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ARTICLE

Detection of Antibiotic Resistance and Resistance Genes in Enterococci Isolated from Sucuk, a Traditional Turkish Dry-Fermented Sausage

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Abstract

The aim of this study was to isolate enterococci in Sucuk, a traditional Turkish dry-fermented sausage and to analyze isolates for their biodiversity, antibiotic resistance patterns and the presence of some antibiotic resistance genes. A total of 60 enterococci strains were isolated from 20 sucuk samples manufactured without using a starter culture and they were identified as *E. faecium* (73.3%), *E. faecalis* (11.7%), *E. hirae* (8.3%), *E. durans* (3.3%), *E. mundtii* (1.7%) and *E. thailandicus* (1.7%). Most of the strains were found resistant to rifampin (51.67%) followed by ciprofloxacin (38.33%), nitrofurantoin (33.33%) and erythromycin (21.67%). All strains were found susceptible to ampicillin. Only *E. faecium* FYE4 and FYE60 strains displayed susceptibility to all antibiotics. Other strains showed different resistance patterns to antibiotics. *E. faecalis* was found more resistant to antibiotics than other species. Most of the strains (61.7%) displayed resistance from between two and eight antibiotics. The *ermB*, *ermC*, *gyrA*, *tetM*, *tetL* and *vanA* genes were detected in some strains. A lack of correlation between genotypic and phenotypic analysis for some strains was detected. The results of this study indicated that Sucuk manufactured without using a starter culture is a reservoir of multiple antibiotic resistant enterococci. Consequently, Sucuk is a potential reservoir for the transmission of antibiotic resistance genes from animals to humans.

Keywords *Enterococcus*, sucuk, antibiotic resistance, antibiotic resistance gene, PCR

Introduction

Fermentation is one of the oldest known food preservation techniques and has been used since ancient times to produce various fermented meat products to protect the meat. Fermented meat products are important sources of valuable nutrients such as protein, fat, essential amino acids, minerals and vitamins (Ojha *et al.,* 2015). Sucuk is a traditional Turkish dry-fermented sausage that is one of the most popular meat products produced in Turkey. In addition, it is popular in many Middle Asians, Middle Eastern, Southeastern European and Northern European countries (Ercoşkun and Özkal, 2011). Sucuk is produced from a mixture of beef, sheep and/or water buffalo meats, beef fat and/or sheep tail fat, salt, sugar, nitrite/nitrate and various spices. In the traditional Sucuk production process, the prepared Sucuk

batter is stuffed into special casings, air-dried in a bovine small intestine and then fermented and ripened to develop its typical sensory characteristics (Ercoşkun and Özkal, 2011; Kaban, 2013).

Enterococcus is a large genus of lactic acid bacteria (LAB) that is important both in food and clinical microbiology (Yogurtcu and Tuncer, 2013). Some species of this genus, especially *E. faecalis* and *E. faecium*, are the relevant components of the bacterial population of some traditional cheeses (Cariolato *et al.,* 2008; Yogurtcu and Tuncer, 2013) and sausages (Landeta *et al.,* 2013; Yüceer and Özden Tuncer, 2015) produced in different European countries. On the other hand, some enterococci are considered as opportunistic human pathogens that often cause hospital-acquired infections such as endocarditis, bacteremia and urinary tract infection. Enterococcal infections are predominantly associated with *E. faecalis* and *E. faecium* (Ogier and Serror, 2008). Although antibiotic resistance is not in itself a virulence factor, multiple antibiotic resistance of enterococci is a contributing factor to their pathogenesis. Enterococci have intrinsic and acquired antibiotic resistance, which is encoded on the chromosome and plasmids or transposons, respectively (Beceiro *et al.,* 2013; Yogurtcu and Tuncer, 2013). The acquired antibiotic resistance genes can be horizontally transferred by mobile genetic elements from other strains, either distantly or closely related. In recent years, attention to the presence of antibiotic resistance in non-pathogenic bacteria has increased because they may act as reservoirs for antibiotic resistance genes (Talon and Leroy, 2011). The human gastrointestinal tract provides an ideal combination of factors for antibiotic resistance genes to arise and spread through bacterial populations (Huddleston, 2014).

Limited data are available on the antibiotic resistance of enterococci isolated from Sucuk and the presence of their antibiotic resistance genes. The objective of the present investigation was to isolate enterococci in Sucuk produced without using starter culture and to analyze isolates for their biodiversity, antibiotic resistance patterns and the presence of some antibiotic resistance genes.

Materials and Method

Sample collection and processing

A total of 20 Sucuk samples produced without using a starter culture were obtained from 20 different local manufacturers in Afyonkarahisar province, Turkey. Sucuk samples were purchased randomly between May and September 2013. Each 25 g Sucuk sample was homogenized with 225 mL sterile physiological water (0.85% NaCl, w/v) in a Waring blender (8011 ES HGB2WTS3, USA). 100 µL of serial decimal dilutions from homogenized Sucuk samples were inoculated onto Kanamycin Aesculin Azide (KAA) agar (LAB M Ltd., UK) and incubated at 37°C for 24-48 h. Typical enterococcal colonies were randomly selected from KAA agar and transferred into de Man, Rogosa and Sharpe (MRS) broth (LAB M). Stock cultures were stored at -20°C in MRS broth with 20% glycerol.

DNA Extraction

Total DNAs of presumptive enterococci were extracted from overnight cultures, and grown in MRS broth at 37°C, as previously described by Cancilla *et al.* (1992).

