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Research Article

Antimicrobial Resistance and Virulence Characteristics in Enterococcus Isolates from Dogs [1]

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Abstract

Antimicrobial resistant enterococci are among the leading causes of nosocomial infections. Transmission of antimicrobial-resistant enterococci from animals to humans has been shown. For this reason, continuous monitoring of antimicrobial resistance in different animal species is of importance both for animal and human health. In this study, it was aimed to investigate the antimicrobial resistance profiles, resistance mechanisms implicated and virulence traits of 107 enterococci isolated from 125 rectal swab samples taken from dogs. The highest resistance rate was determined against tetracycline (65.4%), followed by ciprofloxacin (19.6%), erythromycin (19.6%), chloramphenicol (8.4%) and ampicillin (3.7%). Fourteen (12.1%) enterococci showed multidrug resistance (MDR) phenotype. The *tet*M gene was predominantly detected among tetracycline isolates. Of 21 erythromycin resistant isolates, 18 harbored the *erm*B gene. The frequently detected virulence genes was *ccf* (54.2%), *efa*A_{fs} (52.3%), *cpd* (45.8%) and *gel*E (44.9%). These results indicate that high level of antimicrobial resistance and virulence genes exist among enterococci from dogs and pose a potential public health concern.

Keywords: *Dog, Enterococcus spp., Antimicrobial resistance, Virulence genes*

Köpeklerden İzole Edilen Enterokok Suşlarının Antimikrobiyal Direnç ve Virulans Özellikleri

Özet

Antimikrobiyal dirençli enterokoklar nozokomiyal enfeksiyonların önde gelen nedenleri arasında bulunmaktadır. Antimikrobiyal dirençli enterokokların hayvanlardan insanlara geçişi gösterilmiştir. Bu nedenle, farklı hayvan türlerinde antimikrobiyal direncin sürekli olarak izlenmesi hem hayvan hem de insan sağlığı için önemlidir. Bu çalışmada, köpeklerden alınan 125 rektal sıvab örneğinden izole edilen 107 enterokok suşunun antimikrobiyal direnç profilleri, dirence aracılık eden mekanizmalar ve virülans özelliklerinin araştırılması amaçlandı. En yüksek direnç oranı tetrasikline (%65.4) karşı belirlenirken; siprofloksasin (%19.6), eritromisin (%19.6), kloramfenikol (%8.4) ve ampisiline (%3.7) ise değişen oranlarda direnç tespit edildi. On dört (%12.1) enterokok suşunda çoğul direnç fenotipi (MDR) görüldü. Tetrasiklin dirençli izolatlarda *tet*M geni ağırlıklı olarak saptandı. Eritromisin dirençli 21 izolatın 18'inin *erm*B geni taşıdığı tespit edildi. İzolatlar arasında *ccf* (%54.2), *efa*A_{fs} (% 52.3), *cpd* (%45.8) ve *jel*E (%44.9) virulens genleri sıklıkla belirlendi. Bu sonuçlar köpeklerden izole edilen enterokok suşlarında yüksek düzeyde antimikrobiyal direncin ve virülans genlerinin mevcut olduğunu ve potansiyel bir halk sağlığı sorunu olduğunu ortaya koymaktadır.

Anahtar sözcükler: *Köpek, Enterococcusspp., Antimikrobiyal direnç, Virulens genleri*

INTRODUCTION

Although enterococci are known as commensal agents residing on intestinal microbiota of animals and common in environmental sources, they emerged as an important agents of nasocomial and community-acquired infections due to their ability for developing high level of antimicrobial resistance [1,2]. Overuse and misuse of antimicrobials in pet animals also increases the likelihood of developement of resistance to these agents [3-5]. Enterococci can develop antimicrobial resistance mainly by two mechanisms: (i) target mutation and (ii) horizontal gene transfer which includes transformation, conjugation and transduction [6]. Furthermore, enterococci may serve as a resevoir to transfer

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resistance genes to other resident bacteria [4,5,7].

Enterococci have the ability to produce numerous virulence factors playing an important role of pathogenesis of diseases caused by these bacteria [8]. These virulence factors include gelatinase *(gel*E), enterococcal surface protein *(esp),* aggregation substance*(agg*A), cytolysin *(cyl*A, *cyl*B, *cyl*M), cell wall adhesins *(efa*A_{fs} and *efa*A_{fm}), sex pheromones *(cpd, cob, ccf, eep)* [9].

