Dissemination and Characteristics of High-Level Erythromycin-Resistant *Enterococcus Faecalis* From Bulk Tank Milk of Dairy Companies in Korea

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Abstract

Background: Enterococci are environmental pathogens that can cause bovine mastitis and macrolides are widely used for the treatment of bovine mastitis caused by staphylococci and streptococci/enterococci. The aim of this study was performed to compare the phenotypic and genotypic characteristics of high-level erythromycin-resistant (HLER) *Enterococcus faecalis* (*E. faecalis*) collected from bulk tank milk of four dairy companies (A, B, C, and D) in Korea.

Results: Although isolates from company D showed the highest prevalence of *E. faecalis*, the prevalence of HLER *E. faecalis* in company A (73.1%) and C (57.0%) was significantly higher than company D (33.9%) ($P < 0.05$). A total of 149 HLER *E. faecalis* isolates showed high rates of resistance to tetracycline (93.3%), followed by doxycycline (70.0%), and chloramphenicol (48.3%). In the distribution of macrolides resistance genes, 147 (98.7%) isolates carried *ermB* gene alone, and two isolates carried both *ermA* and *ermB* genes. No isolates carried *ermC*, *msrA*, *msrC*, or *mef* genes. In the distribution of other resistance genes, 72 (48.3%) and 60 (40.3%) isolates carried both *tetM* and *tetL* genes, and *tetM* gene alone, respectively, and 38 (25.5%) isolates carried *optrA* gene. For aminoglycosides resistance genes, the prevalence of both *aac(6')Ie-aph(2")-Ia* and *ant(6')-Ia* genes (43.0%) was the highest. Moreover, 104 (70.0%) isolates harbored *Int-Tn* gene carrying the *Tn916/1545*-like transposon. Although the distribution of *ermB* gene showed no significant difference between the dairy companies, the prevalence of other resistance genes and transposons showed a significant difference between the dairy companies ($P < 0.05$). Virulence genes, such as *ace* (99.3%), *cad1* and *efaA* (each 98.7%), and *gelE* (83.9%), were also highly conserved in the 149 HLER *E. faecalis* isolates.

Conclusions: Our results indicated that HLER *E. faecalis* isolates from bulk tank milk showed significant differences in phenotypic and genotypic characteristics between the dairy companies. In addition, the prevalence of resistance genes and virulence factors was also high in HLER *E. faecalis* isolates.

Background

Although enterococci are harmless commensal inhabitants of the gastrointestinal tract of humans and animals, they are most frequently implicated in human nosocomial infections [1, 2]. In food-producing livestock, enterococci may occur as a secondary infection in a variety of poultry diseases, and are considered environmental mastitis-causing pathogens in bovines [2, 3]. In particular, bovine mastitis is one of the most costly diseases because an antimicrobial approach should be the first option to inhibit pathogen growth in the mammary gland, although a major herd infection caused by enterococci is relatively rare [4].

Macrolides, including erythromycin (ERY), tylosin, and azithromycin, are potential inhibitors of protein synthesis that bind to the 50S subunits in the bacteria ribosomal 23S rRNA [5, 6], and are classified by the World Health Organization as “critically important in human medicine” [7]. However, in Korea, the usage of macrolides to livestock has constantly been increasing since 2016 and also increased by approximately 7% in 2019 compared with 2018 [8, 9].

Enterococci are intrinsically susceptible to ERY due to domain II and domain V of 23S rRNA [10–12], but resistance to ERY has been continuously reported due to acquired ERY resistance genes, such as *ermB* gene [6, 13]. In particular, Kim et al., (2012) [14] reported that enterococci from bovine milk in Korea showed the high resistance to ERY, and 97.4% of ERY-resistant enterococci possessed resistance genes. Moreover, Kim et al.,

(2018) [15] reported that enterococci could easily acquire resistance genes against many antimicrobials, and Chajęcka-Wierzchowska et al., (2019) [16] also reported that enterococci could possess highly effective gene transfer mechanisms, such as conjugative transposons. Thus, the aim of this study was performed to compare the phenotypic and genotypic characteristics of high-level ERY-resistant (HLER) *Enterococcus faecalis* (*E. faecalis*) isolates from bulk tank milk of dairy companies to assess its threat to public health.