Identification of the isolates and phylogenetic analysis

The isolates were identified at the genus level, using Gram staining, catalase and cultural tests such as, growth in MRS broth at 10°C, 45°C, pH 9.6 and 6.5% NaCl (w/v). In addition, isolates were tested for resistance to heat at 60°C for 30 min (Morandi *et al.,* 2006). Identification of isolates at species level was done by 16S rDNA sequencing with pA and pE' universal bacterial primers (Edwards *et al*., 1989). Polymerase chain reaction (PCR) was performed in 50 µL reaction mixtures, using 3 µL of DNA solution, 1 µL of each primer, 20 µL nuclease-free water and 25 µL PCR master mix (Fermentas, Lithuania). PCR was performed using the following cycling parameters: a cycle denaturation (94°C, 2 min); 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 1 min) and extension (72°C, 90 s), followed by a final extension step (72°C, 10 min). The PCR products were analyzed on 1.5% (w/v) agarose gel in Tris-acetate-EDTA buffer. The gels were stained with 0.2 µg/mL of ethidium bromide (Amresco Cat no. 0492, USA) and photographed under UV light. Sequencing of the PCR products was done by Ref Gen Ltd. (Ankara University Technopolis, Turkey) using an automated gene sequencer ABI PRISM 3730XL (Perkin Elmer, USA). The sequences of the PCR products were compared to the 16S rDNA gene sequences of Genbank using the BLAST program for detection of the closest relatives. Phylogenetic analysis was conducted with MEGA software version 4.0 (MEGA; http://www.megasoftware.net). The tree was generated by neighbor-joining using the maximum composite likelihood model (Tamura *et al.,* 2007).

Antibiotic resistance patterns of enterococci

Antibiotic resistance patterns of isolates were detected by the disc diffusion method on Mueller-Hinton agar (Cariolato *et al.,* 2008). A total of 18 commercial antibiotic discs of ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (120 μg), levofloxacin (5 μg), linezolid (30 μg), minocycline (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), penicillin G (10 U), quinupristin/dalfopristin (15 μg), rifampin (5 μg), streptomycin (300 μg), teicoplanin (30 μg), tetracycline (30 μg) and vancomycin (30 μg) were used. All antibiotics were obtained from Oxoid Ltd. (UK). Susceptibility or resistance of enterococci was determined according to the Clinical and Laboratory Standards Institute (2012).

PCR detection of antibiotic resistance genes

The presence of chloramphenicol (*cat*), ciprofloxacin (*gyrA*), erythromycin (*ermA*, *ermB, ermC*), tetracycline (*tetM*, *tetL*, *tetK*, *tetS*, *tetO*) and vancomycin (*vanA*, *vanB*, *vanC*) resistance genes in enterococci was investigated by PCR. PCR primers and annealing temperatures for detection of antibiotic resistance genes are listed in Table 1. PCR was performed in 50 μL reaction mixtures. PCR conditions involved the following cycling parameters: initial denaturation cycle at 94ºC for 2 min (95ºC for 5 min for gene *cat*), next 30 cycles of denaturation at 94ºC for 60 s (95ºC for 30 s for gene *cat*), annealing at an appropriate temperature for 60 s (30 s for gene *cat*) and elongation at 72ºC for 60 s (30 s for gene *cat*), and a final extension cycle at 72ºC for 10 min (7 min for gene *cat*) (Dutka-Malen *et al.,* 1995; Kim *et al.,* 2005; Ouoba *et al.,* 2008; Reviriego *et al.,* 2005).

Results and Discussion

Identification of isolates

A total of 63 presumptive enterococci isolates, which appear surrounded by a black halo on KAA agar, were isolated from 20 Sucuk samples obtained from Afyonkarahisar province, Turkey. Three isolates Gram-positive rods were eliminated and the remaining 60 isolates Gram-positive cocci (single, pairs or in short chains) were selected for further analyses. All the Gram-positive cocci isolates

Table 1. PCR primers and annealing temperatures for detection of antibiotic resistance genes

Genes	Primer sequence (5' to 3')	Annealing temperature (°C)	Reference
cat	GCGAACGAAAAACAATTGCA TGAAGCTGTAAGGCAACTGG	55	(Kim *et al.,* 2005)
gyrA	GAYTATGCWATGTCAGTTATTGT GGAATRTTRGAYGTCATACCAAC	45	(Ouba *et al.,* 2008)
ermA	AAGCGGTAAAACCCCTCTGAG TCAAAGCCTGTCGGAATTGG	55	(Ouba *et al.,* 2008)
ermB	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG	52	(Ouba *et al.,* 2008)
ermC	CAAACCCGTATTCCACGATT ATCTTTGAAATCGGCTCAGG	48	(Ouba *et al.,* 2008)
tetM	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	45	(Ouba *et al.,* 2008)
tetL	GTTGCGCGCTATATTCCAAA TTAAGCAAACTCATTCCAGC	54	(Ouba *et al.,* 2008)
tetS	TGGAACGCCAGAGAGGTATT ACATAGACAAGCCGTTGACC	55	(Ouba *et al.,* 2008)
tetK	TTAGGTGAAGGGTTAGGTCC GCAAACTCATTCCAGAAGCA	55	(Ouba *et al.,* 2008)
tetO	GATGGCATACAGGCACAGAC CAATATCACCAGAGCAGGCT	55	(Ouba *et al.,* 2008)
vanA	GGGAAAACGACAATTGC GTACAATGCGGCCGTTA	54	(Dutka-Malen *et al.,* 1995)
vanB	GTGCTGCGAGATACCACAGA CGAACACCATGCAACATTTC	54	(Reviriego *et al.,* 2005)
vanC	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	54	(Dutka-Malen *et al.,* 1995)

Y, C or T; R, A or G; W, A or T.

