A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

Antibiotic resistance patterns and genetic relatedness of *Enterococcus faecalis* and *E. faecium* isolated from military working dogs in Korea

국내 군견에서 분리한 *Enterococcus faecalis* 와 *E. faecium* 의 항생제 내성양상 및 유전적 연관성

February 2016

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이 논문을 수의학 석사 학위논문으로 제출함

2015 년 10 월

서울대학교 대학원

수의학과 수의병인생물학 및 예방수의학전공

방기만

방기만의 수의학 석사 학위논문을 인준함

2015 년 12 월

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ABSTRACT

Antibiotic resistance patterns and genetic relatedness of *Enterococcus faecalis* and *E. faecium* isolated from military working dogs in Korea

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Enterococcus spp. are normally present in the gastrointestinal tracts of animals and humans, but can cause opportunistic infections that can be transmitted to other animals or humans while harboring antibiotic resistance genes. To investigate whether this is a potential risk for handlers of military working dogs (MWDs), we analyzed antibiotic resistance patterns and genetic relatedness of *Enterococcus* spp. isolated from fecal samples of MWDs of various ages (n = 65). The isolation rate of *Enterococcus* spp., and specifically of *E. faecalis* and *E. faecium*, was 87.7% (57/65), 59.6% (34/57), and 56.1% (32/57), respectively, as determined by bacterial culture and multiplex PCR. The isolation rate of *E. faecalis* gradually decreased with age. Rates of resistance to the antibiotics ciprofloxacin, gentamycin,

streptomycin, sulfamethoxazole–trimethoprim, imipenem, and kanamycin among *Enterococcus* spp. increased in adolescents and adults and decreased in senior dogs, with some isolates showing three different antibiotic resistance patterns. We also confirmed transmission of antibiotic resistant strain by pulsed-field gel electrophoresis. These results suggest that antibiotic resistance genes are transmitted among MWDs and that surveillance studies should be periodically carried out to monitor changes in antibiotic resistance that may necessitate modification of antibiotic regimens to manage antibiotic resistance transmission.

Key words: *Enterococcus faecalis*, *Enterococcus faecium*, Large-breed dog, Antibiotic resistance, PFGE

Student Number: 2014-21034

CONTENTS

LIST OF ABBREVIATIONS

MWD	Military working dog
BGM	Belgium malinois
GS	German shepherd
ATCC	American Type Culture Collection
AM	Ampicillin
AMC	Amoxicillin-clavulanic acid
C	Chloramphenicol
TE	Tetracycline
CIP	Ciprofloxacin
E	Erythromycin
IPM	Imipenem
SXT	Sulfamethoxazole / trimethoprim
GM	Gentamycin
S	Streptomycin
HLGM	High-level gentamycin
HLS	High-level streptomycin
K	Kanamycin
SYN	Quinupristin / dalfopristin
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
MDR	Multi Drug Resistance

PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis

INTRODUCTION

Enterococcus spp. are normally present in the gastrointestinal tracts of animals and humans. *E. faecalis* and *E. faecium* are among the predominant species (Robert C. Moellering, Jr., 1992) and can cause opportunistic infections such as urinary tract and nosocomial infection, bacteremia, and endocarditis in humans. (Byappanahalli *et al*., 2012). Several factors including virulence genes and biofilm formation can increase the pathogenicity of *Enterococcus* spp., and their ability to acquire antibiotic resistance and transfer it to other bacterial species makes it difficult to control and treat enterococcal infections (Arias *et al*., 2012).

Enterococci are considered as having relatively high antibiotic resistance, and have been reported in livestock as well as companion animals (Bortolaia *et al*., 2015, Hammerum *et al*., 2010). In terms of public health and veterinary medicine, it is important to prevent the transmission of multidrug-resistant (MDR) *Enterococcus* strains between animals or from animals to humans (Lloyd DH., 2007; Jackson *et al*., 2009; Kataoka *et al*., 2014; Bogaard *et al*., 2000). Enterococci are considered as having relatively high antibiotic resistance, and various strains have been reported in livestock as well as companion animals that can be transmitted to humans via direct contact (Guardabassi *et al*., 2005; Ryoko *et al*., 2005). One study found that animals that were treated with antibiotics in the intensive care unit were a source or zoonotic transmitter of MDR *Enterococcus* (Ghosh *et al*., 2011).

Specialized training centers breed and train military working dogs

(MWDs). These animals live in close contact with their handlers and other soldiers on army bases, thereby increasing the possibility of horizontal transmission of zoonotic pathogens between animals or from animals to their handlers. It is therefore necessary to investigate the characteristics of *Enterococcus* spp. and the risk of antibiotic resistance transmission. *Enterococcus* spp. are a useful indicator of host antibiotic resistance. To date, no studies have investigated the characteristics and genetic relatedness of *Enterococcus* spp. from large breeds of dogs, such as those that serve as MWD.

The present study addressed this issue by monitoring the antibiotic resistance of *E. faecalis* and *E. faecium* (*Enterococcus* spp.) in MWDs, and by evaluating differences in antibiotic resistance rates in dogs of different ages as well as the possibility of transmission of antibiotic resistance among dogs.

