Characteristics of linezolid-resistant *Enterococcus faecalis* isolates from broiler breeder farms

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ABSTRACT Linezolid is an oxazolidinone class antibiotic used for treatment infections caused by various multidrug-resistant gram-positive pathogens including enterococci. However, recently, linezolid-resistant isolates in animals are considered as a human health hazard. In a broiler operation system, antimicrobial resistance can be transferred to the environment and commercial broiler via the fecal–oral route. Therefore, this study was conducted to investigate the prevalence and characteristics of linezolid-resistant *Enterococcus faecalis* (*E. faecalis*) from broiler parent stock in a broiler operation system. Among 297 *E. faecalis* isolates from 85 flocks in 8 broiler breeder farms, the prevalence of chloramphenicol- and linezolid-resistant isolates was 0 to 12.1% and 0 to 8.0%, respectively; however, there were no significant differences between farms. Therefore, a total of 14 (4.7%) chloramphenicol- and/or linezolid-resistant *E. faecalis* showed resistance to 7 or more antimicrobial classes. The drug-resistance gene *optrA*, which can confer resistance to linezolid, tedizolid, and phenicols, was found in 8 (2.69%) isolates, and 7 (2.36%) of the 8 *optrA*-positive isolates co-carried the phenicol exporter gene *fexA*. However, *E. faecalis* isolates from 3 of 8 broiler breeder farms only carried the *optrA* and/or *fexA* genes. As linezolid is one of the last antimicrobial treatments of choice for multidrug-resistant gram-positive pathogens including *E. faecalis*, the presence of antibiotic-resistant *E. faecalis* in broiler breeder farms should be monitored to prevent the introduction of linezolid-resistant strains to the food chain.

Key words: linezolid-resistant, *optrA*, broiler breeder, *Enterococcus faecalis*, antimicrobial resistance

2020 Poultry Science 99:6055–6061
https://doi.org/10.1016/j.psj.2020.06.087

INTRODUCTION

Enterococci are part of the normal microbiota of the gastrointestinal tract of animals and humans. However, the enterococci in animals may transfer their antimicrobial resistance genes to other animals or humans via the food chain (Ogier and Serror, 2008), and they are generally considered as a representative indicator of the antimicrobial resistance of gram-positive organisms (APQA, 2017). In particular, *Enterococcus faecalis* (*E. faecalis*) of animal origin seems to be a human health hazard as the isolates have been found to express the same phenotype in animals and humans (Hammerum, 2012), and the increasing prevalence of multidrug-resistant *E. faecalis* is a great concern in many countries (Diekema et al., 2019; Na et al., 2019).

Linezolid is the first oxazolidinone antibiotic widely used for treatment against a wide range of multidrug-resistant gram-positive pathogens including enterococci (Leong et al., 2018). It inhibits bacterial growth by suppressing bacterial protein synthesis via interaction with domain V of 23S ribosomal RNA (**rRNA**) (Aoki et al., 2002). The presence of linezolid-resistant enterococci in human isolates has been reported since 2001, shortly after the commercial use of linezolid in the United States in 2000 (Gonzales et al., 2001). Although linezolid is not used in food-producing animals, the resistance to this antimicrobial agent in animals has been reported in the United States (Tyson et al., 2018a), Europe (De Jong et al., 2019), and Asia (Tamang et al., 2017; Shang et al., 2019).

Linezolid resistance in gram-positive bacteria is usually the result of a point mutation of the genes coding for 23S rRNA (Bourgeois-Nicolaos et al., 2007; Ntokou et al., 2012). In addition, a multidrug-resistance gene, *cfr*,

Received January 23, 2020.
Accepted June 17, 2020.