Pet animals are owned by people at increasing rates in Turkey, and this has made these animals a member of the family. Pet animals are known to transfer antimicrobial-resistant bacteria to humans due to their close physical contact with humans [4]. This situation requires continuous surveillance to track antimicrobial resistance among commensal, zoonotic and pathogenic bacteria from these animals, and to monitor changes in antimicrobial resistance over time. Therefore, the objective of this study was to investigate the prevalence of *Enterococcus* spp. and their antimicrobial resistance proflies and to determine the antimicrobial resistance and virulence genes by polymerase chain reaction (PCR).

MATERIAL and METHODS

Sampling

A total of 125 rectal swab samples from dogs admitted to private veterinary clinics in Hatay were collected from January 2014 to June 2014 for different purposes (medical check up, vaccination, treatment etc.). Rectal swabs were transferred to laboratory in Stuart transport medium. Age of dogs was ranging from two month to 13 year (median age 2 years), including 17 breeds. Among the dogs sampled, 61.6% (n=77) were males and 38.4% (n=48) were females. The study was approved by the Animal Ethical Committe of Mustafa Kemal University (2013/7-6).

Isolation and Identification

Rectal swabs were subjected to pre-enrichment procedure in Enterococcosel™ Broth (BD, USA) at 37°C for 24 h. Following enrichment procedure, a loopful of broth was streaked on Vancomycin Resistant Enterococci (VRE) agar (Oxoid, CM0985, UK) plates with and without vancomycin (6 mg/L). Plates were incubated at 37°C for 24 h and one typical colony randomly selected and passaged to blood agar (Merck, 110886, Germany) supplemented with 5% defibrinated sheep blood for obtaining pure culture. These isolates were presumptively identified as *Enterococcus* spp. by Gram staining and catalase test. Identification of the isolates was done by 16S rRNA sequencing by using universal primers [10,11]. The PCR products were sequenced from both ends and compared with published nucleotide sequences in GenBank using the BLAST program *(http://blast.ncbi.nlm.nih.gov/)*. The sequencing data were compared with the previously published sequences in GenBank using the BLAST program *(http://blast.ncbi.nlm.nih.gov/)* and a similarity score of 97% or higher was accepted as criterion for establishing species-level identification.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was determined using disk diffusion method in accordance with Clinical Laboratory Standards Institute guidelines (CLSI) [12] on Mueller Hinton Agar (MHA) plates. Antibiotic disks used were: ampicillin (10 µg), chloramphenicol (30 µg), vancomycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), teicoplanin (30 µg), ciprofloxacin (30 µg), trimethoprim-sulfamethaxozole (1.25 µg /23.75 µg) and gentamicin (120 µg). *Enterococcus faecalis* ATCC29212 was used as control strain. The isolates resistant to at least three different antimicrobial classes were deemed as multidrug resistant (MDR).

Detection of Antimicrobial Resistance Genes

Presence of antimicrobial resistance genes responsible for phenotypic resistance in disk diffusion assay was investigated by PCR. Erythromycin and tetracycline resistant isolates were screened for the presence of *tet*K, *tet*L, *tet*M, *tet*O, *tet*S, *erm*A, *erm*B and *mef*A/E [13], high level gentamicin resistant isolates for *aac(6)-Ie-aph(2)-Ia, aph(2)-Ib, aph(2)-Ic, aph(2)-Id, aph(3)-IIIa* and *ant(4)-Ia* [14], chloramphenicol resistant isolates for *cat* [15], vancomycin resistant isolates for *van*A, *van*B, *van*C1/2, *van*D, *van*E and *van*G [16]. Following resistant reference strains were used as controls: *E. faecium* BM4147 *(van*A), *E. faecalis* V583 *(van*B), *E. gallinarum* BM 4174 *(van*C1), E. *casseliflavus* ATCC 25788 (*van*C2), *S. pyogenes* BM137 *(tet*M, *erm*B), *S. pyogenes* UR1092 *(erm*A), *S. aureus* R-16794 *(tet*K).

Detection of Virulence Genes

The genes responsible for the expression of gelatinase *(gel*A), cytolysin *(cyl*A, *cyl*M and *cyl*B), cell wall adhesins *(efa*A_{fs} and *efa*A_{fm}), enterococcal surface protein *(esp),* sex pheromones *(cpd*, *cob*, *cad*, *ccf* and *eep)* and the aggregation substance *(agg*A) were investigated as previously described by Eaton and Gasson [17], Marques and Suzart [18] and Shankar et al.[19].