Results

Distribution of HLER E. faecalis isolates and antimicrobial resistance

Comparative antimicrobial resistance of the 149 HLER *E. faecalis* isolates from bulk tank milk of four dairy companies is shown in Table 1. Although isolates from company D showed the highest prevalence of *E. faecalis*, the prevalence of HLER *E. faecalis* in company A (73.1%) and C (57.0%) was significantly higher than company D (33.9%) ($P < 0.05$). All HLER *E. faecalis* isolates showed high rates of resistance to TET (93.3%), followed by DOX (70.0%), and CHL (48.3%). In particular, the resistance against these three antimicrobials showed significant differences between dairy companies ($P < 0.05$). Resistance against AMP, CIP, PEN, RIF, and VAN was only 0.7–5.4% without significant differences between dairy companies.

Table 1
Antimicrobial resistance of 149 high-level erythromycin-resistant *E. faecalis* from bulk tank milk in dairy companies

Company (No. of farms)	No. of *E. faecalis* [1]	No. (%) of HLER [2]	No. (%) of antimicrobial resistant HLER isolates by company [3]							
			AMP	CHL	CIP	DOX	PEN	RIF	TET	VAN
A (106)	52	38 (73.1)$_{a}$	0 (0.0)	30 (78.9)$_{a}$	0 (0.0)	27 (71.1)$_{a,b}$	1 (2.6)	3 (7.9)	37 (97.4)$_{a}$	0 (0.0)
B (47)	39	20 (51.3)$_{a,b}$	1 (5.0)	13 (65.0)$_{a,b}$	0 (0.0)	11 (55.0)$_{b}$	0 (0.0)	0 (0.0)	15 (75.0)$_{b}$	1 (5.0)
C (120)	86	49 (57.0)$_{a}$	0 (0.0)	20 (41.0)$_{b,c}$	2 (4.1)	29 (59.2)$_{b}$	0 (0.0)	5 (10.2)	45 (91.8)$_{a,b}$	0 (0.0)
D (123)	124	42 (33.9)$_{b}$	0 (0.0)	9 (21.4)$_{c}$	0 (0.0)	37 (88.1)$_{a}$	0 (0.0)	0 (0.0)	42 (100.0)$_{a}$	0 (0.0)
Total (396)	301	149 (49.5)	1 (0.7)	72 (48.3)	2 (1.4)	104 (70.0)	1 (0.7)	8 (5.4)	139 (93.3)	1 (0.7)

Values within a column not having the same small subscripts are significantly different ($P < 0.05$).

[1]All *E. faecalis* were isolated from bulk tank milk samples collected each time in the summer and winter seasons by farms.

[2]HLER, high-level erythromycin-resistance.

[3]Abbreviation: AMP, Ampicillin; CHL, Chloramphenicol; CIP, Ciprofloxacin; DOX, Doxycycline; PEN, Penicillin; RIF, Rifampin; TET, Tetracycline; VAN, Vancomycin.

Distribution Of Mdr Patterns

The prevalence of MDR in the 149 HLER *E. faecalis* isolates is shown in Fig. 1. Although the prevalence of MDR in HLER *E. faecalis* showed no significant differences between dairy companies, HLER *E. faecalis* isolates from company D showed the highest MDR (100.0%), followed by company A (92.1%), C (85.7%), and B (85.0%). All MDR isolates were resistant against three to five antimicrobial classes. Interestingly, HLER *E. faecalis* isolates from company A (57.9%) and B (55.0%) showed significantly higher resistance against four antimicrobial classes, and isolates from company D (78.6%) showed the highest MDR against three classes ($P < 0.05$). MDR against five classes was only observed in HLER *E. faecalis* isolates from company A (7.9%) and C (10.2%).

