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PATHOGENICITY DETERMINANTS AND ANTIBIOTIC RESISTANCE PROFILES OF ENTEROCOCCI FROM FOODS OF ANIMAL ORIGIN IN TURKEY

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In this study, the presence of genes responsible for the pathogenicity and antibiotic resistance profile of enterococci isolated from various foodstuffs of animal origin was investigated. The percentage prevalence of enterococci was 54.1% (203/375) and the average count was found to be 3.81 log cfu/ml-g. Species-specific primers revealed *Enterococcus faecalis* as the predominant species carrying one or more virulence-associated traits of *efa*, *gel*E, *ace*, *esp* and *agg* genetic markers. Only one *E. faecium* isolate (from milk) was positive for the *esp* gene. Regarding antibiotic resistance, the highest frequency of resistance was observed for tetracycline (21.7%), followed by quinupristin/dalfopristin (13.3%), ciprofloxacin (2.0%), penicillin (2.0%), linezolid (1.0%), ampicillin (1.0%), streptomycin (1.0%), and gentamicin (0.5%). *Enterococcus faecalis* showed a higher prevalence of antibiotic resistance than other enterococci. The percentage of multidrug resistance among the isolates was 3.4%. Twenty-nine *E. faecalis* isolates (26.6%) carrying one of the virulence-associated traits were at the same time resistant to at least one antibiotic. Our results show that foods of animal origin, including ready-to-eat products, may be reservoirs of antibiotic-resistant and potentially virulent enterococci.

Key words: Food, enterococci, incidence, antibiotic resistance, virulence genes

Enterococci are indicator bacteria of possible faecal contamination and are isolated frequently from food, plants, water and soil. They are included in different types of cheese and fermented meat products to improve organoleptic quality and to extend shelf life during production (Pesavento et al., 2014). It has been

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documented that some strains of enterococci, especially *E. faecium*, may inhibit the growth of food-borne pathogens in fermented foods (Barbosa et al., 2014). The probiotic potential of some enterococcal strains is being successfully applied to improve human or animal health (Pieniz et al., 2015). Furthermore, the commercial *E. faecium* NCIMB 10415 strain (Cylatin®) has been safely used in livestock rearing and fattening (EFSA, 2013*a*).

Enterococci were not recommended in the QPS (qualified presumption of safety) list by the European Food Safety Authority (EFSA) because of their potential role in human clinical infections associated with endocarditis, bacteraemia, urinary tract infections and antimicrobial resistance (EFSA, 2013*b*). Antimicrobial resistance, especially multidrug resistance, is one of the main public health concerns due to treatment failures of infections, particularly in immunosuppressed individuals (Fisher and Phillips, 2009; Pesavento et al., 2014). *Enterococcus* species have an intrinsic resistance to several antibiotics such as streptogramins and beta-lactams. Mobile genetic elements were found to be responsible for acquired resistance to glycopeptides, tetracyclines, lincosamides, macrolides and aminoglycosides in enterococci isolated from different sources including livestock and food strains (Hollenbeck and Rice, 2012). Adhesion and secreted virulence factors are the main pathogenicity factors of enterococci. Aggregation substance (*agg*), extracellular surface protein (*esp*), *Enterococcus faecalis* antigen (*efa*A), adhesin to collagen *(ace),* endocarditis and biofilm-associated pili *(ebp)* are the major adhesion factors. Secreted enterococcal virulence factors are cytolysin *(cyl),* gelatinase (*gel*E) and hyalorinidase *(hyl)* (Kafil et al., 2013; Chajecka-Wierzchowska et al., 2017). The safety assessment of enterococcal strains is a complicated task because of their possession of highly prevalent virulence and antimicrobial resistance traits (Klibi et al., 2013).

Enterococci may show tolerance to antimicrobial pressure because of their ability to easily acquire resistance to new classes antimicrobials by expressing new resistance genes. Antimicrobial-resistant enterococci may be present in foods of animal origin, and these foods may be responsible for the transmission of resistant bacteria to humans through consumption. Transfer of the streptomycin resistance gene (aadA) of E. faecium from a food strain into a clinical strain by class 1 integron has been reported recently (Jahan et al., 2015). Although enterococci were considered non-pathogenic for a long period of time, over the last few decades they have been recognised as one of the most common emerging nosocomial pathogens, causing a mortality rate of up to 61% (Fisher and Phillips, 2009).

In the present study, enterococci isolated from foodstuffs of animal origin (including ready-to-eat products) were tested for the presence of virulence genes including *agg*, *esp*, *ace*, *gel*E and *efa*. The antimicrobial susceptibility profiles of the isolates were also determined.

