

## Antibiotic Resistance of *Enterococcus* Isolated from the Processed Grain Foods, *Saengsik* and *Sunsik*

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**Abstract** To evaluate the vancomycin resistance of *Enterococcus* spp. (VRE) from *Saengsik* and *Sunsik*, *Enterococcus* were isolated and identified from 25 *Saengsik* and 35 *Sunsik* samples, and resistance of *Enterococcus* to other antibiotics was also assessed. Thirty nine *Enterococcus*, 16 strains from *Saengsik*, and 23 strains from *Sunsik*, were ultimately isolated. The most frequently collected *Enterococcus* isolates in *Saengsik* were *E. casseliflavus* and *E. hirae*, and were *E. casseliflavus* and *E. faecium* in *Sunsik*. However, *E. faecalis* was not detected in those foods. Minimum inhibitory concentrations of vancomycin against the isolates were below 4 µg/mL and no strains evidenced profound levels of resistance. The isolates were found to be susceptible to vancomycin with the exception of eight *E. casseliflavus* and three *E. gallinarum*. All *Enterococcus* isolates proved resistant to streptomycin and chloramphenicol. 23% of the isolates were resistant to penicillin; however, all of the isolates were sensitive to tetracycline. Six and 48%, respectively, of the strains from the *Saengsik* and *Sunsik* proved resistant to erythromycin. All of *E. mundtii* and *E. hirae* isolates from *Saengsik*, and 20% of *E. gallinarum* and *E. casseliflavus* isolates from *Sunsik* were found to be ampicillin-resistant. All of *E. gallinarum*, *E. casseliflavus*, and *E. faecium* were rifampin-resistant. The antibiotic resistances of *Enterococcus* were relatively low, and this low vancomycin resistance was similar to that evidenced by *Enterococcus* isolates obtained from the other foods. However, there may be a need for some review of the accepted antibiotics criteria for *Enterococcus* and VRE in ready-to-eat foods.

**Keywords:** *Enterococcus*, vancomycin resistance, prevalence, processed grain food, *Saengsik*, *Sunsik*

### Introduction

*Enterococcus* are Gram-positive, facultative anaerobic cocci, and are generally classified under Lancefield's group D streptococci. In 1984, the results of DNA-DNA hybridization and 16S RNA sequencing studies demonstrated that some *Streptococcus* species were sufficiently distinct from other *Streptococcus* species, and Schleifer *et al.* (1) proposed their transfer to the genus *Enterococcus*. *Enterococcus* are ubiquitous microorganisms that inhabit the gastrointestinal tracts of humans and animals, and are also detected in certain foods, including meats, milk, and cheeses (2). Thus far, 28 species have been added to the genus *Enterococcus* on the basis of phylogenetic evidence bolstered by the findings of 16S rDNA sequencing and DNA-DNA hybridization studies (3-5). *E. faecalis* and *E. faecium* were the predominant species, and constituted 80-90 and 5-10% of the isolates identified in the clinical samples, respectively. However, *E. faecium* was identified as a primary cause of nosocomial infection, and was accompanied by the appearance of *E. faecium* species evidencing resistance against a variety of antibiotics (6).

The most important feature of *Enterococcus* is its resistance against glycopeptides, such as vancomycin and teicoplanin. Moreover, the antibiotic resistance evidenced by *Enterococcus* involves both natural intrinsic resistance and acquired transferable resistances mediated by plasmids and transposons (2). Recently, vancomycin-resistant *Enterococcus* (VRE) has been increasingly recognized as a significant nosocomial pathogen. Additionally, it is becoming

increasingly difficult to treat VRE, due to the appearance of resistance against new antibiotics, such as vancomycin (7, 8). An observed increase in VRE has been recognized as a mounting problem, as the majority of VRE have also been shown to be highly resistant to a variety of other antibiotics, and also because vancomycin resistance can be transferred to other Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) (1, 9).