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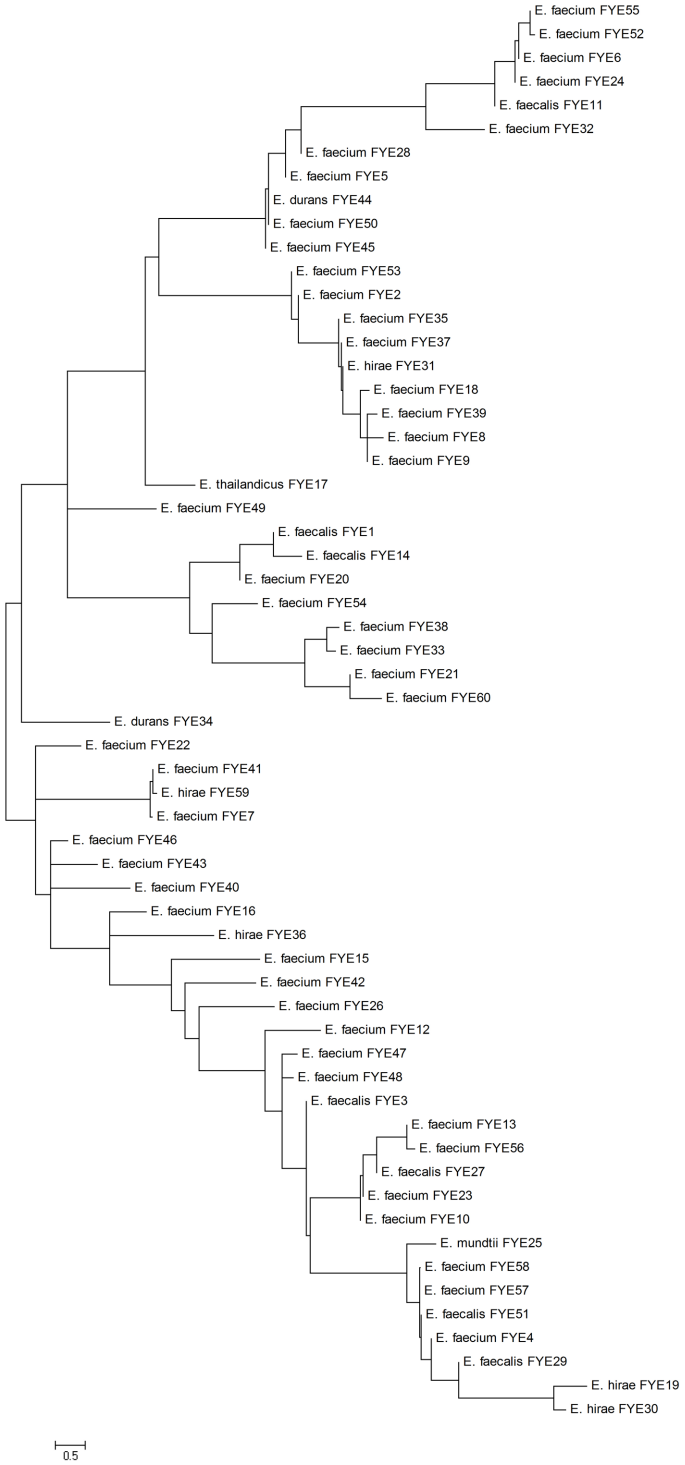

Fig. 1. Phylogenetic analysis of 16S rRNA gene sequences of *Enterococcus* strains.

were catalase-negative and resistant to heat at 60°C for 30 min. In addition, they were grown in MRS broth at 10°C, 45°C, pH 9.6 and presence of 6.5% NaCl. The results of Gram staining, catalase and cultural tests showed that these 60 strains isolated from Sucuk samples were members of the *Enterococcus* genus. 60 presumptive *Enterococcus* strains were identified at species level by 16S rDNA sequence analyses. Presumptive *Enterococcus* strains were identified as 44 *E. faecium* (73.33%), 7 *E. faecalis* (11.67%), 5 *E. hirae* (8.33%), 2 *E. durans* (3.33%), 1 *E. mundtii*

(1.67%) and 1 *E. thailandicus* (1.67%). Phylogenetic analysis of 16S rRNA gene sequences of *Enterococcus* strains was shown in Fig. 1. Enterococci are associated with some traditional sausages produced in different European countries (Jahan *et al.,* 2013; Landeta *et al.,* 2013; Yüceer and Özden Tuncer, 2015), as confirmed in this study. Dominant *Enterococcus* species in Sucuk samples were determined as *E. faecium* (73.33%). Similar to our results, most previous studies reported *E. faceium* as a dominant microbiota in fermented sausages (Landeta *et al.,* 2013; Valenzuela *et al.,* 2009; Yüceer and Özden Tuncer, 2015). In contrast, some researchers found a higher percentage of *E. faecalis* than other species in animal originated foods (Jahan *et al.,* 2013; Peters *et al.,* 2003).

Antibiotic resistance patterns of *Enterococcus* strains

Sixty *Enterococcus* strains were tested for their resistance to 18 different antibiotics using the disc diffusion method. Antibiotic resistance test results of *Enterococcus* strains were given in Table 2. Only *E. faecium* FYE4 and FYE60 strains displayed susceptibility to all antibiotics. All of the strains were found sensitive only to ampicillin. Similar to our result, some researchers showed that enterococci isolated from different traditional fermented sausages and cheeses were completely sensitive to ampicillin (Jahan *et al.,* 2013; Landeta *et al.,* 2013; Yogurtcu and Tuncer, 2013; Yüceer and Özden Tuncer, 2015). On the other hand, Chajecka-Wierzchowska *et al*. (2012) showed that low numbers of enterococci isolated from animal originated food were resistant to ampicillin. *Enterococcus* strains showed different susceptibility patterns to the other antibiotics used in this study. Antibiotic susceptibility and resistance percentage of *Enterococcus* strains were given in Table 3.

