LWT - Food Science and Technology 66 (2016) 20–26

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey

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ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form
18 August 2015
Accepted 2 October 2015
Available online 8 October 2015

Keywords:
Enterococcus spp.
Multi-drug resistance
Chicken meat
Virulence genes

ABSTRACT

The objective of this work was to investigate the antibiotic susceptibility, the mechanisms implicated and the potential virulence genes (gelatinase [*gel*E], cytolysins [*cyl*A, *cyl*M, *cyl*B], cell wall adhesins [*efa*Afs and *efaAfm*], enterococcal surface protein [*esp*], sex pheromones [*cpd*, *cob*, *ccf*], enhanced expression of pheromone [*eep*], aggregation substance [*agg*A]) in enterococci isolated from retail chicken and beef meat samples in Hatay, Turkey. Hundred-one (96%) isolates from chicken meat and sixty-three (63%) from minced meat isolates showed resistance to at least one of the 12 antimicrobial agents tested. The highest frequency of resistance was against tetracycline (89.5% and 53%), erythromycin (59% and 2%), ciprofloxacin (35.2% and 12%) and trimethoprim/sulfamethoxazole (34.3% and 7%) for isolates from chicken and beef samples, respectively. The *erm*B, *tet*M and *tet*L genes were predominantly detected by PCR. Five enterococci from chicken meat were found to be phenotypically resistant to vancomycin and carried the *vanA* gene. The presence of virulence genes including *gelE*, *ccf*, *cpd*, *efa*Afs, and *agg*A were frequently detected. The results of this study show that retail chicken and beef meat is source of concern for public health due to having high prevalence of antibiotic resistance and as well as harbouring virulence factors.

1. Introduction

Enterecocci are Gram-positive, facultative anaerobe bacteria that normally widespread in the intestine of animals and humans. In addition, enterococci are present in a variety of fermented meat and dairy products as a starter culture without affecting human health (Foulquié Moreno, Sarantinopoulos, Tsakalidou, & De Vuyst, 2006). Moreover, some enterococci are able to produce bacteriocins called as enterocins which have already been reported to have antimicrobial activity against food spoilage bacteria such as *Listeria monocytogenes* (Ahmadova et al., 2013). Enterecocci have, however, been recognised as an emerging cause of nosocomial infections (Leavis, Bonten, & Willems, 2006) including bacteraemia, septicaemia, endocarditis and urinary tract infections (Hidron et al., 2008) which could be life threatening in immunocompetent and severely ill individuals.

Enterococci have an intrinsic antibiotic resistance to semi-synthetic penicillins, aminoglycosides (low level), vancomycin (low level resistant in *E. gallinarum*, *E. casseliflavus/E. flavescens*), lincosamides (mostly), polymyxines and streptogramins (*Enterococcus faecalis*) (Klare, Konstabel, Badstübner, Werner, & Witte, 2003). Enterococci can also develop acquired resistance to many other antibiotics by carrying various resistant traits through plasmids, integrongs and transposons (Hollenbeck & Rice, 2012). Enterococcal infections have been traditionally treated with glycopeptides antibiotics, mostly vancomycin, since it was approved for human use. However, because of extensive clinical use of vancomycin in hospitals, frequency of vancomycin resistance (Va^R) was dramatically increased (Kirst, Thompson, & Nicas, 1998). In addition to this extensive usage in hospitals, using growth promoters in livestock could potentially lead to the development of resistant strains. For example, in 1986, avoparcin, a glycopeptide analog, was approved to use as a growth promoter of food animals in Norway (Borgen et al., 2000). There was evidence to show an association between

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http://dx.doi.org/10.1016/j.lwt.2015.10.009
0023-6438/

injudicious use of this class of antibiotics in food animals and a substantial rise in the prevalence of Va^R Enterococci (VRE) recovered from farm animals, foods of animal origin including chicken meat, pork, and beef and from infected humans. Resistance was found to be plasmid-borne and could be transferred to other enterococci (Flannagan et al., 2003). These authors suggested that use of avoparcin in livestock allowed for selection and persistence of resistant strains. Because of the increase prevalence of Va^R in *Enterococcus* isolates, the use of avoparcin as a feed additive in food animals was banned in 1997 in all European Union countries (Borgen et al., 2000).