RESULTS

Isolation and Identification of Enterococcus spp.

Overall, 107 (85.6%) *Enterococcus* spp. were isolated from 125 rectal swab samples. *E. faecalis* was the most frequently detected species (96; 89.7%), followed by *E. faecium* (9; 8.4%), *E. hirae* (1; 0.9%) and *E. durans* (1; 0.9%).

Antimicrobial Resistance Profiles of Enterococcus spp.

Of 107 enterococci isolates, 15 (14.02%) were susceptible to all antimicrobial tested. None of the isolates were resistant to vancomycin, teicoplanin and high level gentamicin

(HLG). Various rates of resistance were observed to tetracycline (70; 65.4%), ciprofloxacin (21; 19.6%), erythromycin (21; 19.6%), chloramphenicol (9; 8.4%) and ampicillin (4; 3.7%). MDR were detected among 14 (13.1%) enterococci. MDR to six, five, four and three antimicrobials was detected in one (0.9%), one (0.9%), five (4.7%) and six (5.6%) isolates, respectively *(Table 1)*.

Presence of Resistance Genes Among Resistant Enterococci

Antimicrobial resistant isolates were screened for the presence of resistance genes acquired by horizantal gene transfer. Of the 70 tetracycline resistant isolates, *tet*M was the most common, found in 45 isolates (64.3%), both *tet*L and *tet*M in 12 (17.1%), *tet*O in 4 (5.7%) isolates, *tet*K in 2 (2.9%) and *tet*L in 2 (2.9%) isolates, which encode proteins involved in ribosomal protection and efflux, respectively. Of 21 erythromycin resistant isolates, 18 harbored *ermB* gene. *cat* gene was detected only two resistant isolates *(Fig.1, Fig. 2)*.

Characterization of Virulence Genes

The percentage of virulence genes among the tested

Table 1. *Resistance phenotypes determined in Enterococcus spp.*

Resistance Phenotype*	*E. faecalis*	*E. faecium*	*E. durans*	*E. hirae*
AMP, TE, E, CIP, C	1			
TE, E, CIP, C	1			
AMP, TE, E		3		
TE, E, CIP	6			
TE, E, C	2			
TE, CIP	3			
TE, C	3			
TE, E	6			
CIP	9			1
E	3			
TE	41	3	1	
C	2			
Susceptible	19	3		
Total	96	9	1	1

* ***AM:*** *ampicillin,* ***TE:*** *tetracycline,* ***CIP:*** *ciprofloxacin,* ***E:*** *eryhtromycin,* ***C:*** *chloramphenicol*

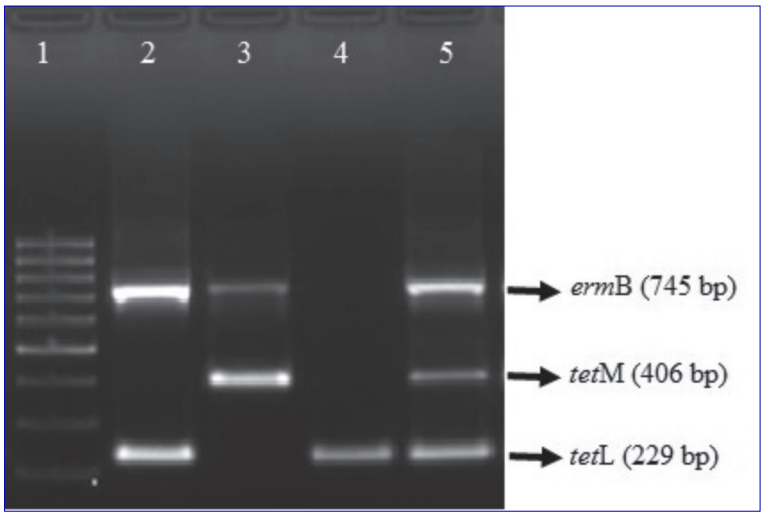

Fig 1. Agarose gel electrophoresis of tetracycline and macrolide resistance genes. Lane 1: 100 bp molecular marker, Lane 2: *erm*B and *tet*L, Lane 3: *erm*B and *tet*M, lane 4: *tet*M, Lane 5: *erm*B, *tet*L and *tet*M

