International Journal of Food Microbiology 293 (2019) 53–59

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Detection of oxazolidinone and phenicol resistant enterococcal isolates from duck feces and carcasses

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ARTICLE INFO

Keywords:
Enterococcus
Linezolid
Phenicol
optrA
fexA

ABSTRACT

The heavy use or abuse of antimicrobials in food animals has caused an increase in antimicrobial resistance in enterococci of animal origin, which could get transmitted to those of human origin via the food chain. Since duck meat consumption has been on the rise in Korea, we conducted this study to provide information about the antimicrobial resistance of the enterococci obtained from healthy ducks and their carcasses. A total of 82 *Enterococcus faecium* and 174 *E. faecalis* isolated from duck fecal and carcass samples were investigated for antimicrobial resistance to 16 agents, using broth dilution method, and were further characterized using molecular methods. Most of *E. faecium* (84.1%) and *E. faecalis* (87.9%) isolates were resistant to one or more antimicrobials. Multi-drug resistant (MDR) isolates were observed in both *E. faecium* (40.2%) and *E. faecalis* (33.9%) with high frequencies. High rate of resistance was observed for tetracycline, ciprofloxacin, chloramphenicol, and erythromycin in both *E. faecium* and *E. faecalis*. Resistance to gentamicin, vancomycin, and daptomycin, in both *E. faecium* and *E. faecalis*, was, if at all, very rare. However, linezolid resistance was observed in nine *E. faecium* (11.0%) and one *E. faecalis* (0.6%). All, but one, Linezolid resistant (LR) isolates were also resistant to chloramphenicol and florfenicol. The novel transferable oxazolidinone and phenicol resistant gene, *optrA*, was found in six *E. faecium* isolates. All of them co-carried phenicol exporter gene *fexA*. None of the LR isolates had mutation in the 23S ribosomal RNA and in the ribosomal protein L3. Six LR *E. faecium* isolates had Asn130Lys mutation in the ribosomal protein L4, of which five also carried *optrA* gene. None of the isolates carried the multi-resistance gene *cfr*. Transfer of oxazolidinone and phenicol resistance was observed in five among the 10 LR isolates; two of them had *optrA* and *fexA* genes. Multi-drug resistant *Enterococcus* that also carried the resistance gene to a last-resort antimicrobial is a major concern for public health. Thus, to prevent the introduction of last-resort antimicrobial resistance into food chain, continuous surveillance of antimicrobial resistance in duck is imperative.

1. Introduction

Enterococci are part of the normal microbiota in the gastrointestinal tract of animals and humans, and have been widely accepted as an indicator for the detection of antimicrobial resistance of Gram-positive bacteria (APQA, 2016; de Jong et al., 2018; FAO/OIE/WHO, 2004; NARMS, 2013). The heavy use or abuse of antimicrobials in food animals has caused an increase in antimicrobial resistance in enterococci of animal origin. Furthermore, the enterococci from animals have potential risks of spreading this resistance to those of human origin via the food-chain (Ogier and Serror, 2008). Due to nosocomial infection and prevailing resistance to antimicrobials, *Enterococcus* species, especially *E. faecium* and *E. faecalis*, have become a particular clinical concern. In Korea, the *Enterococcus* species was one of the common bacteria responsible for approximately 6–8% of all nosocomial infection (Yong et al., 2014).

Linezolid has been used for the treatment of complicated skin and soft tissue infections, nosocomial pneumonia, and community-acquired pneumonia caused by Gram-positive bacteria including *Enterococcus* (Peppard and Weigelt, 2006). However, linezolid resistant (LR) *Enterococcus* has appeared since 2001, shortly after exposure to linezolid (Gonzales et al., 2001). Therefore, LR staphylococci and enterococci pose an urgent clinical challenge in treating Gram-positive cocci infection (Flamm et al., 2013). Linezolid resistance in *Enterococcus* spp.