Besides antibiotic resistance, enterecocci are able to produce potential virulence factors that may enhance their pathogenicity, in another word responsible for causing diseases (Biswas, Dey, Adhikari, & Sen, 2014). These include haemolysin, gelatinase, enterococcal surface protein (Esp), aggregation substance, serine protease, capsule, cell wall polysaccharide and superoxide (Elsner et al., 2000). For example, several studies suggest that haemolysin is important for *Enterococcus* infectivity in animals and humans (Chow et al., 1993; Johnson, 1994). Gelatinase has been shown to be an important virulence factor for aiding to endocarditis in an animal model (Thurlow et al., 2010). It has been shown that *E. faecalis* producing Enterococcal surface protein Esp is more persistence in urinary bladder in experimentally infected animals (Shankar et al., 2001). Enterococci from food of animal origin have been shown to produce these abovementioned virulence factors suggesting that these animal products could act as potential reservoirs for human infections.

There is little data about the incidence of microbial resistance of *Enterococcus* strains in foods of animal origin in Turkey. Therefore, monitoring antimicrobial sensitivity is not only necessary for choosing appropriate antimicrobial agents but also important to monitor antimicrobial resistance development. In this view, the aim of the current study was to investigate the prevalence of enterococci in retail meat samples (chicken and beef) and their antimicrobial resistance profile. The detection of virulence genes and antimicrobial resistance genes were also examined.

2. Materials and methods

2.1. Sample collection, isolation and identification of Enterococcus species

A total of 200 samples of chicken (100) and beef (100) were collected from butcher shops and supermarkets in and around Hatay province in Turkey. All samples were collected in sterile plastic bags, stored in ice packs and transported immediately to the laboratory within 2 h for microbiological analysis. Each sample was screened for the presence of *Enterococcus* spp. using previously published protocols with some modifications (Hayes et al., 2003; Klibi et al., 2013). Meat samples (25 g) were placed in sterile plastic bags containing 225 mL buffered peptone water and mixed with stomacher for 3 min. Rinsate samples (50 mL) were then incubated at 37 °C for 24 h. Following incubation, 10 μl was subcultured into Enterococcosel Broth and further incubated at 37 °C for 24 h. After enrichment, a loopful of broth culture was aseptically streaked on VRE agar and VRA agar including 6 mg/L vancomycin. Plates were incubated at 37 °C for 24 h after which one colony per sample with typical enterococci morphology was then transferred onto blood agar plates in order to obtain pure culture. These isolates were then subjected to Gram staining and catalase test. Identification of the isolates was done by 16S rRNA sequencing. Bacterial 16S rRNA was amplified by using universal primers 16S 20 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1390 (5′-GAC GGG CGG TGT GTA CAA-3′) (Sghir, Antonopoulos, & Mackie, 1998; Suau et al.,1999). The PCR products were sequenced and analysed with the BLAST program available at the National Center for Biotechnology Information (NCBI).

2.2. Antibiotic sensitivity testing

Antimicrobial susceptibility was determined using disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2012) recommendations. Mueller Hinton Agar (MHA) was used for susceptibility testing and the plates were incubated in incubator at 37 °C for 20–24 h. The isolates were screened for susceptibility to 10 antibiotics including penicillin (P; 10 U/disc), ampicillin (AMP; 10 μg/disc), vancomycin (VA; 30 μg/disc), teicoplanin (TEC; 30 μg/disc), erythromycin (E; 15 μg/disc), tetracycline (TE; 30 μg/disc), ciprofloxacin (CIP; 5 μg/disc), chloramphenicol (C; 30 μg/disc), gentamycin (CN; 10 μg/disc) and trimethoprim/sulfamethoxazole (SXT; 1.25–23.75 μg/disc). Antibiotics tested in this study were selected based on their usage in veterinary practice among those classified as “critically important” (P, AMP, VA, TEC, CIP, CN and E) or “highly important” (SXT, C and TE) in human medicine (WHO, 2011). The minimum inhibitory concentration (MIC) values for vancomycin and teicoplanin were determined for Va^R strains using E-Test (Oxoid UK).

