Kocatepe Veterinary Journal

*Kocatepe Vet J (2017) 10(2): 76-82*
**DOI:** 10.5578/kvj.54067
**Submittion**: 27.02. 2017
**Accepted**: 03.05.2017

**RESEARCH ARTICLE**

# Detection of Prevalence, Antibiotic Resistance and Virulence Factors of *Enterococcus* spp. Isolated From Ready to Eat Foods

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**ABSTRACT**

In this study, we identified the prevalence of *Enterococcus* spp., antibiotic resistance and several virulence factors of some ready-to-eat foods. Totally 114 *Enterococcus* spp. were isolated in 112 (59.90 %) of the 187 food samples analysed. *Enterococcus* spp. isolates were obtained from 39 samples of meat products (34.80 %), 42 samples of cheese brands (37.50 %), 25 samples of salads (22.30 %) and eight samples of halva (7.10 %). According to the results of the antibiotic resistance test, the Enterococci isolates obtained were determined to show resistance to at least 4 of the antibiotics used in the study. While no gelatinase activity was observed in any of the isolates, haemolysin activity was observed to be positive in 36 of them (31.60 %). As a result, having been regarded for years as harmless and reported likely to be used as a starter culture, some *Enterococcus* spp. pose a risk to public health and to food safety since they have virulence factors and strong antimicrobial resistance. For this reason, the *Enterococcus* spp. to be used as a starter in the food industry should be chosen from among those that don't have pathogenicity and antibiotic resistance genes.

**Key Words:** Antibiotic resistance, *Enterococcus* spp., Ready-to-eat foods, Virulence factors.

## Tüketime Hazır Bazı Gıdalarda *Enterococcus* spp. Prevalansı, Antibiyotik Dirençlilik ve Virülens Faktörlerinin Tespiti

**ÖZ**

Bu çalışmada tüketime hazır bazı gıdalarda Enterokok türlerinin prevalansı, antibiyotik dirençliliği ve virülans faktörleri belirlendi. Analize alınan 187 gıda örneğinin 112 (%59,9)'sinde 114 *Enterococcus* spp. izole edildi. Et ürünlerinden 39 (%34,8), peynirlerden 42 (%37,5), salatalardan 25'i (%22,3) ve helva örneklerinden 8 (%7,1)'inde *Enterococcus* spp. izolatı elde edildi. Antibiyotik dirençlilik testi sonuçlarına göre, elde edilen Enterokok izolatlarının çalışmada kullanılan antibiyotiklerden en az dördüne dirençlilik gösterdiği tespit edildi. İzolatların hiçbirinde gelatinaz aktivitesi gözlenmezken, 36'sında (%31,6) hemolizin aktivitesi pozitif tespit edildi. Sonuç olarak starter kültür olarak kullanılabileceği ve insanlar için zararsız olduğu düşünülen bazı Enterokok türlerinin, virülens faktörler ve sahip olabilecekleri antimikrobiyal direnç bakımından halk sağlığı ve gıda güvenliği açısından bir risk oluşturabilmektedir. Bu nedenle gıda endüstrisinde starter olarak kullanılabilecek Enterokok türleri, patojenite özelliği bulunmayan ve antibiyotik direnç genlerine sahip olmayanlardan seçilmelidir.

**Anahtar Kelimeler:** Antibiyotik dirençlilik, *Enterococcus* spp., Tüketime hazır gıda, Virülens faktörler.

To cite this article: **Gokmen M. Onen A. Ektik N. Kara R. Torlak E. Metli M.** Detection of Prevalence, Antibiotic Resistance and Virulence Factors of *Enterococcus* spp. Isolated From Ready to Eat Foods. *Kocatepe Vet J. (2017) 10(2): 76-82.*