Most of the strains were found sensitive to gentamicin (93.33%), streptomycin (93.33%), chloramphenicol (90.00%), minocycline (90.00%), penicillin G (90.00%),

Table 2. Antibiotic resistance of *Enterococcus* strains isolated from Sucuk and positive PCR for resistance genes in these strains

Strains	Antibiotic susceptibility[1)]																		Positive PCR/ resistance genes
	AMP	DO	E	CN	C	LEV	LZD	QD	MH	F	NOR	P	RD	CIP	S	TEC	TE	VA	
E. faecalis FYE1	S[2)]	I	R	R	R	S	S	R	I	S	I	S	S	R	R	I	I	S	*ermB*, *tetM*
E. faecium FYE2	S	S	I	S	S	I	S	S	S	R	I	S	R	R	S	I	S	S	ermC
E. faecalis FYE3	S	I	R	R	R	I	I	R	R	S	I	S	S	R	R	R	I	S	*ermB*, *tetM*
E. faecium FYE4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	ermB
E. faecium FYE5	S	S	R	S	S	R	I	S	S	R	R	S	R	R	S	I	S	S	*ermC*, *gyrA*
E. faecium FYE6	S	S	I	S	S	S	S	S	S	S	S	S	S	I	S	S	S	S	none
E. faecium FYE7	S	S	R	S	S	S	S	S	S	S	R	S	S	S	S	I	S	S	none
E. faecium FYE8	S	S	I	S	S	S	S	S	S	S	S	S	S	I	S	I	S	S	ermC
E. faecium FYE9	S	S	S	S	S	S	S	S	S	I	S	S	S	I	S	I	S	S	none
E. faecium FYE10	S	S	I	S	S	I	S	S	S	S	I	S	S	I	S	I	S	S	none
E. faecalis FYE11	S	S	I	S	S	I	I	S	S	R	I	S	R	R	S	I	S	S	ermC
E. faecium FYE12	S	S	I	S	S	I	I	S	S	R	I	S	R	R	S	I	S	S	none
E. faecium FYE13	S	S	I	S	S	S	S	S	S	R	S	S	S	S	S	I	S	S	none
E. faecalis FYE14	S	R	R	S	R	I	I	R	R	S	I	S	R	I	R	I	R	I	*ermB*, *tetL*
E. faecium FYE15	S	S	I	S	S	I	S	S	S	R	I	S	R	I	S	I	S	S	tetM
E. faecium FYE16	S	S	S	S	S	R	S	I	S	R	S	S	S	R	S	R	S	I	*ermC*, *vanA*
E. thailandicus FYE17	S	S	R	S	S	I	I	I	S	S	I	S	R	I	S	I	S	S	gyrA
E. faecium FYE18	S	S	I	S	S	I	I	I	S	R	I	S	R	I	S	I	S	I	none
E. hirae FYE19	S	S	S	S	S	S	S	I	S	I	S	S	I	I	S	S	S	S	gyrA
E. faecium FYE20	S	S	I	S	S	S	I	S	S	R	R	S	R	R	S	I	S	S	ermC

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Table 2. Antibiotic resistance of *Enterococcus* strains isolated from Sucuk and positive PCR for resistance genes in these strains (Continued)

Strains	Antibiotic susceptibility[1)]																		Positive PCR/ resistance genes
	AMP	DO	E	CN	C	LEV	LZD	QD	MH	F	NOR	P	RD	CIP	S	TEC	TE	VA	
E. faecium FYE21	S	S	I	S	S	I	S	S	S	R	R	R	R	R	S	I	S	S	none
E. faecium FYE22	S	S	I	S	S	I	I	S	S	R	I	R	R	R	S	I	S	S	ermC
E. faecium FYE23	S	S	I	S	S	I	S	S	S	R	I	R	R	R	S	I	S	S	ermC
E. faecium FYE24	S	S	I	S	S	S	S	S	S	R	I	S	R	I	S	I	S	S	ermC
E. mundtii FYE25	S	S	I	S	S	S	S	S	S	S	S	S	S	I	S	I	S	S	none
E. faecium FYE26	S	S	I	S	S	I	S	S	S	I	I	S	R	R	S	I	S	S	none
E. faecalis FYE27	S	I	R	I	I	S	I	R	I	S	I	S	I	I	S	I	I	I	ermB
E. faecium FYE28	S	S	I	S	S	I	I	I	S	R	I	R	S	R	S	R	S	S	ermC
E. faecalis FYE29	S	S	I	S	S	I	R	R	S	S	I	S	R	I	S	R	S	R	none
E. hirae FYE30	S	S	S	S	S	S	S	I	S	R	S	S	I	I	S	I	S	S	gyrA
E. hirae FYE31	S	S	S	S	S	S	S	I	S	R	S	S	S	I	S	I	S	S	gyrA
E. faecium FYE32	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	R	S	tetL
E. faecium FYE33	S	S	I	S	S	S	I	I	S	S	I	S	S	I	S	I	S	I	*tetM*, *gyrA*
E. durans FYE34	S	S	I	S	S	I	I	I	S	S	I	R	R	I	S	I	S	S	none
E. faecium FYE35	S	S	I	S	S	R	I	S	S	I	R	S	S	R	S	I	S	I	none
E. hirae FYE36	S	S	I	S	S	S	S	S	S	S	S	S	I	S	S	I	S	S	gyrA
E. faecium FYE37	S	I	R	S	R	I	I	I	I	S	R	S	S	R	S	I	R	S	*ermB*, *tetL*
E. faecium FYE38	S	S	I	S	S	S	S	S	S	S	S	S	R	S	S	I	S	S	tetM
E. faecium FYE39	S	S	I	S	S	I	S	S	S	I	S	S	I	I	S	I	S	S	none
E. faecium FYE40	S	S	I	S	S	S	S	S	S	R	I	S	R	I	S	S	S	S	ermC
E. faecium FYE41	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	I	S	S	*ermC*, *tetM*, *tetL*
E. faecium FYE42	S	S	I	S	S	I	S	S	S	S	S	S	R	I	S	I	S	S	none
E. faecium FYE43	S	S	I	S	S	I	I	S	S	S	I	S	R	R	S	I	S	S	none
E. durans FYE44	S	S	R	S	S	I	I	I	S	R	S	S	R	I	S	R	S	S	gyrA
E. faecium FYE45	S	S	R	S	S	I	S	S	S	S	I	S	R	R	S	I	S	S	none
E. faecium FYE46	S	S	R	S	S	R	S	S	S	R	I	R	I	R	S	I	S	S	ermC
E. faecium FYE47	S	S	I	S	S	S	S	I	S	S	S	S	S	I	S	I	S	S	none
E. faecium FYE48	S	S	I	S	S	S	S	I	S	S	S	S	S	I	S	I	S	S	none
E. faecium FYE49	S	S	I	S	S	S	S	I	S	S	S	S	S	I	S	S	S	S	none
E. faecium FYE50	S	S	I	S	S	S	S	I	S	S	S	S	S	I	S	I	S	S	none
E. faecalis FYE51	S	I	R	R	R	S	S	R	R	S	I	S	I	I	R	I	I	I	*ermB*, *tetM*
E. faecium FYE52	S	S	I	S	S	I	S	S	S	I	I	S	R	R	S	S	S	S	none
E. faecium FYE53	S	S	I	S	S	S	S	S	S	I	I	S	R	R	S	S	S	S	none
E. faecium FYE54	S	S	I	S	S	S	S	S	S	S	I	S	R	I	S	S	S	S	none
E. faecium FYE55	S	S	I	S	S	I	S	S	S	I	I	S	R	R	S	S	S	S	none
E. faecium FYE56	S	S	I	S	S	I	S	S	S	S	S	S	R	I	S	S	S	S	none
E. faecium FYE57	S	S	I	S	S	S	S	S	S	I	I	S	R	R	S	S	S	S	none
E. faecium FYE58	S	S	I	S	S	S	S	S	S	I	I	S	R	R	S	S	S	S	none
E. hirae FYE59	S	S	S	S	S	S	S	I	S	R	S	S	I	I	S	I	S	S	*gyrA*
E. faecium FYE60	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	none