MATERIALS AND METHODS

Collection of fecal samples

Fecal samples of MWDs (n = 65) from a military working dog training center located in Chuncheon, Korea were collected from September 2015 to October 2015. The medical history of each dog was obtained through a questionnaire. The breeds were Belgian Malinois (BGM; n = 44), German Shepherd (GS; n = 20), and mixed (BGM/GS; n = 1). Samples were categorized into four groups according to age: 3–6 weeks (puppies; n = 12), 9–28 weeks (adolescents; n = 13), 2–6 years (adults; n = 27), and ≥ 9 years (senior; n = 13), including five dogs with diseases. BGM samples were collected from all age groups but GS samples were collected only from adult and senior dogs since younger GS dogs were not available.

Fresh fecal samples were collected using sterile devices from dogs' individual cages, except in the case of puppies, for which samples were obtained by rectal swab. The samples were transported in an ice box to the laboratory and processed within 6 h.

Isolation and identification of *Enterococcus* spp.

One gram of fecal Samples were homogenized with 9 ml Enterococcosel broth (BD Biosciences, Franklin Lakes, NJ, USA). After incubation for 16–18 h at 37℃, samples were streaked onto Enterococcosel agar (BD Biosciences) and incubated at 37℃ for 16–18 h. Three to six suspected colonies were selected from each plate and used to confirm *Enterococcus* spp. were identified by multiplex

PCR previously described [9, 15]. Briefly, DNA was prepared by resuspending a single colony in 200 µl InstaGene Matrix (Bio-Rad, Hercules, CA, USA), incubating in a 56℃ water bath incubation for 30 min, and then boiling for 8 min. The solution was incubated for 3 min on ice and centrifuged at 13,000 × *g* for 3 min; the supernatant was used as the template for the multiplex PCR amplification; *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 (American Type Culture Collection, Manassas, VA, USA) were used as the positive control and nuclease-free distilled water as the negative control.

Antimicrobial susceptibility test

Antimicrobial susceptibility was assessed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10, 18]. For 34 *E. faecalis* and 32 *E. faecium* isolates, 14 antibiotics were used to test susceptibility: ampicillin (AM), 10 µg; amoxicillin-clavulanic acid (AMC), 30 µg; chloramphenicol (C), 30 µg; tetracycline (TE), 30 µg; ciprofloxacin (CIP), 5 µg; erythromycin (E), 15 µg; imipenem (IPM), 10 µg; sulfamethoxazole/trimethoprim (SXT), 23.75 µg/1.25 µg; gentamycin (GM), 10 µg; streptomycin (S), 10 µg; high-level gentamycin (HLGM), 120 µg; high-level streptomycin (HLS). 300 µg; kanamycin (K), 30 µg; and quinupristin/dalfopristin (SYN), 15 µg (BD Biosciences). Each cultured single isolates were suspended in 0.85% NaCl and turbidity were adjusted to 0.5 McFarland. Five antibiotic disks were placed on each Mueller-Hinton agar (BD Biosciences) plate followed by incubation at 37℃ for 24 h. The diameter of inhibitory zone of the disks were measured and tested isolates were determined to be susceptible, intermediate, or resistant based on CLSI M100-

S24 *E. faecalis* and *E. faecium* breakpoint guidelines. Interpretive criteria were adapted from Enterobacteriaceae guidelines as published in CLSI M100-S23 when the breakpoint was unavailable. *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were used as controls for the test.

Pulsed-field gel electrophoresis (PFGE)

PFGE were performed with a minor modification of the Center for Disease Control's *Listeria monocytogenes* PulseNet protocol. Briefly, Enterococcus isolates were suspended in TE buffer, adjusted to McFarland scale 4.0, and then the suspension was embedded in 1.0% agarose plugs. After lysis of cells in agarose plugs and washing steps, plug slices were digested with 20 U of *Sma*I (Takara Bio, Otsu, Japan) at 25℃ for 2 h. Electrophoresis was carried out at 6.0 V for 19 h with a ramped pulse time of 1–20 s in 0.5× Tris-Borate-EDTA buffer at 14℃ using CHEF Mapper (Bio-rad). Gels were visualized using a Gel Doc XR imager (Bio-Rad). *Salmonella* Braenderup ATCC BAA664 was used as the size marker.

Data analysis

PFGE band patterns were analyzed with BioNumerics software ver. 6.6 (Applied Maths, Austin, TX, USA). Dendrograms of PFGE were generated for cluster analysis based on the Dice similarity coefficient using the Unweighted Pair Group Method with Arithmetic mean (UPGMA). Isolates showing ≥ 90% similarity were grouped as the same genotype. Differences between groups were assessed with the χ^2 test using SPSS v.23 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Isolation of *Enterococcus* spp.

A total of 66 *Enterococcus* spp. (34 *E. faecalis* and 32 *E. faecium*) were isolated from 65 MWD. The isolation rate of *Enterococcus* spp., *E. faecalis*, and *E. faecium* was 87.7% (57/65), 59.6% (34/57), and 56.1% (32/57), respectively; nine samples contained both *E. faecalis* and *E. faecium* (Table 1). The isolation rate in *E. faecalis* decreased ($P < 0.05$) with age (puppy, 100%; adolescent, 91.7%; adult, 36.4%; and senior, 14.3%), whereas that of *E. faecium* increased with age (puppy, 8.3%; adolescent, 58.3%; adult, 72.7%; senior, 85.7%). There were no differences in terms of isolation rate between sexes.

Antibiotic resistance rates of *Enterococcus* spp.

