Molecular characterization of antimicrobial resistance and virulence factors of *Enterococcus faecalis* from ducks at slaughterhouses

Jiakang Li,[*,†] Lei Yang,[*,†] Xuelin Huang,[*,†] Yiping Wen,[*,†] Qin Zhao,[*,†] Xiaobo Huang,[*,†] Jing Xia,[*,†] Yong Huang,[*,†] Sanjie Cao,[*,†] Senyan Du,[*,†] Rui Wu,[*,†] Likou Zou,[‡] Qigui Yan,[*,†] and Xinfeng Han[*,†,1]

[*]*College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, PR China;* [†]*Key Laboratory of Animal Diseases and Human Health of Sichuan Province, Chengdu 611130, PR China; and* [‡]*College of Resources, Sichuan Agricultural University, Chengdu 611130, PR China*

ABSTRACT This study investigated the prevalence of antimicrobial resistant *Enterococcus faecalis* (***E. faecalis***) from ducks at slaughterhouses, analyzed antimicrobial resistance genes and virulence-associated genes of the isolates. Multilocus sequence typing (**MLST**) was performed to characterize their molecular characteristics. A total of 227 *E. faecalis* isolates (67.8%) were obtained from cecum ($n = 114$), cloaca ($n = 50$), skin ($n = 59$), and rinsed water ($n = 4$). These *E. faecalis* exhibited high level of resistance against tetracycline (95.6%), doxycycline (94.3%), linezolid (75.8%), erythromycin (72.2%), followed by norfloxacin (56.8%), vancomycin (38.3%), penicillin (36.1%), teicoplanin (30.8%). Lower level of resistance was found to high-level streptomycin (19.8%), imipenem (15.9%) and high-level gentamicin (5.7%). The vast majority of isolates (90.3%) were multidrug resistant (**MDR**). Moreover, the commonly observed resistance genes were *optrA* (90.7%) and *ermB* (90.3%), followed by *aph(3')-III* (86.8%), *tetM* (84.6%), *acc(6')-aph(2)* (77.5%), *blaZ* (76.7%) and *aac(6')-Ie-aph(2")-Ia* (75.8%). The less frequently observed genes were *vanC* (19.8%), *blaTEM* (4.8%), *vanM* (2.6%), and *vanA* (0.4%). None of the strains carried *aph(2")-Ic* and *vanB* genes. Furthermore, a high prevalence of ten virulence determinants was identified, and *efaA* (99.1%) was predominant, followed by *eep* (97.4%), *srtA* (96.9%), *asa1* (95.6%), *fsrB* (92.1%), *sprE* (89.9%), *aggA* (63.9%), *gelE* (56.4%), *esp* (33.9%), and *cylL* (15.4%). Eleven isolates (4.9%) co-carried all of the tested virulence-associated genes. MLST analysis demonstrated that, *E. faecalis* isolates consisted of 12 known STs and 5 new STs, among which 6 of the identified STs were associated with nosocomial infection. Our data indicated that retail ducks serve as an important source of MDR *E. faecalis* with high pathogenicity potential, and suggested that transmission to humans could not be excluded.

Key words: *Enterococcus faecalis*, antimicrobial resistance, antimicrobial resistance genes, virulence-associated genes, MLST

2022 Poultry Science 101:101646
https://doi.org/10.1016/j.psj.2021.101646

INTRODUCTION

Enterococci are members of the intestinal microbiota of animals and humans, and have been widely regarded as an indicator for the detection of antimicrobial resistance of Gram-positive bacteria (Kim et al., 2019). Moreover, enterococci have also attracted a clinical concern because of their implication in a wide diversity of infections. *Enterococcus* species, especially *E. faecalis*, can cause severe infection when the immunity of human or other warm-blooded animals is low (Chong et al., 2017; Rashid et al., 2017; Gao et al., 2018). As a second reason for nosocomial infection following *Staphylococcus aureus, E. faecalis* can be isolated from human urinary tract infections, bacteremia, endocarditis, and burn wounds (Barros et al., 2019). It could also result in foodborne infection via food chain (Poulsen et al., 2012; Abat et al., 2016). Furthermore, *E. faecalis* has been linked to severe extraintestinal infections in poultry (Olsen et al., 2012). When it inadvertently enters circulation, it can cause endocarditis, as well as urinary (Abat et al., 2016), oviduct (Fang et al., 2021), intra-abdominal, and pelvic infections in poultry.

Antimicrobial resistance of *E. faecalis* is a major concern for public health worldwide. Due to its intrinsic and

Received August 4, 2021.
Accepted December 1, 2021.

[1]Corresponding author: hanxinf@163.com

acquired resistance, *E. faecalis* has rapidly developed resistance to multiple antimicrobials in the last several decades (Sirichoat et al., 2020). In addition, it possesses efficient gene transfer mechanisms, and is able to exchange genes of antibiotic resistance in different environments with a broad-range of bacteria (Lupo et al., 2012), including nonpathogenic species and life-threatening pathogens, which further increases the risk of enterococcal infections and hinders therapeutic options. Furthermore, *E. faecalis* may possess virulence factors that aid in colonization and pathogenesis (Igbinosa and Beshiru, 2019). Certain virulence traits can promote the dissemination of virulence and antimicrobial resistance within or outside hospital environments, especially through contaminated food (Aslam et al., 2012).

China is both the largest producer and the largest consumer of retail duck in the world. Over the past 20 yr, the growth rate of duck industry in China has even exceeded that of chicken industry. With the rapid growth of duck meat consumption, concerns about food safety have also been increased. Several studies have been performed in China on the prevalence and antimicrobial resistance of foodborne pathogens of duck origin, such as *Salmonella* and *Campylobacter* species (Han et al., 2019, 2020). However, the prevalence and antimicrobial resistance of *E. faecalis* in retail ducks has not been documented in China.

The aim of this study was to characterize the antimicrobial resistance phenotypes and genotypes of *E. faecalis* isolates from ducks at slaughter level in Chengdu of China. Moreover, the study explored the presence of their virulence determinants and the clonality of the isolates so as to evaluate their potential threat to public health.

MATERIALS AND METHODS

Sample Collection From Ducks and Slaughterhouses

A total of 335 samples were collected from 2 large-scaled retail duck slaughterhouses during 4 visits in Chengdu of China between October 2018 to March 2020, and birds at slaughter line were 6-wk-old Cherry Valley Pekin Ducks. For each visit, the sampling areas were chosen based on convenience of sampling and a distance no farther than 50 km, to ensure that the samples would arrive at the laboratory within 24 h after collection. Cecal samples ($n = 155$) were collected from ducks after evisceration, while duck cloaca samples ($n = 80$) and skin swabs ($n = 80$) were collected at the end of slaughtering. Rinsed water samples ($n = 20$) were collected from the slaughter line during processing. The cecal samples were placed into sterile containers. The swab samples were placed into 3 mL of sterile buffered peptone water (**BPW**; Hangzhou Microbial Reagent Co. Ltd, China) after swabbing of the cloaca or 25 cm^2 area of the skin (neck or back skin) with sterile cotton swabs. The rinsed water was contained in 25 mL sterile centrifuge tube. After collection, samples were shipped on ice within 24 h to the laboratory.

Isolation of E. faecalis Isolates

The cecal contents (1 g) were diluted with 10 mL of BPW to form a 10% homogenized solution, then streaked onto Bile Esculin Azide agar (**BA**; Qingdao Hi-Tech Industrial Park Hopebio Biological Technology Co., Ltd., China) and incubated at 37°C for 24 h. The swab samples were inoculated in tryptone soya broth (**TSB**; Qingdao Hopebio Biological Technology Co., Ltd, China) and incubated at 37°C for 20 h. Subsequently, they were subcultured onto BA and incubated at 37°C for 24 h. Water samples were firstly centrifuged at 10,000 r/min for 3 min at 4°C, then 4/5 of the supernatant was discarded and an aliquot of 1 mL of the remaining liquid was performed in accordance with the swab samples. Based on the characteristics of typical colonies (black-brown colonies) of *Enterococcus* spp., one or two black-brown colonies were randomly selected and subcultured on BA and incubated at 37°C for another 24 h. In each sample one suspected enterococci colony with black halo was selected randomly and subcultured into high-salt LB broth (6.5% NaCl) at 37°C for 24 h. The turbid LB broths were further purified on BA, and incubated at 37°C for 24 h. Finally, one colony with typical characteristics of enterococci from each sample was cultured in Brain Heart Infusion (**BHI**; Hangzhou Microbial Reagent Co. Ltd, China) broth, incubated at 37°C for 20 h. Then the bacterial suspension was mixed with a final concentration of 20% glycerol and stored at −70°C.