[1)]AMP, Ampicillin (10 μg); C, Chloramphenicol (30 μg); CIP, Ciprofloxacin (5 μg); CN, Gentamisin (120 μg); DO, Doxycycline (30 μg); E, Erythromycin (15 μg); F, Nitrofurantoin (300 μg); LEV, Levofloxacin (5 μg); LZD, Linezolid (30 μg); MH, Minocycline (30 μg); NOR, Norfloxacin (10 μg); P, Penicillin G (10 U); QD, Quinupristin/dalfopristin (15 μg); RD, Rifampin (5 μg); S, Streptomycin (300 μg); TEC, Teicoplanin (30 μg); TE, Tetracycline (30 μg); VA, Vancomycin (30 μg).

[2)]S, susceptible; I, intermediary; R, resistance.

Table 3. Antibiotic susceptibility and resistance percentage of *Enterococcus* strains

Antibiotics	Concentration (μg/disc)	Sensitive (%)	Intermediary (%)	Resistance (%)
Ampicillin	10	100	0	0
Chloramphenicol	30	90	1.67	8.33
Ciprofloxacin	5	13.33	48.33	38.33
Doxycycline	30	88.33	8.33	3.33
Erythromycin	15	15	63.33	21.67
Gentamicin	120	93.33	1.67	5
Levofloxacin	5	51.67	41.67	6.67
Linezolid	30	70	28.33	1.67
Minocycline	30	90	5	5
Nitrofurantoin	300	50	16.67	33.33
Norfloxacin	10	40	50	10
Penicillin G	10[1)]	90	0	10
Quinupristin/dalfopristin	15	63.33	26.67	10
Rifampin	5	35	13.33	51.67
Streptomycin	300	93.33	0	6.67
Teicoplanin	30	23.33	68.33	8.33
Tetracycline	30	88.33	6.67	5
Vancomycin	30	86.67	11.67	1.67

[1)]Penicillin G 10 U/disc.

doxycycline (88.33%), tetracycline (88.33%) and vancomycin (86.67%), followed by other antibiotics. On the contrary, *Enterococcus* strains were found resistant to rifampin (51.67%), ciprofloxacin (38.33%), nitrofurantoin (33.33%) and erythromycin (21.67%), followed by other antibiotics except for ampicillin. Recently, similar observations to our results have been reported by several researchers. Peters *et al*. (2003) indicated that 22.03% of the *Enterococcus* strains isolated from animal originated foods were sensitive to erythromycin. Chajecka-Wierzchowska *et al*. (2012) reported that enterococci from food of animal origin were sensitive to chloramphenicol (96.73%), streptomycin (91.30%), gentamicin (85.86%) and quinupristin/dalfopristin (66.66%). In addition, Yüceer and Özden Tuncer (2015) showed that *Enterococcus* strains from Sucuk were sensitive to chloramphenicol (100%), doxycycline (100%), gentamicin (100%), minocycline (100%), streptomycin (100%), tetracycline (100%), penicillin G (88%), levofloxacin (52%), rifampin (28%) and ciprofloxacin (12%). Conversely to our results, some researchers showed that *Enterococcus* strains isolated from animal originated foods were 100% sensitive to levofloxacin, quinupristin/dalfopristin, teicoplanin or vancomycin (Chajecka-Wierzchowska *et al.,* 2012; Landeta *et al.,* 2013; Peters *et al.,* 2003; Yogurtcu and Tuncer, 2013; Yüceer and Özden Tuncer, 2015).