*Enterococcus* are distributed widely throughout the environment, and principally inhabit the gastrointestinal tracts of humans and animals. *E. faecalis* is frequently determined to be the predominant *Enterococcus* species in the human bowel, although in some individuals and certain countries, *E. faecium* outnumbers *E. faecalis* (10). However, Mundt (11) reported that the common presence of *E. faecalis* in many food products is not always associated with direct faecal contamination. In 1992, EU established a maximum level for the presence of coliforms and *Escherichia coli*, which were considered as indicators of hygiene, whereas no limits were set for *Enterococcus* (12). Furthermore, it has been determined that *Enterococcus* had little value as a hygiene indicator in the food industry. *Enterococcus* is associated not only with warm-blooded animals, but also has been detected in soil, surface waters, plants, vegetables, and insects (11). The resistance of *Enterococcus* to pasteurization temperature, coupled with their adaptability to different substrates and growth conditions (10°C, 45°C, 6.5% NaCl, pH 9.6), implies that they should also be detectable in the food products manufactured from raw materials, and in the heat-treated food products. This indicates that these bacteria could withstand the usual conditions of food production. Therefore, *Enterococcus* may be considered to

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be an important component of food microbiota.

The diversity of vegetable products available on the market is continually increasing, and minimally-processed, ready-to-eat products are popular with consumers. These products may pose occasional health risks associated with foodborne pathogens (13, 14), but *Enterococcus* species are ubiquitous within the environment, and harbor a significant potential for pathogenicity (11). However, few investigations have been conducted regarding the occurrence of vancomycin-resistant *Enterococci* in the foods of vegetable origin. *Saengsik* is generated by a lyophilization process, which involves the freezing and drying of grains, fruits, and vegetables, with minimal physical handling in order to preserve the valuable dietary fibers, vitamins, minerals, enzymes, and other nutritional components contained in the ingredients. *Sunsik* is prepared, basically, with the above listed ingredients, which are roasted during the making of the food.

In this study, we attempted to determine the prevalence of *Enterococcus* spp. in the processed grain foods, *Saengsik* and *Sunsik*, and also attempted to characterize the antibiotic resistances evidenced by the isolated *Enterococcus* spp. This information would be helpful in evaluations of microbial risk for the ready-to-eat foods.

## Materials and Methods

**Bacterial strains and food samples** Sixty samples of *Saengsik* and *Sunsik* were purchased in Korea, and *Enterococcus* spp. were isolated from all samples. The standard strains utilized for biochemical characterization were *E. faecalis* KCTC 2011 and *E. faecium* KCCM 12118.

The instruments were sterilized and the clean bench was also cleaned after each of the preparations. All the samples were treated on clean benches. Twenty five g of each samples were homogenized for 120 sec in the 225 mL of 0.85% NaCl solution, using a stomacher (IUL, Barcelona, Spain).

**Isolation and identification of *Enterococcus* spp.** One mL of the homogenized samples was blended with 9 mL of 0.85 % NaCl solution. The diluted suspension (0.1 mL portions) was spread onto Enterococcosal agar (Difco Lab., Benton Dickinson Co., Sparks, MD, USA) and incubated for 24-72 hr at 37°C.

Black colonies in enterococcosal agar were selected and subcultured on 5% sheep's blood-added tryptic soy agar (Difco Lab.). Gram-positive isolates were identified via negative catalase test, growth at 45°C and 6.5% NaCl, and applied to polymerase chain reaction (PCR) with *Ent1* (5'-TAC TGA CAA ACC ATT CAT GAT G-3') and *Ent2* (5'-AAC TTC GTC ACC AAC GCG AAC-3'). Isolated colonies growing on the nutrient agar (Difco) for 24 hr at 45°C and 6.5% NaCl nutrient agar for 24 hr at 37°C. Gram-positive and catalase-negative isolates were identified using the Micronaut system (MERLIN Diagnostika GmbH, Germany) (15-17).

