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RESEARCH ARTICLE

Comparison of Genotypic Diversity and Vancomycin Resistance of Enterococci Isolated from Foods and Clinical Sources in Adana Region of Turkey [1][2]

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Summary

In this research, genetic diversity and vancomycin resistance patterns were studied and also evaluated as a possible transfusion of vancomycin resistance that may be found from foods to clinical settings between 51 *Enterococcus* spp. isolated from food and 50 human clinical originated *Enterococcus faecium* strains in Adana Region. Identification and antimicrobial susceptibility tests were performed by Vitek-II system and disc diffusion method respectively. Minimum inhibitory concentrations of clinical isolates were confirmed by E-test and the presence of *vanA* and *vanB* genes were investigated by PCR method. Apart from one isolate, none of the food enterococci were resistant to vancomycin, and none of them carried *vanA* and *vanB* resistance genes. All clinical isolates were resistant to vancomycin, and 84% of these isolates carried *vanA*; 2%, *vanB*; and 14% neither *vanA* nor *vanB* genes. Genetic diversity within each group; 6 clusters of colonization isolates and 5 clusters of food isolates were found to be closely related by Pulsed Field Gel Electrophoresis method. Although no genetic relation was found among foods and human clinical infection isolates; 2 clusters of foods and human intestinal isolates were found to be closely related. Finally, vancomycin sensitive *E. faecium* strains from food colonized in humans acquired *vanA* and *vanB* resistance genes and are thought to be a reservoir for vancomycin resistance. These results revealed that food enterococci should be carefully monitored in food industry in terms of their genetic relation to infection species.

Keywords: *Enterococci, Food, PFGE, Vancomycin resistance*

Türkiye'nin Adana Bölgesinde Gıda ve Klinik Kaynaklı Enterokokların Genotipik İlişkilerinin ve Vankomisin Direnç Özelliklerinin Karşılaştırılması

Özet

Bu çalışmada, Adana Bölgesinde gıdalardan izole edilen 51 *Enterococcus* spp. ile klinik orjinli 50 *Enterococcus faecium* türlerinin vankomisin direnç paternleri ve genetik ilişkileri çalışılmış, ayrıca vankomisin direncinin yayılmasında gıda kaynaklı olası bir geçişin olup olmadığı araştırılmıştır. Tanımlama ve antimikrobiyel direnç testleri sırasıyla Vitek-II ve disk diffüzyon metodları ile araştırılmıştır. Klinik izolatların minimum inhibitor konsantrasyonları E-test ile doğrulanmış; tüm izolatlarda *vanA* ve *vanB* direnç genleri PCR metodu ile araştırılmıştır. Bir izolat dışında, tüm gıda izolatlarının vankomisine dirençli olmadığı ve hiçbir suşun *vanA* ve *vanB* geni taşımadığı saptanmıştır. Klinik izolatların tümünün vankomisine dirençli olduğu, %84'ünün *vanA*, %2'sinin *vanB* genlerini taşıdığı; %14'ünün *vanA* ve *vanB* direnç genlerini taşımadığı belirlenmiştir. Pulsed Field Gel Electrophoresis metodu ile yapılan genetik ilişkilendirmede; 6 kolonizasyon kümesi ve 5 gıda kümesi kendi içlerinde yakın ilişkili bulunmuştur. Gıda ve klinik enfeksiyon izolatları arasında genetik ilişki saptanmamışken, 2 adet kolonizasyon ve gıda kümeleri arasında yakın ilişki bulunmuştur. Sonuç olarak, Adana bölgesinde gıda kaynaklı vankomisine duyarlı *E. faecium* türlerinin insanlarda kolonize olabildikleri, *vanA* ve *vanB* direnç genleri kazanarak vankomisin direncinin yayılmasında rezervuar olabilecekleri düşünülmektedir. Bu sonuçlar, gıda endüstrisinde gıda kaynaklı enterokokların enfeksiyon etkeni izolatlar ile genetik olarak ilişkileri açısından dikkatle izlenmesi gerektiği sonucunu ortaya çıkarmıştır.