Antibiotic susceptibility and resistance percentage of enterococci at species level was shown in Table 4. Among the *Enterococcus* species, *E. faecalis* showed the highest percentage of resistance to the tested antibiotics. *E. faecalis* strains displayed resistance to quinupristin/dalfopristin (85.71%), erythromycin (71.43%), chloramphenicol (57.14%) and streptomycin (57.14%). On the other hand, *E. faecium* strains were found resistant to rifampin (56.82%), ciprofloxacin (45.45%) and nitrofurantoin (34.09%). *E. hirae* strains showed resistance only to nitrofurantoin. Three out of five *E. hirae* strains were found resistant to nitrofurantoin. Both of the *E. durans* strains isolated from Sucuk exhibited resistance to rifampin. In addition, *E. durans* FYE44 was found resistant to erythromycin, nitrofurantoin, penicillin G and teicoplanin. *E. mundtii* FYE25 did not display resistance to any antibiotics used in this study. *E. thailandicus* FYE17 showed resistance to erythromycin and rifampin. Similar to our results, Yogurtcu and Tuncer (2013) reported that *E. faecalis* strains isolated from Turkish tulum cheese were found more resistant to antibiotics than other species. On the other hand, Chajecka-Wierzchowska *et al*. (2012) determined that *E. faecalis* and *E. faecium* strains isolated from ready-to-eat food of animal origin showed similar antibiotic resistance patterns. Conversely, some researchers reported that *E. faecium* strains were more resistant to antibiotics than other species (Landeta *et al.,* 2013; Valenzuela *et al.,* 2009).

The *Enterococcus* strains (61.67%) displayed resistance from between two and eight antibiotics. Some of the strains (11.67%) showed resistance to ciprofloxacin and rifampin.

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Table 4. Antıbiotic susceptibility and resistance per cent of *E. faecium*, *E. faecalis*, *E. hirae*, *E. durans*, *E. mundtii* and *E. thailandicus* strains isolated from Sucuk

Antibiotics	*E. faecium* (*n*[1]: 44)			*E. faecalis* (*n*: 7)			*E. hirae* (*n*: 5)			*E. durans* (*n*: 2)			*E. mundtii* (*n*: 1)			*E. thailandicus* (*n*: 1)		
	S[2]	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
Chloramphenicol	97.73	0	2.27	28.57	14.29	57.14	100	0	0	100	0	0	100	0	0	100	0	0
Ciprofloxacin	15.91	38.64	45.45	0	57.14	42.86	20	80	0	0	100	0	0	100	0	0	100	0
Doxycycline	95.46	2.27	2.27	28.57	57.14	14.29	100	0	0	100	0	0	100	0	0	100	0	0
Erythromycin	11.36	75	13.64	0	28.57	71.43	80	20	0	0	50	50	0	100	0	0	0	100
Gentamicin	100	0	0	42.86	14.29	42.86	100	0	0	100	0	0	100	0	0	100	0	0
Levofloxacin	50	40.91	9.09	42.86	57.14	0	100	0	0	0	100	0	100	0	0	0	100	0
Linezolid	77.27	22.73	0	28.57	57.14	14.29	100	0	0	0	100	0	100	0	0	0	100	0
Minocycline	97.73	2.27	0	28.57	28.57	42.86	100	0	0	100	0	0	100	0	0	100	0	0
Nitrofurantoin	45.45	20.45	34.09	85.71	0	14.29	20	20	60	50	0	50	100	0	0	100	0	0
Norfloxacin	38.64	47.73	13.64	0	100	0	100	0	0	50	50	0	100	0	0	0	100	0
Penicillin G	88.64	0	11.36	100	0	0	100	0	0	50	0	50	100	0	0	100	0	0
Quinupristin/ dalfopristin	79.55	20.45	0	14.29	0	85.71	20	80	0	0	100	0	100	0	0	0	100	0
Rifampin	38.64	4.55	56.82	28.57	28.57	42.86	20	80	0	0	0	100	100	0	0	0	0	100
Streptomycin	100	0	0	42.86	0	57.14	100	0	0	100	0	0	100	0	0	100	0	0
Teicoplanin	29.55	65.91	4.55	0	71.43	28.57	20	80	0	0	50	50	0	100	0	0	100	0
Tetracycline	95.45	0	4.55	28.57	57.14	14.29	100	0	0	100	0	0	100	0	0	100	0	0
Vancomycin	90.91	9.09	0	42.86	42.86	14.29	100	0	0	100	0	0	100	0	0	100	0	0

[1]*n*, number of strain.
[2]S, susceptible; I, intermediary; R, resistance.

Multiple antibiotic resistance in enterococci is not surprising. Several researchers reported that multiple antibiotic resistance in enterococci isolated from fermented meat products is common (Jahan *et al.,* 2013; Yüceer and Özden Tuncer, 2015), as confirmed in this study. Jahan *et al*. (2013) showed that *Enterococcus* strains isolated from fermented meat products exhibited a high rate (93.10%) of multiple antibiotic resistance characteristics. Researchers also reported that 58.62% of the strains displayed resistance from between three and eight antibiotics. In addition, Yüceer and Özden Tuncer (2015) determined that 68% of the *Enterococcus* strains isolated from fermented Sucuk exhibited multiple antibiotic resistance. The strains displayed resistance from between two and five antibiotics.

PCR detection of antibiotic resistance genes

A total of 60 *Enterococcus* strains were evaluated for the presence of chloramphenicol (*cat*), ciprofloxacin (*gyrA*), erythromycin (*ermA*, *ermB, ermC*), tetracycline (*tetM*, *tetL*, *tetK*, *tetS*, *tetO*) and vancomycin (*vanA*, *vanB*, *vanC*) resistance genes. Thirty-one out of 60 isolates showed positive PCR for *gyrA*, *ermB, ermC*, *tetM*, *tetL* and *vanA*. A lack of correlation between genotypic and phenotypic analysis was detected (Table 2).