2.3. Screening of antibiotic resistance genes

The presence of genetic determinants in isolates showing antimicrobial resistance by disc assay conferring resistance to macrolide and tetracycline (*erm*A, *erm*B, *mef*A/E, *tet*K, *tet*L, *tet*M and *tet*O)) (Malhotra-Kumar, Lammens, Piessens, & Goossens, 2005), to aminoglycosides (*aac(6)-Ie-aph(2)-Ia*, *aph(2)-Ib*, *aph(2)-Ic*, *aph(2)-Id*, *aph(3)-IIIa*, *ant(4)-Ia* (Vakulenko et al., 2003) and chloramphenicol (*cat)* (Aarestrup, Agrees, Gerner-Smith, Madsen, & Jensen, 2000) was determined by PCR. The presence of vancomycin resistance genes (*vanA*, *vanB*, *vanC1/2*, *vanD*, *vanE*, *vanG*) was also analysed as previously described (Depardieu, Perichon, & Courvalin, 2004). Antibiotic resistance genes, primer sequences and lengths of products are listed in Table 1.

2.4. Detection of genetic determinants related to virulence

The presence of the genes responsible for the expression of gelatinase (*gel*A), cytolysin (*cyl*A, *cyl*M and *cyl*B), cell wall adhesins (*efaAfs* and *efaAfm*), enterococcal surface protein (*esp*), sex-pheromones (*cpd*, *cob*, *ccf* and *eep*), and the aggregation substance (*aggA*) were investigated in all enterecocci isolates (Eaton & Gasson, 2001; Marques & Suzart 2004; Shankar, Baghdayan, Huycke, Lindahl, & Gilmore, 1999). Virulence markers and PCR primers are listed in Table 2.

2.5. Statistical analysis

Pearson's chi–square ($\chi 2$) test was used to determine if there were significant differences ($P < 0.05$) in frequency of antimicrobial resistance profiles, resistance genes and virulence traits among Enterecocci isolates obtained from different meat species.

3. Results

A total of 205 isolates were obtained from chicken ($n = 105$) and beef samples ($n = 100$). Three different species including *E. faecalis* ($n = 103$), *E. hirae* ($n = 1$) and *Enterococcus faecium* ($n = 1$) were isolated from chicken meat, while only *E. faecalis* was identified from beef samples. A total of five VRE, including four *E. faecalis* and one *E. faecium* isolates, were isolated from one-hundred chicken meats.

Table 1
PCR primers, products and references for the detection of antibiotic resistance genes.

