Original Article

Molecular Characterization of *Enterococcus faecalis* and *Enterococcus faecium* Isolated from a Meat Source in Shahrekord Local Markets, Iran

Shahveh, M[1], Tajbakhsh, E[1 *], Momtaz, H[1], Ranjbar, R[2]

1. Department of Microbiology, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
2. Molecular Biology Research Center, Systems Biology and Poisonings Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

How to cite this article: Shahveh M, Tajbakhsh E, Momtaz H, Ranjbar R. Molecular Characterization of *Enterococcus faecalis* and *Enterococcus faecium* Isolated from a Meat Source in Shahrekord Local Markets, Iran. Archives of Razi Institute. 2023;78(4):1387-96.

DOI: 10.32592/ARI.2023.78.4.1387

Article Info:
Received: 13 November 2022
Accepted: 27 November 2022
Published: 31 August 2023

Corresponding Author's E-Mail:
ee_tajbakhsh@yahoo.com

ABSTRACT

Enterococcus faecalis (*E. faecalis*) and *E. faecium* (*E. faecium*) are commensals of the gastrointestinal biota of humans and animals and are considered opportunistic pathogens. This study aimed to improve the knowledge about *E. faecalis* and *E. faecium* isolated from meat. To this end, 104 meat samples were collected from sheep, goats, cattle, and calves from Shahrekord local markets in Iran. Presumptive colonies of *E. faecalis* and *E. faecium* were isolated from the samples and subjected to identification tests. Antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method, and polymerase chain reaction (PCR) was performed to detect some virulence genes. Furthermore, randomly amplified polymorphic DNA typing and repetitive element sequence-based PCR fingerprinting were used to show the clonal relatedness of the isolates. The results revealed that enterococci were present in 90 out of 104 samples. Specifically, *E. faecalis and E. faecium* were the commonly isolated species, with the predominance of *E. faecalis*, which exhibited high resistance to streptomycin (95%) but was susceptible to vancomycin (85.6%). Virulence genes detection showed that *ccf* and *cpd* genes were the most prevalent genes in both species. In addition, the molecular typing method indicated that the isolates belonged to separate subgroups. This study shows the contamination of meat products by potential pathogens and resistant enterococci. There is a need to implement regular surveillance to monitor the emergence of antimicrobial-resistant *E. faecalis* and *E. faecium* in food, particularly in meat production.

Keywords: Antimicrobial susceptibility, Gastrointestinal biota, Meat, Pathogens, Polymerase chain reaction

1. Introduction

Enterococci are spherical Gram-positive bacteria that occur in pairs and belong to humans' and animals' intestinal tracts. As commensals, the *Enterococcus* genus is generally recognized as safe, and its probiotic properties are well-used in the food industry. However, the emergence of opportunistic pathogens harboring resistance and virulence genes makes their use a potential health risk and a significant public health concern (1). The most common enterococcal species with a pathogenic profile are *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) (2). They can be involved in severe nosocomial infections (such as urinary tract infections, peritoneal infections, wound infections, bacteremia, and endocarditis) and various infections for animals (including mastitis, diarrhea, and sepsis) (3). In addition, they can adapt and survive for a long time in new settings with complex conditions (4). When these bacteria are discharged into the environment, they may cause faecal contamination of water, soil, wastewater, fruits, plants, and animal-derived food (5).

The food chain is the main bridge for transmitting resistance between humans and the environment. The entry of enterococci into the food chain is due to poor sanitary conditions (2). Numerous studies reported the presence of enterococci in both fermented and non-fermented foods (5). Enterococci contamination at slaughter is expected because they are present in the intestines of animals (6). They can also rapidly acquire resistance genes from many bacterial species through their mobile genetic elements (7). Some authors reported high antimicrobial resistance rates in enterococci collected from meat in Europe (8) and Korea (9). Therefore, antimicrobial-resistant-enterococci strains from meat may be acquired by humans, which is a genuine human health concern.

Enterococci contain a variety of virulence factors that allow them to invade the gut and attach to various extracellular matrix proteins and epithelial cells (10). These factors have been found in human and animal food strains (11). In Iran, particularly in Shahrekord, few studies have reported the presence of *E. faecalis* harboring resistance and virulence genes isolated from meat (12). In addition, research has only focused on *E. faecalis*, and more data must be collected on the similarity of isolates recovered from meat. In this respect, the current investigation aims to improve the knowledge about *E. faecalis* and *E. faecium* isolated from meat.