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https://doi.org/10.1016/j.ijfoodmicro.2019.01.002
Received 24 April 2018; Received in revised form 26 November 2018; Accepted 4 January 2019
Available online 06 January 2019

can be attributable to mutations in the central loop of domain V of 23S rRNA (Bourgeois-Nicolaos et al., 2007; Ntokou et al., 2012). Mutations in the ribosomal proteins L3 and L4 have also been linked to linezolid resistance (Locke et al., 2009). In addition, multi-resistance genes, *cfr* (Kaminska et al., 2010) and a new variant *cfr* (*B*) (Deshpande et al., 2015) represent a transferable linezolid resistance mechanism. Most recently, *optrA* gene has been detected in enterococcal isolates from pig and chicken as well as from their carcasses in Korea (Tamang et al., 2017), Colombia (Cavaco et al., 2017), and USA (Tyson et al., 2018).

According to the statistics, duck industry has been growing over the past several years in Korea, ever since it was highlighted as a healthy food (http://kosis.kr). With increase of duck meat consumption, concerns about food safety have also increased. Several studies have been performed in Korea on the prevalence and antimicrobial resistance of foodborne pathogens, such as *Salmonella* (Cha et al., 2013) and *Campylobacter* species (Wei et al., 2014), isolated from duck. However, information on antimicrobial resistance of commensal bacteria is very rare both in Korea, and across the world. Thus, the primary aims of this study were to provide information about the antimicrobial resistance of enterococci obtained from healthy duck and their carcasses, and to study the underlying mechanism and molecular epidemiology of LR *Enterococcus*.

2. Materials and methods

2.1. Sample collection and bacterial isolation

We collected a total of 255 fecal samples and 149 duck carcasses (from 85 farms) from four slaughterhouses in the southern part of Korea between March and July 2016. No more than five feces and carcasses were collected from each farm. Sample processing and enterococcal isolation were conducted as described previously, using buffered peptone water and m *Enterococcus* agar media (Becton Dickinson, Sparks, MD) (Lim et al., 2006). Species identification was performed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) using Vitek MS system (bioMerieux, Marcy-l'Etoile, France) or by PCR (Dutka-Malen et al., 1995). For antimicrobial resistance test, one isolate per animal was selected.

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed by determining minimum inhibitory concentrations (MICs) for 16 antimicrobial agents by broth microdilution method using commercially available Sensititre® panel KRVP2F (TREK Diagnostic Systems, West Sussex, UK) according to the manufacturer's instructions. The antimicrobials tested include ampicillin, chloramphenicol, ciprofloxacin, daptomycin, erythromycin, florfenicol, gentamicin, kanamycin, linezolid, streptomycin, tetracycline, tigecycline, tylosin, vancomycin, and salinomycin. Briefly, approximately 5×10^5 cfu/ml inoculums, prepared from overnight cultures, were inoculated on MIC panels and incubated at 35 °C for 20–24 h. Bacterial susceptibility to quinupristin-dalfopristin (SYN) was determined only in *E. faecium*, due to its intrinsic resistance to quinupristin-dalfopristin. The reference strain *E. faecalis* ATCC 29212 was used as quality control strain in MIC determinations. The MIC values were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). When CLSI breakpoints were not available, the MIC interpretation was done according to the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) (2007), (2013), and (2016) or the National Antimicrobial Resistance Monitoring System (NARMS) (2013). Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes.