Materials and methods

Food sampling

A total of 375 samples of foods of animal origin, including 150 milk/dairy product, 150 meat/meat product and 75 chicken/poultry product samples, were collected from several supermarkets, delicatessens and open bazaars in the Marmara Region of Turkey. These consisted of 180 raw food samples (20 milk, 30 meat, 30 minced meat, 25 meatball, 65 chicken meat and 10 poultry product samples such as nugget and schnitzel) and 195 ready-to-eat food samples (85 cheese, 15 yoghurt, 15 kefir, 15 butter, 25 soujuk, 20 pastrami and 20 salami/sausage samples). The samples were immediately transported to the laboratory under refrigerated conditions. All microbiological analyses were performed within the same day.

Bacterial isolation

A 10-g quantity of each food sample was homogenised in 90 ml of pepton water. Decimal dilutions were streaked onto Slanetz and Bartley agar (CM0377, Oxoid, UK) plates and incubated at 37 °C for two days. After incubation, pink- and brown-coloured colonies were subjected to Gram staining, PYR test, esculin hydrolysis and growth at 10 and 45 °C, in 6.5% NaCl and at pH 9.6. The isolates were preserved in Brain Heart Infusion broth (CM1135, Oxoid, UK) supplemented with 20% glycerol at –20 °C for further experiments.

Antibiotic susceptibility and MIC testing

The antibiotic susceptibility of the strains was evaluated by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2014). Penicillin (10 U), ampicillin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), linezolid (30 μg), quinupristin/dalfopristin (15 μg), teicoplanin (30 μg), vancomycin (30 μg) and, for high-level aminoglycoside resistance, gentamicin (120 μg) and streptomycin (300 μg) (Oxoid, Basingstoke, UK) antibiotic discs were studied. *Staphylococcus aureus* ATCC 25293 was used as quality control microorganism. Minimum inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined using the E-test method and interpreted according to the CLSI guidelines. The strains having MIC values of 32 μg/ml were considered resistant to both antibiotics, those having MICs of 8–16 μg/ml and 16 μg/mL were considered intermediately resistant, and those with MICs of ≤ 4 μg/ml and ≤ 8 μg/ml were regarded as susceptible to vancomycin and teicoplanin, respectively. *Enterococcus faecalis* ATCC 29212 was used as control microorganism.

Table 1

Primers used in the identification of enterococci at genus and species level and the determination of virulence-associated genes

Primers	Product size (bp)	Oligonucleotide sequence (5′–3′)	References
Ent (tuf)	112	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	Ke et al., 1999
fcm	215	GAAAAAACAATAGAAGAATTATTGCTTTTTTGAATTCTTCTTTA	Jackson et al., 2004
fls	360	ACTTATGTGACTAACTTAACC TAATGGTGAATCTTGGTTTGG	Jackson et al., 2004
avi	368	GCTGCGATTGAAAAATATCCG AAGCCAATGATCGGTGTTTTT	Jackson et al., 2004
dur	295	CCTACTGATATTAAGACAGCG TAATCCTAAGATAGGTGTTTG	Jackson et al., 2004
gal	173	TTACTTGCTGATTTTGATTCG TGAATTCTTCTTTGAAATCAG	Jackson et al., 2004
cas	288	TCCTGAATTAGGTGAAAAAAC GCTAGTTTACCGTCTTTAACG	Jackson et al., 2004
efaA_{fs}	705	GACAGACCCTCACGAATA AGTTCATCATGCTGTAGTA	Eaton and Gasson, 2001
efaA_{fm}	735	AACAGATCCGCATGAATA CATTTCATCATCTGATAGTA	Eaton and Gasson, 2001
agg	1553	AAGAAAAAGAAGTAGACCAAC AAACGGCAAGACAAGTAAATA	Eaton and Gasson, 2001
esp	432	TTACCAAGATGGTTCTGTAGGCAC CCAAGTATACTTAGCATCTTTTGG	Shankar et al., 1999
*gel*E	402	AGTTCATGTCTATTTTCTTCAC CTTCATTATTTACACGTTTG	Mannu et al., 2003
ace	320	AAAGTAGAATTAGATCCACAC TCTATCACATTCGGTTGCG	Mannu et al., 2003
*van*A	1030	CATGAATAGAATAAAAGTTGCAATACCCCTTTAACGCTAATACGATCAA	Evers et al., 1993
*van*B	433	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCCTGCAAAAAA	Handwerger et al., 1992
*van*C	822	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	Dutka-Malen et al., 1995