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confers transferable resistance against oxazolidinones, phenicols, lincosamides, pleuromutilins, and streptogramin A by encoding an rRNA methyltransferase that methylates adenosine at base pair A2503 and inhibits ribose methylation at C2498 in the 23S rRNA (Kehrenberg et al., 2005; Long et al., 2006). Recently, a novel gene, *optrA*, from *E. faecalis* of human and animal origin was reported in China (Wang et al., 2015), Italy (Brenciani et al., 2016), Ireland, and Malaysia (Mendes et al., 2016). The *optrA* gene also confers resistance against linezolid, tedizolid, and phenicols and encodes for an ATP-binding cassette transporter (Wang et al., 2015).

A broiler operation system has a pyramidal structure with the grandparent stock at the top followed by parent-stock flocks that produce eggs for the production of commercial broiler. Consequently, antimicrobial resistance and drug resistance genes from the organisms isolated from breeder farms can be transferred to the environment and commercial broiler via the fecal–oral route through hatcheries (Kim et al., 2018). Therefore, this study was conducted to investigate the prevalence and characteristics of linezolid-resistant *E. faecalis* from broiler parent stock in the broiler operation system in Korea.

MATERIALS AND METHODS

Sample Collection

Fecal samples were collected from 86 flocks at 20 wk of age from 8 broiler breeder farms between 2016 and 2018. In accordance with the standards set by the National Poultry Improvement Plan (USDA, 2012), feces (approximately 10 g) were sampled in 5 different sites from each flock and transported to the laboratory in a cooler.

Bacterial Isolation

The fecal samples were individually inoculated into buffered peptone water (BD Biosciences, Sparks, MD) and incubated at 37°C for 18 to 24 h. Pre-enriched buffered peptone water was mixed with Enterococcosel broth (BD Biosciences) at a 1:10 ratio and incubated at 37°C for 18 to 24 h. The cultured Enterococcosel broth was streaked onto Enterococcosel agar (BD Biosciences) and incubated at 37°C overnight. At least 3 representative colonies on the Enterococcosel agar were selected, and *E. faecalis* was identified by PCR using primers targeted specifically on the *PBP5* gene as previously described (del Mar Lleó et al., 1999). If isolates from the same origin showed the same antimicrobial susceptibility patterns, only 1 isolate was randomly chosen and included in the study.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was assessed by determining the minimum inhibitory concentrations (**MIC**) for 16 antimicrobial agents by the broth microdilution method using the commercially available Sensititre panel KRVP2F (TREK Diagnostic Systems, West Sussex, UK) according to the manufacturer's instructions. The antimicrobial agents tested were ampicillin (≥16 μg/mL), chloramphenicol (**CHL**, ≥32 μg/mL), ciprofloxacin (≥1 μg/mL), daptomycin (≥8 μg/mL), erythromycin (**ERY**, ≥8 μg/mL), florfenicol (**FFN**, ≥16 μg/mL), gentamicin (≥16 μg/mL), kanamycin (≥16 μg/mL), linezolid (≥8 μg/mL), salinomycin (≥16 μg/mL), quinupristin/dalfopristin (≥4 μg/mL), streptomycin (≥1,000 μg/mL), tetracycline (**TET**, ≥16 μg/mL), tigecycline (**TGC**, ≥0.25 μg/mL), tylosin tartrate (**TYLT**, ≥32 μg/mL), and vancomycin (**VAN**, ≥32 μg/mL). For quality control in MIC determination, the reference strain *E. faecalis* ATCC 29212 was used. The MIC values were interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI, 2019). When the breakpoints were not available from the Clinical and Laboratory Standards Institute guidelines, the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP, 2017) and the National Antimicrobial Resistance Monitoring System (NARMS, 2019) were applied for FFN, salinomycin, TGC, and TYLT, respectively. Multidrug resistance (**MDR**) was defined as acquired nonsusceptibility to at least 1 agent in 3 or more antimicrobial classes (Magiorakos et al., 2011).