## INTRODUCTION

*Enterococci* are the kind of bacteria that can develop in diverse environmental conditions and which can be found abundantly in the digestive tracts of mammals, in the air, in water, in sewage, in the soil and on the vegetative cover (Gardin et al. 2001, Sadowsky and Whitman 2011). As well as in these environments, they are found in many kinds of food including meat, milk and plant-based foods (Ben-Omar et al. 2004). Enterococci can survive in a heat treatment of 30 minutes at 63.5°C (Gardin et al. 2001) and can cause spoilage especially in meat that is put through heat treatment and processed (Franz et al. 1999). Aggregation substance, gelatinase, extracellular superoxide and extracellular surface protein and haemolysin are important virulence factors for enterococci (Foulquié Moreno et al. 2006). They can be used as starters because of their ability lipolytic and proteolytic activity and to supply the desired organoleptic volatile compounds in such specific food as cheese and fermented sausages (Foulquié Moreno et al. 2006, Giraffa 2002). Besides, enterococci that produce such antimicrobial substances as lactic acid, hydrogen peroxide and bacteriocins (enterocins) can be used to prolong shelf-life of foodstuff and to increase hygienic safety (Fracalanzza et al. 2007). However, certain strains such as *Enterococcus faecalis* and *Enterococcus faecium* may lead to serious hospital infections in humans (Biendo et al. 2010). Therefore, they pose a potential risk for human health and result in a high mortality of up to 61% in patients (De Fa´tima Silva Lopes et al. 2005). Consequently, it has become harder to choose these strains in food technology (Chajęcka-Wierzchowska et al. 2012). Enterococci have the capacity to acquire antibiotic resistance through changes in plasmids, transposons and chromosomes (Hegstad et al. 2014). During the formation of antimicrobial resistance, *Enterococcus* spp. can transmit antibiotic resistance genes to their own species and to other pathogens such as *Staphylococcus aureus* and *Listeria* spp. (Charpentier and Courvalin 1999). The biggest threat is that vancomycin-resistant enterococci may transfer their vancomycin resistance to methicillin-resistant *S. aureus* (Michel and Gutmann 1997). The presence of antimicrobial resistant bacteria in animal-based foods arouses concern due to the possibility of these bacteria to be carried to humans by means of the food chain (Chajęcka-Wierzchowska et al. 2012). Antibiotic resistance is a serious public health problem as it may lead to an inadequacy of treatment in multi-resistance, severe urethra diseases in people, especially in those whose immune system is inhibited, urinary tract diseases and in such enterococcus infections as bacteremia and endocarditis (Kayser 2003). The virulence factor is an effector molecule that enhances the capacity to cause a disease among species of microorganism (Mundy et al. 2000). Since the presence of enterococci in foods is an indicator of poor hygiene and poor bacteriological quality during manufacturing, it is necessary to identify their sources (Lopez-Diaz et al. 1995, Gelsomino et al. 2001). In this study, the prevalence of *Enterococcus* spp., antibiotic resistance and several virulence factors in some ready-to-eat foods sold in retail was investigated.

## MATERIALS and METHODS

*Sampling*

In this study, 187 ready to eat food samples (60 meat products, 67 brands of cheese, 15 brands of halva and 45 salads), collected from various supermarkets and stores in the city of Balikesir (Turkey) were analysed for the presence of *Enterococcus* spp. The samples were brought to the laboratory in cold chain and taken into analysis on the same day.

*Isolation and identification of Enterococcus* spp

From each sample, 25 g/ml was weighed and put into sterile stomacher bags. Two hundred twenty-five ml sterile Buffered Peptone Water (Merck, Germany) was added. They were homogenised in a stomacher for 2 minutes. 0.5 ml homogenate from the first amplification was spread as Kanamycin Aesculin Azide Agar (Merck, Germany). It was incubated at 37±1°C for 24±2 hours. Suspected colonies of *Enterococcus* spp. were those with a round, white or grey colonies, about 2 mm in diameter, surrounded by black zones of at least 1 cm diameter. Three-four of the suspected colonies of *Enterococcus* spp. were transferred onto Tryptone Soya Agar (Oxoid, CM0131, UK) and incubated at 37±1 ºC for 24±2 hours. At the end of the incubation, Gram stain and catalase test were carried out. Only RapID STR and (Thermo Fisher Scientific-Oxoid, UK) *Enterococci* spp. were identified from Gram positive and catalase negative cocci (Pesavento et al. 2014). The isolates were frozen at -80 °C in Brain Heart Infusion Broth (Oxoid CM0225, UK) with 20% glycerol.

*Hemolytic activity*

Haemolysin activity was detected in blood agar base (CM0271, Oxoid, UK) plates (with 5% of defibrinated sheep blood after incubation at 37 °C/24 h and 5 °C/48 h. Hemolysis was defined by the presence of a viridant halo round isolate colonies, while ß-hemolysis was defined by translucent halo (Camargo et al. 2014).