Distribution Of Antimicrobial Resistance Genes And Transposon Genes

Distribution of antimicrobial resistance genes and transposon genes

The distribution of resistance genes and transposons in the 149 HLER E. *faecalis* isolates is shown in Table 2. In prevalence of macrolides resistance genes, 147 (98.7%) isolates carried *ermB* gene alone, and two isolates from company C carried both *ermA* and *ermB* genes. No isolates carried *ermC*, *msrA*, *msrC*, or *mef* genes. In the distribution of other resistance genes, 72 (48.3%) and 60 (40.3%) isolates carried both *tetM* and *tetL* genes, and *tetM* gene alone, respectively, which are related with tetracyclines resistance, and 38 (25.5%) isolates carried *optrA* gene, which is related with phenicols resistance. Of the aminoglycosides resistance genes, the prevalence of both *aac(6')Ie-aph(2")-Ia* and *ant(6')-Ia* genes (43.0%) was the highest. Moreover, 104 (70.0%) isolates harbored *Int-Tn* gene carrying the *Tn916/1545*-like transposon. Although the distribution of *ermB* gene showed no significant differences between dairy companies, the prevalence of other resistance genes and transposons showed a significant difference between the dairy companies ($P < 0.05$).

Table 2
Antimicrobial resistance genes in 149 high-level erythromycin-resistant *E. faecalis* from bulk tank milk in dairy companies

Antimicrobial resistance gene	No. (%) of isolates with antimicrobial resistance gene(s) by company [1]				
	A	B	C	D	Total
	(*n* = 38)*	(*n* = 20)	(*n* = 49)	(*n* = 42)	(*n* = 149)
Macrolides					
ermA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ermB	38 (100.0)	20 (100.0)	47 (95.9)	42 (100.0)	147 (98.7)
ermC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ermA + ermB	0 (0.0)	0 (0.0)	2 (4.1)	0 (0.0)	2 (1.3)
msrA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
msrC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
mef	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tetracyclines					
tetL	0 (0.0)	1 (5.0)	2 (4.1)	0 (0.0)	3 (2.0)
tetM	23 (60.5)$_{a}$	2 (10.0)$_{b}$	27 (55.1)$_{a}$	8 (19.0)$_{b}$	60 (40.3)
tetO	0 (0.0)	2 (10.0)	0 (0.0)	0 (0.0)	2 (1.3)
tetL + tetM	12 (31.6)$_{b}$	10 (50.0)$_{a,b}$	16 (32.7)$_{b}$	34 (81.0)$_{a}$	72 (48.3)
Phenicols					
cfr	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
fexA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
optrA	17 (44.7)$_{a}$	6 (30.0)$_{a,b}$	10 (20.4)$_{a,b}$	5 (11.9)$_{b}$	38 (25.5)
Aminoglycosides					
aac(6')Ie-aph(2")-Ia	2 (5.3)	1 (5.0)	4 (8.2)	1 (2.4)	8 (5.4)
aph(2")-Ib	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
aph(2")-Ic	0 (0.0)	1 (5.0)	1 (2.0)	0 (0.0)	2 (1.3)
aph(2")-Id	0 (0.0)	0 (0.0)	0 (0.0)	3 (7.1)	3 (2.0)
ant(3")-Ia	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ant(6')-Ia	6 (15.8)	6 (30.0)	11 (22.4)	4 (9.5)	27 (18.1)
aac(6')Ie-aph(2")-Ia + ant(6')-Ia	11 (28.9)$_{a,b}$	6 (30.0)$_{b}$	18 (36.7)$_{b}$	29 (69.0)$_{a}$	64 (43.0)
aph(2")-Ic + ant(6')-Ia	1 (2.6)	0 (0.0)	1 (2.0)	1 (2.4)	3 (2.0)

Antimicrobial resistance gene	No. (%) of isolates with antimicrobial resistance gene(s) by company [1]				
	A	B	C	D	Total
	(*n* = 38)*	(*n* = 20)	(*n* = 49)	(*n* = 42)	(*n* = 149)
aph(2'')-Id + ant(6')-Ia	1 (2.6)	2 (10.0)	3 (6.1)	0 (0.0)	6 (4.0)
aph(2'')-Ic + aph(2'')-Id + ant(6')-Ia	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	1 (0.7)
Transposon					
Int-Tn	26 $(68.4)_{a,b}$	9 $(45.0)_{b}$	31 $(63.3)_{b}$	38 $(90.5)_{a}$	104 (70.0)
tndX	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

* *n* = No. of high-level erythromycin resistant *E. faecalis* isolated from bulk tank milk by company.