Detection of Antimicrobial Resistance and Virulence Genes

The presence of genes conferring resistance to ERY (*ermA*, *ermB*, and *mef*), TET (*tetL* and *tetM*), and aminoglycoside-modifying enzyme (*aac(6′)-Ie–aph(2″)-Ia* and *ant(6)-Ia*) was investigated by PCR using primers and conditions as previously described (Aarestrup et al., 2000; Vakulenko et al., 2003; Sepúlveda et al., 2007; Cesare et al., 2013). The oxazolidinone and phenicol resistance gene *optrA* was investigated using primers as described by Wang et al. (2015). The MDR gene *cfr*, FFN resistance gene *fexA*, and genes encoding virulence factors such as collagen-binding protein (*ace*), aggregation substance (*asa*), cytolysin (*cylA*), *E. faecalis* endocarditis antigen (*efaA*), Enterococcal surface protein (*esp*), Gelatinase (*gelE*), and Hyaluronidase (*hyl*) were also detected using primers as previously described (Kehrenberg and Schwarz, 2006; Billström et al., 2008). The primers used in this study are shown in Table 1.

Conjugation Experiment

The transferability of plasmids carrying the *optrA* gene was assessed by the broth-mating protocol as described previously (Werner et al., 2008; Tamang et al., 2017) using rifampicin- and fusidic acid-resistant *E. faecalis* FA2-2 as the recipient strain and *optrA*-positive *E. faecalis* as the donor wild strain, respectively. Both the donor and recipient strains were inoculated

with Brain Heart Infusion (**BHI**) broth (Becton Dickinson, Franklin Lakes, NJ) and incubated overnight at 37°C. The cultured bacteria were mated with a donor/recipient ratio of 1:4 (100 μL: 400 μL), and 100 μL of mixture was inoculated on BHI agar (Becton Dickinson) plates. The bacteria on the BHI agar plates were incubated overnight followed by suspension in 100 μL of phosphate-buffered saline. Then, the cells were inoculated on BHI agar plates, which were supplemented with 2 μg/mL linezolid, 25 μg/mL rifampicin, and 25 μg/mL fusidic acid to select putative transconjugants. All transconjugants were subjected to PCR to detect *optrA* genes to confirm conjugation, and the MIC were determined by antibiotic susceptibility tests.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (**PFGE**) was performed to analyze clonal relatedness among the *optrA*-positive *E. faecalis* isolates as previously described (Gambarotto et al., 2000). In brief, genomic DNA samples were digested with 50 U *Sma*I restriction enzyme in agarose plugs and separated by electrophoresis on 1.0% SeaKem Gold agarose (Lonza, Allendale, NJ) in 0.5 × Tris-Borate-EDTA buffer. The CHEF MAPPER apparatus (Bio-Rad Laboratories, Hercules, CA) was used to perform electrophoresis at 14°C for 20 h with the following parameters: initial switch time = 5.3 s, final switch time = 34.9 s, angle = 120, gradient = 6.0 V/cm, ramping factor = linear, and 14°C for 20 h. The results were analyzed using BioNumerics software, version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium). The unweighted pair-group method with arithmetic average algorithm based on the Dice similarity index was used to calculate the relatedness of the PFGE results. *E. faecalis* isolates showing similarities of <85% were considered to be unrelated.

Statistical Analysis

The statistical package SPSS 23 (IBM SPSS Statistics for Windows, Armonk, NY) was used for statistical analysis. Chi-square tests were used to compare the prevalence of drug-resistant isolates between farms. Differences were considered significant at $P < 0.05$.

RESULTS

Distribution of Antimicrobial Resistance

The distribution of the antimicrobial resistance of *E. faecalis* isolates is shown in Table 2. The prevalence of CHL- and linezolid-resistant isolates was 4.7% (0–12.1%) and 3.7% (0–8.0%), respectively, and all linezolid-resistant isolates showed CHL resistance. Although the *E. faecalis* from 5 of 8 (62.5%) broiler breeder farms showed linezolid resistance, there were no significant differences between farms. Among 297 *E. faecalis* isolates, 80 (26.9%) isolates showed MDR, and there were no significant differences between the 8 farms. However, all 14 CHL-resistant isolates showed MDR.