Antibiotic resistance rates were determined for 62 isolates from 60 healthy MWDs (Fig. 1). More than 50% of isolates showed resistance to TE, CIP, S, and SXT. Resistance to AM, IPM, and HLS was detected only in *E. faecium*, while rates of resistance to TE, CIP, and K were more than 2-fold higher in *E. faecium* than in *E. faecalis* isolates. In contrast, *E. faecalis* isolates exhibited higher resistance to SYN. All isolates were susceptible to C, AMC, and HLGM.

Antibiotic resistance among isolates for different age groups is summarized in Table 2. Resistance rates as well as the number of antibiotics towards which resistance was observed increased from puppies to adult dogs, but decreased in senior dogs. Resistance to SYN decreased with age, whereas

resistance to CIP, GM, S, SXT, IPM, and K first increased before decreasing in the senior group (Fig. 2). Resistance to E increased with age but the rates remained at a low level. E, AM, C, AMC, HLGM, and HLS were excluded from Figure since resistance to these antibiotics remained at a low level and was similar across age groups.

Multidrug resistance patterns of *Enterococcus* spp.

MDR patterns were examined in 66 isolates from 65 MWDs (Table 3). A total of 65 isolates (98.5%) were resistant to more than one antibiotic—i.e., exhibited MDR, with four showing resistance to TE/CIP/S/K, three to CIP/SXT/K, and three to TE/CIP/GM/S/SXT/K. One isolate obtained from a MWD that had diarrhea showed resistance to 11 types of antibiotic.

PFGE

The genetic relatedness of 34 *E. faecalis* and 32 *E. faecium* isolates from 65 MWDs was determined by genotyping and PFGE. For *E. faecalis*, 13–18 restriction fragments were obtained by *Sma*I digestion that corresponded to 16 different genotypes (Fig. 3A). Four genotypes were represented in more than two isolates, whereas one isolate contained 12 genotypes. For *E. faecium*, 16–24 restriction fragments were obtained by PFGE corresponding to 21 different genotypes (Fig. 3B). Seven genotypes were represented in more than two isolates, whereas one isolate contained 14 genotypes. The number of samples of each genotype ranged from one to 11. Six boxed clusters contained isolates that were found mainly in breeding dogs, puppies, and adolescents living in a building for

delivery dogs. Eleven of the isolates exhibited similar PFGE patterns; of these, five clusters included isolates that did not originate from the building for delivery dogs.

DISCUSSION

Enterococcus spp. exist as normal gut flora and are constantly exposed to antibiotics; as such, they are widely used as a model for the acquisition of antibiotic resistance in the host (Levin *et al*., 1997). The prevalence and patterns of antibiotic resistance of *Enterococcus* spp. isolated from livestock and companion animals have been previously described (Butaye *et al.*, 2001; Park *et al.*, 2013; Damborg *et al.*, 2008). Enterococci in animal and human intestine can transfer antibiotic resistance genes to other host commensals (Salyers *et al*. 2004). Most previous studies focused on transmission from livestock to humans or on companion animals in relation to veterinary clinics (Chung *et al*., 2013, Ghosh *et al*, 2012), or investigated the prevalence of *E. faecalis* and *E. faecium* in healthy dogs (Kataoka *et al*., 2013, Damborg *et al*., 2008). However, ours is the first study to examine the characteristics of *Enterococcus* spp. in MWDs with respect to their living environment and age. In contrast to a previous study that found a higher prevalence of *E. faecalis* among dogs without prior exposure to antibiotics and a higher prevalence of *E. faecium* in those that had been previously exposed (Kataoka *et al.*, 2013), we found similar isolation rate of *E. faecalis* (59.6%) and *E. faecium* (56.1%) among isolates. We also observed that *E. faecalis* isolation rate decreased with age, whereas that of *E. faecium* increased with age. This may have been due to the increase in antibiotic usage with age. Additional studies are required in order to clarify the reason for this difference in isolation rate of the two species over the course of the animals' life span.

E. faecalis showed higher resistance to SYN than *E. faecium*, which is in

accordance with the known sensitivity of each species to SYN (Kristic *et al*., 2014). *Enterococcus* has intrinsic resistance to β-lactam compounds (Kristic *et al*., 2014); however, the MWDs in the present study did not show high resistance to ampicillin. Consistent with frequent use of enrofloxacin (a fluoroquinolone antibiotic) in MWD hospitals, we observed a 50% resistance rate to CIP among isolates. Another report found high resistance to kanamycin (42.6%), streptomycin (35.1%), and erythromycin (33.0%) among dogs in Japan (Kataoka *et al.*, 2013). The differences between these results and ours may be attributed to differences in antibiotic usage at the MWD hospital.

We observed three antibiotic resistance patterns with respect to age. Resistance to CIP, GM, S, SXT, IPM, and K increased at adolescence and adulthood and then decreased in senior dogs; SYN resistance decreased uniformly with age; and resistance to AM, C, AMC, HLGM, and HLS remained across age groups. In the case of SYN, the constant rate of decrease corresponded to the reduced the isolation rate of *E. faecalis*. Puppies had not been exposed to antibiotics and therefore did not show resistance to AM, E, C, GM, S, AMC, or IPM. However, as they aged they underwent many kinds of training and activities that could lead to injury and surgery, which would require the administration of antibiotics. Senior dogs would participate in fewer activities and training, and therefore the chances of experiencing trauma and antibiotic usage would be decreased. The resistance to certain types of antibiotic (TE, CIP, SXT, K, and SYN) detected in puppies may have been transmitted to them from their parents; this is supported by the finding that *Staphylococcus aureus* present on parental skin was transferred to infant gut (Lindberg *et al*., 2004). Thus, in MWDs living together in

the same environment, resistance to some types of antibiotic but not others changes with age. This implies the need for taking into account the characteristics of resistance according to age when prescribing antibiotics to MWDs.