2. Materials and Methods

2.1. Sampling, Isolation, and Identification

In the summer of 2019, a total of 104 meat samples were collected from sheep (n=26), goats (n=26), cattle (n=26), and calves (n=26) from Shahrekord local markets in Iran. The samples were promptly sealed in a plastic bag, tagged, and transported in a cold cycle to a microbiological laboratory. To isolate enterococci, 5 g of each sample was suspended in 10 ml of Phosphate-buffered saline in a sterile stomacher bag and centrifuged at medium speed for two minutes using a Seward 400 laboratory stomacher. In 0.85% (w/v) NaCl (Merck™, Germany), specific serial dilutions of the homogenates were made up to 10^{-5}. After that, 100 µl of each dilution was inoculated on kanamycin aesculin azide (KAA) agar (Merck™, Germany) and incubated for 48 h at 37°C. Two typical enterococci colonies on KAA were randomly chosen for further examination from the most significant dilution of each sample. For the early detection of enterococci, Gram stain, catalase test, growth at 6.5% NaCl, and Pyrrolidonyl Arylamidase (PYR) test were used. In this investigation, the arabinose fermentation test was used to distinguish *E. faecalis* from *E. faecium*. To confirm the suspected enterococci colonies, *E. faecalis* ATCC 29212 (Pasteur Institute of Iran) was utilized as a reference strain. Colonies suspected of being *Enterococcus* were identified using the growth and hydrolysis of bile-esculin agar, growth in the presence of 6.5% NaCl, the absence of catalase, the presence of PYR test, 0.04% tellurite reduction, arabinose utilization, arginine dihydrolase activity,

methyl-a-d-glucopyranoside acidification, motility, and pigmentation. Polymerase chain reaction (PCR) using *tfu* primers was used to confirm species identification (13).

2.2. DNA Extraction and PCR Assay

The genomic DNAs from *E. faecalis* isolates were extracted using a DNA extraction kit (Cinapure DNA, CinaClon, Iran) according to the manufacturer's instructions. Following Green and Sambrook (14), the total DNA was measured at 260 nm optical density. To identify enterococci and the virulence genes, PCR was used with mainly targeted primers (15-17).

2.3. Identifying Virulence Genes by PCR

PCR was used to detect genes' virulence factors, including genes, using particular primers. The primer sequence, annealing temperature, and the utilized PCR software are all listed in **(table 1).** PCR was conducted using a DNA thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The amplicons were electrophoresed in 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide at 80 V for 30 min. PCR results were seen and photographed using UV doc gel documentation devices (Uvitec, UK). The 100-bp DNA marker (Fermentas, Germany) was used as a molecular size marker.

Table 1. The oligonucleotide primers and the PCR programs used for amplification of virulence genes of enterococci isolates

Target gene	Primer Oligonucleotide sequences (5'-3')	PCR programme	PCR Volume (50 µL)	Size of amplicon (bp)
E. faecalis ddlE	F: ATCAAGTACAGTTAGTCTTTATTAG R: ACGATTCAAAGCTAACTGA	1 cycle: 95 ^{0C} ---------- 5 min. 32 cycle: 94 ^{0C} ---------- 60 s 60 ^{0C} ---------- 60 s 72 ^{0C} ---------- 2 min 1 cycle: 72 ^{0C} ---------- 5 min	5 µL PCR buffer 10X 2 mM Mgcl2 200 µM dNTP (Fermentas) 0.4 µM of each primers F & R 1 U Taq DNA polymerase (Fermentas) 3 µL DNA template	942
E. faecium ddlE	F: TTGAGGCAGACCAGATTGACG R: TATGACAGCGACTCCGATTCC	1 cycle: 94 ^{0C} ---------- 5 min. 32 cycle: 94 ^{0C} ---------- 60 s 58^{0C} ---------- 60 s 72 ^{0C} ---------- 2 min 1 cycle: 72 ^{0C} ---------- 5 min	5 µL PCR buffer 10X 2 mM Mgcl2 200 µM dNTP (Fermentas) 0.4 µM of each primers F & R 1 U Taq DNA polymerase (Fermentas) 3 µL DNA template	658
asA-1 *glE* *ccf,* *cob* *cpd*	F:GCACGCTATTACGAACTATGA R:TAAGAAAGAACATCACCACGA TE9: ACC CCG TAT CAT TGG TTT TE10: ACG CAT TGC TTT TCC ATC TE53:GGG AAT TGA GTA GTG AAG AAG TE54:AGC CGC TAA AAT CGG TAA AAT TE49 AACATTCAGCAAACAAAGC TE49 TTGTCATAAAGAGTGGTCAT TE51 TGGTGGGTTATTTTTCAATTC TE52: TACGGCTCTGGCTTACTA	1 cycle: 95 ^{0C} ---------- 5 min. 30 cycle: 95 ^{0C} ---------- 30 s 59 ^{0C} ---------- 30 s 72 ^{0C} ---------- 60 s 1 cycle: 72 ^{0C} ---------- 6 min	5 µl PCR buffer 10X 2.5 mm Mgcl2 200 µM dNTP (Fermentas) 0.5 µm of each primers F & R 2 U Taq DNA polymerase (Fermentas) 3 µl DNA template	375 419 543 1405 782