PCR identification and detection

DNA extraction was performed by using Chelex 100 (Sigma Aldrich, USA). The PCR process was carried out in a ThermoCycler (Runik, SCM 96G). Each 25-µl reaction mixture contained 1 µl template DNA, 1.25 U of Hot Start Taq DNA polymerase (Bioron, Germany), 10 mM of Tris-HCl pH 8.9, 22 mM of KCl, 1.8 mM of $MgCl_2$ (Fermentas, USA), 200 µM of dNTPs (Biolabs, UK) and 0.5 mM of each primer (Sentegen, Turkey). The *Enterococcus* genus-specific primers and the *E. faecalis, E. faecium, E. durans, E. gallinarum, E. casseliflavus* and *E. avium* species-specific primers are listed in Table 1. The presence of g*el*E, *ace, agg* and *esp* genes was tested for all identified isolates, while *efa*A_{fs} and *efa*A_{fm} genes were searched only in the *E. faecalis* and *E. faecium* isolates. The presence/absence of *van*A, *van*B and *van*C genes was investigated to confirm vancomycin and teicoplanin resistance. The primers, their sequences and related references for PCR conditions are summarised in Table 1.

Results

In the present survey, 203 enterococci were recovered from 375 food samples (54.1%). The average enterococcus count was 3.81 log cfu/ml-g for all tested foodstuffs, 3.48 log cfu/g for meat and meat products, 4.14 log cfu/ml-g for milk and dairy products, and 3.41 log cfu/g for poultry products. The distribution of enterococcus counts as a function of foodstuffs is shown in Table 2.

According to the PCR identification results, the prevalence of *E. faecalis* was the highest with an incidence of 53.7%, followed by *E. faecium* (30.5%), *E. durans* (10.3%), *E. avium* (0.5%) and *E. casseliflavus* (0.5%). Nine isolates were confirmed as *Enterococcus* spp. but could not be identified at the species level using species-specific primers. PCR identification results obtained for the raw and ready-to-eat foods analysed are summarised in Table 3. A total of 114 enterococcal isolates were recovered from ready-to-eat foods, and 71 of these were isolated from retail cheeses.

The frequency of virulence-associated traits was relatively high among *E. faecalis* isolates and at least one trait was demonstrated in 75 out of the 109 tested isolates. The most commonly detected virulence-associated trait was *efa*$_{fs}$ (38.5%), followed by *gel*E (35.7%), *ace* (29.4%), *esp* (9.2%) and *agg* (4.6%). Thirty-eight isolates had multiple virulence-associated genes. Among these, two isolates from cheese samples were positive for four different genes (*efa*$_{fs}$*, agg, gel*E*, ace* and *efa*$_{fs}$*, esp, gel*E*, ace,* respectively), 11 were positive for three genes and 25 harboured two genes (Fig. 1). The prevalence of *E. faecalis* virulence-associated traits is presented in Table 4. Interestingly, only one *E. faecium* isolate from milk was positive for the *esp* gene. Virulence-associated genes were not detected in other *Enterococcus* isolates. Cheese isolates of *E. faecalis* harboured

more virulence determinants than those isolated from other foodstuffs. None of the isolates from poultry products or salamis-sausages was positive for any virulence trait.

Table 2

Distribution and counts of enterococci in foodstuffs

Sample	No. of positive samples (%)	Enterococcus counts (log cfu/ml-g)		
		Min.	Mean ± SD	Max.
Meat and meat products				
Meat (n = 30)	13 (43)	2.0	3.44 ± 0.76	4.82
Minced meat (n = 30)	18(60)	2.30	3.32 ± 0.67	4.51
Meatball (n = 25)	20 (80)	2.30	3.65 ± 0.55	4.46
Soujuk (n = 25)	7 (28)	2.0	3.36 ± 1.57	6.47
Salami-sausage (n = 20)	2 (10)	2.0	3.19 ± 1.70	4.39
Pastrami (n = 20)	9 (45)	2.0	3.62 ± 1.08	4.71
Milk and dairy products				
Milk (n = 20)	14 (70)	2.0	3.70 ± 0.82	5.23
Cheese (n = 85)	62 (73)	2.0	4.51 ± 1.17	6.38
Yoghurt (n = 15)	7 (47)	2.0	2.81 ± 1.14	4.53
Butter (n = 15)	9 (60)	2.47	3.54 ± 0.87	4.82
Kefir (n = 15)	5 (33)	2.0	3.71 ± 1.05	4.58
Chicken and poultry products				
Chicken (n = 65)	21 (32)	2.0	3.37 ± 0.86	4.59
Poultry products (n = 10)	1 (10)	–	4.41 ± –	–
All (n = 375)	188 (50)	2.0	3.81 ± 1.09	6.47