Most of the isolates (98.5%) showed MDR. This was much higher than the rate of 50.6% that was previously reported in dogs (Kataoka *et al*., 2014), and may be explained by the close contact between MWDs in their unique living environment. The patterns of antibiotic resistance—e.g., TE/CIP/S/K, CIP/SXT/K, and TE/CIP/GM/S/SXT/K—suggest that there could be co-transmission of some antibiotic resistance genes. The resistance to 11 different antibiotics observed in one isolate may have arisen from a long history of exposure over the course of treatments for chronic diarrhea and melena.

PFGE analysis of 34 *E. faecalis* revealed four different genotypes (clusters 1, 4, 5, and 13) that had similar band profiles (90% similarity; Fig. 3A). Many breeding dogs, puppies, and adolescent dogs that were recently discharged from the building for delivery dogs showed near-identical molecular patterns, since they were in close contact with each other over an extended period of time. Indeed, seven of 11 isolates from puppies of the same litter showed the same antibiotic resistant pattern (cluster 4). However, in most cases there was no relationship between PFGE and antibiotic resistance patterns. This is in accordance with another study of antibiotic resistance in *Enterococcus* isolates from dogs and cats in the U.S. (Jackson *et al*., 2010), and may be attributed to the fact that the resistance pattern reflects antibiotic resistance gene transfer between bacteria, while PFGE provides an analysis of total DNA and not specific genes.

The analysis of the 32 *E. faecium* isolates revealed seven different clusters (clusters 1, 3, 7, 11, 13, 14, and 15) that included a wider range of ages than *E. faecalis* (Fig. 3B). These clusters included those related to the building for delivery dogs (clusters 11 and 13) and suggested possible transmission between adults (≥ 1 years of age), likely as a result of direct contact with feces during group training. This raises the possibility of transmission of antibiotic resistance genes from MWDs to humans by direct contact. Further studies on the genetic relatedness of *Enterococcus* spp. in MWDs as well as their handlers and other soldiers are needed to assess the risk of this occurrence.

In conclusion, we investigated for the first time the characteristics of *Enterococcus* spp. isolates of large-breed dogs from a MWD training center in Korea. The observed changes in rates of resistance to certain antibiotics with age suggest a need to consider the age of dogs before administering antibiotic treatment. We confirmed by PFGE analysis that the building for delivery dogs was one source of antibiotic resistant strains, but there was also transmission between adult dogs outside this building. Restricting contact between MWDs and immediate disposal of feces in this building and in the training ground can minimize the risk of horizontal transmission. These results suggest that surveillance studies should be carried out periodically to follow changes in antibiotic resistance of *Enterococcus* spp. in MWDs so that antibiotic resistance can be better managed. In addition, molecular epidemiological analyses of MWD handlers and soldiers are required to address the risk of transmission from dogs to humans.

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Table 1. Classification of 65 MWDs by sex, species, and age

Categories	Characteristic	Sample size (%)	*Enterococcus* (%)	*E. faecalis* (%)	*E. faecium* (%)
Sex	Male	32 (49.23)	28 (87.50)	17 (60.71)	16 (57.14)
	Female	33 (50.77)	29 (87.88)	17 (58.62)	16 (55.17)
Species	BGM[a]	44 (67.69)	38 (86.36)	27 (71.05)	20 (52.63)
	GS[b]	20 (37.00)	18 (90.00)	6 (33.33)	12 (66.67)
	Mixed	1 (1.54)	1 (100)	1 (100)	0 (0.00)
Age	Puppy (3-6 weeks)	12 (18.46)	12 (100)	12 (100)	1 (8.33)
	Adolescence (9-28 weeks)	13 (20.00)	13 (100)	12 (92.31)	7 (53.85)
	Adult (1~6 years)	27 (41.54)	24 (88.89)	9 (37.50)	17 (70.83)
	Senior (> 9 years)	13 (20.00)	8 (61.54)	1 (12.50)	7 (87.50)
Subtotal		65 (100)	57 (87.69)	34 (59.64)	32 (56.14)

[a] BGM: Belgian Malinois, [b] GS: German Shepherd; MWD: military working dog.

* Nine samples contained both *E. faecalis* and *E. faecium*.

Table 2. Age-specific antibiotic resistance rates of *Enterococcus* spp. isolated from 60 healthy MWDs

