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Research Article

Determination of Biofilm Production, Genotype and Antibiotic Resistance Profiles of *Enterococcus feacium* Isolates Originated from Dog, Cat and Human

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Abstract

The aim of the study was to determine the biofilm production, genotypes, antibiotics resistance patterns and antibiotypes of 82 E*nterococcus faecium* strains isolated from dog, cat and human. Of examined strains biofilm production detected totally 72 (87.8%) in 35 (97.2%) dog, 22 (78.6%) cat and 15 (83.3%) human isolates. Genotyping of isolates was performed by RAPD-PCR and 16, 3 and 4 different profiles were detected in dog, cat and human isolates, respectively. In total of 98.8% with a maximum resistance to nalidixic acid and 4.9% with the lowest resistance to vancomycin was found. None of vancomycin resistance 4 isolates, vancomycin resistance genes (*vanA, vanB, vanC1/C2* or *vanD*) has been detected. Antibiotyping of isolates was performed with UPGMA and 5 groups of dog, 10 groups of cat and 7 groups of human isolates were determined. The results from this study indicate that healthy dogs and cats are a source of Antibiotic resistant enterococci and may act as a reservoir of resistance that can be transferred from pets to people. Also our results demonstrated that the phenotype and genotype patterns found among enterococci strains from dogs, cats and humans were heterogeneous.

Keywords: *Antibiotic resistance, Antibiotype, Biofilm, E. faecium, Genotype*

Köpek, Kedi ve İnsan Orijinli *Enterococcus feacium* İzolatlarının Biofilm Üretimi, Genotip ve Antibiyotik Direnç Profillerinin Belirlenmesi

Özet

Bu çalışmada köpek, kedi ve insan orijinli 82 *Enterococcus faecium* izolatının biyofilm üretme özellikleri ile genotip ve antibiyotik direnç profilleri araştırıldı. İncelenen izolatların köpek 35 (%97.2), kedi 22 (%78.6) ve insan 15 (%83.3) olmak üzere toplam 72 (%87.8)'sinde biyofilm oluşumu saptandı. RAPD-PCR ile yapılan genotiplendirilmede; köpek, kedi ve insan izolatlarında sırasıyla 16, 3 ve 4 farklı profil belirlendi. Toplamda en yüksek dirençlilik %98.8 ile nalidiksik aside en düşük dirençlilik ise %4.9 ile vankomisine bulundu. Vankomisine dirençli 4 izolatın hiçbirinde vankomisin dirençlilik geni (*vanA, vanB, vanC1/2* veya *vanD*) saptanamadı. UPGMA ile yapılan antibiyotiplendirmede köpek izolatları 5, kedi izolatları 10 ve insan izolatları da 7 grup altında toplandı. Çalışma sonuçları sağlıklı köpek ve kedilerin antibiyotiklere dirençli enterokoklar için kaynak oluşturabileceğini ve insanlara direncin aktarılmasında rezervuar olarak rol oynayabileceğini göstermiştir. Ayrıca, elde edilen sonuçlarla köpek, kedi ve insanlar orijinli enterokoklarda bulunan fenotipik ve genotipik patternlerin heterojen olduğu belirlenmiştir.

Anahtar sözcükler: *Antibiyotik direnci, Antibiyotip, Biyofilm, E. faecium, Genotip*

INTRODUCTION

Enterococci are a dominant bacterial group in the intestinal flora of human and animals. They are increasingly associated with nosocomial infections. The natural ability of enterococci to acquire, accumulate, and share extra chromosomal elements encoding virulence traits or antibiotic resistance genes. Acquired resistance to various antimicrobial agents and available antibiotics currently limits the therapeutic options [1]. Biofilm is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to various biotic and abiotic surfaces irreversibly. Cells in biofilms are highly resistant to antibiotics and phagocytosis, and removal

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of the medical device is frequently the only appropriate cure, which may not always be possible. Because of the importance of biofilm formation to enterococcal infection, isolating the factors involved has been of great interest [2]. Most enterococci have inherent resistance to various antibiotics such as cephalosporins and semi synthetic penicillinase resistant penicillins, aminoglycosides and clindamycin [3]. Studies have recently focused on enterococcal infections in veterinary medicine in parallel with coming out animal factor in transmission of vancomycin resistant enterococci (VRE) to humans [4,5]. To differentiate the *Enterococcus* strains, various typing methods categorized into phenotypic and genotypic methods have been used in epidemiological studies in the past two decades. Antibiotyping of enterococci isolates by several methods were performed based on their different antibiotic resistance profiles [6]. Genotyping of *Enterococcus* species can be made by several methods such as random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) [3,7]. Antibiotic-resistant enterococci were grouped by RAPD-PCR and a scattered distribution was noted, indicating that resistance was not related to a particular clone [1]. The demonstration of diversity in the RAPD patterns on the species level will be essential for understanding the molecular ecology of enterococci in the intestine of animals and humans [8]. The aims of this study were to investigate the biofilm production, genotyping, antibiotic resistance patterns and antibiotyping of *E. faecium* strains isolated from dog, cat and human. The present study also performed for detecting the relationship between biofilm production and antibiotic resistance.

MATERIAL and METHODS

Bacterial Isolates

A total of 82 *E. faecium* isolates, including 36 dogs, 28 cats and 18 human origins, were used in study. All isolates were phenotypically identified to the species level using conventional methods and were confirmed by PCR [1].