2.4. Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion technique was used to perform antimicrobial susceptibility testing using Mueller-Hinton agar (Merck, Germany) according to the Clinical Laboratory Standards Institute (CLSI, 2019) standards. The following antibiotics were used: ciprofloxacin (CP, 5 µg), tetracycline (TE, 30 µg), meropenem (MEN, 10 µg), co-trimoxazole (SXT, 25 µg), amikacin (AN, 30µg), gentamicin (GM, 10 µg), vancomycin (V, 30 µg), chloramphenicol (C, 30 µg), amoxicillin (AM, 10 µg), streptomycin (S, 10 µg), and cefotaxime (CTX, 30 µg) (produced by PadTan-Teb, Iran).

2.5. RAPD Typing

In random amplified polymorphic DNA (RAPD)-PCR analysis using primers, AP4 primers (5 TCA CGC TGC A 3) were used for RAPD typing (18). The amplified products were electrophoresed in 1 X Tris-Acetate-EDTA on 1.2% agarose gel containing 0.5 µg/ml of ethidium bromide (Genei, Bangalore, India) at 60 V for 1 h. UV illumination was used to see the items, and photographs were stored using a MultiImage Light Cabinet (Alpha Innotech Corporation, USA). Molecular weight markers were utilized to designate band sizes, characteristics, and molecular weights. Simpson's Index of Diversity equation was run to construct a numerical index of the discriminating ability of RAPD typing approaches. The NTSYSpc software (version 2.0) of the Unweighted Pair-Group Method and Arithmetic Mean was used to create dendrograms for cluster analysis of all the isolates.

3. Results

3.1. Percentage of Enterococci Isolated per Type of Meat

Out of 104 samples, enterococci were detected in 90 samples. Forty-two samples (46.66%) were infected with *E. faecalis*, and 40 samples (44.44%) were infected with *E. faecium* (Table 2). The Chi-square test showed a statistically significant relationship between meat type and *E. faecalis* infection ($P<0.05$). According to the Chi-square test, there is no statistically significant relationship between meat type and *E. faecium* infection ($P>0.05$).

3.2. Antibiotic Resistance Pattern of the Enterococci

Figure 1 shows the antibiotic resistance patterns of the *E. faecalis* isolates. The results showed high resistance rates for S (95%), CTX (85.6%), and MEN (80.95%). Most of the isolates showed intermediate resistance to AN (76.2%) but high susceptibility rates to V (90.5%), AM (90.4%), and C (85.7%).

Figure 2 shows antibiotic resistance patterns of the *E. faecium* isolates. The results showed that 50% of isolates were resistant to SXT and CXT. At least 25% of strains have intermediate resistance to each antibiotic tested. The more efficient antibiotics were V (65%), TE (52.5%), and CP (47.5%).

Table 2. Number and percentage of positive cases of *enterococcus* spp isolated from meat in Shahrekord, Iran

Samples		No. of collected samples	No. of samples positive for *E. faecalis* (%)	No. of samples positive for *E. Faecium* (%)
Meat	Cow	26	6 (14.28%)	10 (25%)
	Calf	26	16 (38.1%)	11 (27.5%)
	Sheep	26	10 (23.81%)	9 (23.5%)
	Goat	26	10 (23.81%)	10 (25%)