A correlation between the chloramphenicol resistance phenotype and genotype was not detected in the researched strains. The *cat* gene was not detected by PCR in five phenotypically chloramphenicol-resistant strains. This result indicated that other chloramphenicol resistance genes may be influenced in these strains. From this, we presumed that positive PCR results for *cat* gene were not detected in these strains by the primers used in this study. Similar to our results, Kim *et al*. (2005) determined that 17 out of 29 chloramphenicol-resistant *Staphylococcus intermedius* isolates contained the *cat* gene, while 12 isolates did not carry the *cat* gene by the same primer set used in our study. In addition, Jahan *et al*. (2013) reported that only *E. faecium* S15 strain was positive for the *cat* gene among three phenotypically chloramphenicol-resistant enterococci isolated from meat and fermented meat products.

The *gyrA* gene was detected in nine *Enterococcus* strains that are phenotypically one resistant, seven intermediate and one susceptible). Only one strain, *E. faecium* FYE5, was given a positive result for *gyrA* gene among 23 phenotypically ciprofloxacin-resistant *Enterococcus* strains. Similar to our results, Jahan *et al*. (2013) did not detect *gyrA* or *parC* gene in any of three ciprofloxacin-resistant *Enterococcus* strains isolated from meat and fermented meat products. In addition, PCR results showed that phenotypically ciprofloxacin-susceptible *E. hirae* FYE36 strain

contains the *gyrA* gene (Table 2). In Gram positive bacteria, both *gyrA* and *parC* genes are defined to be the primary target in quinolone resistance. Mutations in Ser84 locus of GyrA in *Staphylococcus aureus* resulted in increased of ciprofloxacin-resistance. Similarly, single mutations taking place in the Ser83 and the Glu87 loci of GyrA were related to an increase of minimal inhibitory concentration values of ciprofloxacin in *E. faecalis* strains. The mutations taking place in both *gyrA* and *parC* genes were found in high-level quinolone-resistant *E. faecium* (Petersen *et al.,* 2004).

The erythromycin resistance gene *ermA* was not detected in any strains. However, *ermB* and *ermC* genes were detected in seven (11.67%) and 13 (21.67%) strains, respectively. These strains were showed the expected amplification fragments of 425 bp (Fig. 2) and 295 bp when their DNAs were amplified with specific primers for *ermB* and *ermC* genes, respectively. Conversely to our results, previous studies showed that the *ermB* gene is the most common erythromycin-resistant gene defined in *Enterococcus* species (Aarestrup *et al.,* 2000; Jahan *et al.,* 2013). In our study, the *ermC* gene was found at a higher frequency than the *ermB* gene. A lack of correlation between phenotypic and genotypic analysis for erythromycin resistance was detected (Table 2). The *ermB* gene was detected in six erythromycin-resistant and one erythromycin-susceptible *Enterococcus* strains. In addition, the *ermC* gene was found in nine erythromycin-intermediate, two erythromycin-resistant and one erythromycin-susceptible strains. The erythromycin-resistant strains *E. faecium* FYE7, *E. thailandicus* FYE17, *E. durans* FYE44 and *E. faecium* FYE45 did not present any of the three *erm* genes (Table 2), indicating that other factors influence resistance in these strains. Similar to these results, Jahan *et al*. (2013) demonstrated the presence of *ermB* and/or *mefA/B* genes in seven of 13 phenotypically erythromycin-resistant *Enterococcus* strains isolated from meat and fermented meat products. However, *erm* genes (*ermA*, *ermB*, *ermC* or *mefA/B*) were not found in the remaining six *Enterococcus* strains. In our study, it is interesting that the presence of *ermB* and *ermC* genes was determined in phenotypically erythromycin-susceptible *E. faecium* FYE4 and *E. faecium* FYE16 strains, respectively. A similar observation was recently reported by Ding *et al*. (2012). In this study, researchers determined the presence of *ermB* and *ermB/ermC* genes in six and two erythromycin-susceptible *Staphylococcus* strains, respectively. The researchers showed that the mutation of 23*S rRNA* gives rise to many erythromycin-susceptible isolates when it has *erm* genes, the mutation on 23*S rRNA* possibly disturbing the site of the methylation, allowing erythromycin to bind to the ribosome. The reason for lack of correlation between genotypic and phenotypic analysis in *E. faecium* FYE4 and *E. faecium* FYE16 strains may be due to mutation of the 23*S rRNA* gene in these strains. Further investigations are needed for the determination of erythromycin susceptibility mechanisms in these strains.

The tetracycline resistance genes *tetM* and *tetL* were detected in seven (11.67%) and four (6.67%) strains, respectively. In tetracycline-resistant *E. faecium* FYE32 and *E. faecium* FYE37 strains carried the *tetL* gene and *E. faecalis* FYE14 strain carried both *tetM* and *tetL* genes. More-

