CIP	SYN	TEC	LZD	MDR
Dog patient	*E. faecalis* (*n* = 14)	0 (0.0)	7 (50.0)	9 (64.3)	4 (28.6)	1 (7.1)	1 (7.1)	0 (0.0)	0 (0.0)	11 (78.6)	0 (0.0)	0 (0.0)	8 (56.1)
	E. faecium (*n* = 9)	0 (0.0)	4 (44.4)	6 (66.7)	0 (0.0)	3 (33.3)	2 (22.2)	6 (66.7)	5 (55.6)	0 (0.0)	0 (0.0)	0 (0.0)	5 (55.6)
	Subtotal (*n* = 23)	0 (0.0)	11 (47.8)	15 (65.2)	4 (17.4)	4 (17.4)	3 (13.0)	6 (26.1)	5 (21.7)	11 (47.8)	0 (0.0)	0 (0.0)	13 (56.5)
Pet owner	*E. faecalis* (*n* = 0)	-	-	-	-	-	-	-	-	-	-	-	-
	E. faecium (*n* = 2)	0 (0.0)	2 (100)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
	Subtotal (*n* = 2)	0 (0.0)	2 (100)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
Veterinary staff	*E. faecalis* (*n* = 2)	0 (0.0)	2 (100)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	2 (50.0)
	E. faecium (*n* = 2)	0 (0.0)	2 (100)	2 (100)	0 (0.0)	1 (50.0)	1 (50.0)	2 (100)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100)
	Subtotal (*n* = 4)	0 (0.0)	3 (100)	3 (100)	0 (0.0)	3 (100)	1 (33.3)	3 (100)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)
Environment[b]	*E. faecalis* (*n* = 0)	-	-	-	-	-	-	-	-	-	-	-	-
	E. faecium (*n* = 3)	0 (0.0)	3 (100)	3 (100)	0 (0.0)	3 (100)	1 (33.3)	3 (100)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)
	Subtotal (*n* = 3)	0 (0.0)	3 (100)	3 (100)	0 (0.0)	3 (100)	1 (33.3)	3 (100)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)
Total	*E. faecalis* (*n* = 16)	0 (0.0)	9 (56.3)	11 (68.8)	6 (37.6)	1 (6.25)	1 (6.25)	0 (0.0)	0 (0.0)	12 (75.0)	0 (0.0)	0 (0.0)	10 (62.5)
	E. faecium (*n* = 16)	0 (0.0)	11 (68.8)	13 (81.3)	0 (0.0)	9 (56.3)	6 (37.5)	13 (81.3)	12 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (75.0)
	Subtotal (*n* = 32)	0 (0.0)	20 (62.5)	24 (75.0)	6 (18.8)	10 (31.3)	7 (21.9)	13 (40.6)	12 (37.5)	12 (37.5)	0 (0.0)	0 (0.0)	22 (68.8)

[a]Abbreviations: VA (Vancomycin), E (Erythromycin), TE (Tetracycline), C (Chloramphenicol), HLG (High-level gentamicin), HLS (High-level streptomycin), AM (Ampicillin), CIP (Ciprofloxacin), SYN (quinupristin/dalfopristin), TEC (teicoplanin), LZD (linezolid), and MDR (Multiple drug resistance).

[b]Samples from veterinary hospital environments.

isolates (Table 3), PCR was carried out with the previously established primers (Table 1) specific to individual resistance genes: *vanA* and *vanB* for resistance to vancomycin, *ermB* for erythromycin, *tetM* and *tetL* for tetracycline, *cat* for chloramphenicol, *aac6'*-Ie-*aph2"*-Ia for high-level gentamicin, and *ant6*-Ia for high-level streptomycin. As summarized in Table 4, almost all the resistant *E. faecalis* and *E. faecium* isolates carried the appropriate resistance genes with minor exceptions, implying the existence of alternative resistance mechanisms. In agreement with the disk diffusion assay results, *vanA* and *vanB* genes were not detected (data not shown).