Biofilm Formation

Congo red agar was used to detect biofilm production. Black colonies on Congo red agar were evaluated as biofilm production positive, pink or colorless colonies were evaluated as biofilm production negative [9].

RAPD-PCR Amplification

RAPD-PCR analysis was done using the primer ERIC2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') as described previously [10,11]. The similarities and numbers of the bands between RAPD patterns were determined based on the Dice similarity coefficient. To create a dendrogram that graphed genetic relatedness between *E. faecium* isolates with the cut-off value of 70%, "Unweighted Pair Group Method with Arithmetic Averages (UPGMA)" was employed using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, Hercules, CA).

Antibiotic Susceptibility Test

All isolates were tested against 12 different antibiotics using disc diffusion method. A susceptibility test result of each antibiotic was evaluated according to CLSI interpretive standards [12].

Detection of van Genes

The genes responsible for resistance to vancomycin (*vanA*, *vanB*, *vanC1/2* and *vanD*) were investigated by PCR as described previously [4].

Antibiotyping of Isolates

The antibiotic susceptible/resistance results were recorded as susceptible, intermediate susceptible or resistant. The schematic diagram including these results have been drawn and the similarities of patterns were determined based on the Dice similarity coefficient using CHEF-DR® III, Quantity One® Software (Bio-Rad Laboratories, Hercules, CA) with a cut of value of 70% [13].

RESULTS

Capacity of Biofilm Production

E. faecium isolates from dog, cat and human feces had a high capacity for biofilm production, with 97.2%, 78.6% and 83.3% of isolates, respectively. Dog isolates had higher capacity for biofilm production compared with cat and human isolates *(Table 1)*.

RAPD-PCR and Genotyping

In genotyping by RAPD-PCR 16, 3 and 4 different profiles were determined in dog, cat and human isolates, respectively *(Fig. 1, 2* and *3)*. Analysis of RAPD-PCR patterns in dog isolates revealed the presence of 16 RAPD types (A-P) based on 70 % similarities. When considered genotypic proximity of dog isolates was observed hetero-

Table 1. *Distribution of biofilm formation in E. faecium isolates by origins*
Tablo 1. *E. faecium izolatlarında biyofilm oluşumunun orijinlerine göre dağılımı*

Origin	Biofilm Formation			
	Positive		Negative	
	n	(%)	n	(%)
Dog (n=36)	35	97.2	1	12.8
Cat (n=28)	22	78.6	6	21.4
Human (n=18)	15	83.3	3	16.7
Total (n=82)	72	87.8	10	12.2

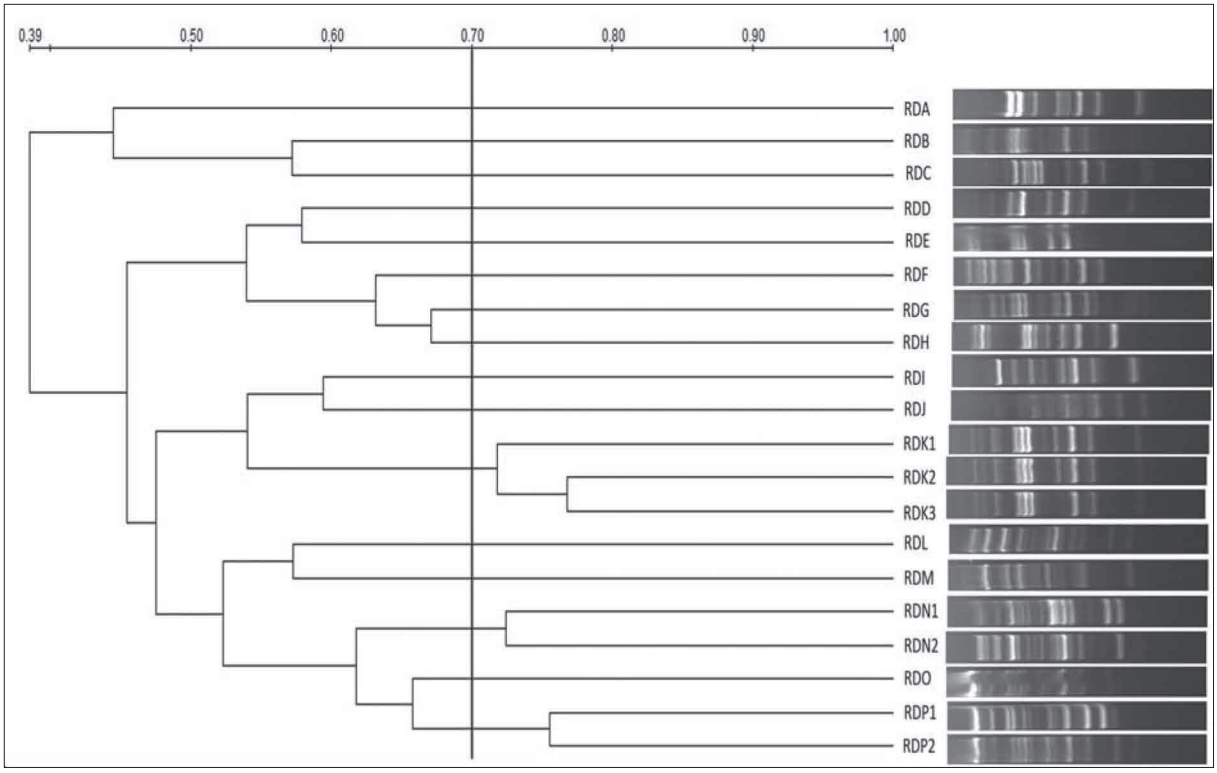

Fig 1. RAPD patterns of *E. faecium* isolated from dog and dendrogram obtained by UPGMA
Şekil 1. Köpek orijinli *E. faecium* izolatlarının RAPD patternleri ve UPGMA ile sağlanan dendrogram