*Gelatinase assay*

Gelatinase production was detected by inoculating the enterococci onto freshly prepared nutrient agar containing 3% gelatin (Merck, Germany). Plates were incubated overnight at 37 ºC and then cooled to

ambient temperature (4 ºC) for 2 h. The appearance of a turbid halo or zone around the colonies was considered to be a positive indication of gelatinase production (Vergis et al. 2002).

*Antimicrobial Susceptibility testing*

All 112 isolates were tested by the standard disk diffusion method of Kirby Bauer (Bauer *et al.,* 1966) on Mueller Hinton Agar (Thermo scientific, Oxoid, UK) incubated at 35±1ºC for 18±2 h. Reference strains were used *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434. Disks containing the following antibiotics (all from Thermo Scientific, Oxoid, UK) were spotted with a 3 cm interval: ampicillin 10 mg, ciprofloxacin 5 mg, chloramphenicol 30 mg, erythromycin 5 mg, gentamicin 10 mg, penicillin G 10 U.I, tetracycline 30 mg. Results were interpreted following EUCAST (2015) breakpoint tables and, where not possible, according to CLSI (2014) indications.

## RESULTS

All of 114 *Enterococcus* spp. were isolated in total in 112 (59.90 %) of the 187 ready-to-eat food samples analysed (60 meat products, 67 brands of cheese, 15 brands of halva and 45 salads). *Enterococcus* spp. isolates were obtained from 39 samples of meat products (34.80%), 42 samples of cheese brands (37.50%), 25 samples of salads (22.30%) and eight samples of halva (7.10 %). The diffusion of *Enterococcus* isolates as genus was that 66 of them (57.90 %) were *E. faecalis*, 36 of them (31.60 %) were *E. faecium*, 8 of them (7.0%) were *E. durans* and 4 of them (3.50 %) were *E. avium*. *Enterococcus* spp. was detected in all the samples from braised meat and bresaola. The most *Enterococcus* isolates in terms of species were detected in cheese samples (Table 1).

*Haemolysin and Gelatinase activity*

Haemolysin and Gelatinase activity test was applied to the 114 *Enterococcus* spp. isolates obtained from the study. While gelatinase activity was not observed in any of the isolates, hemolytic activity was observed to be positive in 36 of them (31.60%). Hemolytic activity was observed in 22 of *E. faecium* isolates and in 14 of *E. faecalis* isolates.

*Antimicrobial resistance Enterococcus* spp. *isolates*

According to the results of the antibiotic resistance test, the *Enterococcus* isolates were determined to show resistance to at least 4 of the antibiotics used in the study. Also, it was determined that *E. faecium* isolates were sensitive to 2 antibiotics (Chloramphenicol and Penicillin G), *E. faecalis* to 1 antibiotic (Ampicillin), *E. durans* to 2 antibiotics (Ciprofloxacin and Penicillin G) and *E. avium* to 3 antibiotics (Ampicillin, Chloramphenicol and Penicillin G) (Table 2).

**Table1:** Prevalence of *Enterococcu*s spp. isolated some from ready-to-eat foods
**Tablo 1:** Tüketime hazır bazı gıdalardan izole edilen *Enterococcus* spp. Yaygınlığı

| Type of products | No. of samples | No. of positive samples | *Enterococci* spp. | *E. faecium* (%) | *E. faecalis (%)* | *E. durans* % | *E. avium* % |
|---|---|---|---|---|---|---|---|
| **Meat products** | | | | | | | |
| Fermented sausage | 15 | 7 (46.6) | 7 | 2 (28.5) | 4(57.1) | 0 | 1(14.3) |
| Salami | 15 | 6 (40.0) | 6 | 2 (33.3) | 3(50.0) | 1(16.7) | 0 |
| Meat Doner | 10 | 6 (60.0) | 6 | 1(16.7) | 5(83.3) | 0 | 0 |
| Braised Meat | 10 | 10 (100) | 10 | 3(30.0) | 6(60.0) | 1(10.0) | 0 |
| Bresaola | 10 | 10 (100) | 10 | 3(30.0) | 5(50.0) | 1(10.0) | 1(10.0) |
| **Milk Products** | | | | | | | |
| White cheese | 42 | 29(69.1) | 31 | 12(38.7) | 17(54.8) | 2(0.6) | 0 |
| Tulum cheese | 25 | 11(44.0) | 11 | 3(27.2) | 7(63.6) | 1(0.9) | 0 |
| **Desserts** | | | | | | | |
| Halva | 15 | 8(53.3) | 8 | 2(25.0) | 6(75.0) | 0 | 0 |
| **Salads** | | | | | | | |
| İtalian salads | 10 | 5(50.0) | 5 | 2(40.0) | 3(60.0) | 0 | 0 |
| Russian Salads | 15 | 8(53.3) | 8 | 3(37.5) | 3(37.5) | 0 | 2(25.0) |
| Vegetable salads | 20 | 12(60.0) | 12 | 3(25.0) | 7(58.3) | 2(16.7) | 0 |
| **Total** | **187** | **112**(59.9) | **114** | 36(31.6) | 66(57.9) | 8(0.70) | 4(0.35) |