In terms of antibiotic resistance, the highest level of resistance was observed for tetracycline (21.7%), followed by quinupristin/dalfopristin (13.3%), ciprofloxacin (2.0%), penicillin (2.0%), linezolid (1.0%), ampicillin (1.0%), streptomycin (1.0%) and gentamicin (0.5%). The *E. faecalis* isolates had a higher resistance rate than other isolates (Table 5). Although one *E. faecalis* and *E. durans* isolate each exhibited resistance to both vancomycin and teicoplanin by the disk diffusion method, their MIC values were lower than 1 μg/ml, suggesting that in reality they were not resistant. This was also observed for one *E. durans* isolate showing intermediate-level resistance to vancomycin. The absence of *vanA, vanB* and *vanC* in these three isolates as tested by PCR confirmed that they were not positive for vancomycin and teicoplanin resistance. The *E. avium* isolates were sensitive to all tested antibiotics. The multidrug resistance patterns among *E. faecalis* (5/109, 4.6%) and *E. faecium* (2/62, 3.2%) isolates demonstrated that, with the exception of one *E. faecalis* isolate which was resistant to four antibiotics (penicillin, ampicillin, quinupristin/dalfopristin, linezolid), all were resistant to three antibiotics. Two *E. faecalis* isolates from butter/chicken showed resistance to quinupristin/dalfopristin, tetracycline and streptomycin. In

Table 3

Number of positive samples and species distribution of enterococci

Sample	Species and number of enterococci in positive samples						
	E. faecalis	*E. faecium*	*E. durans*	*E. casseliflavus*	*E. avium*	*E. gallinarum*	*Enterococcus* spp.[a]
Raw foods							
Meat (n = 30)	9	1	2	–	1	–	2
Minced meat (n = 30)	13	2	1	1	–	–	–
Meatball (n = 25)	13	4	1	–	–	–	1
Milk (n = 20)	6	7	1	–	–	–	2
Chicken (n = 65)	10	3	5	–	–	–	3
Poultry products (n = 10)	–	1	–	–	–	–	–
Ready-to-eat foods							
Soujuk (n = 25)	4	4	–	–	–	–	–
Salami-sausage (n = 20)	1	1	–	–	–	–	–
Pastrami (n = 20)	2	3	4	–	–	–	–
Cheese (n = 85)	39	26	6	–	–	–	–
Yoghurt (n = 15)	4	3	–	–	–	–	–
Butter (n = 15)	5	6	1	–	–	–	–
Kefir (n = 15)	3	1	–	–	–	–	1
All (n = 375)	109	62	21	1	1	–	9

– not detected; [a]not identified at species level

addition, other two *E. faecalis* isolates and one *E. faecium* isolate from chicken were resistant to quinupristin/dalfopristin, tetracycline and ciprofloxacin. One meat isolate of *E. faecium* was found to be resistant to penicillin, ampicillin and tetracycline.

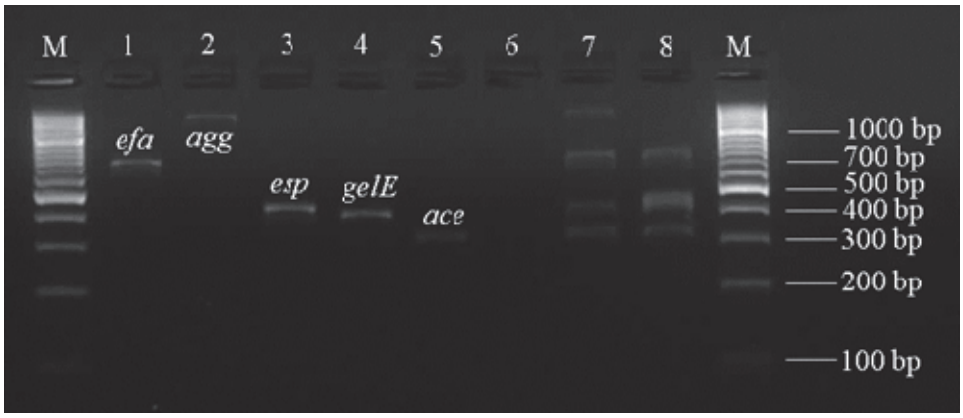

Fig. 1. Virulence-associate genes of enterococci. M: 100 bp DNA ladder; lanes 1: *Enterococcus faecalis* ATCC 29212; lanes 2–4: *E. faecalis* MMH 594; lane 5: *E. faecalis* JH2-2; lane 6: negative control; lane 7: *efa*-, *agg*-, *gel*E- and *ace*-positive *E. faecalis* isolate; lane 8: *efa*-, *esp*-, *gel*E- and *ace*-positive *E. faecalis* isolate

Table 4

Distribution of virulence-associated genes of *Enterococcus faecalis* in foodstuffs