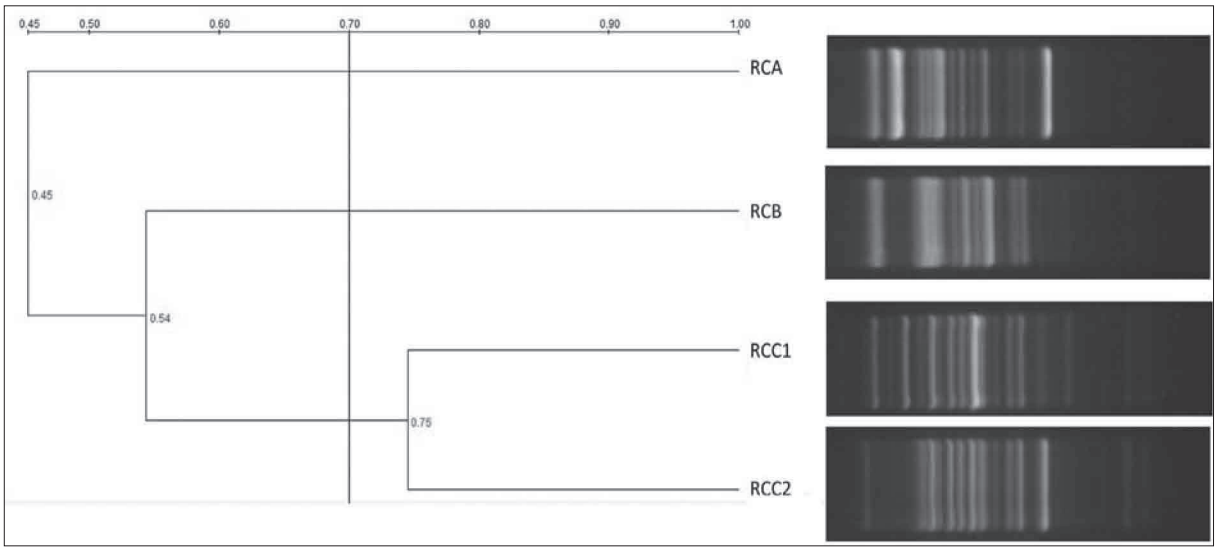

Fig 2. RAPD patterns of *E. faecium* isolated from cat and dendrogram obtained by UPGMA
Şekil 2. Kedi orijinli *E. faecium* izolatlarının RAPD patternleri ve UPGMA ile sağlanan dendrogram

geneity among groups. Isolates were represented in 7 major types: type A (n=2), type D (n=2), type E (n=2), type H (n=7), type K (n=9), type N (n=4) and type P (n=2), and others separate groups (B, C, F,G, I, J, L, M and O). On the other hand, cat isolates were collected in 3 groups (A-C) based on 70% similarities. When considered genotypic proximity of cat isolates was observed homogeneity among groups. Isolates were presented in 3 major types: type A (n=9), type B (n=12) and type C (n=7). Likewise, human isolates were classified into 4 groups (AD) based on 70% similarities. Isolates were presented in 3 major types: type B (n=7), type C (n=6) and type D (n=4), and type A (n=1) separate.