PCR Identification of E. faecalis Isolates

E. faecalis was confirmed at genus and species level with PCR using specific primer sets E1/E2 (Deasy et al., 2000) and FL1/FL2 (Jackson et al., 2004), respectively (Table S1 in Supplementary file). The reaction mixture included: DNA template (1 μL), forward and reverse primers (1 μL each, 10 μM), Premix Taq (12 μL, TaKaRa Taq Version 2.0 plus dye) and bacteria free water (10 μL). PCR was performed in a procedure as following, pre-denaturation at 95°C for 5 min, then 30 cycles of 94°C for 30 s, annealing for 60 s and 72°C for 60 s and a final extension at 72°C for 5 min. Amplicons were electrophoresed in 1% (w/v) agarose gel at 120 V for 20 min, stained with GoldView II Nuclear Staining Dyes (Solarbio Life Sciences, China) and visualized under UV-light. For all reactions, sterile ultrapure water was used as the negative control, and the *E. faecalis* strain ATCC 29213 was used as a positive control.

Antimicrobial Susceptibility Testing of E. faecalis

According to "2019 Animal-Based Bacterial Resistance Surveillance Plan" issued by the Ministry of Agriculture and Rural Affairs of China, a total of 11 antimicrobial agents, including tetracycline (**TE**, 30 μg/disk), doxycycline (**DO**, 30 μg/disk), erythromycin

(**E**, 15 μg/disk), norfloxacin (**NOR**, 10 μg/disk), penicillin (**P**, 10 IU/disk), high-level streptomycin (**S300**, 300 μg/disk), high-level gentamicin (**GM120**, 120 μg/disk), teicoplanin (**TCL**, 30 μg/disk), vancomycin (**VA**, 30 μg/disk), imipenem (**IPM**, 10 μg/disk), and linezolid (**LZD**, 30 μg/disk), were involved in the surveillance for the antibiotic resistance of *E. faecalis* in this study. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method. The testing broth was prepared after culturing at 37°C for 6 h, then the bacterial turbidity degree was regulated to 0.5 McFarland units with bacterial turbidity meter (WGZ-XT; Hangzhou Qiwei Instrument Co., Ltd., China). Subsequently, the tested bacteria were scribbled uniformly on Mueller-Hinton (**MH**) agar and no more than 5 kinds of paper disc were evenly affixed to the center and surrounding of the plate aseptically. After conventional culture at 37°C for 24 h, the diameters of the inhibition zones were measured. According to the standards of Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing, the susceptibility testing results were determined accordingly (Table S2 in Supplementary file). *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were set as quality control. The isolates resistant to 3 or more classes of antimicrobial agents were defined as multidrug-resistant (**MDR**) (Magiorakos et al., 2012).

Detection of Antimicrobial Resistance Genes in E. faecalis *Isolates*

The presence of each resistance gene encoding the resistance phenotypes of 11 tested antimicrobial agents, including *tetM* (Aarestrup, et al., 2000), *ermB, bla*TEM, *blaZ, aac(6')-Ie-aph(2")-Ia* and *aph(2")-Ic* (Donabedian et al., 2003), *acc(6')-aph(2)* (Vliegenthart et al., 1990), *aph(3')-III, vanA, vanB, vanC, vanM* and *optrA* (Wang et al., 2015) was investigated by PCR (Table S3 in Supplementary file). Detection was performed with a final volume of 25 μL, containing 400 nM of each primer, 12 μL Premix Taq (TaKaRa *Taq* Version 2.0 plus dye), 1 μL of DNA template, and added bacteria free water to 25 μL. PCR conditions were the same except annealing temperature, which consisted of a pre-denaturation step at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing temperature for 1 min, and 72°C for 1 min. A final extension step was performed 72°C for 5 min. PCR products were analyzed on 1% (w/v) agarose gels. DNA bands were visualized by staining with GoldView II Nuclear Staining Dyes (Solarbio Life Sciences) and photographed under UV illumination.

Detection of Virulence Determinants of E. faecalis

E. faecalis isolates from ducks were screened by PCR for the presence of virulence determinants (*eep, efaA, gelE, sprE, eep, asa1, aggA, srtA, fsrB* and *cylL*), which play vital roles in pathogenicity (Table S4 in Supplementary file) (Shankar et al., 1999; Eaton and Gasson, 2001; Bittencourt de Marques and Suzart, 2004; Creti et al., 2004). PCR mixture for each specific gene contained 1 μL of 10 pmol of each primer, 12 μL of Premix Taq (TaKaRa *Taq* Version 2.0 plus dye), 1 μL DNA template, and 10 μL bacteria free water. PCR conditions were the same except annealing temperatures. After pre-denaturation at 95°C for 5 min, PCR was conducted for 30 cycles of denaturation (94°C for 1 min), annealing (annealing temperature for 1 min), and extension (72°C for 1 min), followed by one cycle of 10 min at 72°C. Lastly, 5 μL of the PCR products were electrophoresed in 1% (w/v) agarose gel, stained with 0.5 μL/mL GoldView II Nuclear Staining Dyes (Solarbio Life Sciences) and then visualized under ultraviolet light.

Molecular Typing of E. faecalis *Isolates by Multilocus Sequence Typing*

E. faecalis isolates with higher carriage frequency of virulence and antimicrobial resistance genes were further analyzed by Multilocus sequence typing (**MLST**) (Ruiz-Garbajosa et al., 2006; Jolley et al., 2018). Seven house-keeping genes (*gdh, gyd, pstS, gki, aroE, xpt, yqiL*) were amplified by PCR, and then sequenced at TSINGKE Biological Technology (Chengdu, China). The sequence type (**ST**) of *E. faecalis* was obtained by comparing the sequences in the MLST database (https://pubmlst.org/bigsdb?db=pubmlst_efaecalis_seqdef&page=profiles&scheme_id=1). All STs were investigated by goeBURST (http://www.phyloviz.net/goeburst/) to get the smallest clone cluster, and the phylogenetic relationship was analyzed by GrapeTree (https://pubmlst.org/bigsdb?db=pubmlst_efaecalis_isolates&page=plugin&name=GrapeTree). The new STs were analyzed by BioNumerics 7.6 software (Applied Maths, Belgium), then uploaded to website (https://pubmlst.org/bigsdb?db=pubmlst_efaecalis_seqdef&page=submit) to assign its own unique ST number.

Data Analysis

The relationships of categorical variables were examined Chi-Square test. Pearson, Continuity Correction, and Fisher- Freeman-Halton Test were also used. The antimicrobial resistance, antimicrobial resistance gene distribution, and virulence-associated genes were graphically analyzed with Microsoft Office Excel 2019 and SPSS Statistics v22.0.0.0.

RESULTS

Occurrence of E. faecalis *From Duck Slaughterhouses and Ducks*

As a result, 227 *E. faecalis* isolates were obtained from cecum (**C.CZ** and **C.MY**, $n = 114$, 73.5%), cloaca (**Cl** and **Cl.CZ**, $n = 50$, 62.5%), skin (**Ca** and **Ca.CZ**,

$n = 59$, 73.8%), and rinsed water (**W**, $n = 4$, 20.0%), which were confirmed by culture methods and PCR. Overall, the total isolation rate of *E. faecalis* was 67.8% (95% confidence interval [**CI**], 35.5−79.5%). A statistically significant ($P < 0.05$) association between *E. faecalis* contamination and sample types was found ($\chi^2 = 4.388$).

Antimicrobial Resistance Profiles of E. faecalis From Ducks at Slaughterhouses

The antimicrobial susceptibility of the isolates to 11 antimicrobial agents showed that, a high level of resistance was observed against tetracycline (95.6%), doxycycline (94.3%), linezolid (75.8%), erythromycin (72.2%), and norfloxacin (56.8%), followed by vancomycin (38.3%), penicillin (36.1%), teicoplanin (30.8%). In contrast, lower level of resistance was found to high-level streptomycin (19.8%), imipenem (15.9%), and high-level gentamicin (5.7%) (Table 1). Besides, the proportion of Antimicrobial-Intermediate *E. faecalis* among various antimicrobials was shown in Table S5 in Supplementary file. In this study, statistically significant differences were found in same antimicrobial classes between different drugs in terms of resistance except for linezolid from oxazolidinones, erythromycin from macrolides, norfloxacin from quinolone, penicillin from β-lactamase and imipenem carbapenems ($P < 0.05$). Overall, *E. faecalis* isolates from cecum exhibited higher resistance rates than those from other sources. Nearly all of the isolates (98.7%) were resistant to at least one antimicrobial agent, and most of them (90.3%) were MDR. Statistically significant differences ($P < 0.01$) were also found between MDR strains and non-MDR strains ($\chi^2 = 23.275$).

Totally, 61 resistance profiles were found in this study (Table S6 in Supplementary file), among which the dominant resistance profile was P-TE-LZD-VA-TCL-DO-NOR (9.69%), followed by E-TE-LZD-DO (7.49%), E-TE-LZD-DO-NOR (7.05%) and E-TE-DO-NOR (6.17%). Among the isolates, 5 strains (2.20%) were resistant to most of the tested antimicrobial agents ($n = 9$) with resistance profile as P-E-TE-LZD-IPM-VA-TCL-DO-NOR.