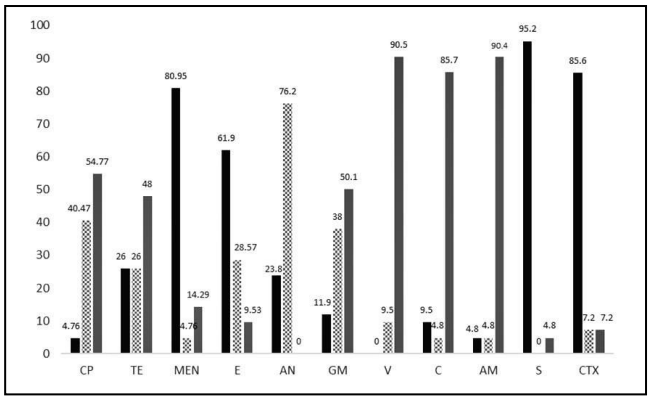

Figure 1. Antibiotic resistance pattern of the *Enterococcus faecalis* isolates. Legend: ciprofloxacin (CP), tetracycline (TE), meropenem (MEN), erythromycine (E), amikacin (AN), gentamicin (GM), vancomycin (V), chloramphenicol (C), amoxicillin (AM), streptomycin (S), and cefotaxime (CTX)

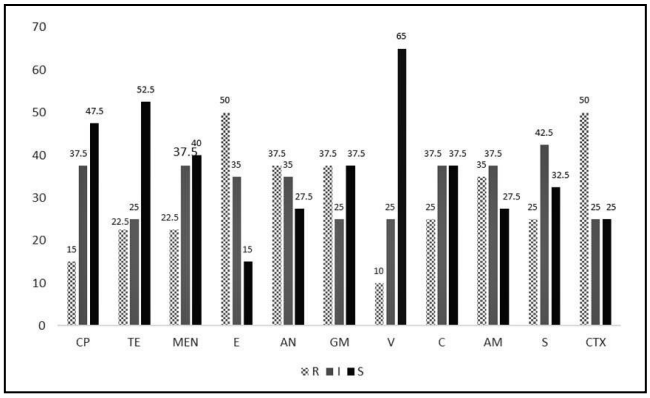

Figure 2. Antibiotic resistance pattern of the *E. faecium* isolates. Legend: ciprofloxacin (CP), tetracycline (TE), meropenem (MEN), erythromycine (E), amikacin (AN), gentamicin (GM), vancomycin (V), chloramphenicol (C), amoxicillin (AM), streptomycin (S), and cefotaxime (CTX)

3.3. Prevalence of Virulence Genes

The frequency of virulence genes in *E. faecalis* and *E. faecium* isolates are shown in table 3. All the tested virulence genes were found in both species. The highest prevalence was obtained for *ccf* and *cpd* genes. Specifically, 76.2% of *E. faecalis* and 75% of *E. faecium* carried the *ccf* gene. The exact prevalence values for both species were also found for the *cpd* gene (Table 4).

Tables 3 and 5 present the prevalence of virulence genes in *E. faecalis* and *E. faecium* isolates by the type of meat. According to the Chi-square test, a statistically significant relationship exists between *asa1* and *cob* genes found in *E. faecalis* and meat type. Concerning *E. faecium*, there is a statistically significant relationship between *asa1* and *ccf* genes and meat type (P<0.05).

3.4. Random Amplified Polymorphic DNA (RAPD)

In the genotyping of *E. faecalis* isolates with RAPD-PCR marker whose dendrogram is shown in figure 3, 30 isolates were studied in 18 profiles (RAPD-PCR), and a 21% to 100% similarity was observed between the isolates. Moreover, a 100% similarity was observed between isolates 1 and 25.

In the genotyping of *E. faecium* isolates with RAPD-PCR marker whose dendrogram is shown in figure 4, 30 isolates were studied in 21 profiles (RAPD-PCR), and a 49% to 100% similarity was observed between the isolates. A 100% similarity was also observed between isolates 19, 22, 21, and 23, as well as between isolates 25 and 26 and isolates 27 and 28.