Sample type (No. of *E. faecalis* positive samples)	Virulence-associated genes				
	efa$_{fs}$	*agg*	*esp*	*gelE*	*ace*
Meat and meat products					
Meat (n = 9)	2	–	–	2	–
Minced meat (n = 13)	3	–	–	4	2
Meatball (n = 13)	1	1	–	3	8
Soujuk (n = 4)	3	–	1	1	–
Salami-sausage (n = 1)	–	–	–	–	–
Pastrami (n = 2)	2	–	–	–	–
Milk and dairy products					
Milk (n = 6)	1	1	2	5	4
Cheese (n = 39)	24	2	5	13	10
Yoghurt (n = 4)	2	–	–	2	2
Butter (n = 5)	1	–	1	3	2
Kefir (n = 3)	1	1	–	1	–
Chicken and poultry products					
Chicken (n = 10)	2	–	1	5	4
Poultry products (–)	–	–	–	–	–
All (n = 109)	42	5	10	39	32

Table 5

Antibiotic resistance patterns of *Enterococcus* species

Antibiotics	*E. faecalis*		*E. faecium*		*E. durans*		*E. casseliflavus*		*E. avium*		*Entero-coccus* spp.	
	R	IM	R	IM	R	IM	R	IM	R	IM	R	IM
Vancomycin	1[a]	–	–	–	1[a]	1[*]	–	–	–	–	–	–
Teicoplanin	1[a]	–	–	–	1[a]	–	–	–	–	–	–	–
Penicillin	1	–	3	–	–	–	–	–	–	–	–	–
Ampicillin	1	–	1	–	–	–	–	–	–	–	–	–
Linezolid	2	1	–	–	–	–	–	–	–	–	–	–
Quinupristin/ Dalfopristin	21	29	2	2	4	–	–	–	–	–	–	3
Ciprofloxacin	2	13	2	14	–	–	–	1	–	–	–	1
Tetracycline	27	6	13	–	2	–	1	–	–	–	1	–
Gentamicin	1	1	–	–	–	–	–	–	–	–	–	–
Streptomycin	2	1	–	–	–	–	–	–	–	–	–	–

R: resistant, IM: intermediately resistant, S: susceptible. [a]Resistance found by disc diffusion method, but MIC results showed susceptibility in relevant isolates

Discussion

The incidence of enterococci in foods of animal origin was 54.13% and the counts varied between 2.0 and 6.5 log cfu/ml-g. Similar incidence rates (around 50%) were also reported by Koluman et al. (2009) and Jahan et al. (2013). Other authors reported lower (23%; Pesavento et al., 2014) or higher (72%; Jamet et al., 2012) incidence levels. The study performed by Jamet et al. (2012) revealed enterococcus counts varying between 10^2 cfu/g and 10^8 cfu/g in cheese samples. Another study indicated that enterococcus counts in fermented foods changed between 4 and 6 log cfu/g (Rehaiem et al., 2016). Overall, 56.2% (114/203) of the enterococcus isolates were from ready-to-eat foods, and the prevalence of enterococci in ready-to-eat meat products was 16.7%. Chajecka-Wierzchowska et al. (2016*b*) reported relatively high prevalence (74.1%) for the same group of foods in Poland. In our studies reported here, 71 isolates of enterococci were obtained from cheese samples (n = 85) collected from the Marmara Region of Turkey. A research conducted in Western Turkey indicated the presence of 95 *Enterococcus* sp. strains among the 129 isolates obtained from 45 traditional home-made cheeses (Buyukyoruk et al., 2014). These results suggest the ability of enterococci to survive or grow at extreme conditions, even in processed ready-to-eat foods.

Species-specific multiplex PCR assays confirmed *E. faecalis* as the most prevalent *Enterococcus* species in the tested foodstuffs, followed by *E. faecium, E. durans, E. casseliflavus* and *E. avium*. Nine *Enterococcus* isolates giving a positive band with genus-specific primers gave no band with species-specific primers. In agreement with our findings, Pesavento et al. (2014) reported *E. faecalis* as the dominant species in food samples. However, they isolated four strains of *E. gallinarum* and found a relatively higher percentage of *E. avium*. In another study performed on retail foods from Turkey (Koluman et al., 2009), the researchers reported the isolation of *E. faecalis* and *E. faecium* only.

Among the meat and meat product samples tested, meatballs were the most contaminated. Moreover, minced meat and fresh meat were also found to be contaminated at relatively high levels. A total of 70 isolates were recovered from meat and meat products (42 *E. faecalis,* 15 *E. faecium,* 8 *E. durans,* 1 *E. casseliflavus,* 1 *E. avium,* 3 *Enterococcus* spp.). Similarly, studies conducted in Portugal (Barbosa et al., 2010) and Tunisia (Belgacem et al., 2010) also revealed contamination of fermented meat products predominantly with *E. faecalis* and *E. faecium* isolates. In this study we did not isolate *E. gallinarum;* however, Jahan et al. (2013) isolated *E. gallinarum* from meat and fermented meat products. The contamination rate of milk and dairy product samples with enterococci was 64.7% (97/150), and the species *E. faecalis, E. faecium* and *E. durans* were identified by multiplex PCR. There was no significant difference in *E. faecalis* and *E. faecium* numbers; however, Buyukyoruk et al. (2014) and Hammad et al. (2015) reported a higher prevalence of *E. faecium* in dairy samples.