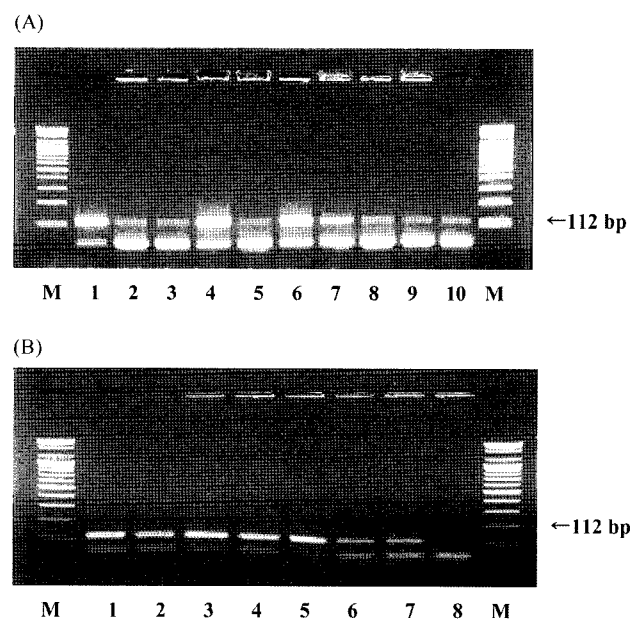
**Antibiotic tests of the isolates** In order to characterize antibiotic resistance, the isolated *Enterococcus* species were tested via agar dilution (18). In brief, the agar media

were treated with a variety of diluted antibiotics. The antibiotics utilized were as follows: vancomycin, penicillin, chloramphenicol, tetracycline, rifampin, streptomycin, ampicillin, and erythromycin, all of which were recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for antibiotic tests of *Enterococcus* (19). The isolated *Enterococcus* species were subcultured in Muller-Hinton (MH) broth (Oxoid, Hampshire, England) for 24 hr at 37°C. The subcultured broth was inoculated on MH agar plates, to which antibiotic solutions corresponding to each concentration was added. The minimum inhibition concentrations (MIC) were determined in media in which growth was not detected with each antibiotic.

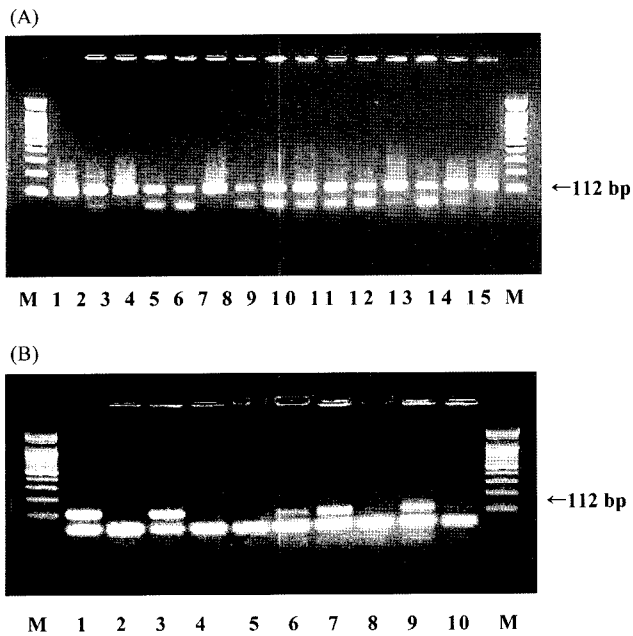
## Results and Discussion

**Isolation and identification of *Enterococcus* spp. in *Saengsik* and *Sunsik*** *Enterococcus* spp. were identified for 16 isolates from 25 *Saengsik* samples and 23 isolates from 35 *Sunsik* samples (Fig. 1, 2). The prevalence of *Enterococcus* was relatively high, largely because *Saengsik* and *Sunsik* are both minimally-processed foods whose ingredients are frequently environmentally contaminated with *Enterococcus* (15). *Enterococcus* in *Saengsik* and *Sunsik* evidenced similar prevalences (64 and 65.7%), and *Enterococcus* species observed herein appeared to be somewhat resistant to heat sterilization (11).

