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

Sunghyun Yoon,* Yeong Bin Kim,* Kwang Won Seo,† Jong Su Ha,‡ Eun Bi Noh,* and Young Ju Lee*,†

*College of Veterinary Medicine & Zoonoses Research Institute, Kyungpook National University, Daegu 41566, Republic of Korea; †Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS 39762, USA; and ‡Quality Management Department, Samhwa GPS Breeding Agri. Inc., Hongseong 32291, Republic of Korea

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,*
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 Enterococcuscel broth (BD Biosciences) at a 1:10 ratio and incubated at 37°C for 18 to 24 h. The cultured Enterococcuscel broth was streaked onto Enterococcuscel agar (BD Biosciences) and incubated at 37°C overnight. At least 3 representative colonies on the Enterococcuscel 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), florenicol (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), tyllosin 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 (CLSI, 2019) and 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), cytolsin (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 rifampcin- 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 SmaI 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 pulsortypes 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 pulsortypes (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(3′)-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.
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 entero-
cocci 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), respec-
tively. 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 1. Primers used in this study.

| Target gene | Primers | Sequence (5'-3') | Amplicon size (bp) | Annealing temperature (°C) | References |
|-------------|---------|------------------|--------------------|---------------------------|------------|
| PBP5        | PBP5F   | CATGCGCAATTAATACCG | 444                | 55                        | (Lleo et al., 1999) |
|             | PBP5R   | CATACGCTTGCCGCAAAC |                   |                           |            |
| optrA       | optrAF  | AGGTTGTCACGCGAATCA | 1,395              | 53                        | (Wang et al., 2015) |
|             | optrAR  | ATCAACGTGTCCCATATCA |                   |                           |            |
| cfr         | cfrF    | TGAAATGATAAACGCTGGTGGTGGA | 746                | 48                        | (Kehrenberg and Schwarz, 2006) |
|             | cfrR    | ACCATATAATGGACCAAGCAGC | 1,272              | 58                        | (Kehrenberg and Schwarz, 2006) |
| fexA        | fexAF   | GTACCTTGAGGAGATCCGATGGTGG |               |                           |            |
|             | fexAR   | ATCAACTGTTCCCATTCA |                   |                           |            |
| ermA        | ermAF   | TAACATCGTACGCGATATTGG | 200                | 54                        | (Di Cesare et al., 2013) |
|             | ermAR   | AGTCTACACTTTGCGGATTTGG |                   |                           |            |
| ermB        | ermBF   | CCGAACACATCTGGGCTTCT | 139                | 54                        | (Di Cesare et al., 2013) |
|             | ermBR   | ATCTGGAACATCTGGGCTTCT |                   |                           |            |
| mef         | mefF    | AGTATCATTAAATCATGTAGTG | 348                | 54                        | (Di Cesare et al., 2013) |
|             | mefR    | TCTTCTGAGTACTAAATTGGA |                   |                           |            |
| tetL        | tetLF   | ATAAAATGGTTTTCGGTCGTTATT | 1,077              | 52                        | (Aarestrup et al., 2008) |
|             | tetLR   | AACCAACCAATCTAGACAAATGAT |                   |                           |            |
| tetM        | tetMF   | GTAAAGATGTTGCCTTGAGGAG | 657                | 53                        | (Aarestrup et al., 2008) |
|             | tetMR   | CTAAGATATGGCTCTAAACCA |                   |                           |            |
| aac(6')-Ia  | aac6F   | CAGAGCCCTTGGGAAAGATGAGA | 348                | 55                        | (Vakulenko et al., 2003) |
|             | aac6R   | CCGTCTGAATTTCTCTGCCTG |                   |                           |            |
| ant(6)-Ia   | ant6IF  | ACTGGCGGACTAACTTTGGGG | 577                | 55                        | (Sepúlveda et al., 2007) |
|             | ant6IR  | GCCCTTCCGCAACTGCAGG |                   |                           |            |
| asa         | asa1F   | CACGCTATTACGAGCATTTG | 375                | 56                        | (Billström et al., 2008) |
|             | asa1R   | TAAAGAACAAACGCTACGCCAGA |                   |                           |            |
| ace         | ace1F   | GGAATGACCGGAAGAGATGGGC | 616                | 58                        | (Billström et al., 2008) |
|             | ace2F   | GTCTGATGTGGCCGCTTTCG |                   |                           |            |
| cyt I       | cyt1F   | ACTCGGCGGATTGATAGGCG | 688                | 56                        | (Billström et al., 2008) |
|             | cyt1R   | GCTGCTAAAGCTGGCCCTT |                   |                           |            |
| cfaA        | cfaA1F  | CCGTGAAAGAAAGATGGAGA | 499                | 56                        | (Billström et al., 2008) |
|             | cfaA2F  | CATCAACGCTGCGAGAATGG |                   |                           |            |
| Esp         | esp14F  | AGATTTCATCTTTGCTTTTGG | 510                | 56                        | (Billström et al., 2008) |
|             | esp12R  | AATTGATCTTCTTCACTTCGGAG |                   |                           |            |
| Gel         | gel11F  | TATGCAATGCTTCTTTGGGAT | 213                | 56                        | (Billström et al., 2008) |
|             | gel12F  | AGATGACCCGAAAAATATTAATATAT |                   |                           |            |
| Hyl         | hyl1F   | ACAGAAGAGCTGAGGAAATG | 276                | 56                        | (Billström et al., 2008) |
|             | hyl2F   | CACGAGCTGCAAGGTTTCCCAA |                   |                           |            |