Primer	Sequence (5′ to 3′)	Product size (bp)	References
erm(A)	CCC GAA AAA TAC GCA AAA TTT CAT CCC TGT TTA CCC ATT TAT AAA CG	590	Malhotra-Kumar et al. 2005
erm(B)	TGG TAT TCC AAA TGC GTA ATG CTG TGG TAT GGC GGG TAA GT	745	
mef(A/E)	CAA TAT GGG CAG GGC AAG AAG CTG TTC CAA TGC TAC GG	317	
tet(K)	GAT CAA TTG TAG CTT TAG GTG AAG G TTT TGT TGA TTT ACC AGG TAC CAT T	155	
tet(M)	GTG GAC AAA GGT ACA ACG AG CGG TAA AGT TCG TCA CAC AC	406	
tet(O)	AAC TTA GGC ATT CTG GCT CAC TCC CAC TGT TCC ATA TCG TCA	515	
tet(L)	TGG TGG AAT GAT AGC CCA TT CAG GAA TGA CAG CAC GCT AA	229	
aac(6)-Ie-aph(2)-Ia	CAG GAA TTT ATC GAA AAT GGT AGA AAA G CAC AAT CGA CTA AAG AGT ACC AAT C	369	Vakulenko et al. 2003
aac(6)-Ie-aph(2)-Ia	CAG AGC CTT GGG AAG ATG AAG CCT CGT GTA ATT CAT GTT CTG GC	348	
aph(2)-Ib	CTT GGA CGC TGA GAT ATA TGA GCA C GTT TGT AGC AAT TCA GAA ACA CCC TT	867	
aph(2)-Ic	CCA CAA TGA TAA TGA CTC AGT TCC C CCA CAG CTT CCG ATA GCA AGA G	444	
aph(2)-Id	GTG GTT TTT ACA GGA ATG CCA TC CCC TCT TCA TAC CAA TCC ATA TAA CC	641	
aph(3)-IIIa	GGC TAA AAT GAG AAT ATC ACC GG CTT TAA AAA ATC ATA CAG CTC GCG	523	
ant(4)-Ia	CAA ACT GCT AAA TCG GTA GAA GCC GGA AAG TTG ACC AGA CAT TAC GAA CT	294	
*Cat*pIP 501	GGA TAT GAA ATT TAT CCC TC CAA TCA TCT ACC CTA TGA AT	505	Aarestrup et al. 2000
vanA	GGG AAA ACG ACA ATT GC GTA CAA TGC GGC CGT TA	732	Depardieu et al. 2004
vanB	ACG GAA TGG GAA GCC GA TGC ACC CGA TTT CGT TC	647	
vanC1/2	ATG GAT TGG TAY TKG TAT TAG CGG GAG TGM CYM GTA A	815/827	
vanD	TGT GGG ATG CGA TAT TCA A TGC AGC CAA GTA TCC GGT AA	500	
vanE	TGT GGT ATC GGA GCT GCA G ATA GTT TAG CTG GTA AC	430	
vanG	CGG CAT CCG CTG TTT TTG A GAA CGA TAG ACC AAT GCC TT	941	

Table 2
PCR primers, products and references for the detection of virulence genes.

Primer name	Sequence (5′ to 3′)	Product size (bp)	References
*gel*E	ACC CCG TAT CAT TGG TTT ACG CAT TGC TTT TCC ATC	419	Eaton and Gasson (2001)
*cyl*A	TGG ATG ATA GTG ATA GGA AGT TCT ACA GTA AAT CTT TCG TCA	517	Eaton and Gasson (2001)
ccf	GGG AAT TGA GTA GTG AAG AAG AGC CGC TAA AAT CGG TAA AAT	543	Eaton and Gasson (2001)
*efa*Afs	GAC AGA CCC TCA CGA ATA AGT TCA TCA TGC TGC TGT AGT A	705	Eaton and Gasson (2001)
*efa*Afm	AAC AGA TCC GCA TGA ATA CAT TTC ATC ATC TGA TAG TA	735	Eaton and Gasson (2001)
*cyl*M	CTG ATG GAA AGA AGA TAG TAT TGA GTT GGT CTG ATT ACA TTT	742	Eaton and Gasson (2001)
cpd	TGG TGG GTT ATT TTT CAA TTC TAC GGC TCT GGC TTA CTA	782	Eaton and Gasson (2001)
*cyl*B	ATT CCT ACC TAT GTT CTG TTA AAT AAA CTC TTC TTT TCC AAC	843	Eaton and Gasson (2001)
esp	TTG CTA ATG CTA GTC CAC GAC C GCG TCA ACA CTT GCA TTG CCG AA	933	Shankar et al. (1999)
eep	GAG CGG GTA TTT TAG TTC GT TAC TCC AGC ATT GGA TGC T	937	Marques and Suzart (2004)
cob	AAC ATT CAG CAA ACA AAG C TTG TCA TAA AGA GTG GTC AT	1405	Eaton and Gasson (2001)
*agg*A	AAG AAA AAG TAG ACC AAC AAC GGC AAG ACA AGT AAA TA	1553	Eaton and Gasson (2001)