Anahtar sözcükler: *Enterokok, Gıda, PFGE, Vankomisin direnci*

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INTRODUCTION

Enterococci live as commensals of the gastrointestinal tract of warm-blooded animals and are the most abundant Gram-positive cocci in humans [1,2] as well as in soil, waters, raw plant and animal products. *E. faecium* and *E. faecalis* are important in food microbiology because of the lypolitic, esterolytic activities, using citrate and making aromatic compounds in foods. They also produce good organoleptic features in some foods. Thanks to these properties, enterococci are used together with lactic acid bacteria in some fermented dairy and meat products as starter cultures as well as probiotics to improve the microbial balance of the intestinal tract in humans and animals and can be used in the treatment of gastroenteritis in humans and animals [2]. However, the *Enterococcus* genus has more beneficial effects in food industry and is not considered "generally recognized as safe" (GRAS) due to its use as an indicator of fecal contamination and the frequent association with food-borne illnesses by biogenic amines production [2,3] but is recognized as important nosocomial pathogens causing endocarditis, bacteremia, and central nervous system infections as well as neonatal, respiratory tract, urinary tract, and other infections [2,4] which may be linked to the presence of antibiotic resistance and virulence properties.

Enterococci are able to acquire resistance determinants through gene transference by mobile genetic elements. Resistance of enterococci to therapeutically important antibiotics gain resistance to the glycopeptides vancomycin and teicoplanin, often associated with high-level resistance to amino glycosides [2]. The emergence of vancomycin-resistant enterococci, belonging predominantly to *E. faecium*, has resulted in cases of untreatable infections [1,2]. Vancomycin resistance is encoded by the *vanA* gene cluster carried on the mobile genetic element Tn1546. Transfer of resistance can be fulfilled by conjugative plasmids [1,2]. Dissemination of antimicrobial resistance genes through clonal expansion and horizontal transmission is so important for infectious disease specialists. Compared to animal products, fruits and vegetable foods are usually consumed raw, so they are more effective in transmission of antibiotic resistance traits since animal products are consumed by cooking, and in this way enterococci are inactivated. However, only few studies on the incidence of antibiotic resistance among enterococci from fruits and vegetable foods have been reported [5,6].

Accurate species identification and strain typing are important to evaluate the genetic diversity among enterococci populations and to select nonpathogenic bacteria for further use in food technology and probiotics [2,7]. Genetic typing techniques such as Pulsed-Field Gel Electro-phoresis [PFGE] analysis of *SmaI* macro restriction profiles are considered to be the "gold standard" for genotyping of enterococci with more discriminative power than other techniques [8,9].

Risk factors for VRE (vancomycin resistant enterococci) colonization may be patient-, hospital-, environment-, and antibiotic-related. Although many studies have been performed in Europe and the USA on the prevalence, incidence, epidemiology, and risk factors of VRE, data obtained in the Middle East and Asia are very rare [1,10].

The objectives of the present study were to determine the genetic diversity and vancomycin resistance of enterococci from food and clinical origins in Adana region of Turkey and to have a better understanding of the different reservoirs in the emergence and the spread of vancomycin resistance.

MATERIAL and METHODS

This study has an ethics report from "Turkish Republic, University of Cukurova, Faculty of Medicine Ethical Board of Scientific Research" with 15 decision code and number 6 on 10.03.2011.

Materials

In this study, a total of 80 food samples [*n*=28 cheese, *n*=10 fruit (raw and pickled olives and tomatoes), *n*=16 vegetables (lectures, packed salads, cabbages and purple cabbages), *n*=21 sucuk (a traditional Turkish meat product) and *n*=5 chicken meat] were purchased from various markets to determine whether enterococci were present in the food samples. The analyses were carried out during the day with an evaluation of a total of 50 enterococci isolates obtained from various clinical specimens provided by the Central Laboratory of Balcalı Hospital, Adana-Turkey during 2010-2011. Of these isolates, 25 were isolated from clinical specimens in patients with nosocomial infection, 25 from stool or rectal specimens in patients with intestinal colonization. All clinical isolates selected at the hospital were VRE.