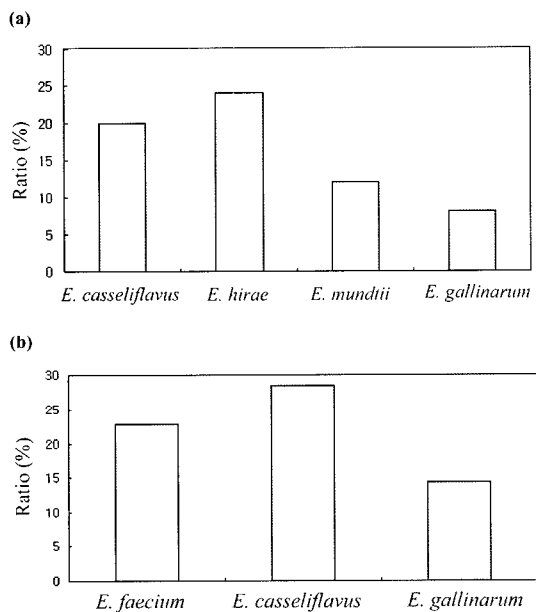
The isolated *Enterococcus* species were identified using an API 20 strep kit and the Micronaut system (Fig. 3, Table 1). Thirty nine *Enterococcus* species were isolated



**Fig. 1.** Agarose gel electrophoresis showing PCR product with *Ent1*, *Ent2* primer from isolated *Enterococcus* spp. in *Saengsik*. Lanes: (A) 1, *Enterococcus faecalis* KCTC 2011; 2, ISa\*-1; 3, ISa-2; 4, ISa-3; 5, ISa-4; 6, ISa-5; 7, ISa-6; 8, ISa-7; 9, ISa-8; 10, ISa-9; (B) 1, *Enterococcus faecium* KCCM 12118; 2, ISa-10; 3, ISa-11; 4, ISa-12; 5, ISa-13; 6, ISa-14; 7, ISa-15; 8, ISa-16; M, 100 bp DNA ladder. ISa\*: *Enterococci* isolated from *Saengsik*.



**Fig. 2.** Agarose gel electrophoresis showing PCR product with *Ent1*, *Ent2* primer from isolated *Enterococcus* spp. in Saengsik. Lanes: (A) 1, *Enterococcus faecalis* KCTC 2011; 2, ISu<sup>-</sup>-1; 3, ISu-2; 4, ISa-3; 5, ISa-4; 6, ISa-5; 7, ISa-6; 8, ISa-7; 9, ISa-8; 10, ISa-9; 11, ISu-10; 12, ISu-11; 13, ISu-12; 14, ISu-13; 15, ISu-14; (B) 1, *Enterococcus faecium* KCCM 12118; 2, ISu-15; 3, ISu-16; 4, ISu-17; 5, ISu-18; 6, ISu-19; 7, ISu-20; 8, ISa-21; 9, ISu-22; 10, ISu-23; M, 100 bp DNA ladder. ISu<sup>-</sup>: Enterococci isolated from Saengsik.



**Fig. 3.** Composition of *Enterococcus* spp. isolated from 25 Saengsik (a) and 35 Sunsik (b).

from 60 samples. The frequency of the identified *Enterococcus* isolates was as follows: *E. casseliflavus* (38.5%) in 15 isolates, *E. faecium* (20.5%) in 8 isolates, *E. gallinarum* (17.9%) in 7 isolates, *E. hirae* (15.4%) in 6