**Table 2:** The distribution of antibiotic-resistant *Enterococcu*s spp. isolates
**Tablo 2:** Antibiyotik dirençli *Enterococcus* spp. izolatların dağılımı

| Antibiotic | *E. faecium* no (%) | | *E. faecalis* no (%) | | *E. durans* no (%) | | *E. avium* no (%) | |
|---|---|---|---|---|---|---|---|---|
| | S | R | S | R | S | R | S | R |
| Ampicillin (10 mg) | 31 (96.9) | 1 (3.1) | 63(100) | 0(0) | 12(92.3) | 1(7.7) | 4(100) | 0(0) |
| Chloramphenicol (30 mg) | 32 (100) | 0(0) | 55 (87.3) | 8(12.7) | 12 (92.3) | 1(7.6) | 4(100) | 0(0) |
| Ciprofloxacin (5 mg) | 23 (71.9) | 9 (28.1) | 47 (74.6) | 16(25.4) | 13(100) | 0(0) | 3 (75) | 1(25) |
| Erytromycin (5 mg) | 26 (81.2) | 6 (18.8) | 53 (84.1) | 10(15.9) | 11 (84.6) | 2(15.4) | 3(75) | 1(25) |
| Gentamicin (10 mg) | 29 (90.6) | 3 (9.4) | 46 (73.0) | 15(27,0) | 11 (84.6) | 2(15.4) | 3(75) | 1(25) |
| Penicillin G (10 mg) | 32 (100) | 0(0) | 59 (93.7) | 4(6.3) | 13(100) | 0(0) | 4(100) | 0(0) |
| Tetracycline (30 mg) | 29 (90.6) | 3 (9.4) | 39 (61.9) | 24(38.1) | 10(76.9) | 3(23.1) | 2(50) | 2(50) |

S: Susceptibility R: Resistance

## DISCUSSION

Ready-to-eat foods are those foods which can be readily consumed, raw or cooked, cooled or hot, without being heated again. Unless rules of hygiene are observed properly, the microorganisms that contaminate them at various stages from production to consumption may lead to food poisoning. *Enterococcus* spp. was the kind of bacteria which can be found in any environment, chiefly in the gut flora of warm-blooded animals. Thought of as harmless by humans for years, *Enterococcus spp.* have become one of the most commonly seen hospital pathogens (De Fa´tima Silva Lopes et al. 2005), with a high mortality rate, due to their strong antimicrobial resistance (Chajęcka-Wierzchowska et al. 2012).