Methods

Identification of Enterococci

For identification purposes; food samples were homogenized before used. After homogenization, samples (10-25 g) were weighed, and their appropriate dilutions were prepared. For *Enterococcus* spp., isolation diluted samples were cultivated on kanamycin aesculin azide agar (KEA agar) (Merck KGaA, Germany) then Slanetz agar (Merck KGaA, Germany) and incubated at 37°C, for 24-48 h [6,11]. For isolation of *Enterococcus* spp., Gram positive cocci isolated from kanamycin aesculin azide agar (KEA) medium were morphologically evaluated, and strains isolated from colonies showing typical enterococci morphology were subject to the following tests to fulfill identification

procedures: Catalase production, Gram staining, gas production from glucose and growing in 6.5% NaCl. Clinical isolates were routinely grown on Columbia agar (Becton-Dickinson, Sparks, MD) supplemented with 5% defibrinated sheep blood (supplied from Experimental Surgery Center of Medical Faculty) at 37ºC for 24 h. All suspected colonies were identified by VITEC automated identification system in Balcali Hospital Central Laboratory, The Faculty of Medicine, Çukurova University (Biomerieux, Durham, North Carolina, USA).

All identified isolates (food and clinical) were stored in Brain Hearth Broth (BHI) including 10% sheep blood and 10% glycerol at -20ºC until the completion of genotypic analysis.

Characterization of Vancomycin Susceptibility Test

Vancomycin susceptibility testing of isolates from food samples was performed using disk diffusion method by CLSI [12] guidelines, using antibiogram discs of vancomycin (Oxoid ltd.)(concentration of vancomycin disc is expressed in 30 µg mL^{-1}). The strains were cultivated on Mueller-Hinton Agar (Merck KGaA, Germany), and then antibiotic discs were located through a dispenser. After incubation (24 h, 37ºC), bacteria strains were classified as resistant, intermediate and sensitive by CLSI document criteria by measuring inhibition zone diameters around the antibiotic discs [12].

Vancomycin resistance patterns of clinical strains and suspected food isolates were evaluated by using Gram-positive antibiotic susceptibility cards (Biomerieux Vitek-2-AST-P534-SA-France) in VITEK-2 automated (Biomerieux, Durham, North Carolina, USA) identification system. The results were recorded following 18-24 h of incubation at 37ºC, and were evaluated by producer instructions using the breakpoints for enterococci proposed by the CLSI [12]. *Enterococcus faecalis* 1047387 (vancomycin sensitive) and *Enterococcus faecium* 1045803 (vancomycin resistant) collected from Balcali Hospital Central Laboratory as reference strains were used in antibiotic resistance tests.

Detection of vanA and vanB Genes

Genomic DNA was extracted mechanically by the "Mickle Sytem" (The Mickle Lab.Engeneering Co. Ltd. Gomshall, Surrey, UK) by producer instructions after overnight cultures in 5% defibrinated sheep-blood agar of enterococci. A spectrophotometer (UV-VIS Spectrophotometer CHEBIOS) was used for quantitation of DNA samples. Extracted DNAs of enterococci were stored at -20ºC until within use as a template for PCR amplifications.

PCR was performed to screen vancomycin resistance genes (*vanA*, *vanB*) as described previously [13,14]. The specific primers and PCR conditions were presented in *Table 1*. Amplicons were analyzed by electrophoresis using 2% agarose gels [PegGOLD Universal Agarose, 91052 Erlangen Deutschland, 2%(w/v)] containing 0.5% ethidium bromide in TBE buffer (40 mM tris, 20 mM boric acid and 1 mM EDTA, pH 8.3) for 30 min at 120 V in the presence of a 50-bp DNA ladder (Fermentas SMo.323-Lithuania). The gel was photographed on a UV transilluminator (Kodak Gellogic-1500 imaging system).

Minimal Inhibitory Concentrations (MICs) Testing

MICs of the antibiotic vancomycin (Va) was determined by VITEK-2 AST compact panel (Biomerieux Vitek-2-AST-P534-SA-France) and E-test (Biodisk, Solana, Sweden) in Mueller-Hinton agar, and the results were interpreted based on CLSI criteria [12].

Hemolytic Activity Testing

For hemolytic activity, all species were cultivated in sheep blood agar. After incubation (24 h, 37ºC), bacteria strains were categorized as α-hemolytic, β-hemolytic and non hemolytic [15].