Among the 11 *E. faecalis* and 13 *E. faecium* isolates resistant to tetracycline, 23 isolates (95.8%) harbored the *tetM* gene. Not surprisingly, the 13 tetracycline-resistant and *tetM*-positive isolates (13/23; 56.5%) also possessed the *tetL* gene (Table 4). The *ermB* gene was widely distributed among the erythromycin-resistant isolates (18/20; 90.0%) (Table 4). Almost all the high-level gentamicin (9/10; 90.0%)- or high-level streptomycin-resistant isolates (6/7; 85.7%) carried the bifunctional gentamicin resistance gene (*aac6'*-Ie-*aph2"*-Ia) or the streptomycin resistance gene (*ant6*-Ia) (Table 4). Interestingly, there was a significant difference in the

Table 4. Detection of the antibiotic resistance genes among the resistant isolates of *E. faecalis* and *E. faecium*.

Species	No. of isolates carrying the antibiotic resistance gene[a]/No. of resistant isolates (%)					
	ermB	*tetM*	*tetL*	*cat*	*aac6′*-Ie-*aph2″*-Ia	*ant6*-Ia
E. faecalis	8/9 (88.9)	11/11 (100)	1/11 (9.1)	6/6 (100)	0/1 (0.0)	1/1 (100)
E. faecium	10/11 (90.9)	12/13 (92.3)	12/13 (92.3)	0/0 (0.0)	9/9 (100)	5/6 (83.3)
Total	18/20 (90.0)	23/24 (95.8)	13/24 (54.2)	6/6 (100)	9/10 (90.0)	6/7 (85.7)

[a]*ermB*, erythromycin resistance gene; *tetM* & *tetL*, tetracycline resistance gene; *cat*, chloramphenicol resistance gene; *aac6′*-Ie-*aph2″*-Ia, high-level gentamicin resistance gene; *ant6*-Ia, high-level streptomycin resistance gene.