[1]Values within a row not having the same small subscripts are significantly different ($P < 0.05$).

Distribution Of Virulence Genes

The distribution of virulence genes in the 149 HLER *E. faecalis* isolates is shown in Table 3. The most prevalent virulence gene was *ace* (99.3%), followed by *cad1* and *efaA* (each 98.7%), *gelE* (83.9%), *asa1* (67.1%), *esp* (13.4%), and *cylA* (8.1%). In particular, the prevalence of *cylA*, *esp*, and *gelE* genes was significantly differences between dairy companies ($P < 0.05$).

Table 3
Virulence genes in 149 high-level erythromycin-resistant *E. faecalis* from bulk tank milk in dairy companies

Company	No. (%) of isolates by company [1]						
	ace	*asa1*	*cad1*	*cylA*	*efaA*	*esp*	*gelE*
A (*n* = 38) *	38 (100.0)	27 (71.1)	38 (100.0)	6 $(15.8)_{a}$	38 (100.0)	3 $(7.9)_{a,b}$	33 $(86.8)_{a,b}$
B (*n* = 20)	19 (95.0)	12 (60.0)	20 (100.0)	2 $(10.0)_{a,b}$	18 (90.0)	2 $(10.0)_{a,b}$	15 $(75.0)_{b}$
C (*n* = 49)	49 (100.0)	30 (61.2)	48 (98.0)	4 $(8.2)_{a,b}$	49 (100.0)	13 $(26.5)_{a}$	35 $(71.4)_{b}$
D (*n* = 42)	42 (100.0)	31 (73.8)	41 (97.6)	0 $(0.0)_{b}$	42 (100.0)	2 $(4.8)_{b}$	42 $(100.0)_{a}$
Total (*n* = 149)	148 (99.3)	100 (67.1)	147 (98.7)	12 (8.1)	147 (98.7)	20 (13.4)	125 (83.9)

* *n* = No. of high-level erythromycin-resistant *E. faecalis* isolated from the dairy company.

[1]Values within a column not having the same subscript letter differ significantly ($P < 0.05$).

Discussion

Macrolides are one of the most commonly used clinically important antibiotics and have been used to treat infections by Gram-positive bacteria, including enterococci [17]. Additionally, they also widely used in food-producing livestock in Korea [9]. Although ERY, which is a first-generation macrolide, is rarely used by dairy companies because it is rapidly inactivated in the stomach due to its extreme acid sensitivity [18], tylosin, which is also one of the macrolides, has been continually used for the treatment of infections caused by staphylococci and streptococci/enterococci. However, pathogen resistance to ERY in food-producing livestock has been conferred by cross-resistance to macrolides, including tylosin [19].

In this study, 149 of the 301 *E. faecalis* isolates from bulk tank milk samples from four dairy companies revealed HLER, with an MIC ≥ 128 μg/mL to ERY. Generally, two principal mechanisms of macrolides resistance have been identified: the methylation of bacteria ribosomal 23S rRNA encoded by a series of structurally related *erm* genes [6, 13], and an efflux pump system mediated by the membrane-bound protein encoded by the macrolide efflux genetic assembly (*mef)* [20] and macrolide- and streptogramin B- resistant (*msr*) genes [21]. In this study, all 149 HLER *E. faecalis* isolates carried *ermB* gene, and two isolates carried both *ermA* and *ermB* genes. Kim et al., (2012) [14] also reported that 97.1% of ERY- resistant *E. faecalis* isolates from bovine milk samples in Korea with an MIC ≥ 64 μg/mL carried *ermB* gene. *ermB* gene is considered the most widespread macrolides resistance gene among enterococci isolated from food-producing animals [22, 23]. Moreover, enterococci showing high-level resistance against an MIC ≥ 64 or ≥ 128 μg/mL to ERY has a strong possibility of carrying *ermB* gene.