Pulsed Field Gel Electrophoresis (PFGE) Analysis

The clonal relationship among isolates was established by PFGE method. Genomic DNA was prepared in agarose plugs according to methods previously described [16] and run on a CHEF-DR II (Bio-Rad Laboratories, Nazared, Belgium) machine, and the electrophoresis conditions used in this study were described previously [17]. In the first block, the initial switch-time was 3.5 s, the final switch-time was 20 s, and the run-time was 12 h, and in the second block, the initial switch time was 1 s, and the final switch time 5 s for 8 h, at 6 V/cm^2. *SmaI* was the enzyme used for cleaving the DNA (Lambda Ladder PFG Marker, New England BioLabs Inc.). Our index strains isolated from blood samples of two bacteriaemic patients in nosocomial infection outbreak were formed in 2011 and placed in our library and used as a molecular size marker and an internal

Table 1. *List of primers and amplification conditions used in the present study*
Tablo 1. *Çalışmada kullanılan primerler ve amplifikasyon şartları*

Gene	Primers sequence (5'-3')	Product Size (bp)	Amplification Conditions	Reference
vanA	TCT GCA ATA GAG ATA GCC GC	375	Initial cycle of 94ºC for 5 min; 30 cycles of 94ºC for 30 s, 48ºC for 30 s, 72ºC for 30 s; 1 cycle of 72ºC for 7 min	[14]
	GGA GTA GCT ATC CCA GCA TT			
vanB	GTG ACA AAC CGG AGG CGA GGA	527	Initial cycle of 94ºC for 5 min; 30 cycles of 94ºC for 40 s, 58ºC for 60 s, 72ºC for 30 s; 1 cycle of 72ºC for 30 min	[13]
	CCG CCA TCC TCC TGC AAA AAA			

control. GelCompar II software system (version 5.0; Applied Maths, Sint-Martens Latem, Belgium) was used to calculate the percentage of similarity (Dice coefficient) of *SmaI* pulsed-field gel electrophoresis (PFGE) banding patterns. Primarily, normalization was fulfilled using three standards bands in every picture of agarose gel. Unweighted pair group method with mathematical averaging (UPGMA) was used for creating dendograms and cluster analysis of PFGE profiles. Band and profile tolerance were used 1.5% for calculating similarity coefficent. Isolates were considered closely related if their PFGE banding patterns were ≥80% similar, and were indicated with capital letters, and if subtypes were in the same cluster, they were shown with numbers.

RESULTS

Among the food samples tested, a total of 51 isolates from 80 food samples were identified as presumptive enterococci. Of these, 51 isolates were identified as the following species; *E. faecium* (20 isolates, 39.2%), *E. faecalis* (12 isolates, 23.5%), *E. casseliflavus* (7 isolates, 13.8%), *E. gallinarum* (4 isolates, 7.8%), *E. durans* (7 isolates, 13.8%) and *E. raffinosus* (1 isolates, 1.9%). From a starting collection of 50 clinical isolates (corresponding to 50 patients), were identified as *E. faecium*. Most of isolates (9, 36%), (2, 8%), (2, 8%), (2, 8%) and (10, 40%) in nosocomial environment were from intensive care internal medicine, pediatric hematology, brain surgery, urology and other clinics respectively, while most of the intestinal isolates (15, 56%), (4, 16%), (2, 8%) and (4, 20%) were from pediatric hematology and oncology, brain surgery intensive care, burn unit and other clinics respectively.

In the present study, 39 (76.4%) of food enterococci were found to be sensitive to vancomycin, and 11 (21.5%) of these were found intermediate sensitive to vancomycin. Only one strain identified as *E. casseliflavus* and collected from a lettuce sample was found to be resistant to vancomycin. It's MIC value was found 64 µg/mL. All clinical *E. faecium* strains were found to be resistant to vancomycin and teicoplanin, and their MIC values were found over 128 µg/mL (*vanA* type).

The results of prevalence of genes encoding vancomycin resistance in enterococci isolated from food and clinical samples showed that, none of the food isolates carried *vanA* and *vanB* resistance genes, and 84% of clinical enterococci carried *vanA*, 2% of them *vanB,* and 14% of them carried neither *van A* nor *van B* genes.

Hemolytic activities of enterococci showed that non-hemolytic, α-hemolytic and β-hemolytic in food isolates were 32 (62.7%), 13 (25.4%) and 6 (11.7%) respectively. All human intestinal isolates were found non-hemolytic; but 50% of clinical *E. faecium* isolates were found to be α-hemolytic and 14% were β-hemolytic.