2.3. Detection of mutations and resistance genes

The central loop of domain V of the 23S rRNA gene was amplified using primers, as described previously (Bourgeois-Nicolaos et al., 2007). The amplified product was sequenced to detect mutations at position 2576 and elsewhere in the amplified gene. The genes encoding ribosomal proteins L3 (*rplC*) and L4 (*rplD*) were PCR amplified as described previously (Diaz et al., 2012), and sequenced to detect the presence of mutations associated with linezolid resistance. The nucleotide and amino acid sequences of *rplC*, *rplD*, and domain V of the 23S rRNA gene, for each of the isolates tested, were compared with those of wild-type linezolid-susceptible *E. faecalis* ATCC 29212 strain (GenBank accession number CP008816.1) and *E. faecium* DO strain (GenBank accession number CP003583.1). Analysis and comparison were performed using BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST) and ExPASY proteomics tools (http://www.expasy.ch/tools/#similarity). Plasmid DNAs were extracted using the QuickGene® plasmid isolation system (FUJIFILM Corporation, Tokyo, Japan), and the novel oxazolidinone and phenicol resistance gene, *optrA* was amplified using primers as described by Wang et al. (2015). The presence of multi-drug resistance gene *cfr* and florfenicol resistance gene *fexA* was determined using previously published primers (Kehrenberg and Schwarz, 2006).

2.4. Conjugation experiment

The transferability of plasmid carrying *optrA* and *fexA* genes was assessed by filter-mating protocol as described previously (Werner et al., 2011; Tamang et al., 2017), using rifampicin and fusidic acid resistant *E. faecalis* FA2-2 and *E. faecium* BM4105RF recipient strains for *optrA*-positive *E. faecalis* and *E. faecium* donor wild strains, respectively. Briefly, overnight cultures of donor and recipient stains were inoculated with fresh Brain Heart Infusion (BHI) broth (Becton Dickinson) and cultured for 4 h at 37 °C. The freshly cultured bacteria were mated with a donor/recipient ratio of 1:9 and trapped on a membrane filter. The bacteria on the filters were incubated overnight and then suspended in phosphate buffered saline (PBS). Appropriate dilutions of the mixture were transferred to BHI agar (Becton Dickinson) plates, supplemented with 2 μg/ml linezolid, 25 μg/ml rifampicin, and 25 μg/ml fusidic acid, to select putative transconjugants. All transconjugants selected were confirmed by the detection of *optrA* and *fexA* genes, using PCR, and investigated for their MICs by antibiotic susceptibility tests. *E. faecium* FM-10 and *E. faecalis* FC-19, having conjugative plasmid as reported previously, were used as positive controls (Tamang et al., 2017).

2.5. Pulsed-field gel electrophoresis (PFGE)

Clonal relatedness among the isolates was analyzed by PFGE, as previously described (Gambarotto et al., 2000). Briefly, chromosomal DNA sample plugs were digested with 50 U of *Sma*I (Takara Bio, Otsu, Japan) and separated by electrophoresis on 1.0% SeaKem Gold agarose (Lonza, Allendale, NJ) in 0.5× Tris-Borate-EDTA buffer at 14 °C for 20 h using a CHEF-Mapper (Bio-Rad, Hercules, CA) 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. Results were analyzed using Bionumerics software, version 4.0 (Applied Maths, Sint-Martens-

Table 1
Minimum inhibitory concentration distribution of *Enterococcus faecium* (n = 82) isolated from duck fecal and carcass samples.