Distribution of MDR Patterns

The MDR patterns of oxazolidinone- and/or phenicol-resistant *E. faecalis* isolates are shown in Table 3. A total of 14 CHL- and/or linezolid-resistant *E. faecalis* showed resistance to 7 antimicrobial classes. In particular, isolates showed the highest resistance to TET (100%), daptomycin (100%), and quinupristin/dalfopristin (100%), followed by ERY (85.7%), TYLT (85.7%), streptomycin (78.6%), ciprofloxacin (64.3%), FFN (64.3%), and kanamycin (50.0%). However, all isolates were susceptible to ampicillin, TGC, and VAN.

Characteristics of optrA-Positive E. faecalis by PCR and PFGE

The clonal relatedness and genetic characteristics of 8 *optrA*-positive isolates from 297 *E. faecalis* are shown in Figure 1. Among 8 *optrA*-positive isolates, 7 of them co-carried the phenicol exporter gene *fexA*. However, *E. faecalis* isolates from 3 of 8 broiler breeder farms only carried the *optrA* and/or *fexA* genes. A total of 8 *optrA*-positive *E. faecalis* isolates were divided into 4 pulsotypes with 85% similarity; however, 5 isolates from 1 farm (D-28-2, D-30-1, D-23-1, D-28-1, and D-66-2) were categorized into 3 different pulsotypes (I, III, IV). Although all 8 *optrA*-positive *E. faecalis* isolates showed MDR, none of the isolates carried the MDR gene *cfr*. However, these isolates carried virulence factor genes such as *ace* (100%, 8/8), *efaA* (100%, 8/8), *gelE* (100%, 8/8), and *asa1* (87.5%, 7/8).

Transferability by Conjugation

In the conjugation experiment, the *optrA* and *fexA* genes were transferred to 5 transconjugants (62.5%, 5/8) with several resistance- and virulence-related genes. The resistance genes related to aminoglycosides (*aac(6′)-Ie–aph(2″)-Ia* and *ant(6)-Ia*) were not detected from the transconjugants. The resistance genes related with macrolide (*ermB*) and TET (*tetL* and *tetM*) and virulence genes from the *optrA*-positive isolates (*ace*, *asaI*, *efaA*, and *gelE*) successfully transferred to the transconjugants.

DISCUSSION

Methicillin-resistant *Staphylococcus aureus* and VAN-resistant enterococci are a serious threat to public health, and linezolid is considered to be one of the last lines of defense against methicillin-resistant *Staphylococcus aureus* and VAN-resistant enterococci. Patel et al. (2013) and Wang et al. (2014) reported that 0.4 and 0.98% of enterococci of human origin in Canada and China, respectively, showed linezolid resistance. Although 11 (3.7%) of 297 *E. faecalis* isolates showed resistance to linezolid in this study, the rate is lower

Table 1. Primers used in this study.