PFGE Analysis

The genome of 25 *E. faecium* strains leading to endogenous colonization strains from stool or perirectal swabs and the genome of 20 *E. faecium* strains from food samples were investigated by PFGE. It was found that band sizes of them ranged from 10 to 300 kb *(Fig. 1)*. According to band patterns, "A1-Gamma" was gathered in the so-called 13 different clonal clusters and 16 unique isolates *(Fig. 2)*. The largest two clusters were composed of four and three subtypes and named "I" and "G". In addition, closely related 11 clusters were included into two subtypes and named "A, B, C, H, J, L, S, T, U, W, Beta," forming clusters of food and human intestinal colonization isolates.

Isolates belonging to PFGE types "A, B, C, W, Beta" were also obtained from samples of different food origins. Human intestinal *E. faecium* isolates were shown to be closely related in PFGE types of "G, H, I, J, S, T," by using PFGE method. Food and intestinal enterococci isolates were found to be closely related in PFGE groups of "L and U" *(Fig. 2)*. Furthermore, this study found no clonal relation between *E. faecalis, E. durans, E. casseliflavus* and *E. gallinarum* isolated from food.

As a result of the PFGE investigation, based on band patterns of 25 *E. faecium* strains of clinical infection and 20 *E. faecium* strains of food origin, the clonal relationship could not be determined between food and clinical infection isolates. Both groups formed a separate group.

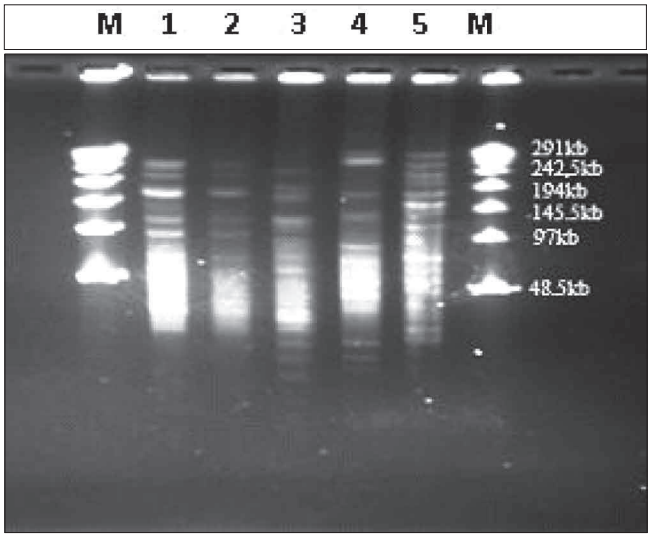

Fig 1. PFGE of *SmaI*-digested genomic DNA from five *Enterococcus* spp. isolates from different food sources. From left to right, M: γ ladder (New England BioLabs Inc.), 1- *E. faecium (cheese)*, 2- *E. faecium*, *(cheese)*, 3- *E. casseliflavus (sucuk)*, 4- *E. faecalis (sucuk)*, 5- *E. faecium (white cheese)*

Şekil 1. Farklı gıda kaynaklarından izole edilmiş beş adet *Enterococcus* spp.'nin *SmaI* ile kesilmiş genomik DNA'larının PFGE ile elde edilen jel görüntüleri. Soldan sağa, M: γ ladder (New England BioLabs Inc.), 1- *E. faecium (peynir)*, 2- *E. faecium (peynir)*, 3- *E. casseliflavus (sucuk)*, 4- *E. faecalis (sucuk)*, 5- *E. faecium (beyaz peynir)*