**Table 1.** Identification of isolated *Enterococcus* spp. by API 20 Strep kit and Micronaut

Sample	Strain	Organism	Relative probability (%)
Saengsik	ISa-1	<i>Enterococcus hirae</i>	87
Saengsik	ISa-2	<i>Enterococcus mundtii</i>	48
Saengsik	ISa-3	<i>Enterococcus hirae</i>	87
Saengsik	ISa-4	<i>Enterococcus mundtii</i>	48
Saengsik	ISa-5	<i>Enterococcus mundtii</i>	48
Saengsik	ISa-6	<i>Enterococcus hirae</i>	87
Saengsik	ISa-7	<i>Enterococcus hirae</i>	87
Saengsik	ISa-8	<i>Enterococcus hirae</i>	87
Saengsik	ISa-9	<i>Enterococcus hirae</i>	87
Saengsik	ISa-10	<i>Enterococcus casseliflavus</i>	92
Saengsik	ISa-11	<i>Enterococcus casseliflavus</i>	99
Saengsik	ISa-12	<i>Enterococcus casseliflavus</i>	92
Saengsik	ISa-13	<i>Enterococcus casseliflavus</i>	96
Saengsik	ISa-14	<i>Enterococcus gallinarum</i>	55
Saengsik	ISa-15	<i>Enterococcus casseliflavus</i>	99
Saengsik	ISa-16	<i>Enterococcus gallinarum</i>	55
Sunsik	ISu-1	<i>Enterococcus casseliflavus</i>	94
Sunsik	ISu-2	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-3	<i>Enterococcus gallinarum</i>	55
Sunsik	ISu-4	<i>Enterococcus gallinarum</i>	55
Sunsik	ISu-5	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-6	<i>Enterococcus gallinarum</i>	99
Sunsik	ISu-7	<i>Enterococcus faecium</i>	88
Sunsik	ISu-8	<i>Enterococcus faecium</i>	83
Sunsik	ISu-9	<i>Enterococcus faecium</i>	98
Sunsik	ISu-10	<i>Enterococcus gallinarum</i>	94
Sunsik	ISu-11	<i>Enterococcus faecium</i>	99
Sunsik	ISu-12	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-13	<i>Enterococcus faecium</i>	91
Sunsik	ISu-14	<i>Enterococcus faecium</i>	90
Sunsik	ISu-16	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-19	<i>Enterococcus gallinarum</i>	83
Sunsik	ISu-20	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-22	<i>Enterococcus casseliflavus</i>	92
Sunsik	ISu-23	<i>Enterococcus faecium</i>	94
Sunsik	ISu-24	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-25	<i>Enterococcus casseliflavus</i>	99
Sunsik	ISu-27	<i>Enterococcus casseliflavus</i>	92
Sunsik	ISu-29	<i>Enterococcus faecium</i>	88

isolates, and *E. mundtii* (7.7%) in 3 isolates. Sixteen *Enterococcus* species (64%) were isolated from 25 Saengsik samples, as follows: six *E. hirae*, five *E.*

*casseliflavus*, three *E. mundtii*, and two *E. gallinarum*. Twenty three isolates (65.%) from 35 *Sunsik* samples were identified as follows: ten *E. casseliflavus*, eight *E. faecium*, and five *E. gallinarum*.

The results of several investigations have demonstrated that the occurrence of *E. faecalis* was higher than that of *E. faecium* in foods derived from animals (20-22). However, there have not yet been any studies conducted regarding the prevalence of *Enterococcus* in minimally processed foods and ready-to-eat foods, including *Saengsik* and *Sunsik*. Klein *et al.* (20) reported that *E. faecium* was frequently isolated in 90% of 111 samples of beef and pork, and Stobbering *et al.* (23) previously isolated *E. faecium* and *E. faecalis* from turkey. Cho *et al.* (24) reported that *E. faecium*, *E. hirae*, and *E. faecalis* were the predominant species isolated in enterococci originating in animals. Randazzo *et al.* (25) identified *E. faecium*, *E. casseliflavus*, and *E. harae* in the olive plant. However, *E. casseliflavus* was more frequently detected in *Saengsik* and *Sunsik* than *E. faecium* and *E. faecalis*. Interestingly, *E. faecalis* was not detected in those processed grain foods.