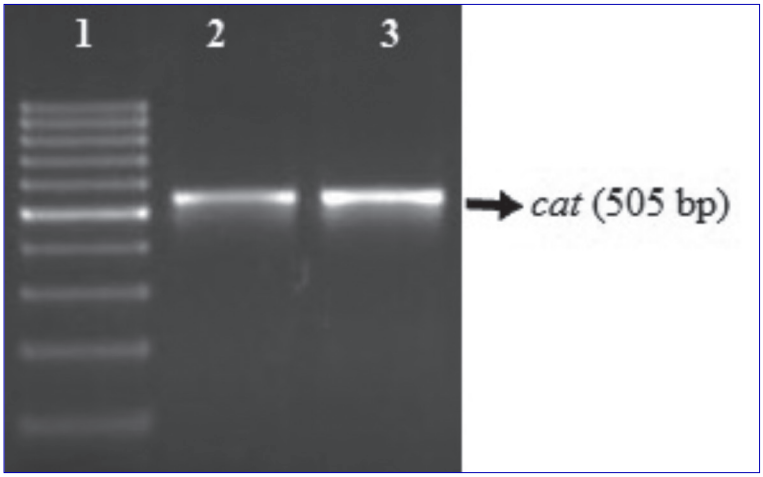

Fig 2. Agarose gel electrophoresis of *cat* gene detected in chloramphenicol resistant isolates. Lane 1: 100 bp molecular marker, Lane 2-3: *cat* gene

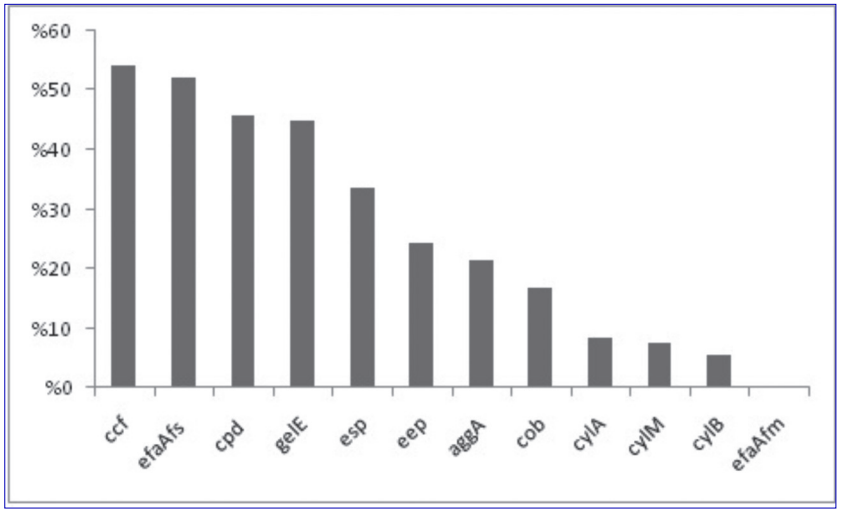

Fig 3. Distribution of virulence genes among enterococci

strains is shown in *Fig. 3*. One hundred and four (97.2%) isolates carried one or more virulence genes. *efa*A_{fm} was not detected in any of the isolates. The *efa*A_{fs} gene, encoding cell wall adhesin in enterococci, was found in 52.3% of the isolates. Sex pheromone genes, *ccf, cpd*, *cob* and *eep*, were detected in 54.2%, 45.8%, 16.8% and 24.3% of the isolates, respectively. Frequency of *cyl*A, *cyl*M and *cyl*B genes were 8.4%, 7.5% and 5.6%, respectively. The *gel*E, *esp* and *agg*A genes were observed in 44.9%, 33.6% and 21.5% of the isolates, respectively.

DISCUSSION

Antimicrobial resistance is a major concern in human and animal health throughout the world. Even tough it is known that enterococci are ubiquitous microorganisms found different habitats including gastrointestinal tract of human and animals, enterococci recently emerged as a leading cause of multiresistant, nosocomial infections in humans [20,21]. A recent study showed that possible transmission of resistant enterococci species between dog, human and hospital environments [22].