Antimicrobials	Sample	Distribution (%) of MICs (μg/ml)															MIC_{50}	MIC_{90}	MIC Range	Resistance (%)
		≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048				
Ampicillin	Feces				58.3	16.7	20.0		5.0								1	4	1 - 16	5.0
	Carcasses				59.1	4.5	22.7	4.5	9.1								1	8	1 - 16	9.1
Chloramphenicol	Feces						45.0	40.0		15.0							8	32	4 - 32	15.0
	Carcasses						40.9	13.6		45.5							8	32	4 - 32	45.5
Ciprofloxacin	Feces		1.7	5.0	6.7	40.0	45.0		1.7								2	4	0.25 - 16	46.7
	Carcasses			9.1		9.1	63.6	4.5	13.6								4	16	0.5 - 16	81.8
Daptomycin	Feces			8.3	6.7	33.3	50.0			1.7							4	4	0.5 - 32	1.7
	Carcasses				9.1	18.2	72.7										4	4	1 - 4	0.0
Erythromycin	Feces				66.7	15.0	3.3	1.7	5.0		8.3						1	16	1 - 64	15.0
	Carcasses				54.5	18.2		18.2			9.1						1	8	1 - 64	27.3
Florfenicol	Feces					50.0	33.3		1.7	15.0							2	32	2 - 32	16.7
	Carcasses					40.9	13.6			45.5							4	32	2 - 32	45.5
Gentamicin	Feces											100.0					128	128	128 - 128	0.0
	Carcasses											100.0					128	128	128 - 128	0.0
Kanamycin	Feces											66.7	26.7	6.7			128	256	128 - 512	0.0
	Carcasses											86.4	9.1			4.5	128	256	128 - 2048	4.5
Linezolid	Feces					85.0	8.3	6.7									2	4	2 - 8	6.7
	Carcasses					68.2	9.1	18.2	4.5								2	8	2 - 16	22.7
Quinupristin /Dalfopristin	Feces				28.3	68.3	1.7	1.7									2	2	1 - 8	3.3
	Carcasses				31.8	63.6	4.5										2	2	1 - 4	4.5
Salinomycin	Feces					100.0											2	2	2 - 2	0.0
	Carcasses					100.0											2	2	2 - 2	0.0
Streptomycin	Feces											73.3	5.0	1.7		20.0	128	2048	128 - 2048	20.0
	Carcasses											77.3				22.7	128	2048	128 - 2048	22.7
Tetracycline	Feces					48.3		3.3	1.7		6.7	40.0					8	128	2 - 128	48.3
	Carcasses					72.7				4.5		22.7					2	128	2 - 128	27.3
Tigecycline	Feces	50.0	15.0	28.3	6.7												0.125	0.5	0.12 - 1	35.0
	Carcasses	59.1	40.9														0.125	0.25	0.12 - 0.25	0.0
Tylosin	Feces				13.3	35.0	41.7				10.0						4	4	1 - 64	10.0
	Carcasses				22.7	22.7	27.3	4.5			22.7						4	64	1 - 64	22.7
Vancomycin	Feces					100.0											2	2	2 - 2	0.0
	Carcasses					100.0											2	2	2 - 2	0.0

The dilution ranges tested are those contained in the white area.
The breakpoints of tested antimicrobial agents are indicated by vertical lines.
MIC_{50} and MIC_{90} are the concentrations at which 50% and 90% of the isolates were inhibited.

Latem, Belgium) and relatedness was calculated using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm, based on the Dice similarity index.

2.6. Multi-locus sequence typing (MLST)

Multi-locus sequence typing (MLST) was performed according to the instructions on MLST website (http://www.mlst.net) for *E. faecalis* (efaecalis.mlst.net) and *E. faecium* (efaecium.mlst.net). Allelic profile and sequence type of *E. faecalis* and *E. faecium* isolates carrying *optrA* gene were determined using the *E. faecalis* MLST database (http://pubmlst.org/efaecalis/) and *E. faecium* MLST database (http://pubmlst.org/efaecium/), respectively.

3. Results

3.1. Antimicrobial susceptibility

A total of 82 *E. faecium* and 174 *E. faecalis* were isolated from 255 duck feces and 149 duck carcasses; 60 *E. faecium* and 77 *E. faecalis* were from duck feces, and 22 *E. faecium* and 97 *E. faecalis* were from duck carcasses. Most of *E. faecium* (84.1%, 69/82) and *E. faecalis* (87.9%, 153/174) isolates were resistant to one or more antimicrobials. The most frequently observed resistance in *E. faecium* and *E. faecalis* isolates was to ciprofloxacin and tetracycline, respectively. In addition, resistance to phenicols (florfenicol and chloramphenicol) and macrolides (erythromycin and tylosin) was common in both *E. faecium* and *E.*

faecalis. However, resistance to gentamicin, vancomycin, and daptomycin was very rarely observed, if at all, in both *E. faecium* and *E. faecalis* (Tables 1 and 2). MDR isolates were observed with high frequencies in both *E. faecium* (40.2%, 33/82) and *E. faecalis* (33.9%, 59/174) (Table S1). 1In total, 53 different resistant patterns were found in *Enterococcus* spp.; ciprofloxacin (13.4%, 11/82) and tetracycline (18.4%, 42/174) being the most common in *E. faecium* and *E. faecalis,* respectively. No co-resistance to aminoglycosides and beta-lactams, or quinupristin-dalfopristin and/or vancomycin, used in combination therapy for enterococcal infection, was observed in this study.