All isolates were tested against twelve antibiotics. Hundred-one (96%) strains from chicken meat and sixty-three isolates (63%) from beef presented resistance to at least one of the twelve antimicrobial drugs tested. Among all *E. feacalis* isolates from chicken carcasses, resistance was observed to tetracycline (89.3%), ciprofloxacin (34.9%), erythromycin (59.2%), trimethoprim/sulfamethoxazole (33%) and chloramphenicol (18.4%). One strain of *E. faecium* isolated from chicken meats had resistance to all antimicrobials tested except chloramphenicol, while *E. hirae* strain was found to be resistance to penicillin, tetracycline and trimethoprim/sulfamethoxazole. Only one isolate from minced meat were resistant to chlorompehinicol. For beef isolates, the highest frequency of resistance was against tetracycline (53%), ciprofloxacin (12%) and trimethoprim/sulfamethoxazole (7%). No resistance to ampicillin, vancomycin, chloramphenicol and gentamycin were observed. The rates of resistance to penicillin (3%) and erythromycin (2%) were low, while only one isolates was found to be resistance to chloramphenicol (Table 3). Overall, 40% of chicken meat isolates were found to be resistant to three of more separate classes of antimicrobials (the multidrug resistance), whereas only 3% of minced meat isolates displayed the multidrug resistance. The antibiotic sensitivity test showed that Va^r enterococci were all resistant to tetracycline, erythromycin, trimethoprim/sulfamethoxazole, ampicillin and penicillin. The proportion of isolates from retail chicken resistant to tetracycline, erythromycin, trimethoprim/sulfamethoxazole, ciprofloxacin and chloramphenicol were significantly higher ($P < 0.05$) than those isolated from retail beef.

In the chicken meat samples, the *erm*B gene was found in 79 (75.2%) isolates and the *mef*A/E gene was found in only one (0.95%) isolate while the *erm*A gene was not detected in any of the isolates. For beef isolates, only three (3%) harboured the *erm*B gene. Among tested aminoglycosides resistance genes; *aph(3′)-IIIa*, *ant(6)-Ia* and *aac(6′)-Ie-aph(2″)-Ia* were observed in six (5.7%), one (0.95%) and one (0.95%) isolates from chicken meat, respectively, whereas none of the isolates from beef samples had these genes. Detection of the *cat* gene encodes resistance to chloramphenicol was confirmed in 5 (4.8%) isolates from chicken meat samples. In addition, the *tet*L gene was the most common, found in 85 (81%) isolates, the *tet*M gene in 80 (76.2%), the *tet*O gene in 18 (17.1%) and the *tet*K gene in one isolate from chicken meat samples. For beef isolates, the *tet*M gene was found in 17 (17%) isolates, the *tet*L gene in 10 (10%), the *tet*K gene in one and the *tet*O gene in one isolate. Sixty isolates carried two and fifteen isolates carried three *tet* genes while twenty four isolates only carried one *tet* genes in chicken isolates. For beef isolates, six isolates carried two and one isolate carried three *tet* genes, however fourteen isolates carried only one *tet* gene. A total of 3 isolates that were resistant to tetracycline did not contain any of the tested genes. All these chicken VRE possessed the *vanA* genotype, *erm*B, *tet*M and *tet*L, but the *van*B, *van*C1 and *van*C2/3 genes were not detected (Table 4).

Table 3
Distribution of antimicrobial resistance among the enterococci.

Antimicrobials	Chicken meat	Minced meat
	E. feacalis, %	*E. feacalis*, %
Ampicillin	4.9	0
Penicillin	4.9	3
Vancomycin	3.9	0
Teicoplanin	3.9	0
Erythromycin	59.2	2
Tetracycline	89.3	53
Gentamycin	4.9	0
Ciprofloxacin	34.9	12
Chloramphenicol	18.4	1
Trimethoprim/sulfamethoxazole	33.0	7
Total (n)	**103**	**100**

Fig. 1 shows the percentage of virulence genes in the isolates recovered from chicken and beef meat samples. The most prevalent virulence determinants were *cpd* (100% and 92.4%), followed by *ccf* (98% and 99%) and *afs* (95% and 95.2%) in isolates obtained from beef and chicken, respectively. The *gel*E and *eep* genes were detected in 82.9% and 88.6% of chicken isolates, and 75% and 84% of beef isolates, respectively. Enterococci from chicken meat samples also carried virulence genes including the *agg*A and *cob* (both genes, 26.7%), which was encountered only in one isolate (both genes, 1%) obtained from beef samples. The *efa*Afm and *cyl*M were not detected in beef isolates and the *cyl*B was not detected in chicken isolates. The enterecocci isolates that harboured the *cpd*, *cyl*A, *cyl*M and *agg*A genes were greater ($P < 0.05$) in retail chicken than in beef. There was no association found between phenotypic antibiotic resistance and the virulence genes detected.