Target gene	Primers	Sequence (5′-3′)	Amplicon size (bp)	Annealing temperature (°C)	References
PBP5	PBP5F	CATGCGCAATTAATCGG	444	55	(Lleo et al., 1999)
	PBP5R	CATAGCCTGTCGCAAAAC			
optrA	optrAF	AGGTGGTCAGCGAACTAA	1,395	53	(Wang et al., 2015)
	optrAR	ATCAACTGTTCCCATTCA			
cfr	cfrF	TGAAGTATAAAGCAGGTTGGGAGTCA	746	48	(Kehrenberg and Schwarz, 2006)
	cfrR	ACCATATAATTGACCACAAGCAGC			
fexA	fexAF	GTACTTGTAGGTGCAATTACGGCTGA	1,272	58	(Kehrenberg and Schwarz, 2006)
	fexAR	CGCATCTGAGTAGGACATAGCGTC			
ermA	ermAF	TAACATCAGTACGGATATTG	200	54	(Di Cesare et al., 2013)
	ermAR	AGTCTACACTTGGCTTAGG			
ermB	ermBF	CCGAACACTAGGGTTGCTC	139	54	(Di Cesare et al., 2013)
	ermBR	ATCTGGAACATCTGTGGTATG			
mef	mefF	AGTATCATTAATCACTAGTGC	348	54	(Di Cesare et al., 2013)
	mefR	TTCTTCTGGTACTAAAAGTGG			
tetL	tetlF	ATAAATTGTTTCGGGTCGGTAAT	1,077	52	(Aarestrup et al., 2000)
	tetlR	AACCAGCCAACTAATGACAATGAT			
tetM	tetmF	GTTAAATAGTGTTCTTGGAG	657	53	(Aarestrup et al., 2000)
	tetmR	CTAAGATATGGCTCTAACAA			
aac(6′) Ie-aph(2″)-la	aac6F	CAGAGCCTTGGGAAGATGAAG	348	55	(Vakulenko et al., 2003)
	aac6R	CCTCGTGTAATTCATGTTCTGGC			
ant(6)-Ia	ant6IaF	ACTGGCTTAATCAATTTGGG	577	55	(Sepúlveda et al., 2007)
	ant6IaR	GCCTTTCCGCCACCTCACCG			
asa	asa11	CACGCTATTACGAACTATGA	375	56	(Billström et al., 2008)
	asa12	TAAGAAAGAACATCACCACGA			
ace	ace1	GGAATGACCGAGAACGATGGC	616	58	(Billström et al., 2008)
	ace2	GCTTGATGTTGGCCTGCTTCCG			
cyt I	cyt I	ACTCGGGGATTGATAGGC	688	56	(Billström et al., 2008)
	cyt IIb	GCTGCTAAAGCTGCGCTT			
efaA	efaA1	CGTGAGAAAGAAATGGAGGA	499	58	(Billström et al., 2008)
	efaA2	CTACTAACACGTCACGAATG			
Esp	esp14 F	AGATTTCATCTTTGATTCTTG	510	56	(Billström et al., 2008)
	esp12 R	AATTGATTCTTTAGCATCTGG			
Gel	gel11	TATGACAATGCTTTTTGGGAT	213	56	(Billström et al., 2008)
	gel12	AGATGCACCCGAAATAATATA			
Hyl	hyln1	ACAGAAGAGCTGCAGGAAATG	276	56	(Billström et al., 2008)
	hyln2	GACTGACGTCCAAGTTTCCAA			

than the previously reported prevalence of 5.7% among isolates from food-producing animals in China (Wang et al., 2015). However, in other studies, only 3 enterococci from 5,000 animal cecal samples and 0.16% of enterococci from food animals have been reported to exhibit linezolid resistance in the United States (Tyson et al., 2018b) and Korea (Tamang et al., 2017), respectively. The increasing rate of linezolid resistance in broiler breeder farms is problematic as commercial broiler may be affected in the pyramidal structure of the industry through hatcheries to hatcheries (Kim et al., 2019).

Although the exact mechanism of linezolid resistance has not been identified, Sierra et al. (2009) reported that modified bacterial membrane permeability or the overexpression of an efflux pump might be associated with linezolid resistance. Wang et al. (2015) indicated that the linezolid resistance gene *optrA* encodes an ATP-binding cassette transporter that can confer resistance to oxazolidinones and phenicols. In addition, Wang et al. (2015) and Tyson et al. (2018a) reported that plasmids carrying *optrA* co-carried the phenicol resistance determinant *fexA* in *E. faecalis* isolates from animals in China and the United States, respectively.

Table 2. Distribution of linezolid-resistant *Enterococcus faecalis* from 8 broiler breeder farms.