Antibiotic Susceptibility and Phenotype

Antibiotic resistance (R) / susceptibility (S) patterns

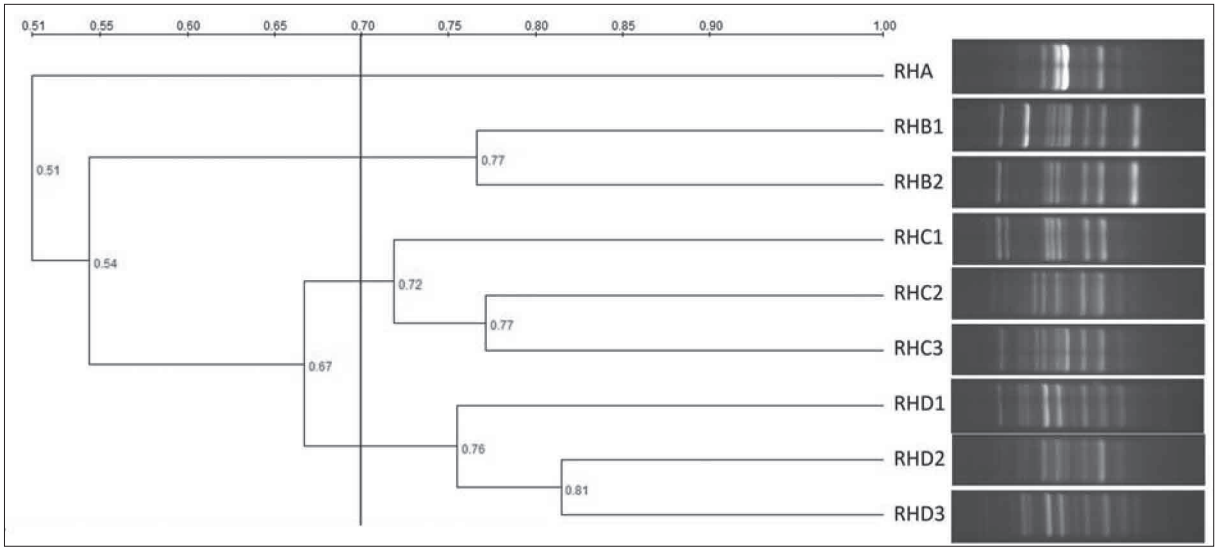

Fig 3. RAPD patterns of *E. faecium* isolated from human and dendrogram obtained by UPGMA
Şekil 3. İnsan orijinli *E. faecium* izolatlarının RAPD patternleri ve UPGMA ile sağlanan dendrogram

Table 2. *Antibiotic resistance/susceptibility patterns of E. faecium isolates*
Tablo 2. *E. faecium izolatlarının antibiyotik direnç/duyarlılık patternleri*

Antibiotics	Dog (n=36)		Cat (n=28)		Human (n=18)		Total (n=82)	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Ampicillin	0 (0)	36 (100)	8 (28.6)	20 (71.4)	1 (5.6)	17 (94.4)	9 (11)	73 (89)
Penicillin G	2 (5.6)	34 (94.4)	11 (39.3)	17 (60.7)	4 (22.2)	14 (77.8)	17 (20.7)	65 (79.3)
Vancomycin	0 (0)	36 (100)	3 (10.7)	25 (89.3)	1 (5.6)	17 (94.4)	4 (4.9)	78 (95.1)
Bacitracin	13 (36.1)	23 (63.9)	15 (53.6)	13 (46.4)	14 (77.8)	4 (22.2)	42 (51.2)	40 (48.8)
Oxytetracyclin	6 (16.7)	30 (83.3)	14 (50)	14 (50)	4 (22.2)	14 (77.8)	24 (29.3)	58 (70.7)
Kanamicin	36 (100)	0(0)	27 (96.4)	1 (3.6)	17 (94.4)	1 (5.6)	80 (97.6)	2 (2.4)
Erythromycin	11 (30.6)	25 (69.4)	15 (53.6)	13 (46.4)	9 (50)	9 (50)	35 (42.7)	47 (57.3)
Amoxicillin	0 (0)	36 (100)	5 (17.9)	23 (82.1)	4 (22.2)	14 (77.8)	9 (11)	73 (89)
Norfloxacin	7 (19.4)	29 (80.6)	3 (10.7)	25 (89.3)	5 (27.8)	13 (72.2)	15 (18.3)	67 (81.7)
Nalidixic acid	36 (100)	0 (0)	27 (96.4)	1 (3.6)	18 (100)	0 (0)	81 (98.8)	1 (1.2)
Cefalotin	22 (61.1)	14 (38.9)	18 (64.3)	10 (35.7)	11 (61.1)	7 (38.9)	51 (62.2)	31 (37.8)
Ciprofloxacin	22 (61.1)	14 (38.9)	6 (21.4)	22 (78.6)	3 (16.7)	15 (83.3)	21 (25.6)	61 (74.4)

of 82 *E. faecium* isolates are presented in *Table 2*. None of vancomycin resistance 4 isolates, vancomycin resistance genes *(vanA*, *vanB*, *vanC1/C2* or *vanD)* has been detected. Multiple antibiotic resistance phenotypes of isolates are presented in *Table 3*. Multi-drug resistance (MDR) was observed to as few as two and as many as twelve antibiotics regardless of class. Our findings showed that cat isolates resistance to multiple antibiotics greater frequency than dog and human isolates.

We also determined that biofilm forming strains showed resistance to antibiotics more frequently than not biofilm forming strains, when examined the relationship biofilm formation and antibiotic resistance in isolates *(Table 4)*.

Antibiotyping

Antibiotyping of isolates performed by UPGMA and were collected in 5 groups of dog, 10 groups of cat and 7 groups of human isolates, respectively *(Fig. 4, 5* and *6)*. According to these results; 36 dog *E. faecalis* isolates were collected in 5 main groups (A-E) based on 70% similarities. Groups were showed as ADA (n=2); ADB1-B7 (n=7); ADC (n=1); ADD (n=1) and ADE1-E17 (n=25). In like manner, 10 main groups (A-J) from 28 cat isolates were generated to 70% similarity rate. Groups were showed as ACA (n=1); ACB1-B2 (n=2); ACC1-C2 (n=2); ACD1-D8 (n=8); ACE (n=1); ACF (n=1); ACG1-G2 (n=2); ACH1-H2 (n=2); ACI (n=1) and ACJ1-J7 (n=8). On the other hand, 18 human isolates were collected in 7 main groups (A-G) to 70% similarity

Table 3. *Multidrug resistance patterns in E. faecium isolates by origin*
Tablo 3. *E. faecium izolatlarında çoklu ilaç direnç patternleri*