Antibiotics (abbrev.)	Concentration of disk (μg)	Diffusion zone break point (mm)	Puppy isolates (n = 13)				Adolescent isolates (n = 18)				Adult isolates (n = 24)				Senior isolates (n = 7)			
			S	I	R	% of R	S	I	R	% of R	S	I	R	% of R	S	I	R	% of R
Ampicillin (AM)	10	≤16	13	0	0	0.00	18	0	0	0.00	23	0	1	4.17	7	0	0	0.00
Tetracycline (TE)	30	≤14	10	0	3	23.08	4	1	13	72.22	7	2	15	62.50	2	0	5	71.43
Erythromycin (E)	15	≤13	3	10	0	0.00	1	15	2	11.11	2	19	3	12.50	1	5	1	14.29
Chloramphenicol (C)	30	≤12	12	1	0	0.00	10	8	0	0.00	23	1	0	0.00	7	0	0	0.00
Ciprofloxacin (CIP)	5	≤15	2	10	1	7.69	1	8	9	50.00	0	7	17	70.83	0	3	4	57.14
Gentamycin (GM)	10	≤12	8	5	0	0.00	2	7	9	50.00	6	7	5	20.83	4	2	1	14.29
Streptomycin (S)	10	≤11	0	4	9	69.23	0	3	15	83.33	0	7	16	66.67	0	4	3	42.86
Sulfamethoxazole-trimethoprim (SXT)	22	≤10	12	0	1	7.69	2	3	13	72.22	4	4	16	66.67	3	3	1	14.29
Amoxicillin-clavulanic acid (AMC)	30	≤13	13	0	0	0.00	17	1	0	0.00	23	1	0	0.00	7	0	0	0.00
Imipenem (IPM)	10	≤13	13	0	0	0.00	16	0	2	11.11	20	3	1	4.17	7	0	0	0.00
High-Level gentamycin (HLGM)	120	≤6	0	0	0	0.00	15	0	0	0.00	5	0	0	0.00	3	0	0	0.00
High-Level streptomycin (HLS)	300	≤6	9	0	0	0.00	9	0	0	0.00	15	0	1	4.17	1	0	0	0.00
Kanamycin (K)	30	≤13	2	7	4	30.77	0	10	8	44.44	5	3	16	66.67	0	5	2	28.57
Quinupristin/dalfopristin (SYN)	15	≤15	1	0	12	92.31	5	4	9	50.00	15	4	5	20.83	6	0	1	14.29

Table 3. Antibiotic resistance patterns of *Enterococcus* spp. isolated from 65 MWDs

Number of antibiotics	Antibiotics resistance pattern	Number of isolates	*E. faecalis*	*E. faecium*
1	SYN	1	P[a] (1)	
2	TE CIP	2		S[d] (2)
	TE S	2	C[b] (1)	S (1)
	TE K	1		P (1)
	CIP SXT	2	A[c] (1)	A (1)
	GM S	1	S (1)	
	S K	1	C (1)	
	S SYN	8	P (8)	
3	TE CIP SXT	1		C (1)
	TE CIP K	1		A (1)
	TE SXT K	1		A (1)
	TE GM S	1		C (1)
	TE E K	1		A (1)
	TE S K	1		A (1)
	TE S SXT	1		A (1)
	TE E SYN	1		S (1)
	TE K SYN	1	C (1)	
	TE S SYN	1	A (1)	
	CIP SXT K	3		A (2), S(1)
	GM S SXT	1	C (1)	
	GM S SYN	2	C (1), A(1)	
	SXT K SYN	1	P (1)	
4	TE CIP SXT K	1		C (1)
	TE CIP S K	4		A (3), S (1)
	TE S SXT SYN	1	C (1)	
	TE CIP S SXT	1		A (1)
	TE E SXT K	1	A (1)	
	TE GM S K	1	A (1)	
	TE CIP K SYN	1	P (1)	

	TE S K SYN	1	P (1)	
	CIP S SXT SYN	1	A (1)	
	GM S SXT SYN	1	C (1)	
5	TE CIP S SXT SYN	1	C (1)	
	TE CIP S SXT K	2		A (2)
	TE CIP S IPM K	1		C (1)
	CIP GM S SXT SYN	2	A (2)	
	CIP S SXT K SYN	1	A (1)	
	E CIP S SXT SYN	1	C (1)	
6	TE CIP GM S SXT K	1	C (1)	
	TE CIP GM S SXT K	3		C (2), A(1)
	TE CIP S SXT IPM K	1		C (1)
	TE GM S SXT K SYN	1	C (1)	
	TE E GM S SXT SYN	1	C (1)	
	TE E CIP S SXT K	1		A (1)
7	AM TE CIP S IPM HLS K	1		A (1)
11	AM TE E CIP GM S SXT AMC IPM HLGM HLS K	1		S (1)
Total		66	34	32

[a]P: Puppy (3–6 weeks), [b]C: Adolescent (9–28 weeks), [c]A: Adults (1–6 years), [d]S: Senior (≥ 9 years) group.

Abbreviations: AM (Ampicillin), CIP (Ciprofloxacin), E (Erythromycin), GM (Gentamycin), HLS (High-level streptomycin), IPM (Imipenem), K (Kanamycin), S (Streptomycin), SXT (Sulfamethoxazole/trimethoprim), SYN (Quinupristin/dalfopristin), TE (Tetracycline).