Five virulence-associated genes were investigated in all the 203 isolates. Virulence traits (except *esp*) were detected only in the *E. faecalis* isolates. The *esp* gene was found only in a *E. faecium* isolate. Similarly, Barbosa et al. (2010) reported the absence of virulence genes in *E. faecium* isolates and the presence of the *efa, esp, agg* and *gel*E genes in *E. faecalis*. However, Jahan and Holley (2014) reported the presence of several virulence traits in *E. faecalis* (*efa, esp, gel*E, *agg, ace*) and *E. faecium* (*efa, esp, gel*E, *agg*) strains isolated from meat and meat products. Furthermore, these authors found the *ace* gene in one *E. gallinarum* isolate. Another study showed that *E. faecium* strains from fermented meat products were positive for the *gel*E and *efa* genes (Belgacem et al., 2010). A study performed by Hammad et al. (2015) in Egypt demonstrated *E. faecalis* isolates carrying the *agg, esp* and *gel*E genes. Buyukyoruk et al. (2014) suggested the existence of *agg, esp* and *gel*E genes in *Enterococcus* species isolated from farmhouse-produced cheeses.

The antibiotic resistance profiles of the 203 isolates were characterised by the disc diffusion test. Resistance was relatively frequent among *E. faecalis* isolates: 39 out of 109 isolates were resistant to one or more antibiotics. However, there was no resistance either to vancomycin or teicoplanin. Resistance to tetracycline was remarkable (21%) among the *E. faecium* isolates. High tetracycline

resistance levels were also reported by Jamet et al. (2012), Klibi et al. (2013), Pesavento et al. (2014), Chajecka-Wierzchowska et al. (2016*a*) and Yılmaz et al. (2016). Studies performed during the last decade in different countries reported resistance to ciprofloxacin (Belgacem et al., 2010; Jahan et al., 2013; Klibi et al., 2013; Chajecka-Wierzchowska et al., 2016*a*; Yılmaz et al., 2016), quinupristin/dalfopristin (Chajecka-Wierzchowska et al., 2016*a*), penicillin (Koluman et al., 2009; Jahan et al., 2013; Klibi et al., 2013), ampicillin (Koluman et al., 2009; Klibi et al., 2013), linezolid (Pesavento et al., 2014; Hammad et al., 2015), and streptomycin (Koluman et al., 2009; Jahan et al., 2013; Klibi et al., 2013). High-level gentamicin resistance is a subject of discrepancy for *Enterococcus* infections, since gentamicin has been used in combination with other antibiotics such as vancomycin, ampicillin and penicillin against enterococcal infections (Choi and Woo, 2013). In the present study, one isolate of *E. faecalis* recovered from yoghurt showed high-level gentamicin resistance. In accordance with these data, isolates from chicken meat (Choi and Woo, 2013) and various foodstuffs (Pesavento et al., 2014) showed resistance to this antibiotic at rates 10.9% and 21.9%, respectively. Resistance of enterococci to glycopeptide antibiotics is a major concern because vancomycin is the last choice for treating infections caused by Gram-positive bacteria (Cetinkaya et al., 2013). In the present survey, a few isolates exhibited some extent of resistance to vancomycin and teicoplanin by the disc diffusion assay but the presence of genes responsible for these phenotypes could not be confirmed by PCR. In contrast with our results, the presence of vancomycin- and/or teicoplanin-resistant isolates was reported in food samples by some other researchers (Ghidán et al., 2008; Koluman et al., 2009; Pesavento et al., 2014; Hammad et al., 2015; Chajecka-Wierzchowska et al., 2016*a*; Yılmaz et al., 2016).

Resistance to multiple antibiotics was widespread among *E. faecalis* and *E. faecium* isolates, with *E. faecalis* (4.6%) having relatively higher multidrug resistance compared to *E. faecium* (3.2%). One cheese isolate of *E. faecalis* showed resistance to four antibiotics (penicillin, ampicillin, quinupristin/dalfopristin, linezolid). Various other studies indicated the presence of multidrug-resistant *E. faecalis* and *E. faecium* isolates from several foods (Koluman et al., 2009; Jamet et al., 2012; Jahan et al., 2013; Chajecka-Wierzchowska et al., 2016*a,b*). Apart from food isolates, *E. faecalis* and *E. faecium* strains obtained from the environment of pig farms were also reported to be resistant to multiple antibiotics (Beshiru et al., 2017).