Num. of Antibiotics	Antibiotic Resistance Phenotype	Number of Isolates with Phenotype		
		Dog	Cat	Human
12	AMP-P-VAN-B-OTET-KAN-ERY-AMX-NOR-NAL-CEF-CIP	-	1	-
10	AMP-P-VAN-B-OTET-KAN-ERY-AMX-NAL-CEF	-	1	-
9	P-B-OTET-KAN-ERY-NOR-NAL-CEF-CIP	1	-	-
8	AMP-P-B-OTET-KAN-ERY-NAL-CEF	-	1	-
	AMP-B- OTET-KAN-ERY-AMX-NAL-CEF	-	1	-
	P-B-OTET-KAN-ERY-NOR-NAL-CIP	-	1	-
7	AMP-P-VAN-B-OTET-KAN-NAL	-	-	1
	AMP-P-OTET-KAN-ERY-NAL-CEF	-	1	-
	AMP-OTET-KAN-ERY-AMX-NAL-CEF	-	1	-
	P-B-OTET-KAN-ERY-NAL-CEF	-	2	-
	P-OTET-KAN-ERY-NAL-CEF-CIP	-	1	-
	B-OTET-KAN-ERY-NOR-NAL-CEF	1	-	-
	B-KAN-ERY-AMX-NOR-NAL-CEF	-	-	1
	B-KAN-AMX-NOR-NAL-CEF-CIP	-	-	1
6	AMP-P-KAN-NAL-CEF-CIP	-	1	-
	P-B-OTET-KAN-ERY-NAL	-	-	1
	P-B-KAN-ERY-NAL-CEF	-	-	2
	VAN-B-OTET-KAN-ERY-NAL	-	1	-
	B-OTET-KAN-ERY-NAL-CEF	-	1	-
	B-OTET-KAN-NOR-NAL-CEF	2	-	-
	B-KAN-NOR-NAL-CEF-CIP	1	-	-
	KAN-AMX-NOR-NAL-CEF-CIP	-	-	1
5	P-OTET-KAN-NAL-CEF	2	-	-
	B-KAN-ERY-NAL-CEF	1	-	1
	B-KAN-AMX-NAL-CEF	-	-	1
	B-KAN-NOR-NAL-CEF	1	-	-
	OTET-KAN-ERY-NAL-CEF	-	-	1
	OTET-KAN-NOR-NAL-CIP	-	-	1
4	P-KAN-NAL-CEF		1	
	B-KAN-ERY-NAL	1	-	2
	B-KAN-NOR-NAL	1	-	1
	B-KAN-NAL-CEF	4	1	1
	B-ERY-NAL-CEF		1	-
	KAN-ERY-NAL-CEF	4	-	1
	KAN-NAL-CEF-CIP	-	1	-
3	AMP-KAN-NAL	-	1	-
	B-KAN-NAL	1	2	1
	KAN-ERY-NAL	5	1	-
	KAN-NAL-CEF	4	-	-
	OTET-KAN-NAL	-	2	-
2	KAN-NAL	8	1	-
	KAN-CEF	-	1	-

AMP: *Ampicillin (30 mg),* ***P:*** *Penicillin G (10 mg),* ***VAN:*** *Vancomycin (30 mg),* ***B:*** *Bacitracin (10 mg),* ***OTET:*** *Oxytetracyclin (30 mg),* ***KAN:*** *Kanamycin (5 mg),* ***ERY:*** *Erythromycin (15 mg),* ***AMX:*** *Amoxicillin (25 mg),* ***NOR:*** *Norfloxacin (30 mg),* ***NAL:*** *Nalidixic acid (30 mg),* ***CEF:*** *Cefalotin (30 mg),* ***CIP:*** *Ciprofloxacin (5 mg)*

Table 4. *Relationship biofilm formation and antibiotic resistance in E. faecium isolates*
Tablo 4. *E. faecium izolatlarında biyofilm oluşturma ve antibiyotik direnç ilişkisi*

Antibiotics	Dog (n=36)		Cat (n=28)		Human (n=18)		Total (n=82)	
	Biofilm Production (n)		Biofilm Production (n)		Biofilm Production (n)		Biofilm Production (n)	
	+ (35/%)	- (1/%)	+ (22/%)	- (6/%)	+ (15/%)	- (3/%)	+ (72/%)	- (10/%)
Ampicillin	0/0	0/0	6/27.3	2/33.3	0/0	1/33.3	6/8.3	3/30
Penicillin G	2/5.7	0/0	8/36.4	3/50	2/13.3	2/66.7	12/16.7	5/50
Vancomycin	0/0	0/0	2/9.1	1/16.7	0/0	1/33.3	2/2.8	2/20
Bacitracin	13/37.1	0/0	12/54.5	3/50	11/73.3	3/100	36/50	6/60
Oxytetracyclin	6/17.1	0/0	10/45.5	4/66.7	2/13.3	2/66.7	18/25	6/60
Kanamicin	35/100	1/100	21/95.5	6/100	15/100	2/66.7	71/98.6	9/90
Erythromycin	11/31.4	0/0	11/50	3/50	8/53.3	1/33.3	30/41.7	4/40
Amoxicillin	0/0	0/0	3/13.6	2/33.3	4/26.7	0/0	7/9.7	2/20
Norfloxacin	7/20	0/0	2/9.1	1/16.7	4/26.7	1/33.3	13/18.1	2/20
Nalidixic acid	35/100	1/100	22/100	5/83.3	15/100	3/100	72/100	9/90
Cefalotin	22/62.9	0/0	13/59.1	5/83.3	11/73.3	0/0	46/63.9	5/50
Ciprofloxacin	2/5.7	0/0	4/18.2	2/33.3	3/20	0/0	9/12.5	2/20