4. Discussion

Even though it is known that enterococci are ubiquitous organism in the gut, it is one of the emerging organism causing nosocomial infections in humans. Recent studies confirmed enterococci contamination in a wide range of foods including cheese, sausages, meat, milk, and cereals due to improper handling (Koluman, Akan, & Akiroglu, 2009). Studies conducted by Olsen, Schønheyder, Christensen, and Bisgaard (2012) have provided strong evidence that enterococci originating from foods of animal origin had a remarkable degree of similarity in virulence characteristics with human isolates implicating animal meat as an important source for virulent enterococci strains for human colonization. To our best knowledge, this is the first report on the presence of virulence and antibiotic resistance genes in enterococci from retail meat samples in Turkey, even though there are some reports available on the prevelance and antimicrobial resistance of enterococci in meat, cheese and fermented Turkish foods (Çitak, Yucel, & Orhan, 2004; Koluman et al., 2009; Togay, Keskin, Acik, & Temiz, 2010). Enterecocci strains were isolated from all samples tested and five chicken samples were contaminated with VRE strains. Among serotypes isolated in this study, *E. feacalis* was the most prevalent, while only one *E. faecium* and *E. hirae* were isolated from chicken samples. The level of contamination and the species distribution found in this study is consistent with the recent reports from Canada and Tunisia where *E. feacalis* is the most commonly reported from chicken meat samples (Aslam, Diarra, Checkley, Bohaychuk, & Masson, 2012; Klibi et al., 2013). Authors also indicated a negligible prevalence in poultry carcasses for *E. faecium* and *E. hirae* (2%). In Tunisa, *E. feacalis* was also reported to be the most frequently reported species from foods of animal origin but there were other species also detected including *E. gallinarum*, *E. casseliflavus*, *E. mundtii*, and *E. sulfureus* (Klibi et al., 2013).

It is well known fact that the percentage of multiple antibiotic resistant enterococci strains is much lower among environmental strains when compared to clinical strains (Abriouel et al., 2008). Enterococci strains are also naturally resistant to aminoglycoside, lincomycine and quinupristin/dalfopristin. In the current study, resistance to three or more class of antibiotics (multidrug resistance) was found to be 40% for chicken derived enteroccoci isolates and only 3% for beef derived isolates which is in agreement with a recent study in Canada in which multidrug resistance was found in 91% of chicken *E. faecalis* and 14% beef *E. faecalis* isolates (Aslam et al., 2012). A high percentage of enterococcal isolates from chicken and beef samples were resistant to critically important antibiotics including ciprofloxacin and erythromycin, as well as tetracycline. A high frequency of resistance to erythromycin and tetracycline in enterococci from various foods was also reported in

Table 4
Main features of VRE isolated in the present study.

Isolate	Source	Virulence traits	Antimicrobial resistance[a]	Resistance gene Varients	MIC (μg/mL)	
					Vancomycin	Teicoplanin
E. faecium	Chicken	*afm*, ccf, *cob*, *gelE*	TE, E, SXT, AMP, P, CIP	*van*A, *erm*B, *tet*M, *tet*L	>256	64
E. faecalis	Chicken	*afs*, *ccf*, *esp*	TE, E, SXT, AMP, P, CIP	*van*A, *erm*B, *tet*M, *tet*L	>256	64
E. faecalis	Chicken	*afs*, *ccf*	TE, E, SXT, AMP, P	*van*A, *erm*B, *tet*M, *tet*L	>256	64
E. faecalis	Chicken	*afs*, *ccf*	TE, E, SXT, AMP, P, CIP	*van*A, *erm*B, *tet*M, *tet*L	>256	64
E. faecalis	Chicken	*afs*, *ccf*	TE, E, SXT, AMP, P, CIP	*van*A, *erm*B, *tet*M, *tet*L	>256	64

[a] TE, tetracycline; E, erithromycin; STX, trimethroprim-sulfamethoxazole; AMC, ampicillin; P, penicillin; CIP, ciprofloxacin.