Interestingly, 139 (93.3%) of 149 HLER *E. faecalis* isolates were resistant to TET, and 137 (98.6%) of the 139 TET-resistant isolates carried tetracycline resistance genes. Many researchers have reported that genes associated with ERY and TET resistance are easily transferred via conjugative transposons, such as those in the *Tn916/1545* and *Tn5397* families [13, 24, 25]. In this study, 132 (88.6%) isolates carried *tetM* gene, including the combination of both *tetL* and *tetM* genes. Moreover, *Int-Tn* gene, the *Tn916/1545*-like family, was also carried by 104 (70.0%) isolates. In particular, all HLER *E. faecalis* isolates from company D harbored both *ermB* and *tetM* genes, and 90.5% of isolates from company D also carried *Int-Tn* gene. Additionally, the dissemination of MDR *E. faecalis* isolates is expected to increase in company D in the future because target modification encoded by *ermB* gene can also confer cross-resistance to macrolide, lincosamide, and streptogramin B antimicrobials [19].

In this study, 72 (48.3%) isolates and 101 (67.8%) isolates carried *aac(6')Ie-aph(2")-Ia* and *ant(6')-Ia* gene, respectively, and both *aac(6')Ie-aph(2")-Ia* and *ant(6')-Ia* genes also showed in 64 (43.0%) of these isolates. The *aac(6')Ie-aph(2")-Ia* and *ant(6')-Ia* gene, which are associated with plasmid-mediated aminoglycoside-modifying enzymes (AMEs), are responsible for resistance to gentamicin and streptomycin, respectively, in enterococci [26, 27], and this study results demonstrated that *erm*, *tet*, and AME genes are commonly disseminated in HLER *E. faecalis* isolates from bulk tank milk in Korea. In this study, 38 (25.5%) isolates carried the phenicols resistance gene, *optrA*, which encodes an ATP-binding cassette transporter [28] and no isolates carried *cfr* or *fexA* genes. Phenicols resistance genes in milk and dairy products have not been reported in Korea until now, therefore, the presence of *optrA* gene in *E. faecalis* isolates from bulk tank milk may significantly accelerate the emergence of novel MDR bacteria.

Virulence genes, such as *ace* (99.3%), *cad1* and *efaA* (each 98.7%), and *gelE* (83.9%) were also highly conserved in the 149 HLER *E. faecalis* isolates. Generally, possessed and expressed virulence factors cause a more severe infection than strains that lacking virulence factors [29, 30]. Moreover, virulence factors accompanied by

resistance genes are of pathogenic importance [29–31]. Therefore, the dissemination of virulence factors in bacteria from bulk tank milk can also lead to public health concern. Our results indicate that although HLER *E. faecalis* isolates from bulk tank milk showed significant differences in phenotypic and genotypic characteristics between dairy companies, there is a high prevalence of isolates that carry a variety of antimicrobial resistance genes and virulence factors. Therefore, a structured management protocol by dairy companies and constant monitoring are necessary to minimize public health hazards.

Conclusion

In this comprehensive research on HLER *E. faecalis* from bulk tank milk of four dairy companies in Korea, the distribution of antimicrobial resistance and the phenotypic and genotypic characteristics of HLER *E. faecalis* showed a significant difference between the dairy companies. Therefore, our results indicate that a structured management protocol by companies and constant monitoring are necessary to minimize publid health hazards.

Methods

HLER E. faecalis **isolates**

A total of 1,584 batches of bulk tank milk, which were sampled twice each in the summer and winter seasons, were collected from 396 dairy farms managed by four dairy companies in Korea. The isolation and identification of *Enterococcus* spp. were performed following the standard microbiological protocols published by the Ministry of Food and Drug Safety (Korea) [32]. Briefly, one mL of the milk sample was cultured in nine mL of buffered peptone water (BPW; BD Biosciences, San Jose, CA, USA). Then, the pre-enriched BPW was transferred to Enterococcosel broth (BD Biosciences) at a 1:10 ratio and steaked onto Enterococcosel agar (BD Biosciences) after incubation at 37°C for 18–24 h. *E. faecalis* isolates were finally confirmed using polymerase chain reaction (PCR) with a specific primer for *ddl* gene as previously described [33]. Although a total of 301 *E. faecalis* were isolated in this study, 149 HLER *E. faecalis* strains with a minimum inhibitory concentration (MIC) ≥ 128 μg/mL against ERY by the standard agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) as previously described [34, 35]. *E. faecalis* ATCC 29212 was used as a standard strain for the MIC tests.

