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Investigation of antimicrobial susceptibility for enterococci isolated from cats and dogs and the determination of resistance genes by polymerase chain reaction

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Abstract: The aim of this study was to investigate the antimicrobial susceptibility and to determinate the resistance genes by Polymerase Chain Reaction (PCR) among enterococci isolated from healthy cats and dogs in İzmir, Turkey. From a total of 114 rectal swabs, 91 (31 cats and 60 dogs) stains were isolated as enterococci. Twenty eight isolates were identified as *Enterococcus faecalis* and 63 as *Enterococcus faecium* with the API 20 IDStrep system. Antimicrobial susceptibilities were determined by disc diffusion. Susceptibility rates of all enterococci strains were found as 100.0%, 95.6%, 94.5%, 87.9%, 84.6%, 30.8%, 29.7%, and 0.0% for teicoplanin, penicillin, vancomycine, chloramphenicol, gentamycin, erythromycin, tetracycline, and clindamicin, respectively. Of 91 enteroccocci isolates 14 (15.4%) were found susceptible to all antimicrobials tested while 77 (84.6%) strains were resistant. All isolates were found resistant to clindamicin while 5 *E. faecium* strains had an intermediate resistance to vancomycin. None of the intermediate resistant vancomycin strains has any *vanA, vanB,* or *vanC* genes. From 91 isolates 4, 11, 14, 63, and 64 were found resistant to penicillin, chloramphenicol, gentamycin, erythromycin, and tetracycline, respectively. Sixty two tetracycline resistant strains had *tet*(M) gene. From erythromycin resistant strains 2 and 54 had *erm*(A) and *erm*(B). Gentamycin resistance was detected for 14 isolates from which 10 had *aac*(6')-*aph*(2') genes. Seven strains have the *cat* gene of 11 chloramphenicol resistant strains. The results revealed that there was a high level of antimicrobial resistance among enterococci isolated from healthy cats and dogs and they pose a potential risk for transmission of enterococci to humans.

Key words: Dog, cat, enterococci, antimicrobial susceptibility, resistance genes, PCR

Kedi ve köpeklerden izole edilen enterokokların antibiyotik duyarlılıklarının incelenmesi ve direnç genlerinin polimeraz zincir reaksiyonu ile saptanması

Özet: Bu çalışmanın amacı Türkiye'de İzmir'de sağlıklı kedi ve köpeklerden izole edilen enterokokların antibiyotik duyarlılıklarının incelenmesi ve direnç genlerinin Polimeraz Zincir Reaksiyonu (PZR) ile belirlenmesidir. Toplam 114 rektal sıvap örneğinden 91 enterokok (31 kedi ve 60 köpekten) izolasyonu yapılmıştır. API 20 IDStrep sistemi kullanılarak

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izolatlardan 28'i *Enterococcus faecalis* ve 63'ü *Enterococcus faecium* olarak identifiye edilmiştir. İzolatların antibiyotik duyarlılıkları disk difüzyon yöntemi ile belirlenmiştir. Tüm enterokok suşlarının teikoplanin, penisilin, vankomisin, kloramfenikol, gentamisin, eritromisin, tetrasiklin ve klindamisine duyarlılıkları sırasıyla % 100,0, % 95,6, % 94,5, % 87,9, % 84,6, % 30,8, % 29,7 ve % 0,0 bulunmuştur. Doksan bir enterokok izolatının 14 (% 15,4)'ü kullanılan tüm antibiyotiklere duyarlı bulunurken, 77 (% 84,6)'sinde direnç belirlenmiştir. Tüm izolatlar klindamisine karşı dirençli bulunurken, 5 *E. faecium* izolatının vankomisine orta derecede dirençli olduğu tespit edilmiştir. Vankomisine orta derecede dirençli suşların hiçbirisinde *vanA, vanB,* veya *vanC* genleri belirlenmemiştir. Doksan bir izolatın sırasıyla 4, 11, 14, 63 ve 64'ü pensilin, kloramfenikol, gentamisin, eritromisin ve tetrasikline dirençli bulunmuştur. Tetrasiklin dirençli 62 suşun *tet*(M) genine sahip olduğu saptanmıştır. Eritromisin dirençli suşların ise 2'sinin *erm*(A), 54'ünün *erm*(B) geni taşıdıkları belirlenmiştir. Ondört izolatta gentamisin direnci saptanırken, bunların 10'unun *aac*(6')-*aph*(2') geni taşıdıkları belirlenmiştir. Kloramfenikol dirençli 11 suşun 7'sinin *cat* genine sahip olduğu bulunmuştur. Bu çalışmada, sağlıklı kedi ve köpeklerden izole edilen enterokoklarda antimikrobiyal direncin yüksek olduğu görülmüştür ve bu türlerin enterokokların insanlara bulaştırılmasında potansiyel bir risk taşıdıkları düşünülmektedir.