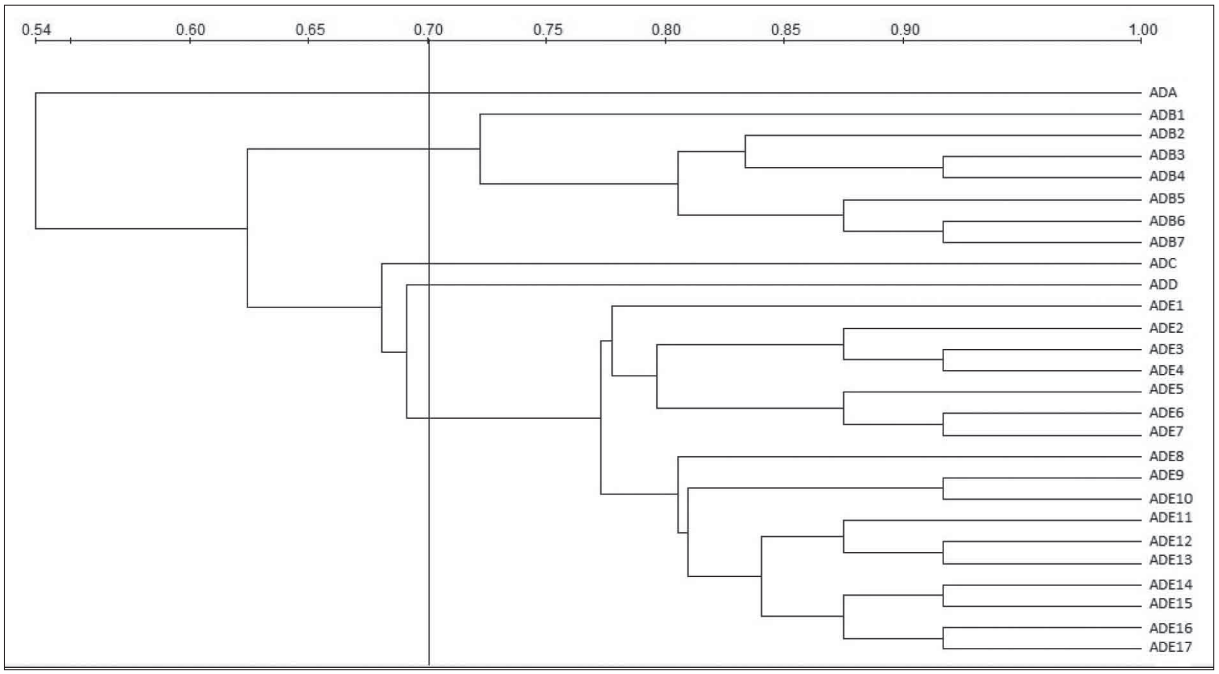

Fig 4. Antibiotype patterns of *E. faecium* isolated from dog and dendrogram obtained by UPGMA
Şekil 4. Köpek orijinli *E. faecium* izolatlarının antibiyotip patternleri ve UPGMA dendrogram

rate. Groups were showed as AHA (n=1); AHB (n=1); AHC (n=1); AHD1-D2 (n=2); AHE1-E2 (n=2); AHF1-F4 (n=4) and AHG1-G7 (n=7).

DISCUSSION

Enterococci are opportunistic pathogens and form part of the normal gastrointestinal flora in humans and animals. Over the last two decades, nosocomial infections caused by enterococci have emerged and their incidence has increased [14].

Biofilm production has been reported in some enterococcal infections. The major clinical infections have been caused by *E. faecium* capable of producing biofilms. Enterococci with biofilms are more highly resistant to antibiotics than planktonically growing enterococci, thus the potential impact of biofilm formation could be significant [7]. The prevalence of biofilm production reported