Antimicrobial Susceptibility Testing

Based on the Clinical and Laboratory Standards Institute (CLSI) guidelines [35], all HLER *E. faecalis* isolates were determined for antimicrobial resistance using the disk diffusion method against eight antimicrobial agents (BD Bioscience, Sparks, MD, USA): ampicillin (AMP, 10 μg), chloramphenicol (CHL, 30 μg), ciprofloxacin (CIP, 5 μg), doxycycline (DOX, 30 μg), penicillin (PEN, 10 units), rifampin (RIF, 5 μg), tetracycline (TET, 30 μg), and vancomycin (VAN, 30 μg). Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial classes [36].

Detection Of Antimicrobial Resistance Genes, Transposons, And Virulence Genes

DNA extraction was prepared by boiling, as described previously [37]. The presence of resistance genes to macrolides (*ermA, ermB*, *ermC*, *msrA, msrC*, and *mef*), tetracyclines (*tetL, tetM*, and *tetO*), phenicols (*cfr, fexA*, and *optrA*), and aminoglycosides [*aac(6')Ie-aph(2")-Ia, aph(2")-Ib, aph(2")-Ic, aph(2")-Id, ant(3")-Ia*, and *ant(6')-Ia*] was detected using PCR as described previously [38–46]. Additionally, *Tn916/1545*-like (*Int-Tn*) and *Tn5397*-like (*tndX*) transposons and virulence genes, such as *ace* (collagen-binding protein), *asa1* (aggregation substance), *cad1* (pheromone cAD1 precursor lipoprotein), *cylA* (cytolysin activator), *efaA* (cell wall-associated protein involved in immune evasion), *esp* (enterococcal surface protein), and *gelE* (gelatinase), were also determined by PCR as described previously [24, 47–49]. All PCR primers are listed in Table 4.

Table 4
Primers used in this study

Description	Target gene	Primer sequence (5'→3')	Size (bp)	References
E. faecalis gene	*ddl1*	F: ATCAAGTACAGTTAGTCT	941	Dutka-Malen et al., 1995
		R: ACGATTCAAAGCTAACTG		
Macrolides resistant gene	*ermA*	F: TAACATCAGTACGGATATTG	200	Di Cesare et al., 2013
		R: AGTCTACACTTGGCTTAGG		
	ermB	F: CCGAACACTAGGGTTGCTC	139	Di Cesare et al., 2013
		R: ATCTGGAACATCTGTGGTATG		
	ermC	F: TCAAAACATAATATAGATAAA	642	Sutcliffe et al., 1996
		R: GCTAATATTGTTTAAATCGTCAAT		
	msrA	F: GCAAATGGTGTAGGTAAGACAACT	402	Singh KV et al., 2001
		R: ATCATGTGATGTAAACAAAAT		
	msrC	F: GCAAATGGTGTAGGTAAGACAACT	399	Sutcliffe et al., 1996
		R: ATCATGTGATGTAAACAAAAT		Singh KV et al., 2001
	mef	F: AGTATCATTAATCACTAGTGC	348	Di Cesare et al., 2013
		R: TTCTTCTGGTACTAAAAGTGG		
Tetracyclines resistant gene	*tetL*	F: TCGTTAGCGTGCTGTCATTC	267	Aarestrup et al., 2000
		R: GTATCCCACCAATGTAGCCG		
	tetM	F: GTGGACAAAGGTACAACGAG	406	Aarestrup et al., 2000
		R: CGGTAAAGTTCGTCACACAC		
	tetO	F: AACTTAGGCATTCTGGCTCAC	515	Aarestrup et al., 2000
		R: TCCCACTGTTCCATATCGTCA		
Phenicols resistant gene	*cfr*	F: TGAAGTATAAAGCAGGTTGGGAGTCA	746	Kehrenberg and Schwarz, 2006
		R: ACCATATAATTGACCACAAGCAGC		
	fexA	F: GTACTTGTAGGTGCAATTACGGCTGA	1272	Kehrenberg and Schwarz, 2006
		R: CGCATCTGAGTAGGACATAGCGTC		
	optrA	F: AGGTGGTCAGCGAACTAA	1395	Wang et al., 2015
		R: ATCAACTGTTCCCATTCA		
Aminoglycosides resistant gene	*aac(6')Ie-aph(2")-Ia*	F: CAGAGCCTTGGGAAGATGAAG	348	Vakulenko et al., 2003