Anahtar sözcükler: Köpek, kedi, enterokok, antimikrobiyal duyarlılık, direnç genleri, PZR

Introduction

Enterococci have been considered as saprophyte inhabitant of the gastrointestinal tracts of humans and animals until a couple of decades ago. These bacteria are largely isolated from environment contaminated by human and animal faecal materials like urban sewage, recipient water, and in fertilized soil, as well as in food products derived from animals (1). However, since 1980, enterococci have emerged as nosocomial pathogens and rates are increasing by years (2).

Although enterococci are commensal bacteria in many animals, including pets under certain conditions, they can cause pathological disorders. Enterococci may be transferred from pets to humans by petting, saliva, or fecal contamination. These bacteria play an important role in the distribution of resistance genes between same species and interspecies (1,3).

Antimicrobial resistance increasingly gain importance worldwide among pathogen bacteria and decrease the options for clinicians in the treatment of infections. Since 1980s hospital infections due to multiple resistant gram positive bacteria has become a growing problem. Multi-drug resistance is one of the most important characteristic of the enterococci. High mortality and morbidity rates of enterococcal infections are due to the difficulties involved in the treatment of multi-drug resistant bacteria (2,4).

Enterococci are naturally resistant to low level beta lactams and aminoglycosides. Recently, multi-drug resistant enterococci, which acquired resistance to many antimicrobial agents in addition to their intrinsic resistance, have caused great concern (5). Glycopeptides, especially vancomycin, are often used to treat enterococcal infections in patients with hypersensitivity to penicillins or in infections due to β-lactam resistant enterococci (6). The therapeutic options for the treatment of infections caused by enterococci with vancomycin resistance in addition to high level penicillin and aminoglycoside resistance are quite limited (7).

To the best of our knowledge, the presence of antibiotic resistant genes among enterococci isolated from cats and dogs has not been studied in Turkey. The aim of this study was to investigate the antimicrobial susceptibility and to determine the resistance genes by Polymerase Chain Reaction (PCR) among enterococci isolated from healthy cats and dogs in İzmir, Turkey.

Materials and methods

Materials

One hundred and fourteen healthy animals admitted to a private veterinary hospital in İzmir from January to November 2006 for medical check-up or vaccination and subjected to rectal swab were included in the present study. Rectal swab samples were transported to laboratory in Amies transport medium.

Isolation and identification of enterococci

In this study, Chromocult® enterococci broth (Merck) and BBL™ enterococcosel agar (BD) were used for isolation, brain heart infusion agar (Merck)

and brain heart infusion broth (Merck) for passage, Mueller-Hinton agar (Difco) for antibiotic susceptibility tests, and 20% glycerinated brain heart infusion broth for storage of the strains were used. Rectal swab samples were immediately inoculated into to broth in the laboratory. Broths were incubated for 24 h at 37 °C until the yellow indicator changed to blue-green. A loop full of culture was streaked from broths to the enterococcosel agar. After 24 h incubation in ambient air, brownish growing colonies on the plates were passaged to 7% sheep blood brain heart infusion agar for further investigation. Catalase test and Gram stain were done for presumptive *Enterococcus* colonies that grew on plates. Gram positive catalase negative cocci were identified as enterococci as described previously (8). Colonies with typical enterococcal morphology and cultural characteristics were subjected to biochemical tests using the API 20 IDStrep system (BioMeriux, France).