Parameter	Broiler breeder farms (no. of flocks)									
	A (5)	B (8)	C (15)	D (18)	E (10)	F (12)	G (8)	H (10)	Total (86)	*P*-value
No. of *E. faecalis*	10	35	49	76	33	34	25	35	297	
No. of MDR[1] (%)	6 (60.0)	8 (22.9)	12 (24.5)	14 (18.4)	8 (24.2)	10 (29.4)	11 (44.0)	12 (34.3)	80 (26.9)	0.065
No. of chloramphenicol-resistance (%)[2]	0 (0.0)	1 (2.9)	0 (0.0)	5 (6.6)	4 (12.1)	0 (0.0)	3 (12.0)	1 (2.9)	14 (4.7)	0.079
No. of linezolid-resistance (%)[3]	0 (0.0)	1 (2.9)	0 (0.0)	5 (6.6)	2 (6.1)	0 (0.0)	2 (8.0)	1 (2.9)	11 (3.7)	0.400

[1]MDR, multidrug resistance.
[2]All chloramphenicol-resistant isolates showed multidrug resistance.
[3]All linezolid-resistant isolates showed chloramphenicol-resistance, simultaneously.

Table 3. Multidrug resistance patterns of 14 oxazolidione-resistant and/or phenicol-resistant *Enterococcus faecalis* isolated from broiler breeder farms.

Strain	Farm	No. antimicrobial classes shown resistance	Resistance pattern[1]
B-24-1	B	8	**CHL**-DAP-ERY-FFN-GEN-KAN-**LZD**-SYN-SAL-STR-TET-TYLT
D-28-2	D	7	**CHL**-DAP-ERY-FFN-GEN-KAN-**LZD**-SYN-STR-TET-TYLT
D-66-2	D	8	**CHL**-CIP-DAP-ERY-FFN-**LZD**-SYN-STR-TET-TYLT
D-28-1	D	8	**CHL**-CIP-DAP-ERY-FFN-KAN-**LZD**-SYN-STR-TET-TYLT
D-23-1	D	8	**CHL**-CIP-DAP-ERY-FFN-GEN-KAN-**LZD**-SYN-STR-TET-TYLT
D-30-1	D	8	**CHL**-CIP-DAP-ERY-FFN-GEN-KAN-**LZD**-SYN-STR-TET-TYLT
E-24-1	E	7	**CHL**-CIP-DAP-ERY-FFN-KAN-SYN-STR-TET-TYLT
E-60-1	E	7	**CHL**-CIP-DAP-ERY-FFN-**LZD**-SYN-TET-TYLT
E-60-2	E	8	**CHL**-CIP-DAP-ERY-FFN-**LZD**-SYN-STR-TET-TYLT
E-69-2	E	7	**CHL**-DAP-ERY-SYN-STR-TET-TYLT
G-12-1	G	7	**CHL**-DAP-ERY-SYN-STR-TET-TYLT
G-12-2	G	7	**CHL**-DAP-ERY-**LZD**-SYN-STR-TET-TYLT
G-14-2	G	7	**CHL**-CIP-DAP-FFN-KAN-**LZD**-SYN-TET
H-21-2	H	7	**CHL**-CIP-DAP-KAN-**LZD**-SYN-TET

[1]Abbreviations: CHL, chloramphenicol; CIP, ciprofloxacin; DAP, daptomycin; ERY, erythromycin; FFN, florfenicol; GEN, gentamicin; KAN, kanamycin, LZD, linezolid; SYN, quinupristin/dalfopristin; SAL, salinomycin; STR, streptomycin; TET, tetracycline; TYLT, tylosin tartrate. CHL and LZD are marked in bold.

In this study, all 11 linezolid-resistant isolates also showed co-resistance to CHL, and most of the *optrA*-positive isolates co-carried the phenicol exporter gene *fexA* (87.5%, 7/8) as previously reported (Wang et al., 2015; Na et al., 2019). Notably, *optrA* and *fexA* were successfully transferred to the transconjugants in this study. Furthermore, as the plasmids harboring *optrA* and *fexA* can carry additional resistance genes, they may contribute to the persistence and/or distribution of MDR genes even in the absence of oxazolidinones, resulting in selective pressure (Tamang et al., 2017). Although the MDR gene *cfr* also confers transferable resistance to linezolid (Kaminska et al., 2009), none of the isolates carried *cfr* in this study, which is consistent with previous studies (Wang et al., 2015; Na et al., 2019).