### 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) |
| No. of E. faecalis | 10 | 35 | 49 | 76 | 33 | 54 | 25 | 35 | 297 |
| No. of MDR (%) | 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 (%) | 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 (%) | 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.
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 gelB (100%, 8/8). Although these virulence genes do not necessarily cause diseases in hosts, they may contribute to the severity of the infection (Yilmaz and Özçengiz, 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).

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 |
|--------|------|-------------------------------------------|-------------------|
| 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-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| D-66-2 | D    | 8                                         | CHL-CIP-DAP-ERY-FFN-LZD-SYN-SAL-STR-TET-TYLT |
| D-28-1 | D    | 8                                         | CHL-CIP-DAP-ERY-FFN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| D-23-1 | D    | 8                                         | CHL-CIP-DAP-ERY-FFN-GEN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| D-30-1 | D    | 8                                         | CHL-CIP-DAP-ERY-FFN-GEN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| E-24-1 | E    | 7                                         | CHL-CIP-DAP-ERY-FFN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| E-60-1 | E    | 7                                         | CHL-CIP-DAP-ERY-FFN-LZD-SYN-SAL-STR-TET-TYLT |
| E-69-1 | E    | 7                                         | CHL-CIP-DAP-ERY-FFN-LZD-SYN-SAL-STR-TET-TYLT |
| G-12-2 | G    | 7                                         | CHL-CAP-DAP-ERY-FFN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| G-14-2 | G    | 7                                         | CHL-CAP-DAP-ERY-FFN-KAN-LZD-SYN-SAL-STR-TET-TYLT |
| H-21-2 | H    | 7                                         | CHL-CAP-DAP-ERY-FFN-KAN-LZD-SYN-SAL-STR-TET-TYLT |

1Abbreviations: 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.

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. *Resistance gene cfr was tested but not detected. **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.

REFERENCES

Aarestrup, F. M., Y. Agero, P. G. Smidt, M. Madsen, and L. B. Jensen. 2000. Comparison of antimicrobial resistance phenotypes and resistance genes in Enterococcus faecalis and Enterococcus faecium from humans in the community, broilers, and pigs in Denmark. Diagn. Microbiol. Infect. Dis. 37:127–137.

Animal and Plant Quarantine Agency (APQA). 2017. National Antimicrobial Resistance Monitoring Program. Ministry of Food and Drug Safety, Republic of Korea.

Aoki, H., L. Ke, S. M. Poppe, T. J. Poel, E. A. Weaver, R. C. Gadwood, R. C. Thomas, D. L. Shimagbaru, and M. C. Ganoza. 2002. Oxazolidinone antibiotics target the P site on Escherichia coli ribosomes. Antimicrob. Agents Chemother. 46:1080–1085.

Billström, H., B. Lund, A. Sullivan, and C. E. Nord. 2008. International Journal of antimicrobial agents virulence and antimicrobial resistance in clinical Enterococcus faecium. Int. J. Antimicrob. Agents 32:374–377.

Bourgeois-Nicolaos, N., L. Massias, B. Couson, M. Butel, A. Andremont, and F. Doucet-Populaire. 2007. Dose Dependence of Emergence of resistance to linezolid in Enterococcus faecalis in Vivo. J. Infect. Dis. 195:1480–1488.

Brecianii, A., G. Morroni, C. Vincenzi, E. Manso, M. Mingoa, E. Giovannetti, and P. E. Varaldo. 2016. Detection in Italy of two clinical Enterococcus faecium isolates carrying both the oxazolidinone and phenicol resistance gene optrA and a silent multidrug resistance gene cfr. J. Antimicrob. Chemother. 71:1118–1119.

Cesare, A. Di, G. M. Luna, C. Vignaroli, S. Pasquaroli, S. Tota, P. Paroncini, and F. Biavasco. 2013. Aquaculture can Promote the presence and spread of antibiotic-resistant enterococci in marine Sediments. PLoS One 8:1–8.