Antibiotic susceptibility

Penicillin (10 IU), gentamycin (120 µg), teicoplanin (30 µg), clindamicin (2 µg), erythromycin (15 µg), chloramphenicol (30 µg), vancomycin (30 µg), and tetracycline (30 µg) were used for disc diffusion (9). All antimicrobial susceptibility discs were provided from Oxoid. *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as control strains. Isolates were categorized as susceptible (S), intermediate resistant (I), and resistant (R), based on interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI) (10).

DNA extraction

DNA was extracted from colonies grown on blood agar by simple boiling. In short, few colonies were removed and suspended in 100 µL of sterile distilled water in 0.2 mL tube and boiled 15 min at 94 °C in thermal cycler (Eppendorf AG, Hamburg, Germany). After centrifugation at 16,000 ×g for 5 min, 2 µL of supernatant was used for the PCR (7). Lysates were stored at -20 °C until use.

PCR: PCR experiments were carried out at the following conditions: 1 U AmpliTaq polymerase (Applied Biosystems, Foster City, CA), 2.5 µL 10× Taq buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM $MgCl_2$, 0.5 µM primers, and 200 µM deoxynucleoside triphosphates, 2 µL of template sample DNA in a final volume of 30 µL. Amplification was obtained with an initial denaturation step at 94 °C for 3 min followed by 32 cycles at 94 °C for 30 s, and at 60 °C for 45 s and at 72 °C for 2 min, followed by a final extension step at 72 °C for 10 min (7). PCR products (19 µL) were separated on a 2% agarose gel with 0.5 µg/mL ethidium bromide. The DNA fragments were visualized by UV. The molecular sizes of the PCR products were compared with a 100 bp DNA ladder.

Resistance genes

Known resistance genes for tetracycline, erythromycin, gentamycin, chloramphenicol, and vancomycin were studied by PCR in all resistant enterococci using specific primers as described previously (11-14) (Table 1).

Statistical evaluation

Significant differences in the isolation rates between *E. faecium* and *E. faecalis* and the distribution of the antibiotic resistant strains between cats and dogs were determined by the Chi-square test (15).

Results

Isolation and identification of enterococci

From 114 rectal swabs, 91 (79.8%) enterococci were grown. No enterococci were grown for 23 (20.2%) samples. Enterococci were isolated from 31 cats and 60 dogs. Sixty three strains were identified; 28 as *E. faecalis* (30.8%) and 63 as *Enterococcus faecium* (69.2%) (Table 2).

Antimicrobial susceptibility

Susceptibility rates of 28 *E. faecalis*, 63 *E. faecium,* and 91 enterococci strains were found as 100.0%, 96.4%, 89.3%, 82.1%, 82.1%, 42.9%, 32.2%, 0.0%; 100.0%, 95.2%, 96.8%, 90.5%, 85.7%, 25.4%, 28.6%, 0.0%; and 100.0%, 95.6%, 94.5%, 87.9%, 84.6%, 30.8%, 29.7%, 0.0% for teicoplanin, penicillin, vancomycin, chloramphenicol, gentamycin, erythromycin, tetracycline, and clindamicin, respectively (Table 3, Figure 1).

All isolates were detected as susceptible to teicoplanin but 5 strains were found as intermediate resistant to vancomycin. Among isolates, 4 were found as resistant to penicillin, 11 to

Table 1. Primers used in PCR for the detection of resistance genes.

Gene detected	Sequence of primer (5' to 3')	Amplican (bp)	Reference
tet(M)	F: GGGGGATCCTTACCAATGCTTAATCA R: GGGGAGCTCATAAAATTCTTGAAGAC	576	11
tet(K)	F: TCGATAGGAACAGCAGTA R: CAGCAGATCCTACTCCTT	456	11
erm(A)	F: TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAGT	645	12
erm(B)	F: GAAAAGRTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTAC	639	12
erm(C)	F: TCAAAACATAATATAGATAAA R: GCTAATATTGTTTAAATCGTCAAT	642	12
*van*A	F: GGGAAAACGACAATTGC R: GTACAATGCGGCCGTTA	732	13
*van*B	F: ATGGGAAGCCGATAGTC R: GATTTCGTTCCTCGACC	635	13
*van*C-1	F: GGTATCAAGGAAATC R: CTTCCGCCATCATCT	822	13
*van*C-2/3	F: CTCCTACGATTCTCTTG R: CGAGCAAGACCTTTAAG	439	13
aac(6)-*aph*(2)	F: GAGCAATAAGGGCATACCAAAAATC R: CCGTGCATTTGTCTTAAAAAACTGG	220	14
cat	F: GATAGAAAAGAATATTTTG R:GGAAACATTTCTTCTTTATC	356	From NCBI gb|EF070730.1|

Table 2. Distribution of *E. faecium* and *E. faecalis* strains.