3.2. Molecular mechanism of linezolid resistance

Among the 82 *E. faecium* and 174 *E. faecalis* strains screened, nine (11.0%) and one strain (5.7%), respectively, were found to be resistant to linezolid (≥8 μg/ml). The nine LR *E. faecium* were obtained from duck feces ($n = 4$) and duck carcasses ($n = 5$) collected from six farms. LR isolates were also resistant to phenicols (100%, 9/9), ciprofloxacin (88.9%, 8/9), macrolides (66.7%, 6/9), tetracycline (44.4%, 4/9), and streptomycin (22.2%, 2/9). Among the nine LR *E. faecium* and one *E. faecalis*, six LR *E. faecium* isolates had Asn130Lys mutation in the ribosomal protein L4, of which five also carried *optrA* gene on their plasmid. All of *optrA*-positive isolates co-carried phenicol exporter gene *fexA*. None of the LR isolates had any mutation in the 23S ribosomal RNA or in the ribosomal protein L3; none carried the multi-resistance gene *cfr* (Table 3).

3.3. Transferability of optrA gene

Linezolid resistance was transferred from 55.6% (5/9) of the LR *E. faecium* isolates to *E. faecium* BM4105RF. Among the five transconjugants, two carried the *optrA* gene on their plasmid, consistent with *optrA*-positive donors. The *fexA* gene co-transferred with *optrA* gene in all *optrA*-positive transconjugants. All transconjugants were resistant to linezolid, chloramphenicol, or florfenicol. In addition, transfer of resistance to erythromycin, tylosin, and quinupristin-dalfopristin was observed in one transconjugant (Table 3). None of the LR *E. faecalis* isolate was a conjugational donor.

3.4. Molecular typing by PFGE and MLST

Genetic relatedness of LR *E. faecalis* and *E. faecium* isolates was analyzed by *SmaI*-PFGE and MLST. The dendrogram of nine *E. faecium* isolates is shown in Fig. S1. Based on a similarity value of 0.85, the nine *optrA*-positive *E. faecium* strains represented seven arbitrary (designated as I through VII) pulsotypes. MLST analysis demonstrated that the nine *E. faecium* strains belonged to ST8 ($n = 3$), ST120 ($n = 1$), ST157 ($n = 1$), ST14 ($n = 1$), ST520 ($n = 1$), and ST309 ($n = 1$). Identical MLST (ST8) showed the same PFGE pattern (pattern I). The single *E. faecalis* strain belonged to ST288. Molecular typing showed the heterogeneity of linezolid resistant *E. faecium* from six farms. Different clones were observed across the farms, except in one. Identical ST (ST8) and PFGE (pulsotype I) were found in fecal and carcass samples from the same farm (farm A). In addition, this clone was also observed in a carcass from another farm (farm E), together suggesting that linezolid resistance could be acquired by individual and/or clonal spread.