Distribution of Antimicrobial Resistance Genes of E. faecalis From Ducks at Slaughterhouses

Except resistance genes of *aph(2")-Ic* and *vanB*, all the other genes were present in *E. faecalis* isolates in this study (Figure 1). The occurrence of *optrA* (90.7%) and *ermB* (90.3%) were the most prevalent, followed by *tetM* (84.6%). Moreover, *aph(3')-III* (86.8%), *acc(6')-aph(2)* (77.5%) and *aac(6')-Ie-aph(2")-Ia* (75.8%) encoding aminoglycoside resistance were frequently observed. It was noteworthy that the prevalence of both *blaZ* (76.7%) and *bla*TEM (4.8%) encoding resistance to β-lactamase was quite different. In contrast, the incidence of glycopeptide resistance genes of *vanC* (19.8%), *vanM* (2.6%), and *vanA* (0.4%) showed a lower level.

A total of 88 antimicrobial resistance gene profiles were observed, and 167 isolates (73.6%) harbored 6 or more kinds of tested resistance genes (Table S7 in Supplementary file). The dominant profile was "*aac(6')-Ie-aph(2")-Ia, acc(6')-aph(2), aph(3')-III, blaZ, ermB, tetM, optrA*" (15.42%), followed by "*acc(6')-Ie-aph(2")-Ia, acc(6')-aph(2), aph(3')-III, blaZ, ermB, tetM, optrA*"(4.85%), "*aac(6')-Ie-aph(2")-Ia, acc(6')-aph(2), aph(3')-III, blaZ, ermB, tetM, vanC, optrA*" (3.96%) and "*aac(6')-Ie-aph(2")-Ia, acc(6')-aph(2), aph(3')-III, ermB, tetM, vanC, optrA*" (3.96%). Among the isolates, one isolate co-harbored nine resistance genes with its profile as "*aac(6')-Ie-aph(2")-Ia, acc(6')-aph(2), aph(3')-III, blaZ, ermB, blaTEM, tetM, vanC*, and *optrA*" (0.44%).

Presence of Virulence-Associated Genes in E. faecalis Isolates From Ducks at Slaughterhouses

The carriage and distribution of virulence-associated genes were commonly observed among the isolates. In total, 226 *E. faecalis* isolates (99.6%) carried multiple virulence-associated genes. The occurrence of *efaA* was observed with the highest frequency (99.1%) among the isolates, followed by *eep* (97.4%), *srtA* (96.9%), *asa1* (95.6%), *fsrB* (92.1%), *sprE* (89.9%), *aggA* (63.9%), *gelE* (56.4%). However, *esp* (33.9%) and *cylL* (15.4%)

Table 1. Antimicrobial resistance of *E. faecalis* isolates from ducks at slaughterhouses.

Antimicrobial agent		*E. faecalis*					χ^2 (v)	P
		Cecum ($n^a = 114$)	Cloaca ($n = 50$)	Skin ($n = 59$)	Water ($n = 4$)	Total ($n = 227$)		
Tetracyclines	Tetracycline	114 (100%)	47 (94.0%)	52 (88.1%)	4 (100%)	217 (95.6%)	365.756	< 0.01
	Doxycycline	113 (99.1%)	49 (98.0%)	49 (83.1%)	3 (75.0%)	214 (94.3%)		
Oxazolidinones	Linezolid	110 (96.5%)	26 (52.0%)	35 (59.3%)	1 (25.0%)	172 (75.8%)		
Macrolides	Erythromycin	70 (61.4%)	42 (84.0%)	49 (83.1%)	3 (75.0%)	164 (72.2%)		
Quinolone	Norfloxacin	81 (71.1%)	25 (50.0%)	21 (35.6%)	2 (50.0%)	129 (56.8%)		
β-lactamase	Penicillin	74 (64.9%)	4 (8.0%)	3 (5.1%)	1 (25.0%)	82 (36.1%)		
Glycopeptides	Vancomycin	71 (62.3%)	4 (8.0%)	12 (20.3%)	0	87 (38.3%)	95.229	< 0.01
	Teicoplanin	68 (59.6%)	1 (2.0%)	1 (2.0%)	0	70 (30.8%)		
Carbapenems	Imipenem	33 (28.9%)	1 (2.0%)	2 (3.4%)	0	36 (15.9%)		
Aminoglycosides	High-level streptomycin	14 (23.3%)	17 (39.1%)	12 (20.3%)	2 (50.0%)	45 (19.8%)	9.519	< 0.05
	High-level gentamicin	5 (4.4%)	5 (10.0%)	3 (5.1%)	0	13 (5.7%)		

[a]Numbers of *E. faecalis* isolates tested.

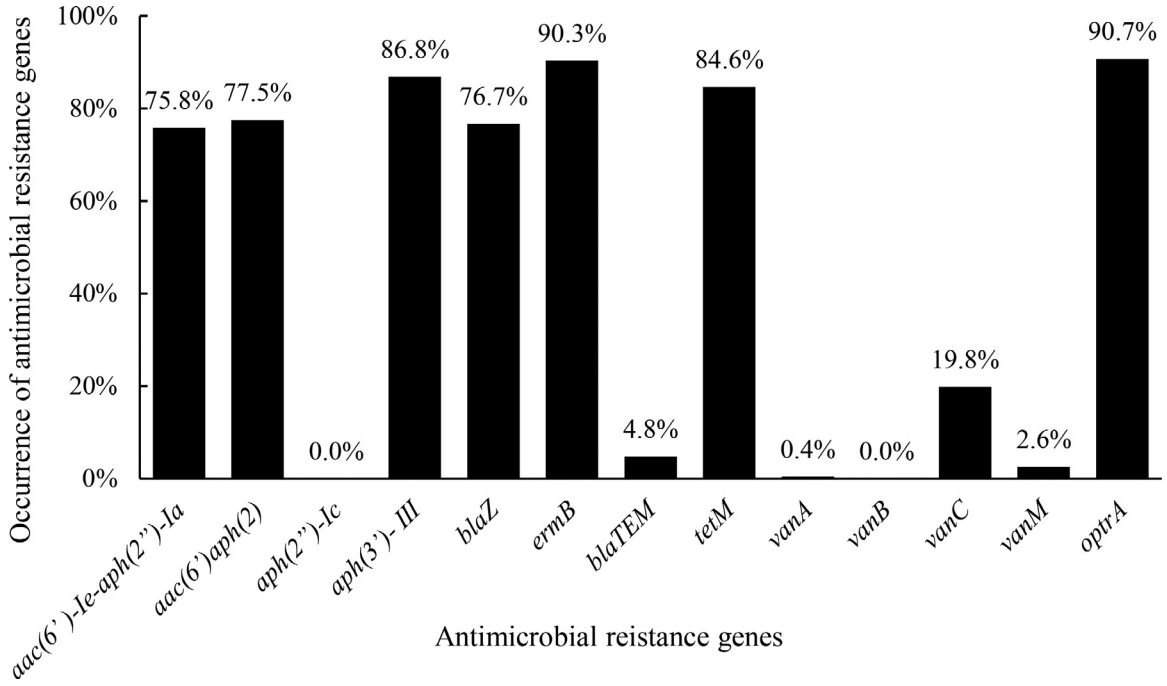

Figure 1. Occurrence of antimicrobial resistance genes of *E. faecalis* isolates ($n = 227$) from ducks and slaughterhouses.

were less frequently detected in the present study (Figure 2).

In addition, 40 virulence-associated genes profiles were observed in this study. All the isolates except one strain (99.6%) co-carried 2 or more of the virulence-associated genes, and the majority (79.3%) co-harbored 7 or more virulence determinants. Notably, 11 strains (4.9%) carried all tested virulence-associated genes in this study (Table S8 in Supplementary file). The dominant virulence-associated genes profile was *aggA-asa1-eep-efaA-fsrB-gelE-sprE-srtA* (19.38%), followed by *aggA-asa1-eep-efaA-esp-fsrB-sprE-srtA* (11.45%), *asa1-eep-efaA-fsrB-gelE-sprE-srtA* (9.69%) and *aggA-asa1-eep-efaA-fsrB-sprE-srtA* (9.25%).