Source	Number of strains				
	E. faecalis	*E. faecium*	Total	No Growth Samples	Total Sample
Cats	8 (25.8%)	23 (74.2%)	31 (100.0%)	8	39
Dogs	20 (33.3%)	40 (66.7%)	60 (100.0%)	15	75
Total	28 (30.8%)	63 (69.2%)	91 (100.0%)	23	114

$X^2 = 0.54$, $P > 0.05$

Table 3. Results of in vitro sensitivity testing of enterococci isolates.

Antibiotic	*E. faecalis* (n = 28) (%)			*E. faecium* (n = 63) (%)			Total (n = 91) (%)		
	R	I	S	R	I	S	R	I	S
Clindamicin	28 (100.0)	0 (0.0)	0 (0.0)	63 (100.0)	0 (0.0)	0 (0.0)	91 (100.0)	0 (0.0)	0 (0.0)
Tetracycline	19 (67.8)	0 (0.0)	9 (32.2)	45 (71.4)	0 (0.0)	18 (28.6)	64 (70.3)	0 (0.0)	27 (29.7)
Erythromycin	16 (57.1)	0 (0.0)	12 (42.9)	47 (74.6)	0 (0.0)	16 (25.4)	63 (69.2)	0 (0.0)	28 (30.8)
Gentamycin	5 (17.9)	0 (0.0)	23 (82.1)	9 (14.3)	0 (0.0)	54 (85.7)	14 (15.4)	0 (0.0)	77 (84.6)
Chloramphenicol	5 (17.9)	0 (0.0)	23 (82.1)	6 (9.5)	0 (0.0)	57 (90.5)	11 (12.1)	0 (0.0)	80 (87.9)
Vancomycine	0 (0.0)	3 (10.7)	25 (89.3)	0 (0.0)	2 (3.2)	61 (96.8)	0 (0,0)	5 (5.5)	86 (94.5)
Penicillin	1 (3.6)	0 (0.0)	27 (96.4)	3 (4.8)	0 (0.0)	60 (95.2)	4 (4.4)	0 (0.0)	87 (95.6)
Teicoplanin	0 (0.0)	0 (0.0)	28 (100.0)	0 (0.0)	0 (0.0)	63 (100.0)	0 (0.0)	0 (0.0)	91 (100.0)

n: Isolated strain number

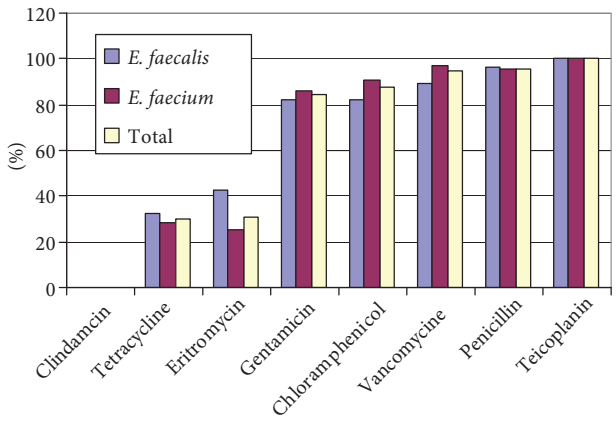

Figure 1. Susceptibility rates of *E. faecalis*, *E. faecium*, and all isolated enterococci.

chloramphenicol, 14 to gentamycin, 63 to erythromycin, and 64 to tetracycline. Of the 91 isolates tested, all were detected as resistant to clindamicin. Among the 91 enterococci isolates, 14 (15.4%) were susceptible to all antimicrobials tested, 77 (84.6%) strains were resistant. From the 77 resistant strains, 36 were found resistant to 1 antibiotic while 41 strains had multi drug resistance. Multi drug resistance was determined in 14, 17, 4, and 6 strains for 2, 3, 4, and 5 antibiotics, respectively.