**Vancomycin minimum inhibition concentration (MIC) of the isolates** The phenotypes of vancomycin-resistant enterococci (VRE) including VanA, VanB, VanC, VanD, and VanE have been reported. VanA and VanB types acquire resistance, which is transferred via conjugation, as has been observed in both *E. faecalis* and *E. faecium*. The

VanA-type confers high-level, inducible resistance to both vancomycin and teicoplanin, whereas the VanB-type evidences variable levels of inducible resistance only to vancomycin. The VanC-type, which evidences a constitutive low-level resistance to vancomycin, appears to be an intrinsic property of motile species of *E. gallinarum*, *E. casseliflavus*, and *E. flavescens* (7).

MIC of vancomycin were calculated in order to determine the distribution of VRE in the isolated *Enterococcus* strains. MIC of vancomycin against the isolates were 0.5-4 µg/mL and no strains were found to evidence high levels of resistance, which normally is indicated by an MIC value in excess of 128 µg/mL (26). All of the isolates were found to be susceptible to vancomycin, with the exception of eight *E. casseliflavus* and three *E. gallinarum* strains (Table 2). The strains evidencing vancomycin resistance constituted 50% of the total *E. casseliflavus* and *E. gallinarum* isolates.

Chadwick *et al.* (27) isolated VRE from chicken, pork, and meat, all of which could transfer the *vanA* gene via food circulation. The VRE isolated from livestock and uncooked chicken were the same ribotyping types as the VRE isolated from the clinical samples (28). Klein *et al.* (20) reported that only 0.5% of *Enterococcus* spp. isolated from 111 beef and pork samples evidenced antibiotic resistance types differing from those of the clinical isolates.

In Korea, as for the VRE originating from animals in 1999, Seo *et al.* (29) isolated 11 VRE isolates via

**Table 2. Minimum inhibitory concentrations (MIC) of vancomycin against *Enterococcus* spp. isolated from *Saengsik* and *Sunsik***

Sample	Species (No. of isolates)	MIC <sup>1)</sup>					Range
		0.25	0.5	1.0	2.0	4.0	
<i>Saengsik</i>	<i>E. gallinarum</i> (2)					2	4.0
	<i>E. casseliflavus</i> (5)				1	4	2.0-4.0
	<i>E. mundtii</i> (3)		1	2			0.5-1.0
	<i>E. hirae</i> (6)		1	5			0.5-1.0
<i>Sunsik</i>	<i>E. gallinarum</i> (5)			3	1	1	1.0-4.0
	<i>E. casseliflavus</i> (10)		1	2	3	4	0.5-4.0
	<i>E. faecium</i> (8)			7	1		1.0-2.0

<sup>1)</sup>MIC, minimum inhibitory concentration (µg/mL).

**Table 3. Minimum inhibitory concentrations (MIC) of tetracycline against *Enterococcus* spp. isolated from *Saengsik* and *Sunsik***

Sample	Species (No. of isolates)	MIC <sup>1)</sup>					Range
		0.25	0.5	1.0	2.0	4.0	
<i>Saengsik</i>	<i>E. gallinarum</i> (2)				2		2.0
	<i>E. casseliflavus</i> (5)				5		2.0
	<i>E. mundtii</i> (3)		3				0.5
	<i>E. hirae</i> (6)			6			1.0
<i>Sunsik</i>	<i>E. gallinarum</i> (5)		4	1			0.5-1.0
	<i>E. casseliflavus</i> (10)		1	7	2		0.5-2.0
	<i>E. faecium</i> (8)		8				0.5

<sup>1)</sup>MIC, minimum inhibitory concentration (µg/mL).

multiplex PCR from 1,091 fecal samples of chickens and pigs. In 1990, Cho *et al.* (24) reported the isolation of *E. gallinarum* and *E. casseliflavus* with relatively low vancomycin resistance, and no strains exhibiting high levels of resistance were detected. However, only a relatively few studies have thus far been conducted with regard to the occurrence of vancomycin-resistant enterococci in processed foods. Minimally processed, ready-to-eat type products that do not require heating are popular among consumers (30, 31). As *Enterococcus* is ubiquitous in the environment, VRE may be expected to be present in minimally processed food. However, the vancomycin resistance of *Enterococcus* in such foods was not determined to be relatively high.