Among 107 enterococcal isolates, *E. faecalis* (89.7%) was the most frequently isolated species followed by *E. faecium* (8.4%). In contrast, *E. faecium* was reported to be most common species isolated from dogs in previous studies carried out in Turkey [23,24]. *E. faecalis* has been reported to be predominantly isolated from urinary tract infections of both dogs KuKanich and Lubbers [25] and humans [26] as a causative agent.

Increased use of antimicrobials in pet animals lead to the emergence of resistant bacteria [4]. The rate of antimicrobial resistance observed among enterococci in this study was lower than those reported of Türkyılmaz et al.[23] in Aydın, but higher than findings of Boynukara et al.[24] in Van, suggesting that resistance rates for enterococci vary regionally and influenced by antibiotic usage. However, Boynukara et al.[24] did not mention the origin of the dogs in their study. Resistance rate to tetracycline (65.4%), which is a widely used in veterinary field in Turkey, was higher than those from findings (41.1%) of Boynukara et al.[24], but lower than findings (70.3%) of Türkyılmaz et al.[23]. The erythromycine resistance rate (19.6%) was similar to findings (21.1%) of Boynukara et al.[24]. But, Türkyılmaz et al.[23] reported higher erythromycin resistance rate (69.2%). In previous studies, no acquired vancomycin resistance mediated by transferable *van*A or *van*B gene was reported in Turkey among enterococci [23,27,28]. Similarly, no resistance was observed against this agent in the current study. However, intrinsic resistance encoded by *van*C gene has been reported among *E. gallinarum* and *E. casseliflavus* species from dogs [23,27,28]. Resistance to ciprofloxacin, which is a clinically important drug, was found high (19.6%), in contrast to findings of Boynukara et al.[24], who reported a resistance rate of 6.7%. The rate of chloramphenicol resistance (8.4%) found in this study was comparable to findings (12.1%) of Türkyılmaz et al.[23]. Ampicillin is a drug of choise to be used for the treatment of multi-drug resistant enterococcal infection in combination with aminoglycosides when the isolates did not exhibite high level aminoglycoside resistance [29]. Furthermore ampicillin resistant enterococci are usually resistant to other classes of antimicrobilals used in dogs [30]. Even tough overall prevalence of ampicillin resistance was low (3.7%), detection of ampicillin resistance poses a significant risk for both human and animal health, and further investigation are, therefore, necessary to determine the true prevalence of ampicillin resistant enterococci in dogs using selective media. In a recent study, Çelik et al.[31] reported higher prevalence (20.9%) of ampicillin resistant enterococcus using selective media.

In current study, the most common resistance gene detected was the *tet*M in tetracycline resistant enterecocci isolates. Previous studies carried out in Turkey showed predominance of *tet*M gene in tetracycline resistant enterococci from different sources such as meat [32], cheese [33] as well as dogs [23]. Reason of wide dissemination of *tet*M gene is that this gene is frequently carried by *Tn*946 in enterococci [5]. Main resistance mechanism against macrolides is the modification of 23S rRNA by *erm* methylases among enterococci [34]. In the current study, *erm*B was the only gene detected in macrolide resistant enterococci. Similarly, Türkyılmaz et al.[23] reported high prevalence of *erm*B gene. The *cat* gene responsible for chloramphenicol resistance was found at a very low

rate (22.2%), suggesting presence of acquired other resistance genes or mechanisms responsible for this resistance phenotype. In a study carried out by Yılmaz et al.[32], lower prevalence rate (4.8%) of *cat* gene among chloramphenicol resistant enterococci from meat was reported. In contrast, Türkyılmaz et al.[22] detected *cat* gene in 63.6% of chloramphenicol resistant enterococci.

Enterococci have the ability to produce various virulence factors contributing the course and severity of infection [35]. The *efa*A_{fs} and *efa*A_{fm} are cell wall adhesins associated with infective endocarditis. In this study, *efa*A_{fs} was detected 58.3% of *E. faecalis* isolates. *efa*A_{fm} was not present in any *E. faecium* isolate. Iseppi et al.[36] found *efa*A_{fs} gene in 33.3% of *E. faecalis* isolates and *efa*A_{fm} gene in 50% of *E. faecium* isolates.

