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ARAŞTIRMA / RESEARCH

Determination of clonal relationships and virulence genes of vancomycin-resistant enterococcus spp. isolated from colonized and infected patients

Kolonize ve infekte hastalardan izole edilen vankomisin dirençli enterococcus spp.'nin virülans genleri ve klonal ilişkilerinin belirlenmesi

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Abstract

Purpose: In this study, we investigated the possible relationship between virulence factors and clonal relationship between Vancomycin-resistant colonization and infection isolates.

Materials and Methods: A total of 156 Vancomycin-resistant Enterococcus spp. (VRE) were collected and grouped as infection and colonization isolates. A multiplex polymerase chain reaction analysis was performed to screen specific virulence genes (esp, hyl, asa1, cylA and gelE) and vancomycin resistance genes (vanA, vanB). The clonal relationship among isolates was investigated by the Pulsed Field Gel Electrophoresis (PFGE) method.

Results: The vanA gene was determined in 86 infection and 64 colonization isolates. esp was the most common virulence gene for both groups (55.8% and 56.25%), followed by hyl (51.1% and 35.9%) that statistically differed between the two groups. The 150 E. faecium isolates carrying the vanA resistance gene were divided into 24 main clusters (A-Y) in PFGE analysis.

Conclusion: When the distribution of virulence genes or genes combinations of the infection and colonization groups was examined, there was no statistical significance of cluster distribution in the two groups. However, hyl gene was found to be more common in infection isolates.

Key words:. VRE, PFGE, esp, hyl, van

Öz

Amaç: Bu çalışmada, Vankomisine dirençli kolonizasyon ve enfeksiyon izolatları arasındaki klonal ilişki ve virülans faktörleri araştırılmıştır.

Gereç ve Yöntem: Toplam 156 Vankomisine dirençli Enterococcus spp. (VRE) izole edildi ve enfeksiyon ve kolonizasyon izolatları olarak gruplandı. Spesifik virülans genlerini (esp, hyl, asa1, cylA ve gelE) ve vankomisin direnç genlerini (vanA, vanB) tespit etmek için multipleks polimeraz zincir reaksiyonu analizi yapıldı. İzolatlar arasındaki klonal ilişki Pulsed-Field jel elektroforezi (PFGE) metodu ile araştırıldı.

Bulgular: vanA geni, 86 enfeksiyon ve 64 kolonizasyon izolatı olmak üzere 150 izolatta belirlendi. Bu izolatlar, PFGE analizinde 24 ana kümeye (A-Y) ayrıldı. Her iki grup için en yaygın virülans geni infeksiyon ve kolonizasyon izolatlarında sırasıyla esp geni (% 55.8 ve% 56.25) olarak belirlendi. İkinci sırada ise iki grup arasında istatistiksel olarak farklılık gösteren hyl (% 51.1 ve% 35.9) bulunmakta idi.

Sonuç: Virülans genlerinin dağılımı ve enfeksiyon ve kolonizasyon gruplarının gen kombinasyonları incelendiğinde, iki grupta küme dağılımının istatistiksel olarak anlamlı olmadığı tespit edilmekle beraber, hyl geninin infeksiyon izolatlarında daha sık görüldüğü tespit edilmiştir.

Anahtar kelimeler: VRE, PFGE, esp, hyl, van

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INTRODUCTION

Enterococci, which is a member of the intestine and vaginal flora, has long been recognized as low virulence pathogens[1]. However, due to the irrational and improper use of disinfectants and glycopeptide antibiotics in hospitals and immusupressive patients, *Enterococcus spp.* has become one of the major infectious agents[2]. Glycopeptides primarily eliminate susceptible *Enterococcus* strains in the intestinal flora[3]. However, glycopeptide-resistant *Enterococcus* (GRE) may become predominant in flora due to various virulence factors, such as the cytolytic toxin, enterococcal surface protein, hyaluronidase, gelatinase, and aggregation factors[4]. Vancomycin-resistant *Enterococcus* (VRE) isolates are translocated from the intestinal lumen to the lamina propria because of corruption of intestinal flora and anatomical structures by chemotherapeutics; then, they pass into the bloodstream and cause endogenous infection. As a result, patient feces are contaminated with VRE, leading to the development of exogenous infections[5]. Therefore, in the control and prevention of the spread of VRE infections, it is important to timely detect patients with VRE colonization, elucidate their relationship with hospital infection outbreaks, and identify virulence factors for colonization and infection[6,7].

In this study, a total of 156 VR *Enterococcus* spp. isolated from the clinical samples were analyzed using the Pulsed Field Gel Electrophoresis (PFGE) and Polymerase Chain Reaction (PCR) methods to determine the clonal relationship and virulence factors.

MATERIALS AND METHODS

In this study, the clinical samples of 156 patients who were registered at a university hospital were collected over a period of 17 months (September 2013-February 2015). The Local Non-Invasive Clinical Trials Ethics Committee declared that approval was not required for this study. Patient consent forms were not used, because the study was not performed directly on humans.