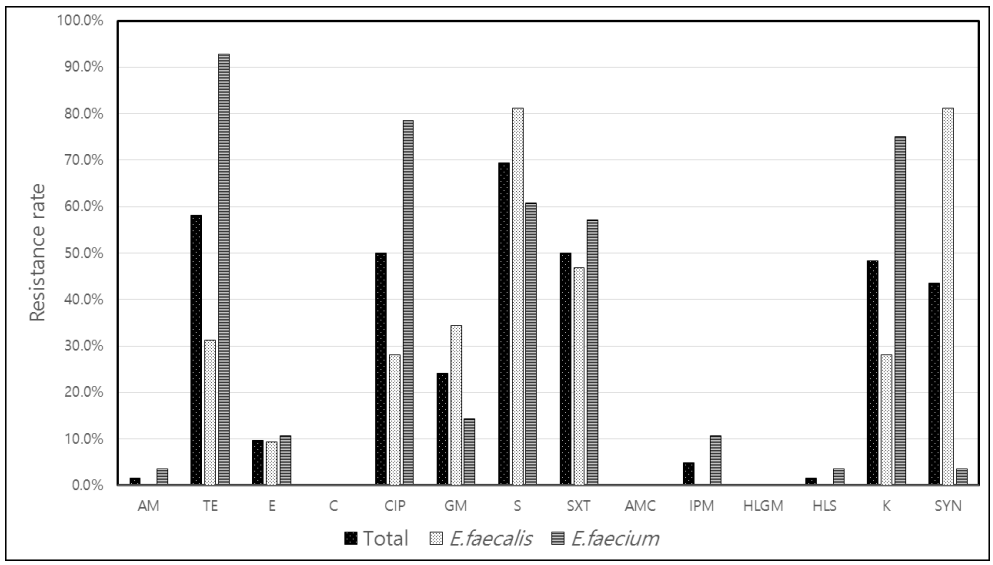


Figure 1. Antibiotic resistance rates among *E. faecalis* and *E. faecium* isolated from 60 healthy MWDs.

Abbreviations: AM (Ampicillin), AMC (amoxicillin-clavulanic acid), C (chloramphenicol), CIP (Ciprofloxacin), E (Erythromycin), GM (Gentamycin), HLGM (High-level gentamycin), HLS (High-level streptomycin), IPM (Imipenem), K (Kanamycin), S (Streptomycin), SXT (Sulfamethoxazole/trimethoprim), SYN (Quinupristin/dalfopristin), TE (Tetracycline).

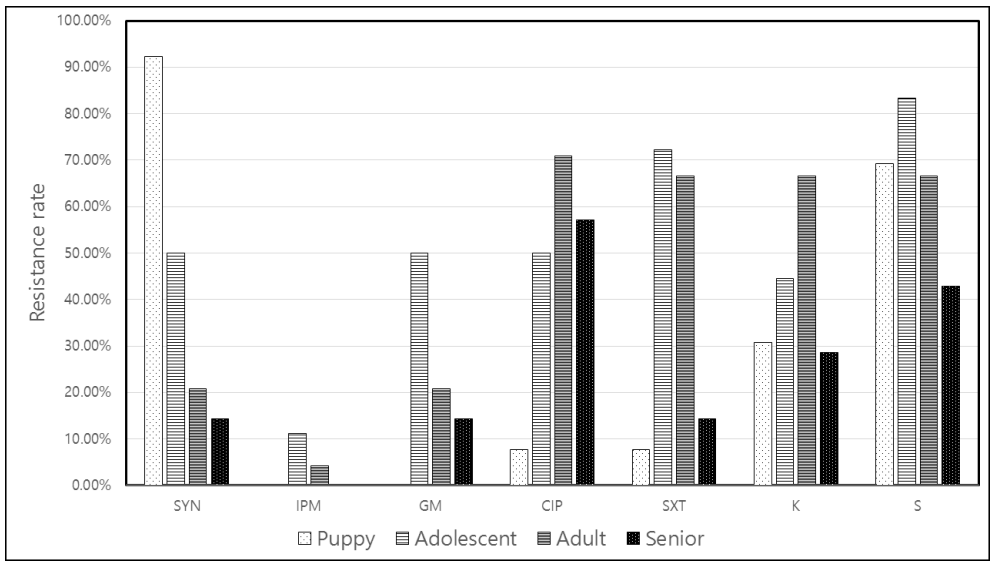
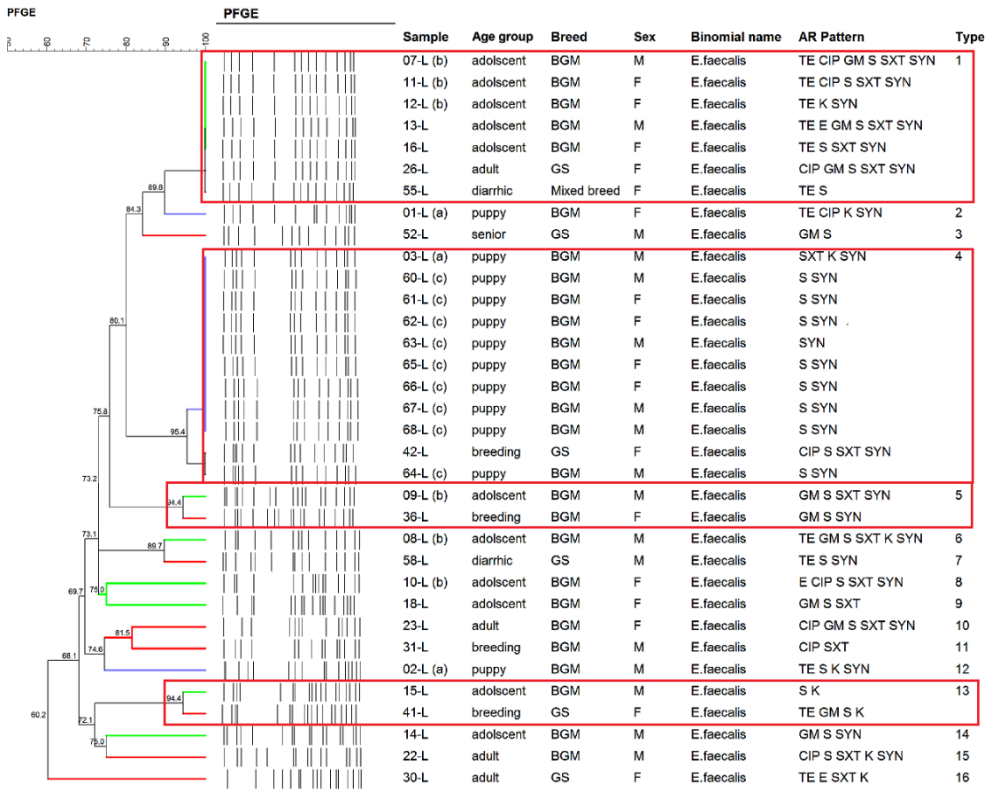


Figure 2. Age-specific changes in antibiotic resistance rates of *Enterococcus* spp. isolated from 60 healthy MWDs.