Molecular Typing of E. faecalis *Isolates From Ducks at Slaughterhouses*

According to the evaluation of antimicrobial resistance and pathogenesis potential, 42 MDR isolates were examined by MLST. They were distributed into 17 sequence types (STs), of which 5 were novel types (ST1073, ST1074, ST1075, ST1076 and ST1077)

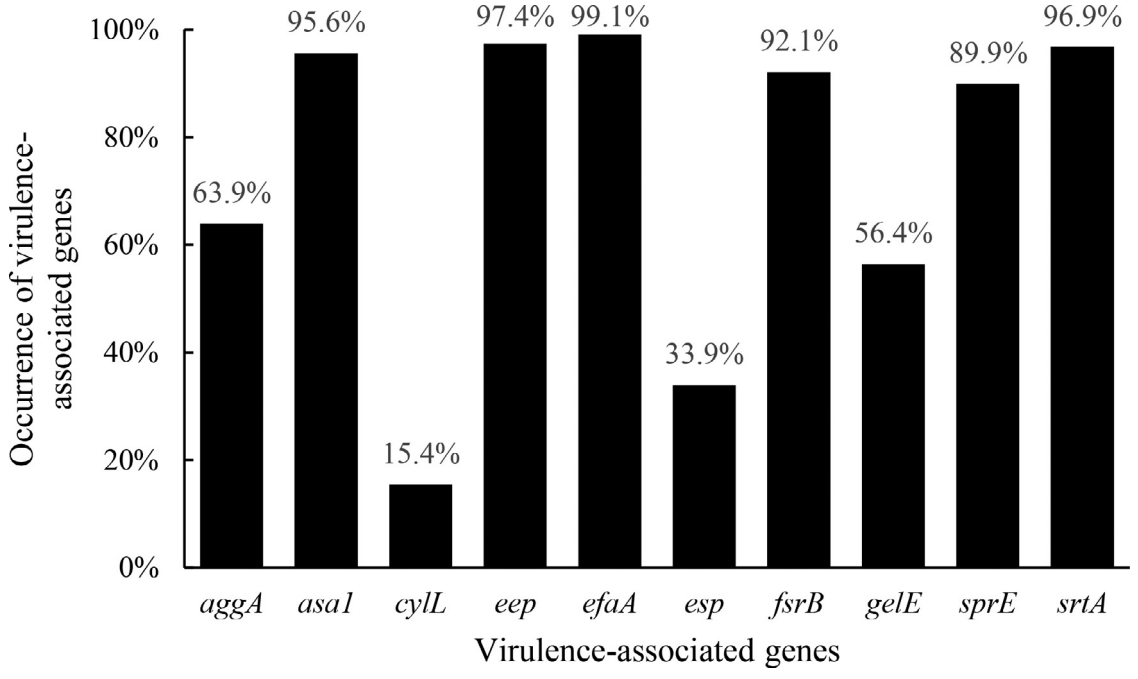

Figure 2. Occurrence of virulence determinants of *E. faecalis* ($n = 227$) isolates from ducks and slaughterhouses.

Table 2. Multilocus sequence typing (MLST), antimicrobial resistance genotype, virulence determinant profile of selected *E. faecalis* isolates from ducks at slaughterhouses.

Strains	ST	MLST allele							CC[a]	Source[b]	No. of AMR[c]	Profile of AMR genes[d]	Profile of virulence genes[e]
		gdh	*gyd*	*pstS*	*gki*	*aroE*	*xpt*	*yqiL*					
C.CZ40	93	25	2	15	7	23	18	26	93	cecum	7	80	35
Ca.CZ14	116	17	2	22	8	14	14	1	476	skin	8	74	23
C.CZ3	170	12	6	28	8	7	1	20	82	cecum	8	39	23
C.MY4	170	12	6	28	8	7	1	20	82	cecum	8	88	31
C.MY19	170	12	6	28	8	7	1	20	82	cecum	8	58	31
C.MY47	170	12	6	28	8	7	1	20	82	cecum	8	78	31
C.MY6	170	12	6	28	7	7	1	20	82	cecum	7	58	31
C.MY11	170	12	6	28	7	7	1	20	82	cecum	7	78	31
C.MY25	170	12	6	28	7	7	1	20	82	cecum	7	78	31
C.MY31	170	12	6	28	7	7	1	20	82	cecum	7	55	31
C.MY37	170	12	6	28	7	7	1	20	82	cecum	7	78	31
C.MY68	170	12	6	28	7	7	1	20	82	cecum	7	78	31
C.CZ33	192	42	1	43	8	39	4	41	192	cecum	8	80	39
C.CZ22	192	42	1	43	7	39	4	41	192	cecum	7	87	35
C.CZ23	192	42	1	43	7	39	4	41	192	cecum	7	65	40
C.MY72	249	15	1	18	7	3	35	53	249	cecum	7	78	31
Cl.CZ24	256	4	6	7	7	8	1	20	256	cloaca	7	82	29
C.MY63	314	9	6	4	8	11	15	61	314	cecum	8	78	31
C.MY80	314	9	6	4	8	11	15	61	314	cecum	8	54	31
C.MY9	314	9	6	4	7	11	15	61	314	cecum	7	78	25
C.MY26	314	9	6	4	7	11	15	61	314	cecum	7	78	31
C.MY38	314	9	6	4	7	11	15	61	314	cecum	7	78	31
C.MY79	314	9	6	4	7	11	15	61	314	cecum	7	78	31
C.CZ21	334	8	1	7	7	4	4	1	4	cecum	7	81	40
C.MY64	593	14	2	17	9	3	3	17	593	cecum	9	78	31
C.MY70	593	14	2	17	9	3	3	17	593	cecum	9	78	31
C.MY7	593	14	2	17	8	3	3	17	593	cecum	8	78	28
C.MY24	593	14	2	17	7	3	3	17	593	cecum	7	78	28
C.MY41	593	14	2	17	7	3	3	17	593	cecum	7	80	31
C.MY62	593	14	2	17	7	3	3	17	593	cecum	7	74	31
C.MY74	593	14	2	17	8	3	3	17	593	cecum	8	39	28
C.MY18	706	64	1	36	9	92	25	30	706	cecum	9	78	28
C.MY5	903	98	2	93	7	3	17	27	903	cecum	7	78	31
C.MY22	903	98	2	93	8	3	17	27	903	cecum	8	78	28
C.MY39	903	98	2	93	7	3	17	27	903	cecum	7	78	31
C.MY44	903	98	2	93	8	3	17	27	903	cecum	8	88	31
C.MY23	1,009	12	6	28	8	106	1	20	82	cecum	8	79	28
C.CZ6	1,073	92	11	43	8	39	1	41	1073	cecum	8	88	34
C.CZ28	1,074	5	1	56	7	7	7	6	1074	cecum	7	79	40
C.MY14	1,075	92	11	6	8	11	32	2	1075	cecum	8	55	28
Ca.CZ22	1,076	20	1	1	7	6	1	5	1076	skin	7	49	23
Ca.CZ32	1,077	5	1	14	7	7	7	6	1077	skin	7	80	29

[a]CC, clonal complex (analyzed by goeBURST).
[b]Source: Samples from ducks at slaughterhouses.
[c]AMR, antimicrobial resistance.
[d]Profile of AMR genes: Detailed information was shown in Table S7 in Supplementary file.
[e]Profile of virulence genes: Detailed information was shown in Table S8 in Supplementary file.

(Table S9 in Supplementary file). The dominant types were ST170 (10 isolates), followed by ST593 (7 isolates), ST314 (6 isolates), ST903 (4 isolates), and ST192 (2 isolates). However, other 12 STs were represented by a single isolate (ST93, ST116, ST249, ST256, ST334, ST706, ST1009 and 5 novel STs) (Table 2).

The results revealed 16 clonal complexes (**CC**s) by the clustering of goeBURST, which were CC4 (ST334), CC82 (ST170 and ST1009), CC93 (ST93), CC246 (ST249), CC256 (ST256), CC314 (ST314), CC476 (ST116), CC706 (ST706), and 8 singletons (ST192, ST593, ST903, ST1073, ST1074, ST1075, ST1076 and ST1077) (Figure 3). ST170 represented a single-locus variant (**SLV**) of ST 82 (from the hospitalized patient in Poland) and ST1009 (ST170 is his group founder) belonging to the CC82. The isolate of C.CZ40 (ST93) belonged to CC93 that has another 4 STs, including ST75 (form the hospitalized patient in Portugal), ST657 (form the hospitalized patient in China), ST226 (from the chicken meat), and ST98. ST116, which was detected from duck skin in 2018 of this study, was a SLV of ST45 that was isolated from hospitalized patients in Spain. In addition, ST192, ST256, ST314, and ST334 were previously isolated from the hospitalized patient.

By the analysis of GrapeTree (Zhou et al., 2018), 42 *E. faecalis* isolates clustered together within the strains "C.CZ33, C.CZ22, and C.CZ23" (Figure 4), which indicated that the isolates were mainly evolved from id663 (ST192). As ST192 was primarily isolated from urine of hospitalized patient, *E. faecalis* isolates of duck origin in this study had a close relation with nosocomial strains which might pose a great challenge to public health.