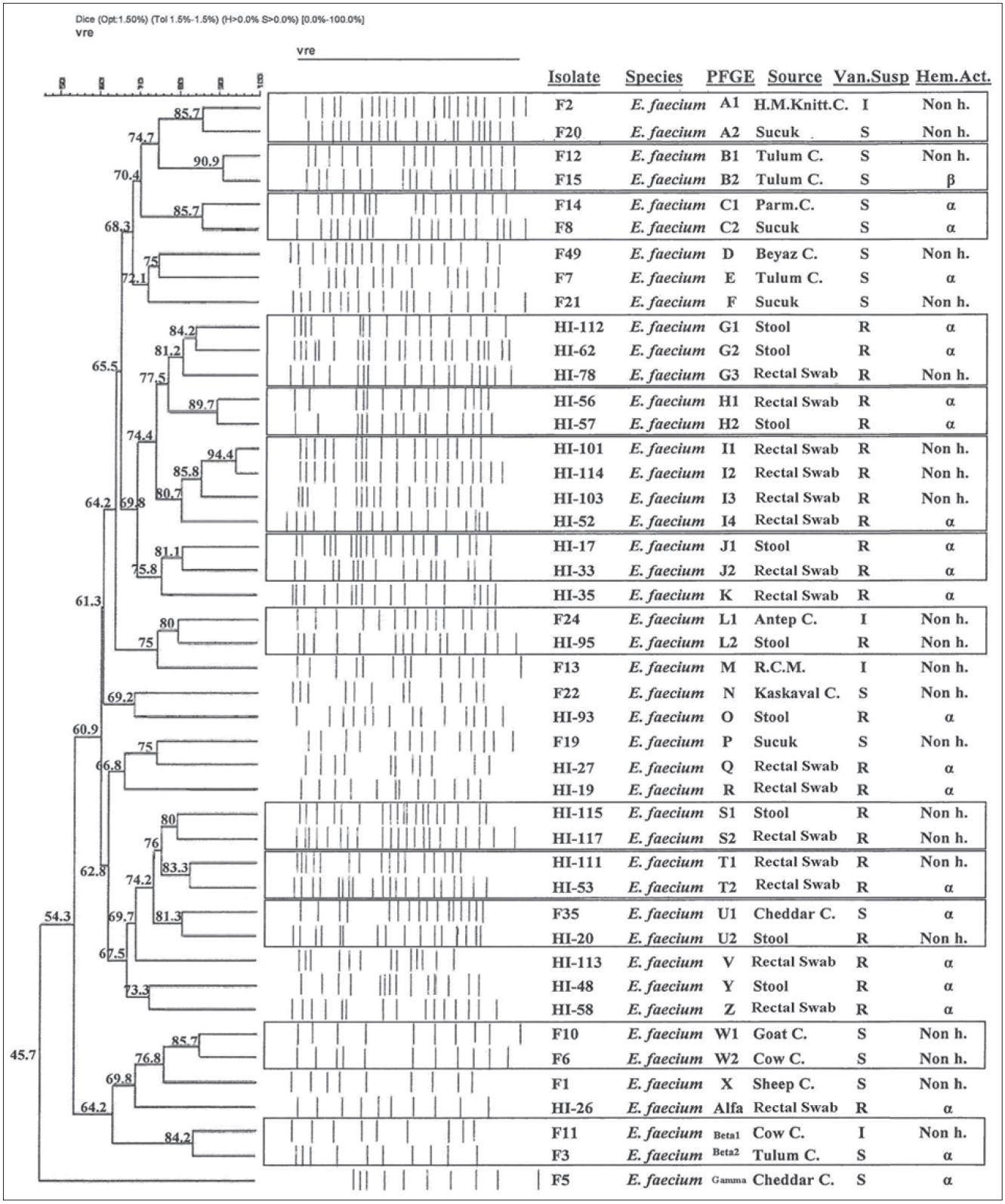

Fig 2. Dendrograms showing the similarity index among the 45 strains of *Enterococcus faecium* from human intestinal (25 strains) and foods (20 strains) are included. PFGE types are indicated for isolates tested by pulsed field gel electrophoresis (PFGE). Clusters sharing 80% or bigger similarity are shown boxed; F; Food isolate, HI; Human Intestinal Isolate, C; Cheese, R.C.M.; Raw Chicken Meat, Hm Knitt.; Home made Knitting Cheese, Van. Susp.; Vancomycin Susceptibility, Hem. Act; Hemolytic Activity, S; Sensitive, I; Intermediate, R; Resistant

Şekil 2. Gıda kaynaklı (20 suş) ve klinik kolonizasyon etkeni (25 suş) olan 45 *E. faecium* suşları arasındaki benzerlik indeksini gösteren dendogramlar. PFGE tipleri, Pulsed Field Gel Elektophoresis yöntemi ile elde edilmiştir. %80 ve üzeri benzerlik gösteren gen kümeleri kutu içine alınmıştır. F; gıda izolatı, HI; klinik kolonizasyon izolatı, C; peynir, R.C.M.; çiğ tavuk eti, Hm Knitt.; ev yapımı örgü Peyniri, Van. Susp.; vankomisin direnç özelliği, Hem. Act; Hemolitik aktivite, S; hassas, I; orta derecede hassas, R; dirençli