Resistance genes

Among the 77 enterococci isolates, resistance genes were studied for tetracycline, erythromycin, gentamycin, chloramphenicol, and vancomycin. Five *E. faecium* strains had an intermediate resistance to vancomycin. None of the intermediate vancomycin strains had any *vanA, vanB,* or *vanC* genes. Of the 64 tetracycline resistant strains, 62 have *tet*(M) and 2 have neither *tet*(M) nor *tet*(K) genes. Among the erythromycin resistant strains, 54 have *erm*(B), 2 have *erm*(A), and 7 have no *erm*(A), *erm*(B), or *erm*(C) genes. Gentamycin resistance was detected against *aac*(6)-*aph*(2) gene in 10 of the 14 isolates. Of the 11 chloramphenicol resistant strains, 7 have *cat* gene by PCR. Distribution of *E. faecalis* and *E. faecium* strains

between cats and dogs is presented in Table 4. PCR analyses of DNA from antibiotic resistant enterococci are shown in Figure 2.

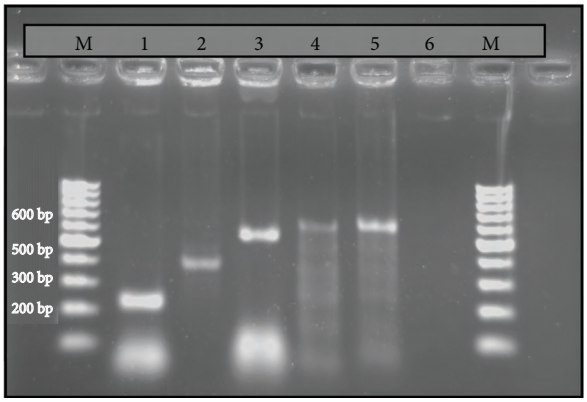

Figure 2. PCR analysis of DNA from antibiotic resistant enterococci. Lanes: M = DNA ladder (100 bp); Lane 1 = Gentamycin resistance gene (*aac*(6)-*aph*(2)), Lane 2 = Chloramphenicol resistance gene (*cat*) , Lane 3 = Tetracycline resistance gene (*tet*(M)), Lane 4 = Erythromycin resistance gene *erm*(B), Lane 5 = Erythromycin resistance gene *erm*(A), Lane 6 = Negative control without DNA.

Statistical evaluation

Differences for isolation rates between *E. faecium* and *E. faecalis* were found as statistically nonsignificant (Table 2) while the distribution of antibiotic resistant strains had no significance between cat and dog species (Table 4).

Discussion

Isolation of enterococci from healthy cats and dogs, investigation of antimicrobial susceptibility of these strains, and the determination of the known resistance genes by PCR in resistant strains were performed in this study. Increasing rates of multi-drug resistant enterococci in animals have been reported and this is important for the dissemination of resistance genes from pet animals to their owners or to other people (4,16).

In this study we were able to isolate enterococci from 79.5% of the cats and 80.0% of the dogs. These rates were found higher than the rates reported from Belgium (64.0%) and Turkey (57.5%) (17,18).

Table 4. Distribution of antibiotic resistant *E. faecalis* and *E. faecium* strains between cats and dogs.

Source	*E. faecalis*	*E. faecium*	Total
Cat	13 (24.1%)	41 (75.9%)	54 (100.0%)
Dog	36 (35.0%)	67 (65.0%)	103 (100.0%)
	49	108	157

$X^2 = 1.95$, $P > 0.05$

When we analysed the enterococci by species, *E. faecium* was found to be the most isolated species. Dominance of *E. faecium* was also reported by Boynukara et al. (18) from Turkey. These results were in contrast with other studies in which *E. faecalis* was reported as the most common isolated species (17,19). Further studies should be carried out to verify if there is a geographic relation for isolation of higher level of *E. faecium* from pet animals.