4. Discussion

High antimicrobial resistance of 82 *E. faecium* and 174 *E. faecalis* isolates was observed in duck feces and their carcasses. Furthermore, resistance to linezolid, one of the last resort antimicrobials was detected in both *E. faecium* and *E. faecalis*. Antimicrobial resistance patterns were similar to that in other food animals. The above-mentioned high-level resistance to tetracycline, macrolides, and phenicols was also found among pigs and chicken in Korea (APQA, 2016), USA (NARMS, 2013) and European countries (de Jong et al., 2018). Although consumption data, from national monitoring program or the duck farms studied, are not available, these antimicrobials are the commonly administered ones among food animals in Korea. In addition, low or no resistance to critically important antimicrobials was also found in other food animals. Notably, in the present study, resistance to ciprofloxacin in *E. faecium* was the highest with 46.7% and 81.8% in fecal and carcass samples, respectively. This is similar to that in chicken in Korea (62.2%) and USA (34.7%); however, much higher than in other food animals. Enrofloxacin, which belongs to the same fluoroquinolone class as ciprofloxacin, may be commonly consumed in poultry industry and duck industry in Korea. In this study, the occurrence of MDR was common in both *Enterococcus* spp. Although the prevalence was lower than in chicken (*E. faecium* 45.6% and *E. faecalis* 55.7%) in Korea, it was much higher than that in EU (*E. faecium* 16.1% and *E. faecalis* 0.6%).

In this study, the rate of linezolid resistant *E. faecium* was high (11%). Linezolid is a critically important antimicrobial drug used in the treatment of enterococcal infections that is constantly being monitored by surveillance programs such as the global Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) and the Linezolid Experience and Accurate Determination of Resistance (LEADER) (USA) (Mendes et al., 2014a, 2014b). Although linezolid resistance commonly develops as a spontaneous mutation, such as G2576T mutation in the 23S rRNA gene, rather than genetic transfer (Patel et al., 2013), the six LR *E. faecium* isolates in this study had N130K mutation in the ribosomal protein L4 instead of G2576T and other mutations in the domain V of 23S rRNA gene. These results are consistent with those of our previous study (Tamang et al., 2017). In this study, the *optrA* gene was found in 60% of the LR enterococci. The novel transferable gene *optrA* was first reported in *E. faecalis* and *E. faecium* of human and animal origin in China (Wang et al., 2015). Subsequently, Bi et al. (2017) reported thirty-two *optrA*-positive LR *Enterococcus* isolates including 27 *E. faecalis* and five *E. faecium*. Recently, seven additional LR *E. faecium* isolates from a tertiary referral hospital in Korea were reported (Cho et al., 2018).

All of the *optrA*-positive isolates in our study co-carried phenicol exporter gene *fexA*. Our previous study had shown that *optrA*-positive isolates co-carried *fexA* with high frequency (30/35, 85.7%) in *Enterococcus* spp., and the presence of both *optrA* and *fexA* in the same isolate has also been described in *Enterococcus* from humans and animals from China (Cai et al., 2015; Wang et al., 2015). Moreover, the *fexA* gene co-transferred with *optrA* in two transconjugants. The *optrA* carrying plasmids were reported in *E. faecium* and *E. faecalis* strains isolated from humans and animals in China (Wang et al., 2015), Italy (Morroni et al., 2018), and the United States (Tyson et al., 2018). Linezolid resistance plasmids frequently carried the other resistance determinants such as *fexA, fexB*, *erm*(A), and *erm*(B). One isolate in this study was also transferred with macrolide, streptogramin, and phenicol resistance, along with linezolid resistance. The mobile genetic element, carrying not only linezolid resistance but also additional other resistance genes, is a matter of concern, since they might facilitate co-selection of the *optrA* gene. Plasmid-mediated oxazolidinone resistance has been linked to animal sources where the use of phenicols, macrolides, and streptogramins might co-select resistance to both antibiotic families (Freitas et al., 2017; Tamang et al., 2017; Wang et al., 2015).

Table 2
Minimum inhibitory concentration distribution of *Enterococcus faecalis* (n = 174) isolated from duck fecal and carcass samples.