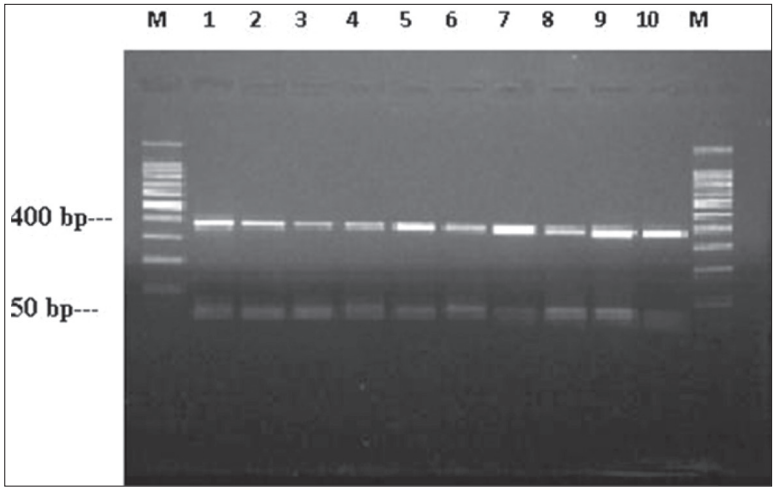

Fig 3. Agarose gel electrophoresis showing positive amplification of 375 base fragments specific for *van*A gene of VRE strains from clinical specimens. Lane M: Size marker (50-bp DNA ladder); lanes 1-10: clinical VR*E. faecium* strains

Şekil 3. Klinik kaynaklı VRE suşlarının 375 bp'de *van*A genine özgü pozitif amplifikasyon fragmentlerini gösteren agaroz jel elektroforezi. M: Moleküler belirteç (50-bp DNA ladder); 1-10: klinik kaynaklı VR*E. faecium* suşları

DISCUSSION

In this study, *E. faecium* was the most abundant species in human and food samples. Our results are different compared to the studies performed previously [6,18]. The species found most abundant in their study were *E. faecalis,* whereas the species found most abundant in our study were *E. faecium*. The studies show that enterococci can also be found in a variety of food products such as dairy products, meat and vegetables. The dominant flora can vary according to products or environment [18].

In sucuk, raw chicken meat, fruit and vegetables, the strains of *E. faecalis* are the second prevalent species, which could be explained by the fact that these foods are manipulated by hands suggesting a possible contamination during manufacturing process. Our results showed different species prevailing in the different reservoirs similar to some other studies [10,19]. As expected, *E. faecium* was the most prevalent species irrespective of the source, whereas *E. faecalis, E. durans, E. gallinarum, E. casseliflavus* and *E. raffinosus* were only isolated from food samples. Some studies show that, *E. faecium* dominant in hospitals by undergoing clonal selection either alone or together with Staphylococci rapidly increases the incidence of nosocomial hospital infections and is accepted as epidemic or hospital-acquired [20,21]. Similarly, all our clinical strains were classified as *E. faecium.*

Only one *E. casseliflavus* isolated from lettuce was resistant to vancomycin and did not carry *vanA* and *vanB* resistance genes. This might be an intrinsic resistance of *E. casseliflavus,* and its MIC value of vancomycin was 64 µg/mL. This study also investigated the prevalence of genes encoding vancomycin resistance in enterococci isolated from food and clinical samples. None of food isolates carried *vanA* and *vanB* resistance genes. Data on the incidence of vancomycin resistance within different types of food (especially dairy and meat products) enterococci occur at different extents depending on the geographical area [22]. In a recent study conducted in Turkey, *vanA* gene was found in only one *E. faecalis* strain isolated from a cheese sample and was intermediately resistant to vancomycin [23].

When all the clinical *E. faecium* strains are analyzed by using PCR for the detection of *vanA* and *vanB* genes, 84% of them carried *vanA*, and 2% of them *vanB,* and 14% of them neither *vanA* nor *vanB* genes *(Fig. 3)*. The results of our clinical isolate genotypes have been found to be similar to the previous studies (generally the most clinical isolates carried *vanA* genes) performed in Turkey [24,25]. However, different studies showed variable results concerning the detection rates of *vanA* and *vanB* genes in Middle East Countries such as; in Kuwait hospitals, all VRE strains carried the *vanA* genotype and *vanB* gene was not detected in any of the isolates [26]. Sharifi et al.[27] reported that high prevalence of *E. faecium* harbored vancomycin resistance with *vanA* genotype (89.5%) in North West Iran, which also showed that all VRE strains carried the *vanA* genotype, and *vanB* gene (6.3%) was detected as phenotypically sensitive in their isolates [27].