In addition, the results indicated that *E. faecalis* isolates of duck origin with new STs were mainly evolved

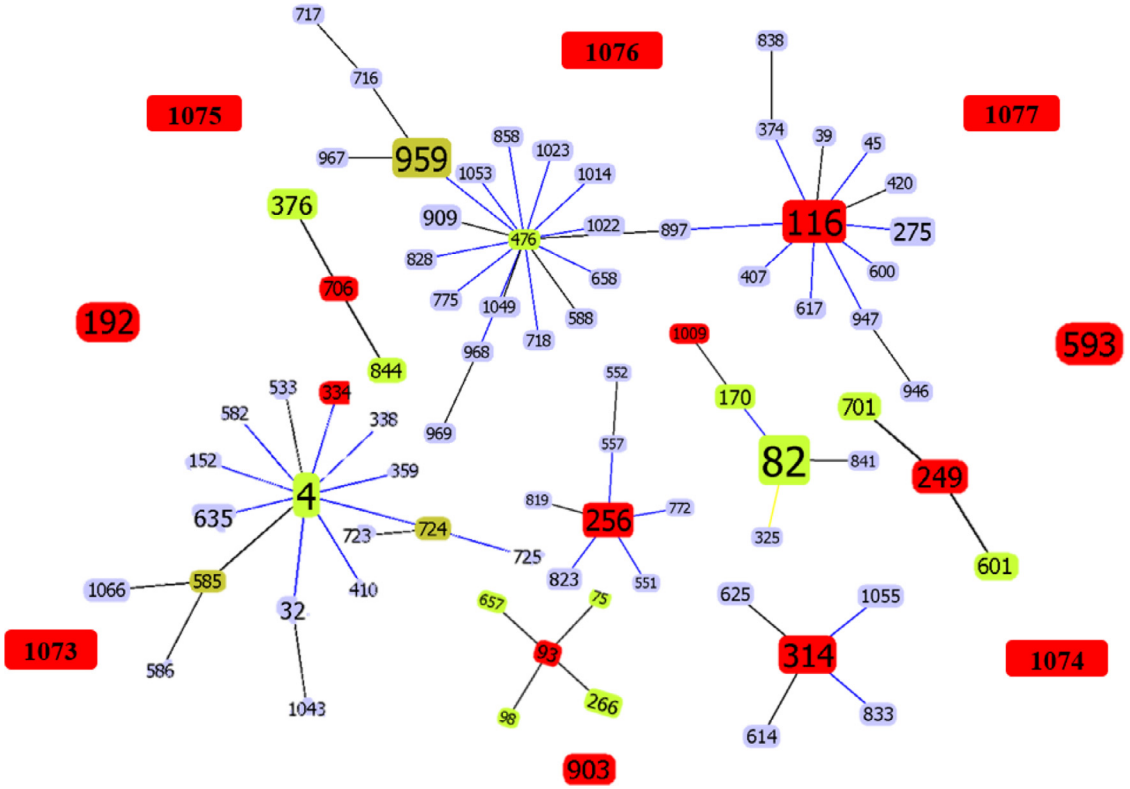

Figure 3. Smallest clonal cluster of 17 STs analyzed by goeBURST. Link colors: Blue - Link drawn using tiebreak rule 1 (number of SLVs); Green - Link drawn using tiebreak rule 2 (number of DLVs); Yellow - Link drawn using tiebreak rule 4 or 5 (Frequency found on the data set and ST number, respectively); Gray - Links drawn at DLV (darker gray) or TLV (lighter gray) if the groups are constructed at DLV/TLV level. ST nodes colors: All items marked in red were the STs confirmed in this study (Do not include ST170); Light green - Group founder; Dark green - Sub-group founder; Light blue - Common node.

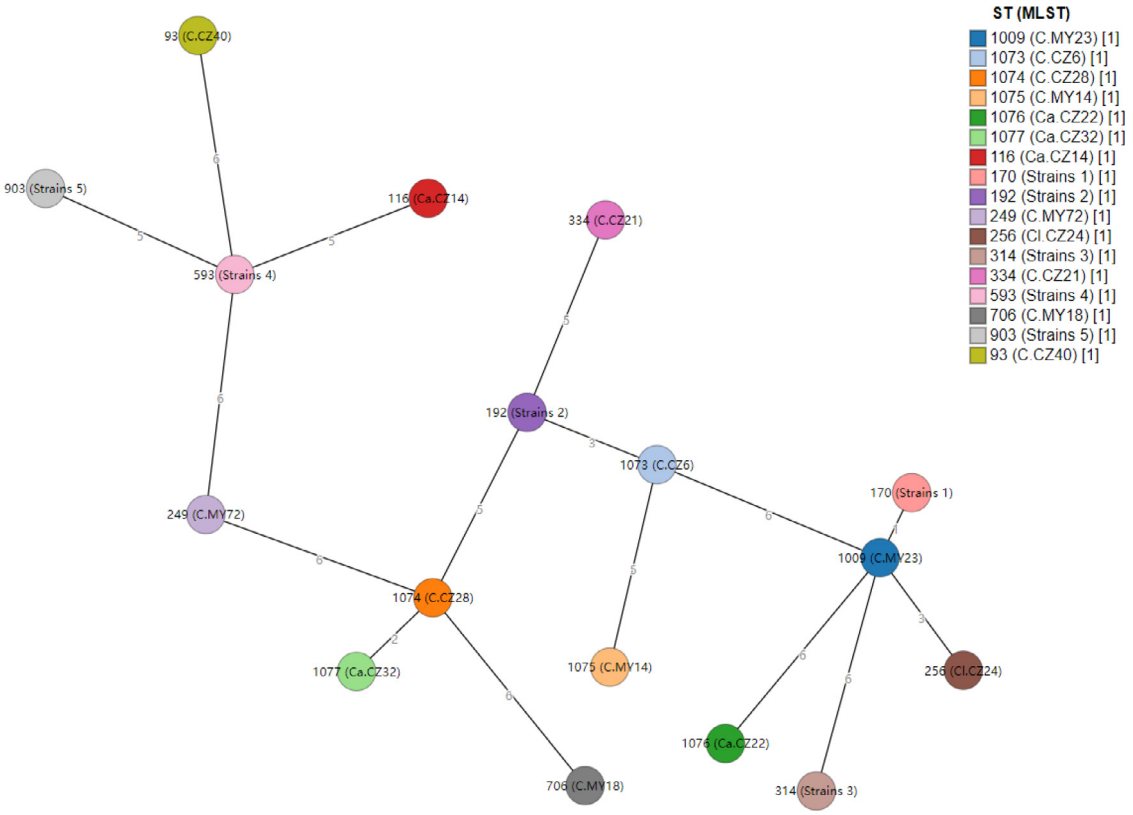

Figure 4. Phylogenetic relationships of *E. faecalis* isolates with 17 ids (Quick positioning number by pubMLST) originating from different geographical regions by GrapeTree. [a] ST (MLST): The STs of 42 strains by MLST. [b] Note: The content in the brackets after each ST is the strains that belong to the ST. [c] Strains 1: C.CZ3, C.MY4, C.MY19, C.MY47, C.MY6, C.MY11, C.MY25, C.MY31, C.MY37 and C.MY68; Strains 2: C.CZ33, C.CZ22 and C. CZ23; Strains 3: C.MY63, C.MY80, C.MY9, C.MY26, C.MY38 and C.MY79; Strains 4: C.MY64, C.MY70, C.MY7, C.MY24, C.MY41, C.MY62 and C. MY74; Strains 5: C.MY5, C.MY22, C.MY39 and C.MY44. [d] The numbers on the evolutionary branches represented evolutionary algebra.

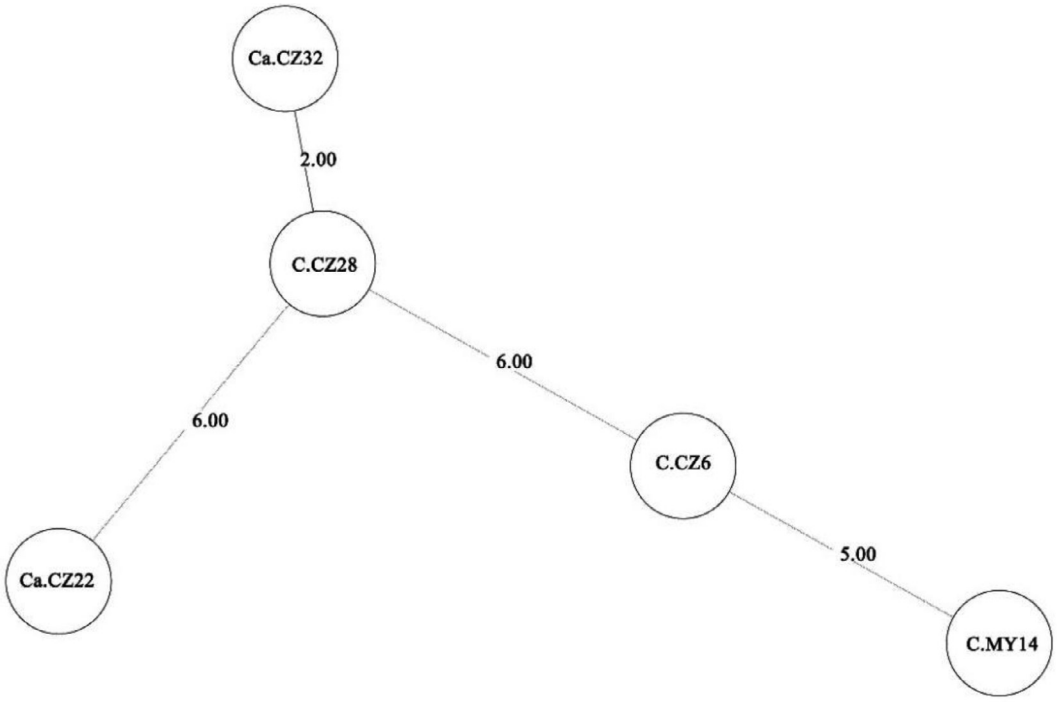

Figure 5. Genetic relationship of *E. faecalis* isolates with novel STs from ducks at slaughterhouses by BioNumerics 7.6 analysis. The numbers on the evolutionary branch represented evolutionary algebra.