Interestingly, 74.4% (29/39) of *E. faecalis* isolates from 16 ready-to-eat foods (cheese, yogurt, butter, kefir, pastrami, soujuk), having resistance to one or more antibiotics, were found to possess at least one virulence trait as well. Our results indicated that all of the 5 *E. faecalis* isolates presenting a multiresistant profile had at least one virulence gene trait, and two of them were isolated from ready-to-eat foods such as cheese and butter. Furthermore, it was found that a

cheese isolate of *E. faecalis* resistant to four different antibiotics harboured virulence traits *ace* and *efa*, while some of the other multidrug-resistant isolates were positive for virulence factors *gelE* and *ace*.

In conclusion, the present research has revealed the presence of *Enterococcus* species, particularly *E. faecalis*, in foods of animal origin and determined the virulence-associated traits and/or antibiotic resistance of the isolates. The detection of isolates having pathogenic traits and antibiotic resistance, especially from ready-to-eat foods, provides useful data for risk assessment and indicates that these foods may present a potential public health risk to consumers. The assessment of this public health hazard would, however, need further comparative studies involving human faecal enterococci tested from the same region for the same pathogenicity and antimicrobial resistance traits.

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References

Barbosa, J., Borges, S. and Teixeira, P. (2014): Selection of potential probiotic *Enterococcus faecium* isolated from Portuguese fermented food. Int. J. Food Microbiol. **191,** 144–148.

Barbosa, J., Gibbs, P. A. and Teixeira, P. (2010): Virulence factors among enterococci isolated from traditional fermented meat products produced in the North of Portugal. Food Control **21,** 651–656.

Belgacem, Z. B., Abriouel, H., Omar, N. B., Lucas, R., Martinez-Canamero, M., Galvez, A. and Mania, M. (2010): Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. Food Control **21,** 462–470.

Beshiru, A., Igbinosa, I. H., Omeje, F. I., Ogofure, A. G., Eyong, M. M. and Igbinosa, E. O. (2017): Multi-antibiotic resistant and putative virulence gene signatures in *Enterococcus* species isolated from pig farms environment. Microb. Pathog. **104,** 90–96.

Buyukyoruk, S., Ayaz, N. D., Gencay, Y. E., Beyaz, D. and Koçak, P. (2014): Species distribution, molecular characteristics and vancomycin resistance gene profiles of *Enterococcus* sp. isolates from farmhouse cheeses in western Turkey. Int. J. Dairy Technol. **67,** 103–109.

Cetinkaya, F., Elal Mus, T., Soyutemiz, G. E. and Cibik, R. (2013): Prevalence and antibiotic resistance of vancomycin-resistant enterococci in animal originated foods. Turk. J. Vet. Anim. Sci. **37,** 588–593.

Chajecka-Wierzchowska, W., Zadernowska, A. and Łaniewska-Trokenheim, L. (2016*a*): Virulence factors, antimicrobial resistance and biofilm formation in *Enterococcus* spp. isolated from retail shrimps. LWT-Food Sci. Technol. **69,** 117–122.

Chajecka-Wierzchowska, W., Zadernowska, A. and Łaniewska-Trokenheim, L. (2016*b*): Diversity of antibiotic resistance genes in *Enterococcus* strains isolated from ready-to-eat meat products. J. Food Sci. **81,** 2799–2807.

Chajecka-Wierzchowska, W., Zadernowska, A. and Łaniewska-Trokenheim, L. (2017): Virulence factors of *Enterococcus* spp. presented in food. LWT-Food Sci. Technol. **75,** 670–676.

Choi, J. M. and Woo, G. J. (2013): Molecular characterization of high-level gentamicin-resistant *Enterococcus faecalis* from chicken meat in Korea. Int. J. Food Microbiol. **165,** 1–6.

CLSI (Clinical and Laboratory Standards Institute) (2014): Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement M100-S24, Wayne, PA. pp. 76–79.

Dutka-Malen, S., Evers, S. and Courvalin, P. (1995): Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. **33,** 24–27.

Eaton, T. J. and Gasson, M. J. (2001): Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. Appl. Environ. Microb. **67,** 1628–1635.

EFSA (European Food Safety Authority) (2013*a*): Scientific opinion on Cylactin® (*Enterococcus faecium*) for calves, lambs and kids for rearing and for fattening. EFSA J. **11,** 3097.

EFSA (European Food Safety Authority) (2013*b*): Scientific opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). EFSA J. **11,** 3449.

Evers, S., Sahm, D. F. and Courvalin, P. (1993): The *van*B gene of vancomycin-resistant *Enterococcus faecalis* V583 is structurally related to genes encoding D-Ala:D-Ala ligases and glycopeptide-resistance proteins *van*A and *van*C. Gene **124,** 143–144.

Fisher, K. and Phillips, C. (2009): The ecology, epidemiology and virulence of *Enterococcus*. Microbiol. **155,** 1749–1757.