Table 3. Virulence genes in *E. faecalis* isolates as a function of meat type

Virulence gene	Cow	Calf	Sheep	Goat	*P*-value
gel E	0 0 %	8 19.05	2 4.76	0 0 %	0.000
asa1	0 0 %	4 9.52	0 0 %	0 0 %	0.013
cob	5 11.9%	16 38.1%	8 19.05%	7 16.67%	0.008
cpd	5 11.9%	12 28.57%	8 19.05%	7 16.67%	0.231
ccf	5 11.9%	12 28.57%	8 19.05%	7 16.67%	0.231

Table 4. Prevalence of virulence genes in *E. faecalis* and *E. faecium* isolates

Virulence gene	*ccf* *n* (%)	*cpd* *n* (%)	*cob* *n* (%)	*gel E* *n* (%)	*asa1* *n* (%)
E. faecalis	32 (76.2%)	32 (76.2%)	31 (73.81%)	10 (23.81%)	4 (9.52%)
E. faecium	30 (75%)	30 (75%)	27 (67.55%)	20 (50%)	6 (15%)

Table 5. Virulence genes in *E. faecium* isolates are more common in certain types of meat

Virulence gene	Cow	Calf	Sheep	Goat	*P*-value
gel E	5 12.5%	9 22.5%	2 5%	4 10%	0.114
asa1	0 0%	6 15%	0 0%	0 0%	0.001
cob	4 10%	11 27.5	6 15	6 15	0.162
cpd	8 20%	12 30	6 15	4 10	0.095
ccf	9 22.5%	13 32.5%	5 12.5%	3 7.5%	0.013

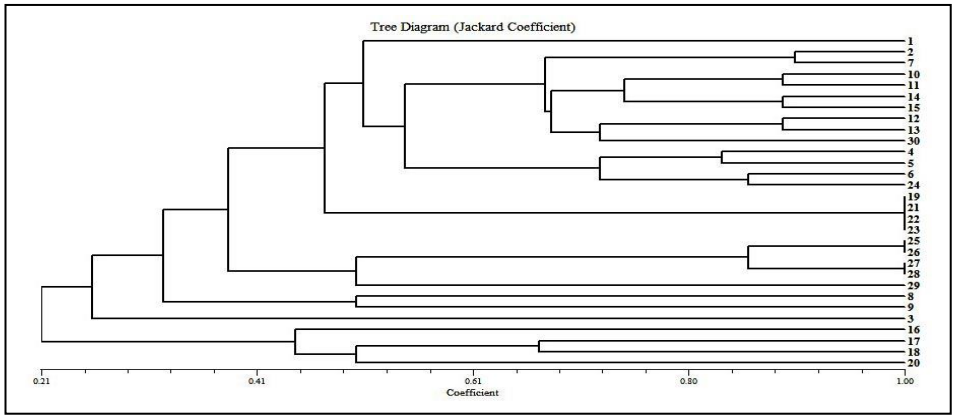

Figure 3. Random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) molecular typing of *E. faecalis* isolates

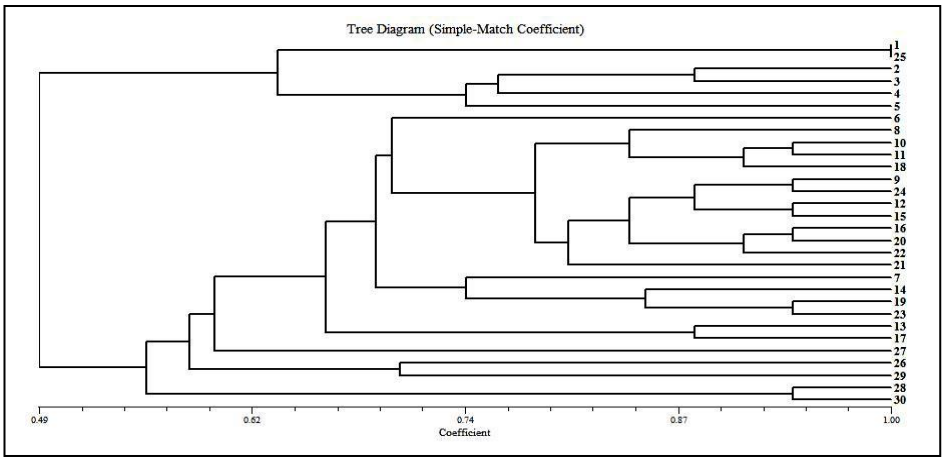

Figure 4. Random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) molecular typing of *E. faecium* isolates