The emergence of antibiotic resistant enterococci in food samples would be the massive use of antibiotic in agriculture (e.g., avoparcin as animal growth promoters). Avoparcin was legally banned in 1998 in European Countries, and VRE isolation rate has increased and showed some differences depending on the geographical areas [28]. Biavasco et al.[19] reported that, the role of different reservoirs in the spread of glycopeptide resistance in European Countries was not obvious. While VRE infections acquired in several research hospitals in Turkey have been reported since 1999, the role and mechanism of the reservoirs in the spread of glycopeptide resistance was not explicit in our country, either. Few studies have been performed on vancomycin resistance and genetic diversity of enterococci clinical isolates in Turkey as well as Middle East Countries [10,24,29].

In this study, we compared GRE (Glycopeptide Resistant Enterococci) isolates of food and clinical origins (a broad

collection of *E. faecium* isolates from different human origins; wound fluid, sterile sites, urine and intestinal origin). PFGE results showed a polyclonal distribution of vancomycin resistance of isolates in the different reservoirs; however, the presence of some clones in different reservoirs was observed. For example, closely related clones were isolated from both foods (PFGE groups of L, U) and intestinal samples, suggesting food isolates may have the ability of human colonization. These results implied that, some *E. faecium* isolates from stools and perirectal swaps were highly related but not indistinguishable from food isolates. This may suggest that some human isolates collected in this study might be the reisolation of commercial starter culture isolates. Similar results were found by Vankerchoven et al.[5] for clinical and probiotic enterococci isolates, and Vancanneyt et al.[9] for (potentially) probiotic *L. rhamnosus* isolates.

Our results are different from those of Vancanneyt et al.[9] in terms of vancomycin susceptibility and hemolytic activities of closely related genomic groups. In their study, they differentiated two main genomic groups (I and II) among *E. faecium* by a combination of RAPD-PCR and AFLP (amplified fragment length polymorphism) methods. They reported that human clinical strains, antibiotic resistant strains, and beta hemolytic strains were found only in genomic group I. By contrast, in our study, vancomycin resistance and hemolytic activities of closely related food and human intestinal colonization isolates were found to be different *(Fig. 2)*. Interestingly, closely related two intestinal *E. faecium* strains (HI-115 and HI-117) carried neither *vanA* and nor *vanB* resistance genes. On the other hand, intestinal *E. faecium* strain (HI-95) carried *vanB* gene and was closely related to a food strain (F24). In addition, other related intestinal *E. faecium* strain (HI-20) carried *vanA* gene and was closely related to another food strain (F35). These results suggested that, food enterococci can acquire *vanA* or *vanB* gene in colonization section or these food isolates might be human originated.

We could not find a relationship between foodborne enterococci isolates and clinical VRE infection isolates. Foodborne and clinical isolates formed a separate group by PFGE. Our results are similar to the results of Abriouel et al.[6]. They found isolates of clinical samples clustered in separate groups with LH-PCR (length heterogenity-polimerase change reaction) typing methods.

As a result, the genetic relation between food and intestinal *E. faecium* isolates show that commercial strains used in the cheese-making production or/and contamination occur via staff during the cheese-making process. It may affect the genetic profile of the strains. The clonal relationship between each food isolates suggested that the same commercial starter cultures may be used in cheese and sucuk- making process. The genetic relation of vanA type (carrying *vanA* gene) in human intestinal isolates suggested that occasional clonal dissemination can occur in hospital environments. Finding 7 *E. faecium* [3 urine, 1 CSF (cerebrospinalfluid) and 3 intestinal isolates] strains with non *vanA* and non *vanB* genotype, but resistant to vancomycin show that other resistant genes (*vanC, vanD,* etc.) might also be important in the vanA phenotype of VRE isolates or some polymorphism might occur in the detection of resistance genes. More genotyping studies are needed to understand the mechanism of VRE dissemination between food (including starter cultures) and clinical origin in our country as well as in the geographical area of Middle East in the future.

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