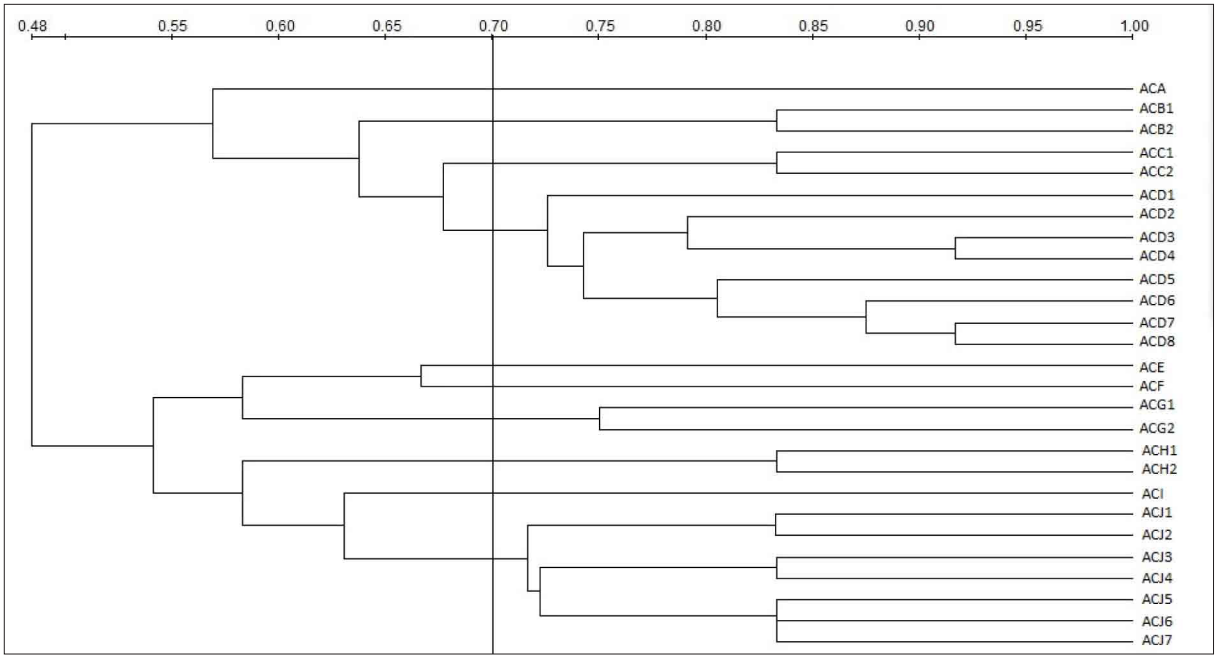

Fig 5. Antibiotype patterns of *E. faecium* isolated from cat and dendrogram obtained by UPGMA
Şekil 5. Kedi orijinli *E. faecium* izolatlarının antibiyotip patternleri ve UPGMA dendrogram

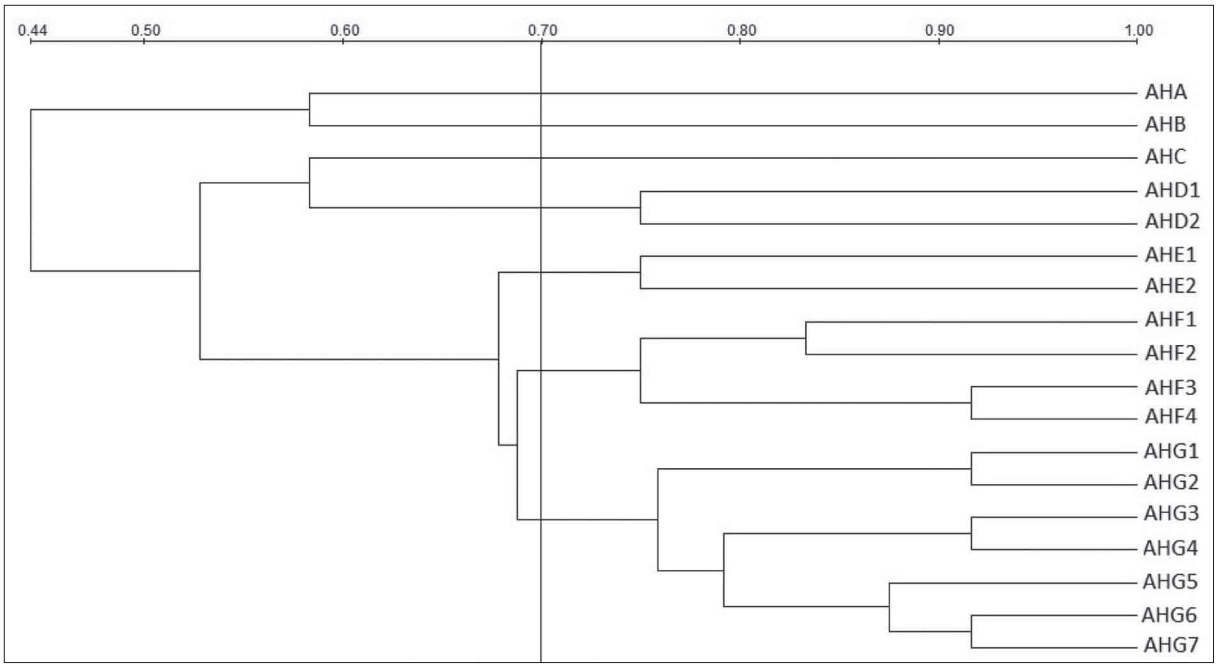

Fig 6. Antibiotype patterns of *E. faecium* isolated from human and dendrogram obtained by UPGMA
Şekil 6. İnsan orijinli *E. faecium* izolatlarının antibiyotip patternleri ve UPGMA dendrogram

previously for commensal isolates has been variable [3,9,15]. For instance, in a study from Italy 48% of *E. faecium* isolates from infected patients were able to form biofilm [16], while study from Greece reported biofilm production among 64.9% of *E. faecium* human and 34.4% of animal isolates [15]. Other investigators have reported similar results [9]. In this study, biofilm production was detected 35 (97.2%) of 36 dog, 22 (78.6%) of 28 cat and 15 (83.3%) of 18 human isolates. These results indicated that there may be more than one factor determining the production of biofilms in enterococci. In this study, we compared directly the biofilm formation between dog, cat and human *E. faecium* isolates and found that the dog isolates exhibited a significantly higher capacity for biofilm formation than isolates from cat and human isolates. Similar results reported previously [10,15].