4. Discussion

The most common enterococci recovered from foods are *E. faecalis* and *E. faecium*, which may readily acquire and spread both resistance and virulence determinants. In this regard, investigations are needed to characterize isolates contaminating foods. Therefore, the present study of antimicrobial resistance investigated virulence genes and the clonal relatedness of *E. faecalis* and *E. faecium* isolated from meat sources in Shahrekord, Iran. In this study, 90 out of 104 samples produced enterococci colonies, which implies using enterococci as an indicator of fecal contamination of meat (19). In addition, *E. faecalis* and *E. faecium* are the main enterococci species isolated, with a slightly higher proportion of *E. faecalis* (46.66%) than *E. faecium* (44.44%). Considering the sample size (104 samples), even *E. faecalis* predomination is not significant, which aligns with Tyson, Nyirabahizi (9) results. Furthermore, a nine-year surveillance of antimicrobial trends of *E. faecalis* and *E. faecium* throughout Korea shows that *E. faecalis* is the most dominant species isolated from healthy cattle, pigs, and chickens (20). Statistical analysis performed in our study shows a statistically significant relationship between meat type and *E. faecalis* infection. No study highlights the relationship between meat type and enterococci, but the high proportion of *E. faecalis* may contribute to this observation.

The antimicrobial susceptibility of *E. faecalis* and *E. faecium* isolates from meats differed significantly for both species. Determination of the resistance pattern of the isolates shows that *E. faecalis* isolates exhibit high-level resistance to the tested antibiotics, compared to *E. faecium*. This is consistent with some studies reporting similar observations (19). Specifically, *E. faecalis* exhibited a high resistance against S (95%), which is in line with the findings of many authors also reporting the high prevalence of *E. faecalis* resistance to aminoglycoside in Asia (21) and Europe (22). The use of an aminoglycoside in animal production can largely contribute to the emergence of resistant enterococci (2). These phenomena cannot be neglected since they threaten the efficacy of the broad-spectrum activity of aminoglycosides. In addition, some *E. faecalis* and *E. faecium* strains are naturally resistant to aminoglycosides, cephalosporins, macrolides, and sulphonamides (6). This is consistent with the high proportion of *E. faecalis* resistant to CTX (85.6%) found in this study and their moderate resistance to AN (76.2%). In a study by Golob, Pate (6), all isolates from meat were susceptible to V, which is consistent with the results of this study.

The prevalence of virulence genes shows that all the tested virulence genes were found in both species. The highest prevalence was obtained for *ccf* and *cpd* genes. Specifically, 76.2% of *E. faecalis* and 75% of *E. faecium* carried the *ccf* gene. The exact prevalence values for both species were also found for the *cpd* gene. The present findings support the results of Abouelnaga, Lamas (23), indicating that *ccf* and *cpd* genes are the most common virulence genes.

The chromosomal *gel E* gene encodes the gelatinase enzyme, an extracellular metalloprotease hydrolyzing collagen, gelatin, and small peptides, and it is involved significantly in endocarditis formation in animal models. Gelatinase damages the host tissue, decreases the immune response, and contributes to the activation of the autolysins and degrading of the peptidoglycans, which subsequently leads to DNA release and biofilm formation. *E. faecalis* with gelatinase genes have been identified in about 33% of patients with endocarditis. Heidari, Emaneini (24), (25) reported *gel E* as the most recurrent virulence factor in *E. faecalis* strains. In contrast, some studies have shown the absence or low rate of this gene in enterococcal isolates (26, 27).

The most frequent virulence genes reported by Shokoohizadeh, Ekrami (28) among 56 enterococci isolates in hospitalized burn patients were *gel E* and *asa1* genes in *E. faecalis* (48.5%) and *E. faecium* (43%).

According to Fisher's exact test, there is a significant relationship between meat type and *gel E* and *asa1*

genes in *E. faecalis* isolates, as well as meat type and *asa1* gene in *E. faecium* isolates (P<0.05). The Chi-square test also showed a significant relationship between meat type and *cob* gene in *E. faecalis* isolates, as well as meat type and *cob* and and *ccf* genes in *E. faecium* isolates (P<0.05).

This study used RAPD-PCR to classify *E. faecalis* and *E. faecium* isolated from meat. Numerous studies used RAPD typing and demonstrated its advantages as being easy to use and requiring less expensive epidemiological surveillance tools (29). The present study used RAPD typing to evaluate distantly related enterococcal isolates from a meat source. The generated dendrogram classified *E. faecalis* isolates into 18 profiles and *E. faecium* isolates into 21. The placement of the isolates in several different profiles shows the acceptable differentiation power of this technique in genotyping *E. faecalis* and *E. faecium* isolates. Moreover, the results of this study show that RAPD-PCR is a simple, fast, and low-cost method to describe the genetic diversity of *E. faecium* strains. However, it is recommended that more research is conducted on the samples and in comparison with methods such as Multilocus sequence typing and Pulsed-field gel electrophoresis.