from C.CZ28 (ST1074, id: 2048) by BioNumerics 7.6. Except Ca.CZ32 (ST1077, id: 2051) which was a triple-locus variant (**TLV**) of C.CZ28, other strains had a relatively distant relationship with C.CZ28 (Figure 5).

DISCUSSION

E. faecalis can cause a wide range of nosocomial infection such as bacteremia and urinary tract infection (Guzman Prieto et al., 2016; Diekema et al., 2019). Moreover, previous studies had revealed close epidemiological links between *E. faecalis* clones isolated from food-producing animals and human *E. faecalis* urinary-tract infections (Abat et al., 2016). As one of the primary meats consumed by Asians, duck meat may also act as an important source for transmission of *E. faecalis*, which could cause infection in immunocompromised people. Due to antibiotics abuse and its inherent resistance, *E. faecalis* is prone to show resistance against a variety of antimicrobial agents, resulting in difficulties for clinically accurate medications. At present, the clinical treatment of MDR *E. faecalis* infection is mainly dependent on penicillin, vancomycin, and new antimicrobial agent like linezolid (Cavaco et al., 2017). Hence, the monitoring and surveillance of antimicrobial resistance of *E. faecalis* are of particular importance to public health.

In this study, 11 antimicrobial agents that commonly used in clinical practice of humans or animal husbandry of *E. faecalis* infection were applied for antimicrobial susceptibility testing. The vast majority of the isolates were resistant to tetracycline (95.6%) and doxycycline (94.3%), and the majority were resistant to erythromycin (72.2%) and norfloxacin (56.8%), which were in accordance with previous studies (Anderson et al., 2018; Farman et al., 2019). The results indicated that traditional antimicrobial agents may have no effect or low effect on the infection caused by these *E. faecalis* isolates. Particularly, the resistance to linezolid (75.8%) and vancomycin (38.3%) in the study were much higher than that of *E. faecalis* isolates from duck feces and skin at four slaughterhouses in the southern part of Korea (11 and 0%, respectively) (Na et al., 2019). In addition, the resistance to linezolid of *E. faecalis* isolated from human urine samples in Egypt is lower than our results, although the isolation rate of Vancomycin-Resistant *Enterococcus* (**VRE**) from hospital is higher (Osman et al., 2020). For immunocompromised patient, the risk of VRE-related bacteremia and other infections is significantly increased. VRE from hospitalized patient infections when compared with vancomycin-susceptible strains have been associated with a 2-fold increase in morbidity and mortality (Reyes et al., 2016). Linezolid can be used for treatment of VRE infection (Hasman et al., 2019). However, when the frequency of Linezolid-Resistant *Enterococcus* (**LRE**) from hospitalized patient gradually increases, linezolid is less effective in treating VRE and LRE complications. Thus, the infection caused by duck-originated *E. faecalis* may be admitted to hospitals, resulting in more serious problems in treatment. On the other hand, the results indicated that *E. faecalis* isolates from ducks were less resistant to high-level streptomycin (19.8%), followed by imipenem (15.9%) and high-level gentamicin (5.7%), which may be applied in the clinical treatment. Athough a high level of resistance to erythromycin, that is widely used in livestock and poultry farming, was found in *E. faecalis* isolates from meat and pigeons (Cordero et al., 2019), it

was lower than the resistance level of a novel antimicrobial agent of linezolid, which was consistent with previous studies of *E. faecalis* isolated from chicken (Kim and Woo, 2017; O'Dea et al., 2019). Furthermore, over half of *E. faecalis* isolates (63.9%) were susceptible to penicillin, thus penicillin may still have an ideal therapeutic effect on foodborne infection of *E. faecalis* (Olawale et al., 2015).

We analyzed 13 kinds of antimicrobial resistance genes that commonly confer antimicrobial resistance of *E. faecalis*. Among 87 *E. faecalis* isolates that were resistant to vancomycin, only one isolate (C.CZ16) harbored high level of vancomycin resistance genes (*vanA* and *vanB*). A low proportion of them (12.6%) carried *vanC*, which is a natural low-level vancomycin resistance gene. In addition, a tiny minority (6.9%) carried *vanM*, which could regulate the resistance to teicoplanin. However, the prevalence of teicoplanin-resistant *E. faecalis* isolates was 30.8% in this study. It was speculated that these strains were regulated by several other high-level vancomycin resistance genes, such as *vanD, vanE* or *vanG* (Fines et al., 1999; Ostrowsky et al., 1999; Boyd et al., 2006). It is noteworthy that the prevalence of aminoglycoside resistance genes was very high except *aph(2")-Ic*, which was in line with other study performed in hospital (Chow, 2000). Furthermore, the results indicated that the isolates were more susceptible to high-level streptomycin and high-level gentamicin, and it was assumed that associated resistance genes were not expressed in the majority of the isolates. Overall, *vanB* and *aph(2")-Ic* genes were not observed in the present study, which may be due to geographical factors and sampling numbers.

As for the resistance genes of β-lactam, it was found that only 4.8% of the isolates were positive for *blaTEM* gene, which was significantly different from that of nosocomial *E. faecalis* from various clinical specimens in previous study (Jia et al., 2014). Nevertheless, the occurrence of *blaZ* gene was as high as 76.7%, which was higher than that of *E. faecalis* from milk, despite their positive rates of higher than 50% (Resende et al., 2018). Considering the rate of resistance to penicillin was 36.1%, it could be concluded that the resistance of penicillin might be mainly regulated by *blaZ* gene in this study. In summary, both animal and nosocomial origin of *E. faecalis* have broad resistance to β-lactam antibiotics. However, their regulatory genes may be different. Further, as the great majority of the isolates were resistant to linezolid in this study, we tested the newly discovered resistance gene of *optrA* and found that its prevalence was as high as 90.7%, which was coincided with the phenotypic results. Although *poxtA* gene also regulates the resistance to linezolid (Elghaieb et al., 2019), it was suggested that *optrA* gene may play an essential role in this study.

The virulence-associated genes were commonly observed in *E. faecalis* isolates from ducks in the present study. In particular, 99.1% of the isolates harbored endocarditis antigen-encoding gene (***efaA***) and the prevalence was in harmony with previous studies (Valenzuela et al., 2010; Aslam et al., 2012), which was associated with infective endocarditis. In addition, 97.4% of the isolates carried pheromone-expressing gene (*eep*), which has the ability to induce genes expression aggregates, promote plasmid binding and adhesion (Clewell, 1993), and the prevalence was higher than previous study (Hasan et al., 2018). Since binding of plasmids is related to the parallel transfer of genes, it is speculated that there might be a high frequency of gene transfer in *E. faecalis* from ducks in this study. It should be pointed out that, the positive rates of cell hemolysin-encoding gene (*cylL*) and enterococci surface protein-coding gene (*esp*) were less than 50%, which was in accordance with previous reports of *E. faecalis* isolated from retail meat (Aslam et al., 2012; Silvetti et al., 2019). Cell hemolysin can cause red blood cell lysis and toxic effects on cells such as neutrophils, platelets and sperm, while *cylL* gene encodes the core fragment of hemolysin that becomes the executor of cytotoxic function. The prevalence of *esp* gene in *Enterococcus* spp. with biofilm formation ability is relatively high, which is associated with colonization of host cells by colonization, adhesion, host immune escape and vancomycin resistance (Willems et al., 2001; Faille et al., 2014). The lower occurrence of both virulence-associated genes in this study might indicate that they could not cause serious blood diseases. From the molecular analysis, plasmid transfer by virulence gene regulation may carry resistance genes, resulting in the parallel transfer of resistance genes (Paoletti et al., 2007). In this study, 162 isolates (71.4%) carrying at least 6 virulence-associated genes simultaneously harbored at least 6 antimicrobial resistance genes. When bacteria virulence and high antimicrobial resistance coexist, clinical treatment will be challenged, seriously threatening life and endangering public health.