The enterococci isolated from pet animals were all susceptible to teicoplanin; however, 5 isolates were intermediate resistant to vancomycin. Resistance to vancomycin is an emerging problem worldwide. Simjee et al. (20) suggested that vancomycin-resistant enterococci may be acquired from dogs. The study did not support this hypothesis, because none of our isolates was resistant to vancomycin. In previous studies, the vancomycin resistance rates were reported to be higher (21) and lower (22) than our study. However, we found no previous report on the intermediate vancomycin resistance in enterococci, which is found in 5.5% of the isolates in our study. None of the intermediate resistant strains had *van* genes tested (*vanA, vanB,* and *vanC*), which may suggest involvement of other mechanisms. Further studies should be carried out for characterization of intermediate resistance mechanism to vancomycin in enterococci.

Resistance to tetracycline among *Enterococcus* spp. is very common. All the tetracycline-resistant 62 enterococcal isolates of this study harboured the *tet*(M) gene, except 2 that did not carry *tet*(M) or *tet*(K) genes. Tetracyclin resistance gene *tet*(M) is usually carried by Tn*916* in enterococci, which plays

an important role in dissemination of antimicrobial resistance (23) and *tet*(M) is the most common tetracycline resistance gene in among enterococci (22).

Erm methylase that modifies the 23S rRNA has been previously found as the most common macrolide resistance gene among enterococci (24). Macrolides resistance level was high among enterococci from pet animals and *erm*(B) was the most common gene found followed by *erm*(A). Further analysis should be performed for identification of resistance mechanism of the 7 strains that are negative for *erm*(A), *erm*(B) and *erm*(C). Methylation of 23S rRNA affects the killing activity of streptogramin antimicrobials. Although enterococci remain susceptible to the association of streptogramins, synergic effect of streptogramin A and streptogramin B does not reach the bactericidal level. High resistance rates against tetracycline and erythromycin were found among isolated strains. Tetracycline resistance followed by macrolide resistance is the most common in enterococci (25).

High level gentamycin resistant strains usually carry double functional enzyme *aac*(6)-*aph*(2). We are currently studying resistance mechanisms in 4 gentamycin resistant strains without *aac*(6)-*aph*(2). Gentamycin resistance is important among *E. faecium,* which affects the synergic activity of penicillin and gentamycin. Penicillin resistance was found among 4 (4.4%) strains. High level expression of PBP5 causes the susceptibility decrease among enterococci. The level of resistance is relatively lower compared to the clinical enterococci isolated from patients in Turkey (26) and in India (21).

Intestinal microbiota of cats and dogs may act as a reservoir of resistance genes for animal or human pathogens (22). In conclusion, relatively high levels of antibiotic resistance were detected in enterococcal isolates from cats and dogs. The resistance genes implicated in resistant isolates were similar to those detected in enterococcal isolates of human origin. The results of this study also indicated that enterococci isolated from cats and dogs could be a potential risk factor for transmission to humans. Further studies of biochemical and molecular characterizations should be conducted to determine other pathogenic properties, antimicrobial susceptibility, resistance genes, and virulence factors of enterococci.

References

1. Franz, C.M., Muscholl-Silberhorn, A.B., Yousif, N.M., Vancanneyt, M., Swings, J., Holzapfel, W.H.: Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. Appl. Environ. Microbiol., 2001; 67: 4385-4389.
2. Çetinkaya, Y., Falk, P., Mayhall, C.G.: Vancomycin-resistant enterococci. Clin. Microbiol. Rev., 2000; 13: 686-707.
3. Koch, S., Hufnagel, M., Theilacker, C., Huebner, J.: Enterococcal infections: host responsible, therapeutic, and prophylactic possibilities. Vaccine, 2004; 22: 822-830.
4. Rodrigues, J., Poeta, P., Martins, A., Costa, D.: The importance of pets as reservoirs of resistant *Enterococcus* strains, with special reference to vancomycin. J. Vet. Med. B. Infect. Dis. Vet. Public Health, 2002; 49: 278-280.
5. Descheemaeker, P.R.M., Chapelle, S., Devriese, L.A., Butaye, P., Vandamme, P., Goossens, H.: Comparison of glycopeptide-resistant *Enterococcus faecium* isolates and glycopeptide resistance genes of human and animal origins. Antimicrob. Agents Chemother., 1999; 43: 2032-2037.
6. Gold, H.S.: Vancomycin-resistant enterococci: mechanism and clinical observations. Clin. Infect. Dis., 2001; 33: 210-219.
7. Tejedor-Junco, M.T., Afonso-Rodríguez, O., Martín-Barrasa, J.L., González-Martín, M.: Antimicrobial susceptibility of *Enterococcus* strains isolated from poultry faeces. Res. Vet. Sci., 2005; 78: 33-38.
8. Facklam, R.R., Collins, M.D.: Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol., 1989; 27: 731-734.
9. Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M.: Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 1966; 45: 493-496.
10. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. 15th Informational Supplement. Approved Standard MS100-S16. 2006; CLSI, Wayne, PA
11. Warsa, U.C., Nonoyama, M., Ida, T., Okamoto, R., Okubo, T., Shimauchi, C., Kuga, A., Inoue, M.: Detection of *tet*(K) and *tet*(M) in *Staphylococcus aureus* of Asian countries by the polymerase chain reaction. J. Antibiot. (Tokyo), 1996; 49: 1127-1132.
12. Sutcliffe, J., Grebe, T., Tait-Kamradt, A. Wondrack L.: Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Chemother., 1996; 40: 2562-2566.