In conclusion, the current study indicates an alarming presence of enterococci species resistant to commonly used antibiotics. Various virulence genes are evidence of the potential threat these strains present to public health. Therefore, the findings support the need to implement regular surveillance to monitor the emergence of antimicrobial-resistant *E. faecalis* and *E. faecium* in food, particularly meat production.

List of Abbreviations

Enterococcus faecalis: *E. faecalis*
Enterococcus faecium: *E. faecium*
kanamycin aesculin azide: KAA
Clinical Laboratory Standards Institute: CLSI
Ciprofloxacin: CP
Tetracycline: TE
Meropenem: MEN
Co-trimoxazole: SXT
Amikacin: AN
Gentamicin: GM
Vancomycin: V
Chloramphenicol: C
Amoxicillin: AM
Streptomycin: S
Cefotaxime: CTX
Random amplified polymorphic DNA: RAPD-PCR
Unweighted Pair-Group Method and Arithmetic: UPGMA

Acknowledgment

The researchers are grateful for the help of the Islamic Azad University's Shahrekord Branch. In addition, the authors would like to thank Manouchehr Momeni Shahraki for his assistance during this work.

Authors' Contribution

All authors collected the data and approved the final version of the manuscript.
Study concept and design: E. T.
Acquisition of data: M. S.
Analysis and interpretation of data: M. S. and R. R.
Drafting of the manuscript: M. S. and E. T.
Critical revision of the manuscript for important intellectual content: H. M. and R. R.
Statistical analysis: M. S. and H. M.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Ethics

Approval for the research study was obtained from the Shahrekord Branch, Islamic Azad University, Shahrekord, Iran ethics board.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Ramsey M, Hartke A, Huycke M. The physiology and metabolism of enterococci. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. 2014.
2. Kim YB, Seo KW, Shim JB, Son SH, Noh EB, Lee YJ. Molecular characterization of antimicrobial-resistant Enterococcus faecalis and Enterococcus faecium isolated from layer parent stock. Poult Sci. 2019;98(11):5892-9.
3. Nowakiewicz A, Ziolkowska G, Troscianczyk A, Zieba P, Gnat S. Determination of resistance and virulence genes in Enterococcus faecalis and E. faecium strains isolated from poultry and their genotypic characterization by ADSRRS-fingerprinting. Poult Sci. 2017;96(4):986-96.
4. Hasanpour F, Neyestani Z, Arzanlou M, Moradi-Asl E, Sahebkar A, Khademi F. Vancomycin-resistant enterococci in Iran: A systematic review and meta-analysis of non-clinical studies. Gene Rep. 2021;24:101265.
5. Bednorz C, Guenther S, Oelgeschlager K, Kinnemann B, Pieper R, Hartmann S, et al. Feeding the probiotic Enterococcus faecium strain NCIMB 10415 to piglets specifically reduces the number of Escherichia coli pathotypes that adhere to the gut mucosa. Appl Environ Microbiol. 2013;79(24):7896-904.
6. Golob M, Pate M, Kusar D, Dermota U, Avbersek J, Papic B, et al. Antimicrobial Resistance and Virulence Genes in Enterococcus faecium and Enterococcus faecalis from Humans and Retail Red Meat. Biomed Res Int. 2019;2019:2815279.
7. Chajęcka-Wierzchowska W, Zadernowska A, Łaniewska-Trokenheim Ł. Virulence factors of Enterococcus spp. presented in food. LWT. 2017;75:670-6.
8. Ogier JC, Serror P. Safety assessment of dairy microorganisms: the Enterococcus genus. Int J Food Microbiol. 2008;126(3):291-301.
9. Tyson GH, Nyirabahizi E, Crarey E, Kabera C, Lam C, Rice-Trujillo C, et al. Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Meats in the United States, 2002 to 2014. Appl Environ Microbiol. 2018;84(1).
10. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology (Reading). 2009;155(Pt 6):1749-57.
11. Franz CM, Stiles ME, Schleifer KH, Holzapfel WH. Enterococci in foods--a conundrum for food safety. Int J Food Microbiol. 2003;88(2-3):105-22.
12. Maleki P, Tajbakhsh E, Rahimi E. Prevalence of ebp A, ebp B and ebp C genes in Enterococcus faecalis producing biofilm isolated from meat in Shahrekord. Navid No. 2018;21(66):41-51.
13. Iweriebor BC, Obi LC, Okoh AI. Virulence and antimicrobial resistance factors of Enterococcusspp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa. BMC Microbiol. 2015;15:136.
14. Green MR, Sambrook J. Molecular cloning. A Laboratory Manual 4th. 2012.
15. Eaton TJ, Gasson MJ. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol. 2001;67(4):1628-35.
16. Tatsing Foka FE, Ateba CN. Detection of Virulence Genes in Multidrug Resistant Enterococci Isolated from Feedlots Dairy and Beef Cattle: Implications for Human Health and Food Safety. Biomed Res Int. 2019;2019:5921840.
17. Togay SO, Keskin AC, Acik L, Temiz A. Virulence genes, antibiotic resistance and plasmid profiles of Enterococcus faecalis and Enterococcus faecium from naturally fermented Turkish foods. J Appl Microbiol. 2010;109(3):1084-92.
18. Banerjee T. Random Amplified Polymorphic DNA (RAPD) Typing of Multidrug Resistant Enterococcus faecium Urinary Isolates from a Tertiary Care Centre, Northern India. J Clin Diagn Res. 2013;7(12):2721-3.
19. Soodmand J, Zeinali T, Kalidari G, Hashemitabar G, Razmyar J. Antimicrobial susceptibility profile of Enterococcus species isolated from companion birds and poultry in the Northeast of Iran. Arch Razi Inst. 2018;73(3):207-13.
20. Kim MH, Moon DC, Kim SJ, Mechesso AF, Song HJ, Kang HY, et al. Nationwide Surveillance on Antimicrobial Resistance Profiles of Enterococcus faecium and Enterococcus faecalis Isolated from Healthy Food Animals in South Korea, 2010 to 2019. Microorganisms. 2021;9(5).
21. Kwon KH, Hwang SY, Moon BY, Park YK, Shin S, Hwang CY, et al. Occurrence of antimicrobial resistance and virulence genes, and distribution of enterococcal clonal complex 17 from animals and human beings in Korea. J Vet Diagn Invest. 2012;24(5):924-31.
22. Seputiene V, Bogdaite A, Ruzauskas M, Suziedeliene E. Antibiotic resistance genes and virulence factors in Enterococcus faecium and Enterococcus faecalis