In this study, 57.1% of representative *E. faecalis* isolates in this study was closely correlated with hospitalized patients. It was revealed by MLST that ST170 was dominant in this study, which was correlated with isolates from chicken joint cavity of amyloid arthritis chickens (Petersen et al., 2009). Thus, it could be inferred that *E. faecalis* from ducks could not only cause serious diseases in poultry, but also has the ability to spread to humans via food chain (ST170 is a SLV of ST82). Besides, ST1009, a ST170 clone of Portuguese vegetable surface origin, were MDR and co-carried several virulence-associated genes and drug resistance genes, which indicated that its pathogenicity and the difficulty in treatment may be further increased. In addition, except ST903 which was first discovered in Sichuan Province of China, the remaining 11 STs were mainly clones that originated from hospital, husbandry, and foods of other countries. Importantly, 4 STs (ST192, ST256, ST314 and ST334) were clonal strains of *E. faecalis* from hospitalized patients. The results indicated that most of the isolates were associated with these 4 STs (SLVs, TLVs or within the identical CCs), which indicated that *E. faecalis* isolates from ducks could cause diseases in immunocompromised people if the germs spread to humans via food chain. Furthermore, the results of

GrapeTree analysis showed that the isolates were mainly evolved from *E. faecalis* of clinical patients, which further provided evidence for the risk of human infection and pathogenicity of *E. faecalis* from ducks. Finally, 5 new STs of *E. faecalis* were mainly evolved from C.CZ28 strain (ST1074, id: 2048). Therefore, the epidemiological investigation on C.CZ28 strain from ducks can reveal more reliable information for public health in the future.

In conclusion, our data demonstrated that duck acts as a source for a diversity of MDR *E. faecalis* strains. Some of them exhibited virulence characteristics associated with human disease, reinforcing the zoonotic potential of *E. faecalis* from ducks. It would be important to provide interventions to reduce the risks of transmission to the human food chain.

ACKNOWLEDGMENTS

This work was supported by the grant from Sichuan Science and Technology Program (2021YFH0192 and 20YYJC0952). We also thank the grant from National Natural Science Foundation of China (31671954).

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101646.

REFERENCES

Aarestrup, F. M., Y. Agerso, P. Gerner-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.

Abat, C., M. Huart, V. Garcia, G. Dubourg, and D. Raoult. 2016. *Enterococcus faecalis* urinary-tract infections: do they have a zoonotic origin? J. Infect. 73:305–313.

Anderson, A. C., H. Andisha, E. Hellwig, D. Jonas, K. Vach, and A. Al-Ahmad. 2018. Antibiotic resistance genes and antibiotic susceptibility of oral *Enterococcus faecalis* isolates compared to isolates from hospitalized patients and food. Adv. Exp. Med. Biol. 1057:47–62.

Aslam, M., M. S. Diarra, S. Checkley, V. Bohaychuk, and L. Masson. 2012. Characterization of antimicrobial resistance and virulence genes in *Enterococcus* spp. isolated from retail meats in Alberta, Canada. Int. J. Food Microbiol. 156:222–230.

Barros, J., L. D. R. Melo, P. Poeta, G. Igrejas, M. P. Ferraz, J. Azeredo, and F. J. Monteiro. 2019. Lytic bacteriophages against multidrug-resistant *Staphylococcus aureus, Enterococcus faecalis* and *Escherichia coli* isolates from orthopaedic implant-associated infections. Int. J. Antimicrob. Agents 54:329–337.

Bittencourt de Marques, E., and S. Suzart. 2004. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. J. Med. Microbiol. 53:1069–1073.

Boyd, D. A., T. Du, R. Hizon, B. Kaplen, T. Murphy, S. Tyler, S. Brown, F. Jamieson, K. Weiss, and M. R. Mulvey. 2006. VanG-type vancomycin-resistant *Enterococcus faecalis* strains isolated in Canada. Antimicrob. Agents Chemother. 50:2217–2221.

Cavaco, L. M., J. F. Bernal, E. Zankari, M. Léon, R. S. Hendriksen, E. Perez-Gutierrez, F. M. Aarestrup, and P. Donado-Godoy. 2017. Detection of linezolid resistance due to the *optrA* gene in *Enterococcus faecalis* from poultry meat from the American continent (Colombia). J. Antimicrob. Chemother. 72:678–683.

Chong, K. K. L., W. H. Tay, B. Janela, A. M. H. Yong, T. H. Liew, L. Madden, D. Keogh, T. M. S. Barkham, F. Ginhoux, D. L. Becker, and K. A. Kline. 2017. *Enterococcus faecalis* modulates immune activation and slows healing during wound infection. J. Infect. Dis. 216:1644–1654.

Chow, J. W. 2000. Aminoglycoside resistance in enterococci. Clin. Infect. Dis. 31:586–589.

Clewell, D. B. 1993. Bacterial sex pheromone-induced plasmid transfer. Cell 73:9–12.

Cordero, J., C. Alonso-Calleja, C. García-Fernández, and R. Capita. 2019. Microbial load and antibiotic resistance patterns of *Escherichia coli* and *Enterococcus faecalis* isolates from the meat of wild and domestic pigeons. Foods 8:536.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Twenty-eighth edition. M100-28. Accessed September 13, 2018.

Deasy, B. M., M. Rea C., G. Fitzgerald F., T. Cogan M., and T. Beresford P.. 2000. A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus*. Syst Appl Microbiol 23:510–522.

Diekema, D. J., P. R. 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: e00355-19.

Donabedian, S. M., L. A. Thal, E. Hershberger, M. B. Perri, J. W. Chow, P. Bartlett, R. Jones, K. Joyce, S. Rossiter, K. Gay, J. Johnson, C. Mackinson, E. Debess, J. Madden, F. Angulo, and M. J. Zervos. 2003. Molecular characterization of gentamicin-resistant Enterococci in the United States: evidence of spread from animals to humans through food. J. Clin. Microbiol. 41:1109–1113.

Eaton, T. J., and M. J. Gasson. 2001. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microbiol. 67:1628–1635.

Elghaieb, H., A. R. Freitas, M. S. Abbassi, C. Novais, M. Zouari, A. Hassen, and L. Peixe. 2019. Dispersal of linezolid-resistant enterococci carrying *poxtA* or *optrA* in retail meat and food-producing animals from Tunisia. J. Antimicrob. Chemother. 74:2865–2869.

Faille, C., T. Bénézech, G. Midelet-Bourdin, Y. Lequette, M. Clarisse, G. Ronse, A. Ronse, and C. Slomianny. 2014. Sporulation of *Bacillus* spp. within biofilms: a potential source of contamination in food processing environments. Food Microbiol. 40:64–74.

Fang, H., H. Quan, Y. Zhang, Q. Li, Y. Wang, S. Yuan, S. Huang, and C. He. 2021. Co-infection of *Escherichia coli, Enterococcus faecalis* and Chlamydia psittaci contributes to salpingitis of laying layers and breeder ducks. Pathogens 10:755.

Farman, M., M. Yasir, R. R. Al-Hindi, S. A. Farraj, A. A. Jiman-Fatani, M. Alawi, and E. I. Azhar. 2019. Genomic analysis of multidrug-resistant clinical *Enterococcus faecalis* isolates for antimicrobial resistance genes and virulence factors from the western region of Saudi Arabia. Antimicrob. Resist Infect Control 8:55.

Fines, M., B. Perichon, P. Reynolds, D. F. Sahm, and P. Courvalin. 1999. VanE, a new type of acquired glycopeptide resistance in *Enterococcus faecalis* BM4405. Antimicrob. Agents Chemother. 43:2161–2164.

Gao, W., B. P. Howden, and T. P. Stinear. 2018. Evolution of virulence in *Enterococcus faecium*, a hospital-adapted opportunistic pathogen. Curr. Opin. Microbiol. 41:76–82.

Guzman Prieto, A. M., W. van Schaik, M. R. Rogers, T. M. Coque, F. Baquero, J. Corander, and R. J. Willems. 2016. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? Front. Microbiol 7:788.

Han, X., X. Guan, H. Zeng, J. Li, X. Huang, Y. Wen, Q. Zhao, X. Huang, Q. Yan, Y. Huang, S. Cao, R. Wu, X. Ma, and L. Zou. 2019. Prevalence, antimicrobial resistance profiles and virulence-associated genes of thermophilic *Campylobacter* spp.

isolated from ducks in a Chinese slaughterhouse. Food Control 104:157–166.

Han, X., J. Peng, X. Guan, J. Li, X. Huang, S. Liu, Y. Wen, Q. Zhao, X. Huang, Q. Yan, Y. Huang, S. Cao, R. Wu, X. Ma, and L. Zou. 2020. Genetic and antimicrobial resistance profiles of *Salmonella* spp. isolated from ducks along the slaughter line in southwestern China. Food Control 107:106805.