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

14. Kao, S.J., You, I., Clewell, D.B., Donabedian, S.M., Zervos, M.J., Petrin, J., Shaw, K.J., Chow, J.W.: Detection of the high-level aminoglycoside resistance gene *aph(2'')-lb* in *Enterococcus faecium*. Antimicrob. Agents Chemother., 2000; 44: 2876–2879.

15. Sümbüloğlu, V., Sümbüloğlu,, K.: Biyoistatistik. Özdemir Yayıncılık, Ankara, 1993; 155-174.

16. Johnson, A.P.: The pathogenicity of enterococci. J. Antimicrob. Chemother., 1994; 33: 1083-1089.

17. Devriese, L.A., Cruz-Colque, J.I., De Herdt, P., Haesebrouck, F.: Identification and composition of the tonsillar and anal enterococcal and streptococcal flora of dogs and cats. J. Appl. Bacteriol., 1992; 73: 421-425.

18. Boynukara, B., Ekin, İ.H., Aksakal, A., Gülhan, T.: Isolation and antibiotic susceptibility of enterococci from human, dog and cat faeces. Vet. Hek. Mikrobiyol. Derg., 2002; 2: 37-42. (article in Turkish, with and abstract in English)

19. Butaye, P., Devriese, L.A., Haesebrouck, F.: Differences in antibiotic resistance patterns of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from farm and pet animals. Antimicrob. Agents Chemother., 2001; 45: 1374-1378.

20. Simjee, S., White, D.G., McDermott, P.F., Wagner, D.D., Zervos, M.J., Donabedian, S.M., English, L.L., Hayes, J.R., Walker, R.D.: Characterization of Tn1546 in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. J. Clin. Microbiol., 2002; 40: 4659-4665.

21. Kapoor, L., Randhawa, V.S., Deb, M.: Antimicrobial resistance of enterococcal blood isolates at a pediatric care hospital in India. Jpn. J. Infect. Dis., 2005; 58: 101-103.

22. De Leener, E., Decostere, A., De Graef, E.M. , Moyaert, H., Haesebrouck, F.: Presence and mechanism of antimicrobial resistance among enterococci from cats and dogs. Microb. Drug Resist., 2005; 11: 395-403

23. Rice, L.B.: Tn*916* family conjugative transposons and dissemination of antimicrobial resistance determinants. Antimicrob. Agents Chemother., 1998; 42: 1871-1877

24. Del Campo, R., Ruiz-Garbajosa, P., Sánchez-Moreno M.P., Baquero, F., Torres, C., Cantón, R., Coque T.M.: Antimicrobial resistance in recent fecal enterococci from healthy volunteers and food handlers in Spain: genes and phenotypes. Microb. Drug Resist., 2003; 9: 47–60.

25. De Graef, E.M., Decostere, A, Devriese, L.A., Haesebrouck, F.: Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb. Drug Resist., 2004; 10: 65-69.

26. Ersoy, Y., Bayraktar, M., Fırat, M., Yağmur, M., Durmaz, R.: Antimicrobial susceptibility of *Enterococcus* strains isolated from clinical specimens. Ankem Derg., 2005; 19: 92-96. (article in Turkish, with an abstract in English).