Ghidán, Á., Kaszanyitzky, É. J., Dobay, O., Nagy, K., Amyes, S. G. B. and Rozgonyi, F. (2008): Distribution and genetic relatedness of vancomycin-resistant enterococci (VRE) isolated from healthy slaughtered chicken in Hungary from 2001 to 2004. Acta Vet. Hung. **56,** 13–25.

Hammad, A. M., Hassan, H. A. and Shimamoto, T. (2015): Prevalence, antibiotic resistance and virulence of Enterococcus spp. in Egyptian fresh raw milk cheese. Food Control **50,** 815–820.

Handwerger, S., Perlman, D. C., Altarac, D. and McAuliffe, V. (1992): Concomitant high level vancomycin and penicillin resistance in clinical isolates of enterococci. Clin. Infect. Dis. **14,** 655–661.

Hollenbeck, B. L. and Rice, L. B. (2012): Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence **3,** 421–433.

Jackson, C. R., Fedorka-Cray, P. J. and Barrett, J. B. (2004): Use of genus and species specific multiplex PCR for identification of enterococci. J. Clin. Microbiol. **42,** 3558–3565.

Jahan, M. and Holley, R. A. (2014): Incidence of virulence factors in enterococci from raw and fermented meat and biofilm forming capacity at 25 °C and 37 °C. Int. J. Food Microbiol. **170,** 65–69.

Jahan, M., Krause, D. O. and Holley, R. A. (2013): Antimicrobial resistance of *Enterococcus* species from meat and fermented meat products isolated by a PCR-based rapid screening method. Int. J. Food Microbiol. **163,** 89–95.

Jahan, M., Zhanel, G. G., Sparling, R. and Holley, R. A. (2015): Horizontal transfer of antibiotic resistance from *Enterococcus faecium* of fermented meat origin to clinical isolates of *E. faecium* and *Enterococcus faecalis*. Int. J. Food Microbiol. **199,** 78–85.

Jamet, E., Akary, E., Poisson, M. A., Chamba, J. F., Bertrand, X. and Serror, P. (2012): Prevalence and characterization of antibiotic resistant *Enterococcus faecalis* in French cheeses. Food Microbiol. **31,** 191–198.

Kafil, H. S., Mobarez, A. M. and Moghadam, M. F. (2013): Adhesion and virulence factor properties of enterococci isolated from clinical samples in Iran. Indian J. Pathol. Microbiol. **56,** 238–242.

Ke, D., Picard, F. J., Martineau, F., Menard, C. H., Roy, P. H., Ouellette, M. and Bergeron, M. (1999): Development of a PCR assay for rapid detection of enterococci. J. Clin. Microbiol. **37,** 3497–3503.

Klibi, N., Ben Said, L., Jouini, A., Slama, K. B., Lopez, M., Sallem, R. B., Boudabous, A. and Torres, C. (2013): Species distribution, antibiotic resistance and virulence traits in enterococci from meat in Tunisia. Meat Sci. **93,** 675–680.

Koluman, A., Akan, L. S. and Cakiroglu, F. P. (2009): Occurrence and antimicrobial resistance of enterococci in retail foods. Food Control **20,** 281–283.

Mannu, L., Paba, A., Daga, E., Comunian, R., Zannetti, S., Dupre, I. and Sechi, L. A. (2003): Comparison of the incidence of virulence determinants and antibiotic resistance between *Enterococcus faecium* strains of dairy, animal and clinical origin. Int. J. Food Microbiol. **88,** 291–304.

Pesavento, G., Calonico, C., Ducci, B., Magnanini, A. and Lo Nostro, A. (2014): Prevalence and antibiotic resistance of *Enterococcus* spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat. Food Microbiol. **41,** 1–7.

Pieniz, S., De Moura, T. M., Cassenego, A. P. V., Andreazza, R., Frazzon, A. P. G., Camargo, F. A. O. and Brandelli, A. (2015): Evaluation of resistance genes and virulence factors in a food isolated *Enterococcus durans* with potential probiotic effect. Food Control **51,** 49–54.

Rehaiem, A., Fhoula, I., Slim, A. F., Ben Boubaker, I. B., Chihi, A. B. and Ouzari, H. I. (2016): Prevalence, acquired antibiotic resistance and bacteriocin production of *Enterococcus* spp. isolated from Tunisian fermented food products. Food Control **63,** 259–266.

Shankar, V., Baghdayan, A., Huycke, M. M., Lindahl, G. and Gilmore, M. S. (1999): Infection-derived *Enterococcus faecalis* strains are enriched in *esp,* a gene encoding a novel surface protein. Infect. Immun. **67,** 193–200.

Yilmaz, E. S., Aslantas, O., Pehlivanlar Onen, S., Turkyilmaz, S. and Kurekci, C. (2016): Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. LWT-Food Sci. Technol. **66,** 20–26.