**Antibiotic resistances of the isolates** Antibiotic resistances of the isolates from *Saengsik* and *Sunsik* to penicillin, chloramphenicol, tetracycline, rifampin, streptomycin, ampicillin, and erythromycin were evaluated. All *Enterococcus* isolates were resistant to both streptomycin (>16 µg/mL) and chloramphenicol (8 µg/mL) (Table 6). Twenty three % of the isolates were found to be penicillin-resistant (Table 4). However, all of the isolates were determined to be sensitive to tetracycline at concentrations below 2 µg/mL (Table 3). The susceptibility of *E. faecalis* and *E. faecium* to penicillin was associated with the synthesis of a particular low-affinity penicillin-binding protein (PBP) (5), which was a normal component of the PBP repertoire of these bacteria (32). The resistance in *Enterococcus* was associated with the overproduction of

**Table 6. Minimum inhibitory concentrations of chloramphenicol and streptomycin against *Enterococcus* spp. isolated from *Saengsik* and *Sunsik***

Sample	Species (No. of isolates)	MIC <sup>1)</sup> <sub>chloramphenicol</sub>		MIC <sub>streptomycin</sub>	
		4.0	8.0	16	>16
<i>Saengsik</i>	<i>E. gallinarum</i> (2)		2		2
	<i>E. casseliflavus</i> (5)		5	1	4
	<i>E. mundtii</i> (3)		3		3
	<i>E. hirae</i> (6)		6		6
<i>Sunsik</i>	<i>E. gallinarum</i> (5)	2	3	1	4
	<i>E. casseliflavus</i> (10)	5	5	2	8
	<i>E. faecium</i> (8)	5	3		8

<sup>1)</sup>MIC, minimum inhibitory concentration (µg/mL).

PBP5 and a lower affinity for penicillin (33). One *E. faecium* (12.5%) and five *E. hirae* (83%) strains among the isolates were susceptible to penicillin. Six and 48% of the strains from the *Saengsik* and *Sunsik* samples were erythromycin-resistant (Table 5). All of the isolates of *E. mundtii* and *E. hirae* from the *Saengsik* samples, and 20% of *E. gallinarum* and *E. casseliflavus* isolates from *Sunsik* samples were found to be resistant to the β-lactam antibiotic, ampicillin (Table 7). All *E. mundtii* and *E. hirae* strains among the isolates were susceptible to rifampin, but 71, 40, and 88% of *E. gallinarum*, *E. casseliflavus*, and *E.*

**Table 4. Minimum inhibitory concentrations (MIC) of penicillin against *Enterococcus* spp. isolated from *Saengsik* and *Sunsik***

Sample	Species (No. of isolates)	MIC <sup>1)</sup>					Range
		0.25	0.5	1.0	2.0	4.0	
<i>Saengsik</i>	<i>E. gallinarum</i> (2)			2			1.0
	<i>E. casseliflavus</i> (5)	3	1	1			0.25-1.0
	<i>E. mundtii</i> (3)				2	1	2.0-4.0
	<i>E. hirae</i> (6)				1	5	2.0-4.0
<i>Sunsik</i>	<i>E. gallinarum</i> (5)		1	1	1	2	0.5-4.0
	<i>E. casseliflavus</i> (10)	4	2	3	1		0.25-2.0
	<i>E. faecium</i> (8)				7	1	2.0-4.0

<sup>1)</sup>MIC, minimum inhibitory concentration (µg/mL).

**Table 5. Minimum inhibitory concentrations of erythromycin against *Enterococcus* spp. isolated from *Saengsik* and *Sunsik***

Sample	Species (No. of isolates)	MIC <sup>1)</sup>							Range
		0.25	0.5	1.0	2.0	4.0	8.0	16.0	
<i>Saengsik</i>	<i>E. gallinarum</i> (2)					2			4.0
	<i>E. casseliflavus</i> (5)				2	3			2.0-4.0
	<i>E. mundtii</i> (3)				2	1			2.0-4.0
	<i>E. hirae</i> (6)	1					4	1	0.25-16.0
<i>Sunsik</i>	<i>E. gallinarum</i> (5)			1		1	2	1	1.0-16.0
	<i>E. casseliflavus</i> (10)		1	1	5	3			0.5-4.0
	<i>E. faecium</i> (8)	3		1		1	3		0.25-8.0

<sup>1)</sup>MIC, minimum inhibitory concentration (µg/mL).