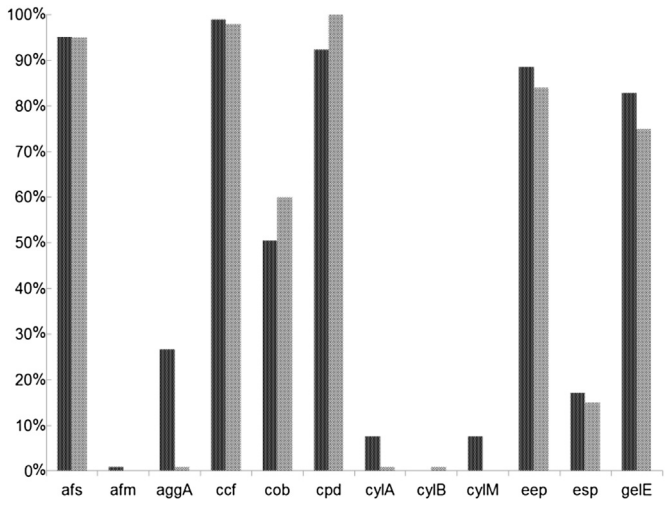

Fig. 1. Distribution of virulence genes in enterococci. Dark bars and grey bars represent the percentage of isolates obtained from chicken and beef meat samples, respectively. Vertical bar represents percentage of isolates, horizontal bar represents virulence genes.

Canada, Turkey and Tunisia(Aslam et al., 2012; Hammad, Shimamoto, & Shimamoto, 2014; Klibi et al., 2013; Koluman et al., 2009; Togay et al., 2010). 37% and 12% of isolates of enterococci obtained from chicken and beef samples were also found to be resistant to ciprofloxacin in this study. However, there was no enterococcal isolates found to be resistant to ciprofloxacin in Canada (Aslam et al., 2012), suggesting that this class of antibiotics might be still used in animal production in Turkey. In this study, there was no enterococci showed high level aminoglycoside resistance. However, high level of aminoglycoside resistant isolates were obtained from chicken isolates and this situation was attributed to the use of this antibiotics in poultry breeding in Tunisia (Klibi et al., 2013). Chicken isolates showed significantly higher rates of resistance ($P < 0.05$) to tetracycline, erythromycin, trimethoprim/sulfamethoxazole, ciprofloxacin and chloramphenicol than did beef isolates, suggesting that antibiotic resistance profile of food borne pathogens in broiler and chicken meat samples has to be regularly monitored in Turkey.

Vancomycin, which is one of the few alternatives in treating enterocooccal infections is ranked as critically important in human medicine and the presence of Va^R strains in foods is of great importance (WHO, 2011). There is significant evidence showing the reduced number of VRE were isolated since 1995 when avoparcin was first banned for use in livestock (Borgen et al., 2000; Bortolaia, Mander, Jensen, Olsen, & Guardabassi, 2015). In our study, 5% of chicken meat samples were found to have VRE strains. This is similar to results of a recent study from Spain in which 4.7% of chicken samples at retail level were also found to harbour VRE enterococci with acquired mechanisms of resistance ten years after the ban (Lopez et al., 2009). The continuance of Va^R was attributed to the co-existence of the *erm*B gene encoding erythromycin resistance and vancomycin resistance genes on the same plasmid (Lopez et al., 2009). This observation was also obtained in our study as all Va^R isolates carried the both *van*A and *erm*B genes together. In comparison, no VRE was detected in food of animal origins in studies done in Canada which can be explained with the implementation of strict no vancomycin usage in livestock.