In this study, the occurrence of MDR was common; 26.9% (80/297) of *E. faecalis* isolates from breeder farms showed MDR. The prevalence of *E. faecalis* isolates with MDR from chicken and duck in Korea has been reported to be as high as 55.7 and 33.9%, respectively; however, the prevalence in the EU is much lower at 0.6% (De Jong et al., 2019; Na et al., 2019). Therefore, the high prevalence of *E. faecalis* with MDR in broiler breeder farms is of great concern considering that broiler breeders could be a source for the strains, and they could play a crucial role in the transmission and dissemination of strains with MDR in the broiler production pyramid.

Most *E. faecalis* isolates harbor common virulence genes including *ace* (100%, 8/8), *asa1 asa1* (87.5%, 7/8), *efaA* (100%, 8/8), and *gelE* (100%, 8/8). Although these virulence genes do not necessarily cause diseases in hosts, they may contribute to the severity of the infection (Yılmaz and Özcengiz, 2017; Kim et al., 2019).

In PFGE analysis, 8 *optrA*-positive *E. faecalis* isolates were clustered into 4 pulsotypes. Surprisingly, 5 isolates from a farm consisted of 3 different pulsotypes. Although 3 of 5 isolates belonged to the same pulsotype, *optrA*-positive *E. faecalis* with different genetic characteristics might be distributed on the same farm.

Linezolid- and/or CHL-resistant *E. faecalis* isolates were detected in 5 of 8 farms, which is a matter of great concern. Although there were no significant differences in the prevalence of CHL- and linezolid-resistant isolates between farms, the increasing presence of CHL- and linezolid-resistant isolates in farms should be monitored periodically by surveillance programs such as the Linezolid Experience and Accurate Determination of Resistance (LEADER) program in the United States and the global Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) program in 33 countries including Korea (Mendes et al., 2014).

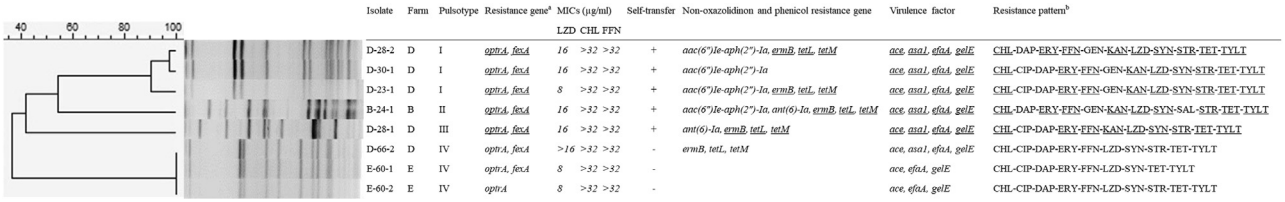

Figure 1. Dendrogram of *SmaI*-PFGE patterns of 8 *optrA*-positive *E. faecalis* isolates. *E. faecalis* isolates showing similarities of <85% were considered to be unrelated. Underline indicated that was found in the transconjugant strains. [a]Resistance gene *cfr* was tested but not detected. [b]LZD, linezolid; CHL, chloramphenicol; FFN, florfenicol; CIP, ciprofloxacin; DAP, daptomycin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; SYN, quinupristin/dalfopristin; SAL, salinomycin; STR, streptomycin; TET, tetracycline; TYLT, tylosin tartrate. Abbreviations: MIC, minimum inhibitory concentrations.

ACKNOWLEDGMENTS

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Agriculture, Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA; 716002-7).

Conflict of Interest Statement: The authors declare no conflict of interest.

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