Cytolysin is a bacteriocin-type exotoxin, exerts its effects towards erythrocytes, leukocytes and macrophages [8]. *cyl*A, *cyl*M and *cyl*B genes were detected in 8.4%, 7.5% and 5.6% of the isolates, respectively. A similar finding was also reported by Iseppi et al.[36], who detected these genes in 8.3%, 8.3% and 8.3% of *E. faecalis* isolates. In this study, the authors detected only *cyl*M gene (7.1%) in *E. faecium* isolates. Gülhan et al.[37] investigated cytolysin activity of *E. faecalis* and *E. faecium* from humans and pet animals, and detected no cytolytic activity in dog isolates, except one *E. faecium* isolate from cat.

*gel*E is zinc-dependent metalloendopeptidase, is capable of hydrolysing gelatine, elastin, collagen, haemoglobin [8]. Geletinase has also been reported to play an important role in the developement of endocarditis and decrease neutrophil accumulation to the site of infection by splitting complement C5a fragment, which has anaphylotoxin activity [38]. In the current study, the *gel*E was detected in 44.9% of the isolates. However, Iseppi et al.[36] reported higher prevalence rates 57.1% and 80.3% of *E. faecium* and *E. faecalis* isolates, respectively. Gülhan et al.[37] found 26.6% and 60% of *E. faecium* and *E. faecalis* isolates positive for gelatinase activity.

Aggregation substance (AS) is enterococcal surface protein that contributes the formation of mating aggregates facilitating bacterial conjugation [8]. In the current study, 21.5% of the isolates were positive for *agg*A gene. In contrast, Iseppi et al.[36] did not find this gene, and speculated that the isolates had a reduced capability to transfer resistance virulence genes by mating. A very low rate was also reported by Gülhan et al.[37], who detected a positivity rate of 1.6% and 6.7% for *E. faecium* and *E. faecalis* isolates, respectively.

Enterocci have the ability to produce sex pheromones (*cpd*, *cob*, *ccf*, *cad*) facilitating conjugative plasmid transfer betwen cells [8]. Sex pheromone genes, *ccf, cpd*, *cob* and *eep*, were detected in 54.2%, 45.8%, 16.8% and 24.3% of the isolates. In a recent study, higher prevalence of sex pheromones was reported among enterococci from meat samples by Yılmaz et al.[32].

The *esp* gene is known to be involved in biofilm formation. Furthermore, biofilm production has been shown to play an important role in the exchange of antibiotic resistance genes between cells and to increase their resistance to antibiotics [8]. The *esp* gene was detected in 33.6% of the isolates. In contrast, Iseppi et al.[36] did not find this gene among the isolates. Also, Oliveira et al.[39] reported a lower prevalence rate (10%) among enterococci from dogs with periodontal disease.

In conclusion, the current study reveals that dogs are colonized with potentially virulent and antibiotic resistant enterococci. Therefore, the possibility of transmission of enterococci from dogs to humans, particularly those in the risk group, can not be excluded. Also, further studies are needed to elucidate the risk of transmission to humans by analyzing the clonal relationship in enterococci from dogs and dog owners.

REFERENCES

1. Martone WJ: Spread of vancomycin-resistant enterococci: Why did it happen in the United States ? *Infect Control Hosp Epidemiol*, 19, 539-545, 1998. DOI: 10.2307/30141777

2. Çetinkaya Y, Falk P, Mayhall CG: Vancomycin resistant enterococci. *Clin Microbiol Rev*, 13, 686-707, 2000. DOI: 10.1128/CMR.13.4.686-707.2000

3. De Graef EM, Decostre A, Devriese LA, Haesbrouck F: Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. *Microb Drug Resist*, 10, 65-69, 2004. DOI: 10.1089/107662904323047826

4. Guardabassi L, Schwarz S, Lloyd DH: Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother*, 54, 321-332, 2004. DOI: 10.1093/jac/dkh332

5. Leener ED, Decostere A, De Graef EM, Moyaert H, Haesebrouck F: Presence and mechanism of antimicrobial resistance among enterococci from cats and dogs. *Microb Drug Resist*, 11, 395-403, 2005. DOI: 10.1089/mdr.2005.11.395

6. Palmer KL, Kos VN, Gilmore MS: Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr Opin Microbiol*, 13, 632-639, 2010. DOI: 10.1016/j.mib.2010.08.004

7. Murray BE: Diversity among multidrug resistant enterococci. *Emerg Infect Dis*, 4, 37-47, 1998. DOI: 10.3201/eid0401.980106