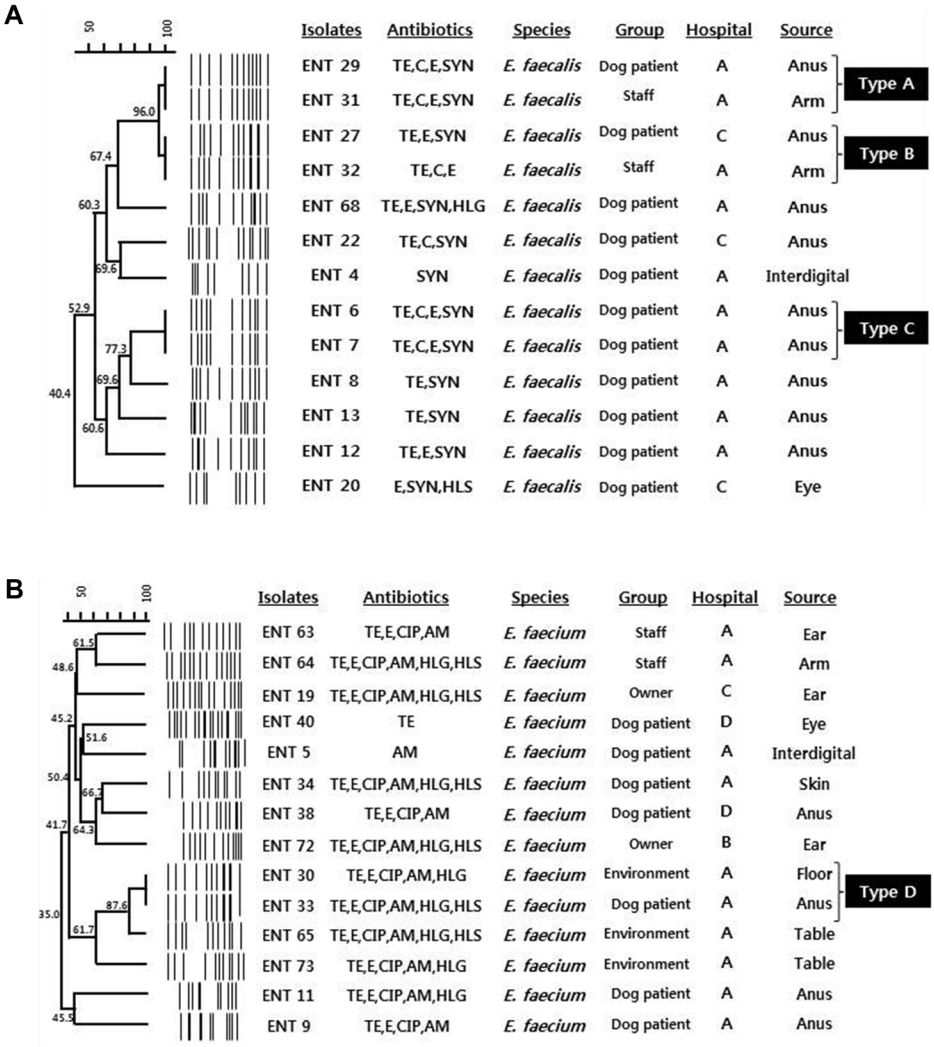

Fig. 1. PFGE analysis of the 13 *E. faecalis* (**A**) and 14 *E. faecium* (**B**) isolates resistant to antibiotics.
All the genomic DNAs were digested with *Sma*I followed by standard PFGE analysis (see Materials and Methods). Levels of similarity were determined using Dice coefficient (0.5% optimization, 1.0% tolerance) and the unweighted pair group method. Individual PFGE patterns are summarized with their antibiotic resistance profiles, sample groups, veterinary hospitals where the samples were collected, and sample sources. Abbreviations: E, erythromycin; TE, tetracycline; C, chloramphenicol; HLG, high-level gentamicin; HLS, high-level streptomycin; AM, ampicillin; CIP, ciprofloxacin; SYN, quinupristin/dalfopristin.

presence of the *aac6'*-Ie-*aph2"*-Ia gene between *E. faecalis* and *E. faecium* isolates (Table 4; $p < 0.05$). However, such a difference between *E. faecalis* and *E. faecium* would not be a species-specific pattern because the same resistance gene has been detected among the gentamicin-resistant *E. faecalis* isolates from dog and cats in the US [17, 18].

Genetic Relatedness Between the Antibiotic-Resistant Isolates of *E. faecalis* or *E. faecium*

To determine the genetic relatedness between the veterinary hospital-associated *Enterococcus* isolates, 27 antibiotic-resistant *E. faecalis* and *E. faecium* isolates were analyzed by PFGE (see Materials and Methods) because of their clinical importance. The analysis with the 13 *E. faecalis* isolates revealed three different sets (Type A to C), which were almost identical in their molecular patterns (Fig. 1A). Types A and C originated at the same veterinary hospital from different sample groups (Type A) or dog patients (Type C) (Fig. 1A). They also shared their own antibiogram profiles (Fig. 1A). These results indicate that Type A or C might be the same clonal sets. PFGE analysis with the 14 *E. faecium* isolates showed the single identical set in their molecular patterns (Type D; Fig. 1B); Type D was isolated from the different sample groups at the same veterinary hospital and showed a slight difference in antibiogram profiles (Fig. 1B).

In conclusion, our experimental analysis revealed a low contamination of enterococci among veterinary hospital-associated swab samples in Korea. Although no VRE isolates were identified, 68.8% of the *E. faecalis* and *E. faecium* isolates displayed the MDR phenotypes. More importantly,the PFGE data strongly indicate the possibility for cross-transmission of antibiotic-resistant *Enterococcus* clones among veterinary hospital-associated environments, such as dog patients, their owners, veterinary staff, and hospital environments. To the best of our knowledge, this is the first report on the existence of a potential clonal set of the antibiotic-resistant *E. faecalis* isolates from different sample groups, namely dog patients and veterinary staff, at the same animal hospital in Korea. Proper hygiene, effective infection control, and restricted movement of companion animal patients in veterinary hospitals would be prudent.

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