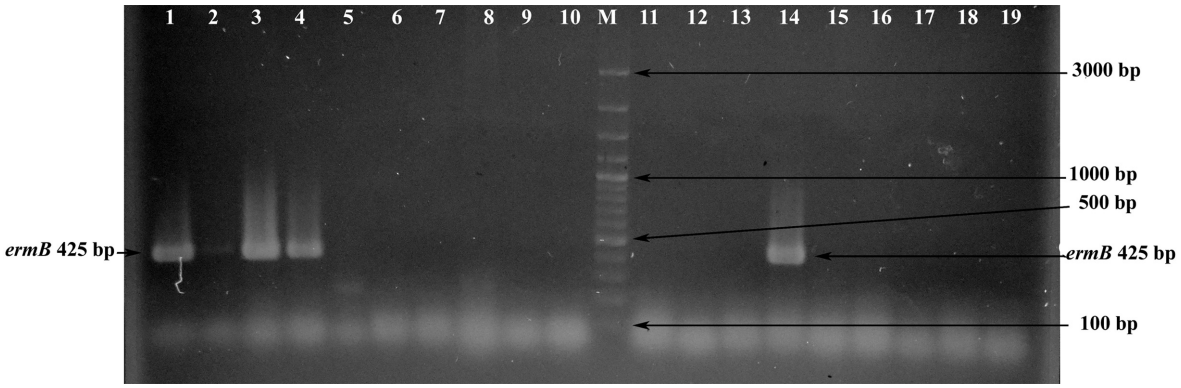

Fig. 2. PCR screen for *ermB* gene from *Enterococcus* strains. Order line 1, *E. faecalis* FYE1; line 2, *E. faecium* FYE2; line 3, *E. faecalis* FYE3; line 4, *E. faecium* FYE4; line 5, *E. faecium* FYE5; line 6, *E. faecium* FYE6; line 7, *E. faecium* FYE7; line 8, *E. faecium* FYE8; line 9, *E. faecium* FYE9; line 10, *E. faecium* FYE10; line M, 100 bp DNA ladder (Fermentas); line 11, *E. faecalis* FYE11; line 12, *E. faecium* FYE12; line 13, *E. faecium* FYE13; line 14, *E. faecalis* FYE14; line 15, *E. faecium* FYE15; line 16, *E. faecium* FYE16; line 17, *E. thailandicus* FYE17; line 18, *E. faecium* FYE18; line 19, negative control.

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over, *tetM* gene was detected in three out of four tetracycline-intermediate strains. The *tetK*, *tetS* and *tetO* genes were not detected in any strains. Different researchers have reported that the *tetM* gene is more common in tetracycline-resistant *Enterococcus* strains (Aarestrup *et al.,* 2000; Cauwerts *et al.,* 2007; Jahan *et al.,* 2013), as confirmed in this study. Similar to our results, Aarestrup *et al*. (2000) detected *tetM* and *tetL* genes in tetracycline-resistant *E. faecalis* and *E. faecium* strains isolated from humans, broilers and pigs. Researchers did not detect *tetK* and *tetS* genes in any animal-origin isolates. Hummel *et al*. (2008) reported that 94%, 63% and 56% of tetracycline-resistant *Enterococcus* strains isolated from cheese contained *tetL*, *tetM* and *tetK* genes, respectively. In addition, researchers reported that none of the strains contained *tetO* or *tetS* genes, as confirmed in this study. In another study, Jahan *et al*. (2013) also detected *tetM* (16 strains), *tetL* (six strains), and *tetK* (six strains), genes in 19 *Enterococcus* strains isolated from meat and fermented meat products, respectively. The *tetO* and *tetS* genes were not found in any of the strains. A lack of correlation between phenotypic and genotypic analysis for tetracycline resistance was detected (Table 2). In our study, the presence of both *tetM* and *tetL* genes was determined in the phenotypically tetracycline-susceptible *E. faecium* FYE41 strain. Similar observations were detected by Cauwerts *et al*. (2007). Researchers determined that two strains contained *tetM* and one strain contained *tetL* among three phenotypically tetracycline-sensitive *E. faecium* strains isolated from broilers.

The vancomycin resistance genes *vanB* and *vanC* were not detected in any tested strains. However, *vanA* gene was detected only in vancomycin-intermediate *E. faecium* FYE16 strain. One vancomycin-resistant and six vancomycin-intermediate *Enterococcus* strains did not present any of the three vancomycin resistance genes (Table 2). The *vanA* is the most common glycopeptide resistance gene in enterococci and it is usually related to high-level vancomycin resistance. Most of the VanA type resistant strains are also teicoplanin resistant (Garrido *et al.,* 2014). In this study, the phenotypically vancomycin-resistant strain, *E. faecalis* FYE29, was also found to be teicoplanin-resistant but, none of the vancomycin resistance genes researched in this study were found, indicating that other factors influence resistance in this strain. Baylan *et al*. (2011) reported that one out of eight phenotypically vancomycin- and teicoplanin-resistant urinary *E. faecium* isolates did not contain *vanA* or *vanB* genes, as confirmed in this study. The *vanA*, *vanB* and *vanC* genes were detected in none of the phenotypically vancomycin-sensitive *Enterococcus* strains as expected. Conversely to our findings, Szakacs *et al*. (2014) determined the presence of the *vanA* gene in clinical isolate of vancomycin-sensitive *E. faecium*.

Conclusion

The results of this study indicated that *E. faecium* is the dominant *Enterococcus* species present in Sucuk. *Enterococcus* strains were found resistant to the clinically relevant antibiotics except ampicillin. Most of *Enterococcus* strains displayed multiple antibiotic resistance. The *ermB*, *ermC*, *gyrA*, *tetM*, *tetL* and *vanA* genes were detected in some strains. These strains may play a role in the spread of antibiotic resistance among bacteria, and so could create a health risk for consumers. The results of this study indicated that Sucuk manufactured without using a starter culture is a reservoir of multiple antibiotic resistant enterococci. Consequently, Sucuk is a potential reservoir for the transmission of antibiotic resistance genes from animals to humans. Other potential risk factors (biogenic amine production and virulence factors) of enterococci isolated from Sucuk should be researched to protect consumer health. Moreover, further investigations are also needed for the determination of the lack of correlation between antibiotic resistance genotypes and phenotypes in enterococci to clarify antibiotic resistance and susceptibility mechanisms of these bacteria.

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