**Table 7. Minimum inhibitory concentrations of ampicillin against *Enterococcus* spp. isolated from Saengsik and Sunsik**

Sample	Species (No. of isolates)	MIC <sup>1)</sup>					Range
		0.25	0.5	1.0	2.0	4.0	
Saengsik	<i>E. gallinarum</i> (2)		1	1			0.5-1.0
	<i>E. casseliflavus</i> (5)		2	2	1		0.5-2.0
	<i>E. mundtii</i> (3)					3	4.0
	<i>E. hirae</i> (6)			1	1	4	1.0-4.0
Sunsik	<i>E. gallinarum</i> (5)			2	1	2	1.0-4.0
	<i>E. casseliflavus</i> (10)		3	3	3	1	0.5-4.0
	<i>E. faecium</i> (8)					8	2.0

<sup>1)</sup>MIC, minimum inhibitory concentration ( $\mu\text{g}/\text{mL}$ ).

**Table 8. Minimum inhibitory concentrations of rifampin against *Enterococcus* spp. isolated from Saengsik and Sunsik**

Sample	Species (No. of isolates)	MIC <sup>1)</sup>							Range
		0.25	0.5	1.0	2.0	4.0	8.0	16	
Saengsik	<i>E. gallinarum</i> (2)			1		1			1.0-4.0
	<i>E. casseliflavus</i> (5)				1	2	2		2.0-8.0
	<i>E. mundtii</i> (3)	3							0.25
	<i>E. hirae</i> (6)	5		1					0.25-1.0
Sunsik	<i>E. gallinarum</i> (5)				1		3	1	2.0-16
	<i>E. casseliflavus</i> (10)	2	2	1	3	1	1		0.25-8.0
	<i>E. faecium</i> (8)		1			1	1	5	0.5-16

<sup>1)</sup>MIC, minimum inhibitory concentration ( $\mu\text{g}/\text{mL}$ ).

*faecium* strains, respectively, were resistant (Table 8).

Klare *et al.* (34) reported that three vancomycin-resistant strains of *E. faecium* from animal origin were also resistant to penicillin, and were susceptible to chloramphenicol and rifampin. Klein *et al.* (20) and Pavia *et al.* (35) isolated *Enterococcus* from cow, chicken, turkeys, and pigs were found to be susceptible to ampicillin, rifampin, and resistant to tetracycline. Therefore, the antibiotic sensitivity of *Enterococcus*, according to each country and location studied, evidenced a variety of differences, with regard both to degree and to the antibiotics applied.

In this study, all *Enterococcus* isolates were resistant to streptomycin, chloramphenicol, and sensitive to tetracycline at concentrations below 2  $\mu\text{g}/\text{mL}$ . However, the sensitivity of the isolates from the processed grain foods to penicillin, rifampin, ampicillin, and erythromycin were different from those of the *Enterococcus* strains isolated from animals.

The prevalence of *Enterococcus* in the Saengsik and Sunsik samples were approximately 60%. The prevalence of *E. faecium* among the isolates from Saengsik and Sunsik were 20%. However, interestingly, no *E. faecalis* was detected. The antibiotic resistances of *Enterococcus* were not relatively high, and the low levels of vancomycin resistance were similar to those of the *Enterococcus* isolated from the other evaluated foods. Therefore, it appears that Saengsik and Sunsik are relatively safe with regard to VRE. However, there may be a further need for some review of the accepted criteria for *Enterococcus*, as

well as VRE resistance analyses of other ready-to-eat foods.

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