In this study, *Enterococcus* spp. was found to be positive in 59.9 % of the samples taken from 187 ready-to-eat foods (in 112 of them). Fracalanza et al. (2007) detected enterococci positive in 86.6 % of 50 milk and meat samples; Camargo *et al.* (2014) detected them in 95.20 % of 105 food samples. Chajęcka-Wierzchowska et al. (2012) detected them in 82.10% of 122 diverse food samples and Gomes *et al.* (2008) detected them in 52.5 % of 120 food samples. The results obtained in this study were found to be lower than the results of certain researchers (Fracalanza et al. 2007, Chajęcka-Wierzchowska et al. 2012, Camargo et al. 2014) and consistent with the results of Gomes et al. (2008). Camargo et al. (2014) reported that the low results might result from the absence of the amplification stage in the study in which low results were obtained. Therefore, no amplification was conducted in this study. When we look at the distribution of the food samples, we see that the highest rate of *Enterococcus* spp. is seen in cheese samples (37.50%), followed by meat products (34.80%), salads (22.30 %) and halva (7.10%). In this study, a high level of *E. faecalis* and *E. faecium* but a low level of *E. durans* and *E. avium* was identified in *Enterococcus* spp. (Table 1). In a study, Chajęcka-Wierzchowska et al. (2012) identified a higher rate of *Enterococcus* spp. in cheese (89.90%) than in meat products (69.80%). *Enterococcus* spp. was the bacteria commonly found especially in various animal-based foods such as meat, milk and cheese (Jamet et al., 2012). *E. faecalis, E.faecium* and to a lesser extent *E. durans* are found mostly in cheese and other milk products (Franz et al. 1999). *Enterococcus* spp. can be found in many different foods owing to their resistance to pasteurization temperature and their ability to show resistance to differing substrates and conditions of development (low and high temperature, extreme pH, salinity, etc.) and to reproduce in these environments (Foulquié Moreno et al. 2006, Biendo et al. 2010). The presence of *Enterococci* in cheese that is produced from raw and pasteurised milk is associated with the level of contamination in the milk, the type of cheese and whether a starter is used during the production (Maietti et al. 2007). Also, the presence of Enterococci in cheese made from pasteurized or thermalized milk indicates that they aren't eliminated as a result of recontamination or heat treatment (Jamet et al. 2012). Besides these factors, the contamination of the milk used to produce cheese with Enterococci results from the bacteria which are on the breasts of the animals or in their manure, in the water used on the farm, or which cannot be cleaned from the farm workers or from the milking

machines and storage tanks (Gelsomino et al. 2001). On the other hand, some strains of *E. faecalis* and *E. faecium* species may lead to degradation in the texture and taste of the cheese even when the cheese is kept in a cool place, due to their photolytic activity (Marra et al. 2007). In our study, *Enterococcus* spp. were detected, in varying degrees, both in fermented meat products (fermented sausages) and in heat-treated meat products (salami, doner, braised meat, bresaola) (Table 1). Some researchers (Ben-Omar et al. 2004, Aslam et al. 2012, Klibi et al. 2013) have reported that identified *Enterococcus* spp. in meat and meat products. It is reported that the presence of *Enterococcus* spp. in meat may be because of a contamination stemming from the digestive system during the slaughter. *Enterococcus* spp. survive and reproduce due to their resistance to heat especially in fermented products during fermentation in which no starter is used (Giraffa 2002) or in meat products that are processed after being cooked. Also, cross contamination may occur at the final stages of production, such as slicing and packaging of the food (Hugas et al. 2003). In 36 of the *Enterococcus* spp. obtained in this study (31.60%), hemolytic activity was observed to be positive. haemolytic activity was observed in 22 of the *E. faecium* isolates and in 14 of the *E. faecalis* isolates. Trivedi et al. (2011) established that *E. faecalis* (29%) has a higher β-hemolytic activity than *E. faecium* (10%). Franz et al. (1999) reported that the absence of hemolytic activity in *Enterococcus* spp. Gelatinase activity wasn't detected in any of the *Enterococcus* spp. we isolated in our study. Comerlato et al. (2013) detected the presence of *gelE* gene in *E. faecalis and E. faecium* species in their study. However, Marra et al. (2007) reported that there was no direct correlation between the presence of *gelE* gene in *Enterococcus* spp. and gelatinase activity. In our study, it was determined that the *Enterococci* isolates show resistance to at least 4 of the antibiotics, but that *E. faecium* was sensitive to two antibiotics, *E. faecalis* to one antibiotic, *E. durans* to four antibiotics and *E. avium* to four antibiotics. Ristori et al. (2012) determined the resistance of *Enterococcus* spp. to several antibiotics as follows at the following rates; to tetracycline at 89.20 %, to erythromycin at 83.50 %, to ciprofloxacin at 65 %, to chloramphenicol at 55.40 %, and to ampicillin at 0.20 %. Dahlen et al. (2012) reported that ampicillin has a strong effect on *Enterococci,* that 57.20 % of the isolates were sensitive to this antibiotic.

## CONCLUSIONS

In conclusion, unless rules of hygiene are observed properly, the microorganisms that contaminate them at various stages from production to consumption of ready-to-eat foods may lead to food-based diseases. Reported likely to be used as a starter in fermented foods and having been regarded for years as harmless, some *Enterococci* spp. have become one of the most commonly seen hospital pathogens recently since they have virulence factors and strong antimicrobial resistance. For this reason, enough care should be taken while choosing *Enterococcus* spp. that will be used as a starter in food industry so that they don't have any pathogenic affinity and antibiotic resistance genes.

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