from diseased farm animals: pigs, cattle and poultry. Pol J Vet Sci. 2012;15(3):431-8.

23. Abouelnaga M, Lamas A, Quintela-Baluja M, Osman M, Miranda JM, Cepeda A, et al. Evaluation of the extent of spreading of virulence factors and antibiotic resistance in Enterococci isolated from fermented and unfermented foods. Ann Microbiol. 2016;66(2):577-85.

24. Heidari H, Emaneini M, Dabiri H, Jabalameli F. Virulence factors, antimicrobial resistance pattern and molecular analysis of Enterococcal strains isolated from burn patients. Microb Pathog. 2016;90:93-7.

25. Sharifi Y, Hasani A, Ghotaslou R, Varshochi M, Hasani A, Aghazadeh M, et al. Survey of Virulence Determinants among Vancomycin Resistant Enterococcus faecalis and Enterococcus faecium Isolated from Clinical Specimens of Hospitalized Patients of North west of Iran. Open Microbiol J. 2012;6:34-9.

26. Cobo Molinos A, Abriouel H, Omar NB, Lopez RL, Galvez A. Detection of ebp (endocarditis- and biofilm-associated pilus) genes in enterococcal isolates from clinical and non-clinical origin. Int J Food Microbiol. 2008;126(1-2):123-6.

27. Tendolkar PM, Baghdayan AS, Gilmore MS, Shankar N. Enterococcal surface protein, Esp, enhances biofilm formation by Enterococcus faecalis. Infect Immun. 2004;72(10):6032-9.

28. Shokoohizadeh L, Ekrami A, Labibzadeh M, Ali L, Alavi SM. Antimicrobial resistance patterns and virulence factors of enterococci isolates in hospitalized burn patients. BMC Res Notes. 2018;11(1):1.

29. Werner G, Willems RJ, Hildebrandt B, Klare I, Witte W. Influence of transferable genetic determinants on the outcome of typing methods commonly used for Enterococcus faecium. J Clin Microbiol. 2003;41(4):1499-506.