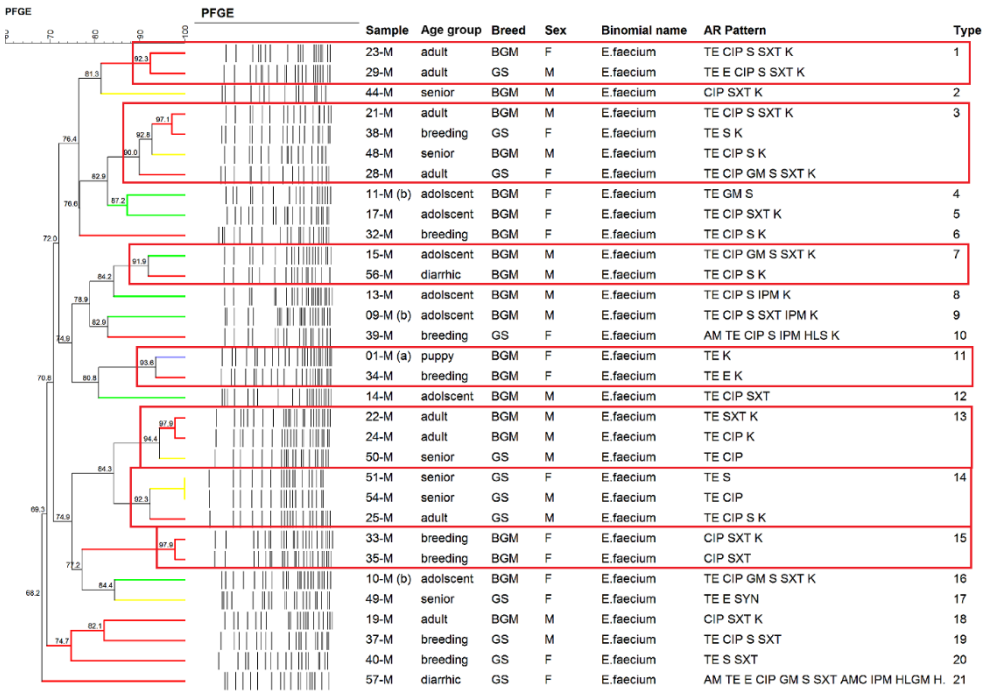
Abbreviations: Refer to Figure 1 for abbreviation list of antibiotics.

(A)



Sample	Age group	Breed	Sex	Binomial name	AR Pattern	Type
07-L (b)	adolscent	BGM	M	E.faecalis	TE CIP GM S SXT SYN	1
11-L (b)	adolscent	BGM	F	E.faecalis	TE CIP S SXT SYN	
12-L (b)	adolscent	BGM	F	E.faecalis	TE K SYN	
13-L	adolscent	BGM	M	E.faecalis	TE E GM S SXT SYN	
16-L	adolscent	BGM	F	E.faecalis	TE S SXT SYN	
26-L	adult	GS	F	E.faecalis	CIP GM S SXT SYN	
55-L	diarrhic	Mixed breed	F	E.faecalis	TE S	
01-L (a)	puppy	BGM	F	E.faecalis	TE CIP K SYN	2
52-L	senior	GS	M	E.faecalis	GM S	3
03-L (a)	puppy	BGM	M	E.faecalis	SXT K SYN	4
60-L (c)	puppy	BGM	M	E.faecalis	S SYN	
61-L (c)	puppy	BGM	F	E.faecalis	S SYN	
62-L (c)	puppy	BGM	F	E.faecalis	S SYN	
63-L (c)	puppy	BGM	M	E.faecalis	SYN	
65-L (c)	puppy	BGM	F	E.faecalis	S SYN	
66-L (c)	puppy	BGM	F	E.faecalis	S SYN	
67-L (c)	puppy	BGM	M	E.faecalis	S SYN	
68-L (c)	puppy	BGM	M	E.faecalis	S SYN	
42-L	breeding	GS	F	E.faecalis	CIP S SXT SYN	
64-L (c)	puppy	BGM	M	E.faecalis	S SYN	
09-L (b)	adolscent	BGM	M	E.faecalis	GM S SXT SYN	5
36-L	breeding	BGM	F	E.faecalis	GM S SYN	
08-L (b)	adolscent	BGM	M	E.faecalis	TE GM S SXT K SYN	6
58-L	diarrhic	GS	M	E.faecalis	TE S SYN	7
10-L (b)	adolscent	BGM	F	E.faecalis	E CIP S SXT SYN	8
18-L	adolscent	BGM	F	E.faecalis	GM S SXT	9
23-L	adult	BGM	F	E.faecalis	CIP GM S SXT SYN	10
31-L	breeding	BGM	M	E.faecalis	CIP SXT	11
02-L (a)	puppy	BGM	M	E.faecalis	TE S K SYN	12
15-L	adolscent	BGM	M	E.faecalis	S K	13
41-L	breeding	GS	F	E.faecalis	TE GM S K	
14-L	adolscent	BGM	M	E.faecalis	GM S SYN	14
22-L	adult	BGM	M	E.faecalis	CIP S SXT K SYN	15
30-L	adult	GS	F	E.faecalis	TE E SXT K	16