Description	Target gene	Primer sequence (5'→3')	Size (bp)	References
		R: CCTCGTGTAATTCATGTTCTGGC		
	aph(2")-Ib	F: CTTGGACGCTGAGATATATGAGCAC	867	Vakulenko et al., 2003
		R: GTTTGTAGCAATTCAGAAACACCCTT		
	aph(2")-Ic	F: CCACAATGATAATGACTCAGTTCCC	444	Vakulenko et al., 2003
		R: CCACAGCTTCCGATAGCAAGAG		
	aph(2")-Id	F: GTGGTTTTTACAGGAATGCCATC	641	Vakulenko et al., 2003
		R: CCCTCTTCATACCAATCCATATAACC		
	ant(3")-Ia	F: TGATTTGCTGGTTACGGTGAC	284	Clark et al., 1999
		R: CGCTATGTTCTCTTGCTTTTG		
	ant(6')-Ia	F: ACTGGCTTAATCAATTTGGG	577	Sepúlveda A. et al., 2007
		R: GCCTTTCCGCCACCTCACCG		
Transposon	*Int-Tn*	F: GCGTGATTGTATCTCACT	1028	Doherty et al., 2000
		R: GACGCTCCTGTTGCTTCT		
	tndX	F: ATGATGGGTTGGACAAAGA	610	AgersΦ et al., 2006
		R: CTTTGCTCGATAGGCTCTA		
Virulence gene	*ace*	F: GGAATGACCGAGAACGATGGC	616	Billström et al., 2008
		R: GCTTGATGTTGGCCTGCTTCCG		
	asa1	F: CACGCTATTACGAACTATGA	375	Billström et al., 2008
		R: TAAGAAAGAACATCACCACGA		
	cad1	F: TTCCAAAACTACGCACAACA	423	Song et al., 2019
		R: CTTTTTCAGCAGCATTCACTAATT		
	cylA	F: GACTCGGGGATTGATAGGC	688	Billström et al., 2008
		R: GCTGCTAAAGCTGCGCTTAC		
	efaA	F: CGTGAGAAAGAAATGGAGGA	499	Billström et al., 2008
		R: CTACTAACACGTCACGAATG		
	esp	F: AGATTTCATCTTTGATTCTTG	510	Billström et al., 2008
		R: AATTGATTCTTTAGCATCTGG		
	gelE	F: TATGACAATGCTTTTTGGGAT	213	Billström et al., 2008

Description	Target gene	Primer sequence (5'→3')	Size (bp)	References
		R: AGATGCACCCGAAATAATATA		

Statistical analysis

Statistical analysis by Pearson's chi-square tests and Fisher's exact tests with Bonferroni correction was performed using Statistical Package for the Social Science version 25 (SPSS; IBM, Korea). Significant differences were considered at $P < 0.05$.

List Of Abbreviations

AMP: Ampicillin, CHL: Chloramphenicol, CIP: Ciprofloxacin, CLSI: Clinical and Laboratory Standards Institute, DOX: doxycycline, ERY: Erythromycin, *E. faecalis*: *Enterococcus faecalis*, HLER: High-Level Erythromycin-Resistant, MDR: Multi – drug resistance, PEN: Penicillin, RIF: Rifampin, TET: Tetracycline, VAN: Vancomycin, WHO: World Health Organization

Declarations

Ethics approval and consent to particiate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The complete data sets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, Y.J. and Y.J.L.; methodology, Y.J., K.K and Y.J.L.; validation, Y.J., K.K. and Y.J.L.; formal analysis, Y.J. and K.K.; investigation, Y.J.; resources, Y.J. and Y.J.L.; data curation, Y.J. and K.K.; writing – original draft preparation, Y.J.; writing – review and editing, Y.J. and Y.J.L; visualization, Y.J.; supervision, Y.J.L.; project administration, Y.J.L. All authors have read and agreed to the published version of the manuscript.