In this study, the *tet*(M) or *tet*(L) genes encoding tetracycline resistance were found to be the most common resistance traits detected in enterecocci isolates. Other earlier studies from Canada (Aslam et al., 2012) and Tunisia (Klibi et al., 2013) were also reported that tetracycline resistant Enterococci isolates from meat samples harboured the *tet*(M) or *tet*(L) genes mostly. A number of tetracycline resistance determinants including the *tet*(M), *tet*(L), *tet*(K), *tet*(O) have been described so far. Several studies from all around the worlds showed that the *tet*(M) gene is the most frequently detected in tetracycline resistant enterococci isolates due to the transfer of Tn*916*-type transposons (Wilcks, Andersen, & Licht, 2005). In the current study, the *erm*B gene conferring erithromycine resistance was most frequently determined in Enterococci isolates, and none of the erithromycine resistant isolates harboured the *erm*A, *erm*C and *msr*C genes. There are also reports of the *erm*B gene in erythromycin resistant enterococci from foods of animal origin, animal and humans (Aslam et al., 2012; Diarra et al., 2010). Only 4.8% of isolates were found to carry the *cat* gene in chloramphenicol resistant chicken isolates. The *aph(3′)-IIIa*, *ant(6)-Ia* and *aac(6′)-Ie-aph(2″)-Ia* gene encoding aminoglycosides were only detected in chicken isolates in low frequency.

The presence of virulence factors does not necessarily mean that the strains isolated from the foods of animal origin cause diseases in humans, but may have pathogenic potential as these factors have been found to contribute to the severity of infection (Biswas et al., 2014; Elsner et al., 2000; Thurlow et al., 2010). A number of genes including *gelE*, *ccf*, *cpd*, *cob*, *afs*, and *aggA* were frequently detected in enterecococci strains isolated from chicken and beef samples, which is similar to previous studies (Aslam et al., 2012; Jahan & Holley, 2014; Klibi et al., 2013). The genes, *gel*E and *cpd*, associated with toxin production and sex pheromones were also reported to be commonly found in commensal isolates (Nueno-Palop & Narbad, 2011). Virulence traits (*cyl*A, *cyl*B and *cyl*M) associated with the production of the active cytolysin were reported to be the most important genes and were present in small number of isolates. Of the twenty nine enterococcal strains from raw and fermented meat, only two hourboured the *cyl*A gene (Jahan & Holley, 2014). Domann et al., (2007) reported that virulence trait for aggregation (*agg*A) may support the strain's probiotic characters. The *esp* gene is known to be associated with the biofilm production, endocarditis and nosocomial infections (Hayes, English, Carr, Wagner, & Joseph, 2004; Heikens et al., 2011) were detected in both chicken and beef isolates at low frequency which is in agreement with a previous study carried by Olsen et al., (2012). It was also previously speculated that this gene might play a role in the attachment to the equipment in the slaughter

house which might cause the contamination of meat products (Hayes et al., 2004). A recent report found that virulence genes carried by enterococci isolates in human and poultry isolates had similar gene sequences, supporting the zoonotic potential of this organism (Olsen et al., 2012). A recent study found an association between the genes encoding aggregation substances and cytolysin and the *tet*M resistance genes (Aslam et al., 2012). However, there were no such an association detected in our study. Moreover, some virulence traits (*cyl*A, *cyl*M and *agg*A) were statistically more prevalent in chicken isolates which was also demonstrated by Aslam et al., (2012).

In conclusion, the current study reveals the presence of Va^R enterococci in chicken carcasses in Turkey. The presence of Va^R enterococci on meats creates a major risk for public health which might cause severe infections due to consumption of this contaminated product. In addition, a high level of resistance against clinically important class of antibiotics was found in enterecocci which is also important concern for the effective treatment of infections and the potential transfer of this resistance to other intestinal organisms. Further research is needed to establish the risk of transmission of these organisms from foods of animal origin to humans and also to monitor the increasing antimicrobial resistance as well as virulence and resistance genes.

Acknowledgements

This project is financially supported by MKU Scientific Research Project Fund (Project Number: BAP-413). We thank Dr. Errol Hassan (The University of Queensland) for his valuable comments on the manuscript.

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