Antimicrobials	Sample	Distribution (%) of MICs (μg/ml)															MIC_{50}	MIC_{90}	MIC Range	Resistance (%)
		≤0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	≥2048				
Ampicillin	Feces				98.7	1.3											1	1	1 - 2	0.0
	Carcasses				94.8	4.1	1.0										1	1	1 - 4	0.0
Chloramphenicol	Feces						5.2	72.7	1.3	20.8							8	32	4 - 32	20.8
	Carcasses						3.1	87.6	1.0	8.2							8	8	4 - 32	8.2
Ciprofloxacin	Feces		1.3	2.6	42.9	22.1	19.5		11.7								2	16	0.25 - 16	31.2
	Carcasses			7.2	53.6	24.7	9.3		5.2								1	4	0.5 - 16	14.4
Daptomycin	Feces			1.3	58.4	35.1	3.9	1.3									1	2	0.5 - 8	1.3
	Carcasses			9.3	43.3	40.2	6.2	1.0									1	2	0.5 - 8	1.0
Erythromycin	Feces				61.0	11.7		3.9	2.6	2.6	18.2						1	64	1 - 64	27.3
	Carcasses				69.1	7.2	2.1	1.0	1.0		19.6						1	64	1 - 64	21.6
Florfenicol	Feces					11.7	67.5		1.3	19.5							4	32	2 - 32	20.8
	Carcasses					8.2	85.6			6.2							4	4	2 - 32	6.2
Gentamicin	Feces											98.7		1.3			128	128	128 - 512	0.0
	Carcasses											97.9		1.0	1.0		128	128	128 - 1024	1.0
Kanamycin	Feces											87.0				13.0	128	2048	128 - 2048	13.0
	Carcasses											87.6			1.0	11.3	128	2048	128 - 2048	11.3
Linezolid	Feces				11.7	72.7	15.6										2	4	1 - 4	0.0
	Carcasses				5.2	91.8	2.1	1.0									2	2	1 - 8	1.0
Salinomycin	Feces					98.7	1.3										2	2	2 - 4	0.0
	Carcasses					100.0											2	2	2 - 8	0.0
Streptomycin	Feces											71.4	1.3			27.3	128	2048	128 - 2048	27.3
	Carcasses											66.0		1.0		33.0	128	2048	128 - 2048	33.0
Tetracycline	Feces					20.8			2.6	1.3	35.1	40.3					64	128	2 - 128	79.2
	Carcasses					22.7		2.1		9.3	29.9	36.1					64	128	2 - 128	75.3
Tigecycline	Feces	54.5	22.1	20.8	2.6												0.125	0.5	0.12 - 1	23.4
	Carcasses	55.7	18.6	21.6	4.1												0.125	0.5	0.12 - 1	25.8
Tylosin	Feces				24.7	49.4					26.0						2	64	1 - 64	26.0
	Carcasses				23.7	52.6	2.1				21.6						2	64	1 - 64	21.6
Vancomycin	Feces					98.7	1.3										2	2	2 - 4	0.0
	Carcasses					89.7	10.3										2	4	2 - 4	0.0

The dilution ranges tested are those contained in the white area.
The breakpoints of tested antimicrobial agents are indicated by vertical lines.
MIC_{50} and MIC_{90} are the concentrations at which 50% and 90% of the isolates were inhibited.

In our study, no known resistance mechanism was observed in the two *E. faecium* isolates. Previous studies in Canada (Patel et al., 2013) and Korea (Tamang et al., 2017) also failed to identify linezolid resistance mechanisms. The most recently described linezolid resistance gene, *poxtA*, of *S. aureus* encodes a protein that is 32% identical to OptrA. Expression of *poxtA* in *E. faecalis* was able to decrease susceptibility to phenicols and oxazolidinones. This gene was found by a BLAST search in *E. faecium* and *E. faecalis* isolates (Antonelli et al., 2018; Sadowy, 2018). Another study on LR *S. epidermidis* suggested that changes in bacterial membrane permeability or over-expression of an efflux pump might be associated with linezolid resistance (Sierra et al., 2009). Thus, our data, together with earlier reports, suggest the possibility of additional mechanisms that could lead to linezolid resistance in enterococci and warrant further investigations.