8. Sava IG, Heikens E, Huebner J: Pathogenesis and immunity in enterococcal infections. *Clin Microbiol Infect*, 16, 533-540, 2010. DOI: 10.1016/j.lwt.2016.10.026

9. Chajęcka-Wierzchowska W, Zadernowska A, Łaniewska-Trokenheimł: Virulence factors of *Enterococcus* spp. presented in food. *LWT - Food Sci Technol*, 75, 670-676, 2017. DOI: 10.1016/j.lwt.2016.10.026

10. Sghir A, Antonopoulos D, Mackie RI: Design and evaluation of a *Lactobacillus* group-specific ribosomal RNA-targeted hybridization probe and its application to the study of intestinal microecology in pigs. *Syst Appl Microbiol*, 21, 291-296, 1998. DOI: 10.1016/S0723-2020(98)80036-2

11. Suau A, Bonnet R, Sutren M, Godon J J, Gibson G, Collins MD, Dore´J: Direct rDNA community analysis reveals a myriad of novel bacterial lineageswithin the human gut. *Appl Environ Microbiol*, 65, 4799-4807, 1999.

12. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI Document M100-S22, 2012.

13. Malhotra-Kumar S, Lammens C, Piessens J, Goossens H: Multiplex

PCR for simultaneous detection of macrolide and tetracycline resistance determinants in streptococci. *Antimicrob Agents Chemother*, 49, 4798-4800, 2005. DOI: 10.1128/AAC.49.11.4798-4800.2005

14. Vakulenko SB, Zervos MJ, Donabedian SM, Lerner SA, Voskresenskiy AM, Chow JW: Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. *Antimicrob Agents Chemother*, 47, 1423-1426, 2003. DOI: 10.1128/AAC.47.4.1423-1426.2003

15. Aarestrup FM, Agrees Y, Gerner-Smith P, Madsen M, Jensen LB: Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers and pigs in Denmark. *Diagn Microbiol Infect Dis*, 37, 127-137, 2000. DOI: 10.1016/S0732-8893(00)00130-9

16. Depardieu F, Perichon B, Courvalin P: Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. *J Clin Microbiol*, 42, 5857-5860, 2004. DOI: 10.1128/JCM.42.12.5857-5860.2004

17. Eaton TJ, Gasson MJ: Molecular screening of e*nterococcus* virulence determinants and potential for genetic exchange between food and medical isolates. *Appl Environ Microbiol*, 67, 1628-1635, 2001. DOI: 10.1128/AEM.67.4.1628-1635.2001

18. Marques BE, Suzart S: Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. *J Med Microbiol*, 53, 1069-1073, 2004. DOI: 10.1099/jmm.0.45654-0

19. Shankar V, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS: Infection-derived *Enterococcus faecalis* strains are enriched in *esp*, a gene encoding a novel surface protein. *Infect Immun*, 67, 193-200, 1999.

20. Lebreton F, Willems RJL, Gilmore MS: *Enterococcus* Diversity, Origins in Nature, and Gut Colonization. **In,** Gilmore MS, Clewell DB, Ike Y, Shankar N (Eds): Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary. https://www.ncbi.nlm.nih.gov/pubmed/24649513; *Accessed:* 10.03.2017.

21. Agudelo Higuita NI, Huycke MM (2014): Enterococcal Disease, Epidemiology, and Implications for Treatment. **In,** Gilmore MS, Clewell DB, IkeY, Shankar N (Eds): Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary. https://www.ncbi.nlm.nih.gov/pubmed/24649513; *Accessed:* 10.03.2017.

22. Chung YS, Kwon KH, Shin S, Kim JH, Park YH, Yoon JW: Characterization of veterinary hospital-associated isolates of Enterococcus species in Korea. *J Microbiol Biotechnol*, 24 (3): 386-393, 2014.

23. Türkyılmaz S, Erdem V, Bozdoğan B: Investigation of antimicrobial susceptibility for enterococci isolated from cats and dogs and the determination of resistance genes by polymerase chain reaction. *Turk Vet J Anim Sci*, 34, 61-68, 2010. DOI: 10.3906/vet-0807-17

24. Boynukara B, Ekin İH, Aksakal A, Gülhan T: Isolation and antibiotic susceptibility of enterococci from human, dog and cat faeces. *Vet Hek Mikrobiyol Derg*, 2 (1): 37-42, 2002. (Article in Turkish with English Abstract)