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Figures

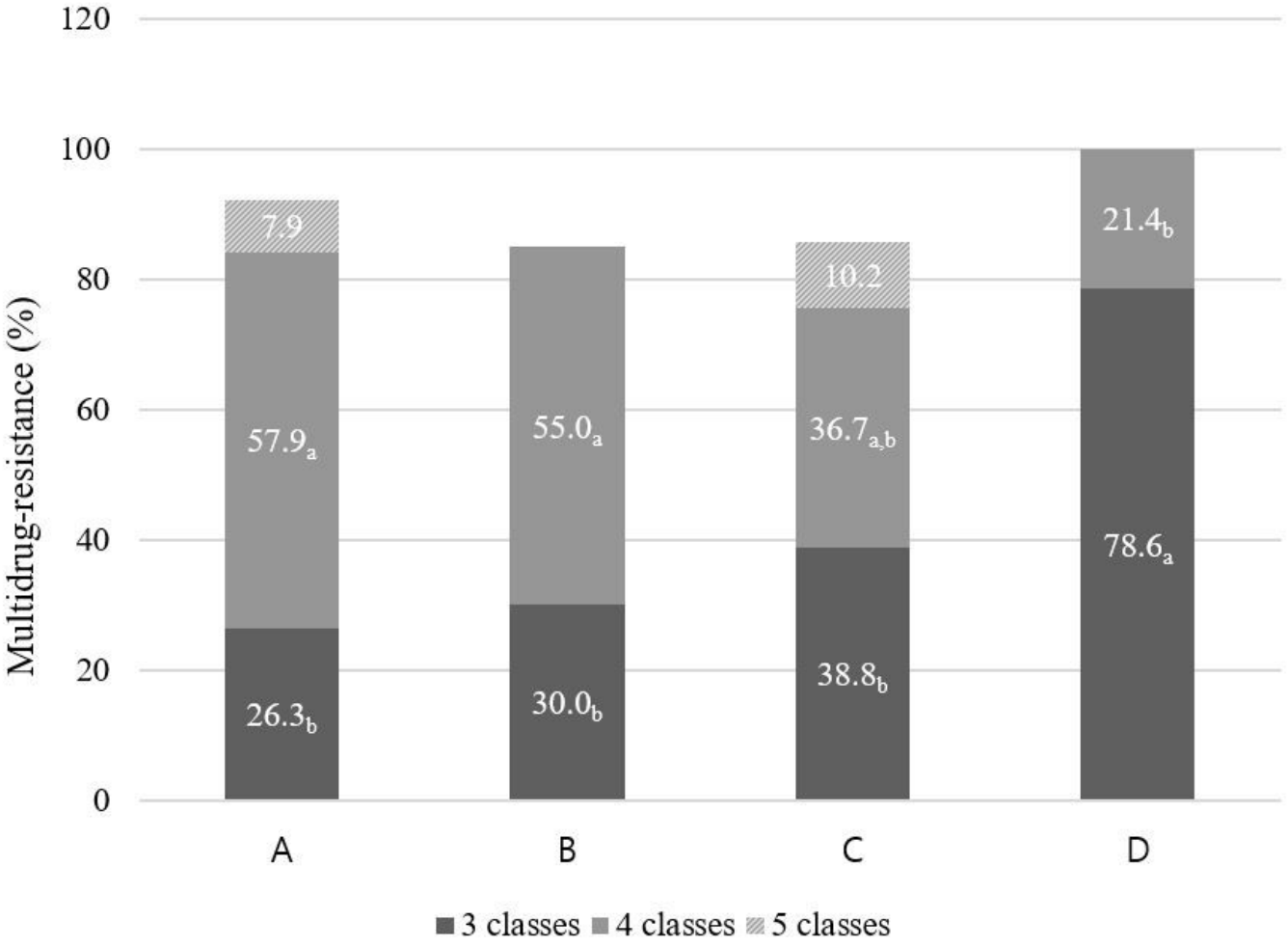

Figure 1

Distribution of multidrug resistant 149 high-level erythromycin-resistant E. faecalis from bulk tank milk in dairy companies Values not having the same subscript letter differ significantly by company ($P < 0.05$).