Hasan, K. A., S. A. Ali, M. Rehman, H. Bin-Asif, and S. Zahid. 2018. The unravelled *Enterococcus faecalis* zoonotic superbugs: emerging multiple resistant and virulent lineages isolated from poultry environment. Zoonoses Public Health 65:921–935.

Hasman, H., P. T. L. C. Clausen, H. Kaya, F. Hansen, J. D. Knudsen, M. Wang, B. J. Holzknecht, J. Samulioniené, B. L. Røder, N. Frimodt-Møller, O. Lund, and A. M. Hammerum. 2019. LRE-Finder, a Web tool for detection of the 23S rRNA mutations and the *optrA, cfr, cfr(B)* and *poxtA* genes encoding linezolid resistance in enterococci from whole-genome sequences. J. Antimicrob. Chemother. 74:1473–1476.

Igbinosa, E. O., and A. Beshiru. 2019. Antimicrobial resistance, virulence determinants, and biofilm formation of enterococcus species from ready-to-eat seafood. Front. Microbiol. 10:728.

Jackson, C. R., P. J. Fedorka-Cray, and J. B. Barrett. 2004. Use of a genus- and species-specific multiplex PCR for identification of enterococci. J. Clin. Microbiol. 42:3558–3565.

Jia, W., G. Li, and W. Wang. 2014. Prevalence and antimicrobial resistance of Enterococcus species: a hospital-based study in China. Int. J. Environ. Res. Public Health 11:3424–3442.

Jolley, K. A., J. E. Bray, and M. C. J. Maiden. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res. 3:124.

Kim, M. C., and G. J. Woo. 2017. Characterization of antimicrobial resistance and quinolone resistance factors in high-level ciprofloxacin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolates obtained from fresh produce and fecal samples of patients. J. Sci. Food Agric. 97:2858–2864.

Kim, Y. B., K. W. Seo, J. B. Shim, S. H. Son, E. B. Noh, and Y. J. Lee. 2019. Molecular characterization of antimicrobial-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from layer parent stock. Poult. Sci. 98:5892–5899.

Lupo, A., S. Coyne, and T. U. Berendonk. 2012. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front. Microbiol. 3:18.

Magiorakos, A. P., A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268–281.

Na, S. H., D. C. Moon, M. J. Choi, S. J. Oh, D. Y. Jung, H. Y. Kang, B. H. Hyun, and S. K. Lim. 2019. Detection of oxazolidinone and phenicol resistant enterococcal isolates from duck feces and carcasses. Int. J. Food Microbiol. 293:53–59.

O'Dea, M., S. Sahibzada, D. Jordan, T. Laird, T. Lee, K. Hewson, S. Pang, R. Abraham, G. W. Coombs, T. Harris, A. Pavic, and S. Abraham. 2019. Genomic, antimicrobial resistance, and public health insights into *Enterococcus* spp. from Australian chickens. J. Clin. Microbiol 57: e00319-19.

Olawale, A. K., O. M. David, A. O. Oluyege, R. T. Osuntoyinbo, S. A. Laleye, and O. Famurewa. 2015. Histopathological changes induced in an animal model by potentially pathogenic *Enterococcus faecalis* strains recovered from ready-to-eat food outlets in Osun State, Nigeria. Infect. Drug Resist. 8:181–187.

Olsen, R. H., H. C. Schønheyder, H. Christensen, and M. Bisgaard. 2012. *Enterococcus faecalis* of human and poultry origin share virulence genes supporting the zoonotic potential of *E. faecalis*. Zoonoses Public Health 59:256–263.

Osman, K., T. R. Zolnikov, J. Badr, H. Naim, M. Hanafy, A. Saad, and A. Elbehiry. 2020. Vancomycin and florfenicol resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from human urine in an Egyptian urban-rural community. Acta Trop. 201:105209.

Ostrowsky, B. E., N. C. Clark, C. Thauvin-Eliopoulos, L. Venkataraman, M. H. Samore, F. C. Tenover, G. M. Eliopoulos, R. C. Moellering Jr, and H. S. Gold. 1999. A cluster of VanD vancomycin-resistant *Enterococcus faecium*: molecular characterization and clinical epidemiology. J. Infect. Dis. 180:1177–1185.

Paoletti, C., G. Foglia, M. S. Princivalli, G. Magi, E. Guaglianone, G. Donelli, C. Pruzzo, F. Biavasco, and B. Facinelli. 2007. Co-transfer of *vanA* and aggregation substance genes from *Enterococcus faecalis* isolates in intra- and interspecies matings. J. Antimicrob. Chemother. 59:1005–1009.

Petersen, A., H. Christensen, H. C. Philipp, and M. Bisgaard. 2009. Clonality of *Enterococcus faecalis* associated with amyloid arthropathy in chickens evaluated by multilocus sequence typing (MLST). Vet. Microbiol. 134:392–395.

Poulsen, L. L., M. Bisgaard, N. T. Son, N. V. Trung, H. M. An, and A. Dalsgaard. 2012. *Enterococcus faecalis* clones in poultry and in humans with urinary tract infections, Vietnam. Emerg. Infect. Dis. 18:1096–1100.

Rashid R., Cazenave-Gassiot A., Gao I. H., Nair Z. J., Kumar J. K., Gao L., Kline K. A., Wenk M. R. Comprehensive analysis of phospholipids and glycolipids in the opportunistic pathogen *Enterococcus faecalis*. PLoS One. 2017;12:e0175886.

Resende, J. A., C. O. Fontes, A. B. Ferreira-Machado, T. C. Nascimento, V. L. Silva, and C. G. Diniz. 2018. Antimicrobial-resistance genetic markers in potentially pathogenic gram positive cocci isolated from Brazilian soft cheese. J. Food Sci. 83:377–385.

Reyes, K., A. C. Bardossy, and M.. Zervos. 2016. Vancomycin-resistant enterococci: epidemiology, infection prevention, and control. Infect. Dis. Clin. North Am. 30:953–965.

Ruiz-Garbajosa, P., M. J. Bonten, D. A. Robinson, J. Top, S. R. Nallapareddy, C. Torres, T. M. Coque, R. Cantón, F. Baquero, B. E. Murray, R. del Campo, and R. J. Willems. 2006. Multilocus sequence typing scheme for *Enterococcus faecalis* reveals hospital-adapted genetic complexes in a background of high rates of recombination. J. Clin. Microbiol. 44:2220–2228.

Shankar, V., A. S. Baghdayan, M. M. Huycke, G. Lindahl, and M. S. Gilmore. 1999. Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. Infect. Immun. 67:193–200.

Silvetti, T., S. Morandi, and M. Brasca. 2019. Does *Enterococcus faecalis* from traditional raw milk cheeses serve as a reservoir of antibiotic resistance and pathogenic traits? Foodbor. Pathog. Dis. 16:359–367.

Sirichoat, A., A. B. Flórez, L. Vázquez, P. Buppasiri, M. Panya, V. Lulitanond, and B. Mayo. 2020. Antibiotic resistance-susceptibility profiles of *Enterococcus faecalis* and *Streptococcus* spp. from the human vagina, and genome analysis of the genetic basis of intrinsic and acquired resistances. Front. Microbiol. 11:1438.

The European Committee on Antimicrobial Susceptibility Testing. EUCAST disk diffusion method for antimicrobial susceptibility testing. v 6.0. 2017. Accessed September 13, 2018.

Valenzuela, A. S., N. Benomar, H. Abriouel, M. M. Cañamero, and A. Gálvez. 2010. Isolation and identification of *Enterococcus faecium* from seafoods: antimicrobial resistance and production of bacteriocin-like substances. Food Microbiol. 27:955–961.

Vliegenthart, J. S., P. A. Ketelaar-van Gaalen, and J. A. van de Klundert. 1990. Identification of three genes coding for aminoglycoside-modifying enzymes by means of the polymerase chain reaction. J. Antimicrob. Chemother. 25:759–765.

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. Feßler, 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.

Willems, R. J., W. Homan, J. Top, M. van Santen-Verheuvel, D. Tribe, X. Manzioros, C. Gaillard, C. M. Vandenbroucke-Grauls, E. M. Mascini, E. van Kregten, J. D. van Embden, and M. J. Bonten. 2001. Variant *esp* gene as a marker of a distinct

genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. Lancet 357:853–855.

Zhou, Z., N. F. Alikhan, M. J. Sergeant, N. Luhmann, C. Vaz, A. P. Francisco, J. A. Carriço, and M. Achtman. 2018. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Res. 28:1395–1404.

FURTHER READING

Creti, R., M. Imperi, L. Bertuccini, F. Fabretti, G. Orefici, R. Di Rosa, and L. Baldassarri. 2004. Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. J Med Microbiol 53:13–20.