25. KuKanich KS, Lubbers BV: Review of enterococci isolated from canine and feline urine specimens from 2006 to 2011. *J Am Anim Hosp Assoc,* 51, 148-154, 2015. DOI: 10.5326/JAAHA-MS-6070

26. Etiz P, Kibar F, Ekenoğlu Y, Yaman A: İdrar kültüründen izole edilen Enterokok türlerinin antibiyotik direnç profillerinin değerlendirilmesi. *Türk Mikrobiyol Cem Derg*,44, 107-113, 2014. DOI: 10.5222/TMCD.2014.107

27. Bağcıgil AF, Koenhemsi L, Çelik B, Metiner K, Or ME, Ak S: Examination of vancomycin resistant Enterococci (VRE) isolated from canine and feline rectal swabs. *J Fac Vet Med Istanbul Univ*, 42, 111-116, 2016. DOI: 10.16988/iuvfd.2016.15364

28. Bağcıgil AF, İkiz S, Ak S, Özgür NY: Isolation of vancomycin resistant Enterococci from animal faeces, detection of antimicrobial resistance profiles and vancomycin resistant genes. *Kafkas Univ Vet Fak Derg*, 21 (1): 87-94, 2015. DOI: 10.9775/kvfd.2014.11805

29. Arias CA, Contreras GA, Murray BE (2010): Management of multidrug-resistant enterococcal infections. *Clin Microbiol Infect,* 16, 555-562, 2010. DOI: 10.1111/j.1198-743X.2010.03214.x

30. Damborg P, Sørensen AH, Guardabassi L: Monitoring of antimicrobial resistance in healthy in healthy dogs: First report of canine ampicillin-resistant *Enterococcus faecium* clonal complex 17. *Vet Microbiol,* 132, 190-196, 2008. DOI: 10.1016/j.vetmic.2008.04.026

31. Çelik B, Bağcıgil AF, Koenhemsi L, Adıgüzel MC, Or ME, Ak S: Determination of ampicillin resistant Enterococci (ARE) ısolated from canine and feline rectal swabs. *J Fac Vet Med Istanbul Univ*. 43 (1): 1-6, 2017. DOI: 10.16988/iuvfd.265324

32. Yılmaz EŞ, Aslantas Ö, Pehlivanlar Onen S, Süheyla Türkyılmaz S: Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey**.** *LWT - Food Sci Technol*, 66, 20-26, 2016. DOI: 10.1016/j.lwt.2015.10.009

33. Kürekci C, Önen SP, Yipel M, Aslantaş Ö, Gündoğdu A: Characterisation of phenotypic and genotypic antibiotic resistance profile of enterococci from cheeses in Turkey. *Korean J Food Sci Anim Resour*, 36, 352-388, 2016. DOI: 10.5851/kosfa.2016.36.3.352

34. Leclercq R: Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Review. *Clin Infect Dis*, 34, 482-492, 2002. DOI: 10.1086/324626

35. Mundy LM, Sahm DF, Gilmore M: Relationships between enterococcal relationships between enterococcal virulence and antimicrobial resistance. *Clin Microbiol Rev*, 13, 513-522, 2000. DOI: 10.1128/CMR.13.4.513-522.2000

36. Iseppi R, Messi P, Anacarso I, Bondi M, Sabia C, Condò C, de Niederhausern S: Antimicrobial resistance and virulence traits in Enterococcus strains isolated from dogs and cats. *New Microbiol,* 38 (3): 369-378, 2015.

37. Gülhan T, Aksakal A, Ekin İH, Savaşan S, Boynukara B: Virulence factors of Enterococcus faecium and *Enterococcus faecalis* strains isolated from humans and pets. *Turk J Vet Anim Sci*, 30, 477-482, 2006.

38. Thurlow LR, Thomas VC, Narayanan S, Olson S, Fleming SD, Hancock LE: Gelatinase contributes to the pathogenesis of endocarditis caused by *Enterococcus faecalis*. *Infect Immun*, 78,4936-43, 2010. DOI: 10.1128/IAI.01118-09

39. Oliveira M, Tavares M, Gomes D, Touret T, São Braz B, Tavares L, Semedo-Lemsaddek T: Virulence traits and antibiotic resistance among enterococci isolated from dogs with periodontal disease. *Comp Immunol Microbiol Infect Dis*, 46, 27-31, 2016. DOI: 10.1016/j.cimid.2016.04.002