The genotypic diversity of enterococcal isolates obtained from different origins was assessed using RAPD-PCR fingerprinting [10,11,17]. The RAPD-PCR analysis classified five profiles were discerned for *E. faecium* [11]. In a study RAPD-PCR analysis, *E. faecium* isolates (n=23) were grouped in four RAPD-types (clusters 1,4,6 and 7) at a similarity level of ca. 70% [10]. Similar results reported by Ben Omar et al.[17]. Getachew et al.[7] reported that VRE species showed diverse RAPD profiles with some clustering of strains based on the individual's background. In the other hand, in a study performed cats reported cats showed that clonal matches based on PFGE clearly demonstrate cross contamination between the resident cats and the hospital environment [3]. In this study RAPD-PCR profiles in dog isolates showed 16 types, of which 7 were predominant. When considered genotypic proximity of dog isolates was observed heterogeneity among groups. This suggests that isolates were polyclonally disseminated in our setting. On the basis of RAPD-PCR, 3 main groups could be distinguished in cat and 4 in human isolates. These findings imply that enterococci are genetically and phenotypically diverse. Our results are in good agreement with previous reports in which RAPD was found to allow rapid identification of unknown isolates [8,11,17].

Antibiotic resistances have been reported to better define the links between animals and humans [6,18,19]. Cats and dogs have played an important role in the human community [20,21], which allows them to have a good relationship with humans and contribute to their welfare; however, this relationship also poses serious risks of transmission of infectious agents to human [14]. Some enterococci are inherently resistant to some penicillins; and in the past few years, they have also shown increased resistance to vancomycin, cephalosporins, and amino-glycosides in nosocomial infections [3]. Vancomycin is, in some cases, the only antibiotic still effective in the treatment of nosocomial enterococcal infection in humans and is often considered the last treatment available in serious MDR infections [4].

Clinical cases involving VRE in companion animals are rare. Beside the several existing reports of VRE in animals [5], there are a limited number of studies dealing with the colonization of VRE in companion animals [4], even though VRE have been recorded in the intestinal tract of dogs and cats [6]. Simjee et al.[22] described the isolation of a high-level gentamicin-resistant (HLGR) and vancomycin-resistant *E. faecium* (VREfm) from a canine urinary tract infection in the USA, while Manson et al.[23] isolated a gentamicin-sensitive VREfm from a canine in New Zealand. Similar results cited by recently [5]. In contrast, no resistance to vancomycin was found several studies on enterococci from dogs and cats [3,14,21,24]. In our study only 3 cat and one human isolates were found to be resistant to vancomycin by phenotypically. However, all isolates were negative for *van* genes as recently reported [1].

In present study almost all isolates were found to be resistant to kanamycin and nalidixic acid. Cefalotin, bacitracin and erythromycin resistances were observed most frequently as compared to the other antibiotics. Similar results have been reported by other researchers [3,24]. As these antibiotics are habitually employed for treatment of a variety of infections in dogs and cats, their use could be the cause of a selective pressure for the resistance phenotype. Tetracycline, ciprofloxacin, penicillin and norfloxacin resistance was also common among isolates exhibiting MDR. These drags are used in dogs and cats for treatment of a variety of infections including urinary tract infections, periodontitis, upper respiratory tract infections and conjunctivitis [21].

The present study showed that cat isolates resistance to multiple antibiotics greater frequency than dog and human isolates as previously reported [18]. Fortunately, our isolates remain highly susceptible to ampicillin and amoxicillin as similar by Ossiprandi et al.[24]. Our findings contribute to the refinement of future therapeutic decisions in the management infections by enterococci of animals.

Antibiotyping of enterococci isolates by several methods were performed based on their different anti-biotic resistance profiles [1,3,6,10]. Antibiotic-resistant *E. faecium* isolates were grouped by RAPD-PCR and a scattered distribution was noted, indicating that resistance was not related to a particular clone as cited previously [1,23]. The spread of virulence/resistance traits in isolates of species and different RAPD-types suggest the pathogenic potential of species [10]. In a recently study, evaluated genetic similarities of the enterococcus isolates using the RAPD-PCR analysis and fingerprinting revealed no clonal lineage among tested isolates [1]. In the present study antibiotyping of isolates performed by UPGMA and were collected in 5, 10 and 7 groups of dog, cat and human isolates, respectively. These findings differ from the study by Jackson et al.[21] where they found that healthy domestic cats harbored MDR enterococcal strains of diverse clonal origin.

Previous reports demonstrated that there is a relationship between biofilm production and antibiotic resistance [9,10,15]. On the other hand, it has been proved that the very high concentrations of ampicillin, vancomycin and linezolid required inhibiting enterococcal biofilms in vitro and may explain why monotherapy with these agents frequently fails to eradicate biofilm infections. In the present study we determined that biofilm forming strains showed resistance to antibiotics more frequently than not biofilm forming strains, when examined the relationship biofilm formation and antibiotic resistance in isolates as cited previously [10].

In conclusions, we compared directly the biofilm formation between dog, cat and human *E. faecium* isolates and found that the dog isolates exhibited higher capacity

for biofilm formation than from cat and human isolates. The results demonstrated that the RAPD-PCR patterns found among enterococci strains from dog, cat and human were heterogeneous and considerably diverse. The demonstration of diversity in the RAPD patterns on the species level will be essential for understanding the molecular ecology of enterococci in the intestine of animals and humans. Further studies on the molecular typing and clinical significance of these isolates are needed. The results from this study indicate that healthy dogs and cats are a source of antibiotic resistant enterococci and may act as a reservoir of antibiotic resistance that can be transferred from pets to people. This risk is highlighted by Antibiotic resistance by use of the same antibiotics used to treat infections in humans and pets. Furthermore, the enterococcal isolates were MDR exhibiting resistance to as many as twelve antibiotics. Additional studies will address the presence of antibiotic resistance genes harbored by resistant isolates.

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