Clinical and Laboratory Standards Institute (CLSI). 2019. M100 Performance Standards for Antimicrobial Susceptibility Testing, Clinical and Laboratory Standards Institute, Wayne, PA.

DANMAP. 2017. Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark. National Food Institute, Copenhagen, Denmark.

Diekema, D. J., P. Hsueh, R. E. Mendes, M. A. Pfaller, K. V. Rolston, H. S. Sader, and R. N. Jones. 2019. The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY antimicrobial surveillance program. Antimicrob. Agents Chemother. 63:1–10.

Gambetti, K., M.-C. Ploy, P. Turlure, C. Grelaud, C. Martin, D. Bordesoulle, and F. Denis. 2000. Prevalence of vancomycin-resistant enterococci in fecal samples from Hospitalized Patients and Nonhospitalized controls in a cattle-Rearing Area of France. J. Clin. Microbiol. 38:620–624.

Gonzales, R. D., P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn. 2001. Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. Lancet 357:1179.

Hammerum, A. M. 2012. Enterococci of animal origin and their significance for public health. Clin. Microbiol. Infect. 18:619–625.

De Jong, A., S. Simjée, M. Rose, H. Moynaert, F. El Garch, M. Yonula, P. Butty, S. Haag-Diegerman, U. Klein, T. Pellet, G. Schiffer, P. J. Serreyen, and T. Vila. 2010. Antimicrobial resistance monitoring in commensal enterococci from healthy cattle, pigs and chickens across Europe during 2004-14 (EASSA Study). J. Antimicrob. Chemother. 74:921–930.
Sierra, J. M., M. Ortega, C. Tarragó, C. Albet, J. Vila, J. Terencio, and A. Guglietta. 2009. Decreased linezolid uptake in an in vitro-selected linezolid-resistant Staphylococcus epidermidis mutant. J. Antimicrob. Chemother. 64:990–992.

Tamang, M. D., D. C. Moon, S. R. Kim, H. Y. Kang, K. Lee, H. M. Nam, G. C. Jang, H. S. Lee, S. C. Jung, and S. K. Lim. 2017. Detection of novel oxazolidinone and phenicol resistance gene optrA in enterococcal isolates from food animals and animal carcasses. Vet. Microbiol. 201:252–256.

Tyson, G. H., E. Nyirabahizi, E. Crarey, C. Kabera, C. Lam, C. Rice-Trujillo, P. F. McDermott, and H. Tate. 2018a. Prevalence and antimicrobial resistance of enterococci isolated from retail meats in the United States, 2002 to 2014. Appl. Environ. Microbiol. 84:1–9.

Tyson, G. H., J. L. Sabo, M. Hoffmann, C. H. Hsu, S. Mukherjee, J. Hernandez, G. Tillman, J. L. Wasilenko, J. Haro, M. Simmons, W. Wilson Egbe, P. L. White, U. Dessai, and P. F. McDermott. 2018b. Novel linezolid resistance plasmids in Enterococcus from food animals in the USA. J. Antimicrob. Chemother. 73:3254–3258.

USDA. 2012. National Poultry Improvement Plan (NPIP). USDA Animal and Plant Health Inspection Service (APHIS), Riverdale, MD.

Vakulenko, S. B., S. M. Donabedian, A. M. Voskresenskiy, M. J. Zervos, S. A. Lerner, and J. W. Chow. 2003. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. Antimicrob. Agents Chemother. 47:1423–1426.

Wang, L., Y. He, Y. Xia, H. Wang, and S. Liang. 2014. Investigation of mechanism and molecular epidemiology of linezolid-resistant Enterococcus faecalis in China. Infect. Genet. Evol. 26:14–19.

Wang, Y., Y. Lv, J. Cai, S. Schwarz, L. Cui, Z. Hu, R. Zhang, J. Li, Q. Zhao, T. He, D. Wang, Z. Wang, Y. Shen, Y. Li, A. T. Fedler, C. Wu, H. Yu, X. Deng, X. Xia, and J. Shen. 2015. A novel gene, optrA, that confers transferable resistance to oxazolidinones and phenicols and its presence in Enterococcus faecalis and Enterococcus faecium of human and animal origin. J. Antimicrob. Chemother. 70:2182–2190.

Werner, G., T. M. Coque, A. M. Hammerum, R. Hope, W. Hryniewicz, A. Johnson, I. Klare, and K. G. Kristinsson. 2008. Emergence and spread of vancomycin resistance among enterococci in Europe. Eurosurveillance 13:1–11.

Yılmaz, and G. Özçengiz. 2017. Antibiotics: Pharmacokinetics, toxicity, resistance and multidrug efflux pumps. Biochem. Pharmacol. 133:43–62.