In conclusion, we presented a report about the prevalence of antimicrobial resistance in enterococci from duck feces and carcasses. Antimicrobials considered important in human clinical settings, such as daptomycin and vancomycin, could still be a useful treatment. However, our results suggested that the resistance rates against antimicrobials, commonly used in food-animal farm, such as fluoroquinolones and tetracycline, were high. Especially, phenicols might induce the co-selective pressure with oxazolidinones. Animal- and meat-associated enterococci could be a reservoir for antimicrobial resistance. Once the contaminated food is ingested, antimicrobial-resistant enterococci can multiply and colonize the human intestine. Especially linezolid is one of the last-resort antimicrobial agents available for treatment of serious infections caused by MDR Gram-positive bacteria. Therefore, active surveillance of antibiotic abuse and resistant

Table 3
Characteristics of linezolid-resistant *Enterococcus faecium* and *E. faecalis* isolates from duck fecal and carcass samples.[a]

Isolate	Farm ID	Slaughter-house	Sample	MIC (μg/ml)			Resistant gene			Mutation			Pulso-type	MLST	Self-transfer	Non-oxazolidinone and phenicol resistance	Transferred resistance
				LNZ	CHL	FFC	*optrA*	*fexA*	*cfr*	23S rRNA	*rplC*	*rplD*					
E. faecium																	
16-05-DF-44	A	a	Feces	8	32	32	+	+	–	WT	WT	N130K	I	8	+	CIP, ERY, TYL, TET, SYN	ERY, TYL, LNZ, CHL, FFC, SYN
16-05-DF-50	B	a	Feces	8	32	32	–	–	–	WT	WT	WT	V	120	+	CIP	LNZ, CHL, FFC
16-06-DF-29-1	C	b	Feces	8	> 32	> 32	+	+	–	WT	WT	WT	III	157	–	CIP, TYL, TET	
16-06-DF-31	C	b	Feces	8	16	16	–	–	–	WT	WT	N130K	II	14	+	TET	LNZ, FFC
16-05-DM-20	A	a	Carcass	8	32	32	+	+	–	WT	WT	N130K	I	8	–	CIP, ERY, TYL, TET, SYN	
16-04-DM-37	D	a	Carcass	8	32	32	+	+	–	WT	WT	N130K	VI	520	–	STR, CIP, ERY, TYL	
16-07-DM-1-1	E	b	Carcass	8	> 32	> 32	+	+	–	WT	WT	N130K	I	8	–	CIP, ERY, TYL	
16-07-DM-3	E	b	Carcass	16	> 32	> 32	+	+	–	WT	WT	N130K	VII	309	+	CIP	LNZ, CHL, FFC
16-09-DM-46-1	F	b	Carcass	8	32	> 32	–	–	–	WT	WT	WT	IV	7	+	CIP, TET	LNZ, CHL, FFC
E. faecalis																	
16-10-DM-EFC-47	G	c	Carcass	8	> 32	> 32	–	+	–	WT	WT	WT	ND	288	–	STR, CIP, TYL	

[a] Abbreviations: MLST, multi-locus sequence type; MIC, minimum inhibitory concentration; LNZ, linezolid; CHL, chloramphenicol; FFC, florfenicol; STR, streptomycin; CIP, ciprofloxacin; ERY, erythromycin; TYL, tylosin; TET, tetracycline; SYN, quinupristin/dalfopristin; WT, wild type; ND, not done; +, positive; and −, negative.

isolates of both human and food-animal origin is urgently required.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.01.002.

Abbreviations

LR	linezolid resistant
MALDI-TOF	matrix-assisted laser desorption ionization time-of-flight
MDR	multi-drug resistance
BHI	brain heart infusion
MLST	Multi-locus sequence typing
PFGE	Pulsed-field gel electrophoresis

Conflict of interest

None.

Acknowledgements

This study was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural affairs, Republic of Korea [N-1543081-2015-24-01].

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