(B)



Sample	Age group	Breed	Sex	Binomial name	AR Pattern	Type
23-M	adult	BGM	F	E.faecium	TE CIP S SXT K	1
29-M	adult	GS	M	E.faecium	TE E CIP S SXT K	
44-M	senior	BGM	M	E.faecium	CIP SXT K	2
21-M	adult	BGM	M	E.faecium	TE CIP S SXT K	3
38-M	breeding	GS	F	E.faecium	TE S K	
48-M	senior	BGM	M	E.faecium	TE CIP S K	
28-M	adult	GS	F	E.faecium	TE CIP GM S SXT K	
11-M (b)	adolscent	BGM	F	E.faecium	TE GM S	4
17-M	adolscent	BGM	F	E.faecium	TE CIP SXT K	5
32-M	breeding	BGM	F	E.faecium	TE CIP S K	6
15-M	adolscent	BGM	M	E.faecium	TE CIP GM S SXT K	7
56-M	diarrhic	BGM	M	E.faecium	TE CIP S K	
13-M	adolscent	BGM	M	E.faecium	TE CIP S IPM K	8
09-M (b)	adolscent	BGM	M	E.faecium	TE CIP S SXT IPM K	9
39-M	breeding	GS	F	E.faecium	AM TE CIP S IPM HLS K	10
01-M (a)	puppy	BGM	F	E.faecium	TE K	11
34-M	breeding	BGM	F	E.faecium	TE E K	
14-M	adolscent	BGM	M	E.faecium	TE CIP SXT	12
22-M	adult	BGM	M	E.faecium	TE SXT K	13
24-M	adult	BGM	M	E.faecium	TE CIP K	
50-M	senior	GS	M	E.faecium	TE CIP	
51-M	senior	GS	F	E.faecium	TE S	14
54-M	senior	GS	M	E.faecium	TE CIP	
25-M	adult	GS	M	E.faecium	TE CIP S K	
33-M	breeding	BGM	F	E.faecium	CIP SXT K	15
35-M	breeding	BGM	F	E.faecium	CIP SXT	
10-M (b)	adolscent	BGM	F	E.faecium	TE CIP GM S SXT K	16
49-M	senior	GS	F	E.faecium	TE E SYN	17
19-M	adult	BGM	M	E.faecium	CIP SXT K	18
37-M	breeding	GS	M	E.faecium	TE CIP S SXT	19
40-M	breeding	GS	F	E.faecium	TE S SXT	20
57-M	diarrhic	GS	F	E.faecium	AM TE E CIP GM S SXT AMC IPM HLGM H.	21

Figure 3. Genetic relatedness of *E. faecalis* and *E. faecium* isolates resistant to antibiotics.

(A) *E. faecalis* (n = 34)

(B) *E. faecium* (n = 32)

Band profiles generated by PFGE are based on 1.5% band matching tolerance. The box indicates the 90% similarity criterion.

PFGE patterns were clustered with isolate information including age group, breed, sex, and antibiotic resistance profiles. L and M in sample category indicate *E. faecalis* and *E. faecium* isolate, respectively. (a), (b), and (c) indicate siblings. Refer to Table 1 and Figure 1 for abbreviation list of breeds and antibiotics.

국문초록

국내 군견에서 분리한 *Enterococcus faecalis* 와 *E. faecium* 의 항생제 내성양상 및 유전적 연관성

서울대학교 대학원

수의학과 수의공중보건 전공

방기만

(지도교수: 조성범)

장구균은 동물과 사람의 위장관에 정상적으로 존해하지만 기회감염을 일으키고 항생제 내성 유전자와 함께 다른 동물이나 사람에게 전파될 수 있다. 군견의 내성균 전파상의 잠재적 위험을 조사하기 위해 다양한 연령대의 군견 분변샘플 (자견 12, 중견 13 두, 성견 27 두, 노령견 13 두) 총 65 두에서 분리된 장구균의 항생제 내성패턴과 유전적 연관성을 분석하였다. 세균배양과 multiplex PCR 법을 이용하여 밝혀낸 장구균과 *E.*

faecalis, *E. faecalis* 의 분리율은 각각 87.7% (57/65), 59.6% (34/57), 56.1% (32/57)였다. *E. faecalis* 의 분리율은 나이가 들며 점차 감소하였다. 특징적으로 연령에 따른 항생제 내성률의 경우 CIP, GM, S, SXT, IPM, K 에서 중견, 성견기까지 증가하다가 노령기에 감소하였고, 몇몇 분리주들은 3 가지 다른 항생제 내성패턴을 공통적으로 보였다. PFGE 분석을 통해 항생제 내성 균주의 전파도 확인할 수 있었다. 이러한 결과를 통해 군견간의 항생제 내성 유전자와 균주의 전파가 있음을 시사하였고, 주기적인 항생제 내성 감시 연구를 통해 군견에서의 항생제 내성 변화를 파악하여 항생제 처방에 변화를 주고, 항생제 내성 전파를 관리하여야 할 필요가 있다.

주요어: *Enterococcus faecalis, Enterococcus faecium*, 대형견, 항생제 내성, PFGE

학 번: 2014-21034