Patients groups

The patients are divided into two groups. The infection group was formed based on data of the infection control committee. In this group, 90 VRE were isolated from patients detected infection on the third day after hospitalization. In the colonization group, there were 66 VRE isolates from the perirectal swap samples collected from the hospitalized patients for screening purposes with the decision of the infection control committee.

Enterococus spp. Isolation

The clinical samples were inoculated on kanamycin esculin azide agar (Merck, Darmstadt, Germany) and were evaluated by gram staining, catalase test, and growth in 6.5% NaCl. The suspected *Enterococcus* spp. isolates were identified by the VITEK-2 automated identification system (Biomerieux, Basingstoke, UK). All identified isolates were stored in Brain Heart Infusion Broth (Merck, Darmstadt, Germany) at -20ºC.

Antimicrobial susceptibility test

The Kirby-Bauer disk diffusion test was performed and the broth dilution method was applied to determine the minimum inhibitory concentration (MIC) values of vancomycin and teicoplanin according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[8].

Table1. Primer sequences of virulence and van resistance gene.

Genes	Primer sequences
gelE	F:5'-TATGACAATGCTTTTTGGGAT-3' R:5'-AGATGCACCCGAAATAATATA-3'
hyl	F:5'-ACAGAAGAGCTGCAGGAAATG-3' R:5'-GACTGACGTCCAAGTTCCCAA-3'
asa1	F:5'-GCACGCTATTACCAACTATGA-3' R:5'-TAAGAAAGAACATCACCACGA-3'
esp	F:5'-AGATTTCATCTTTGATTCTTGG-3' R:5'-AATTGATTCTTAGCATCTGG-3'
cylA	F:5'-ACTCGGGGATTGATAGGC-3' R:5'-GCTGCTAAAGCTGCGCTT-3'
vanA	F:5'-TCTGCAATAGAGATAGCCGC-3' R:5'-GGAGTAGCTATCCCAGCATT-3'
vanB	F:5'-TCTGCAATAGAGATAGCCGC-3' R:5'-GGAGTAGCTATCCCAGCATT-3'

DNA extraction

The DNA extraction of all isolates was performed mechanically with a Mickle cell disruptor (The Mickle Lab. Engineering Co. Ltd., Surrey, UK)[9]. The DNA

samples were quantified by a spectrophotometer (CHEBIOS, Rome, Italy) and stored at -20ºC.

Polymerase chain reaction

A PCR analysis was carried out to determine the virulence genes (*cylA, hyl, gelE, asaI* and *esp*) and *vanA*/*vanB* vancomycin resistance genes[10,11] (Table 1). The Amplicons were run on 2% agarose gel, which was visualized on a UV transilluminator (Kodak, New York, USA).

Pulsed field gel electrophoresis analysis

The clonal relationship of the isolates was evaluated using the PFGE method as described in a previous study[12]. The *Sma*I restriction enzyme (25 units) was used to cut the DNA molecules (Promega, Southampton, UK). For the PFGE analysis, the CHEF DRII system (Bio-Rad, California, USA) was used (Fig. 1). In electrophoresis the initial and final switch time was 5 and 20 s, respectively, at 200 volts for 21 h, at 4ºC. The band profiles were analyzed using GelCompar II software (version 5.0; Applied Maths, Sint-Martens-Latem, Belgium).

A dendrogram was constructed based on the Dice similarity coefficients using the unweighted pair-group method with arithmetic mean (UPGMA). The isolates with an 80% band profile similarity were evaluated within the same cluster and designated capital letters. The isolates with a similarity percentage of 80% to 100% in the same cluster were presented in a sub-cluster and named with the lower-case letter of the name of their cluster followed by a number. The isolates that were identical (100% similarity) were evaluated within the same sub-cluster.

Statistical analysis

The data were processed using S-PLUS 18 statistical program (Solution Metrics, Sydney, Australia). A chi-square test was used to compare the groups (Fisher's exact test where appropriate). A p value of less than 0.05 was accepted to indicate statistical significance.

RESULTS

In this study, the clonal relationships, antibiotic resistance and potential virulence factors of 156 VRE isolates were evaluated. There were 86 (95.6%) *E. faecium* and four (4.4%) *E. faecalis* isolates in the infection group. All the 66 isolates in the colonization group were identified as *E. faecium*. All the isolates in both groups were resistant to vancomycin with MIC ≥ 256µg/mL. Teicoplanin resistance (MIC ≥ 32µg/mL) was determined in 81.8% and 93.3% of the isolates in the infection and colonization groups, respectively. Four *E. faecalis* isolates in the infection group and two in the colonization group were susceptible to teicoplanin.

The *van* resistant gene was screened in 156 VRE isolates that were determined as vancomycin-resistant by the phenotypic method. The isolation rate of *vanA* gene-containing *Enterococcus* was 95.6% (n=86 *E. faecium*) in the infection group and 97% (n=64) in the colonization group. All the vanA-resistant isolates (n=150) were *E. faecium*. Two *E. faecalis* isolated from blood and urine samples in the infection group and one of colonization isolates carried the *vanB* resistance gene. Similarly, two *E. faecalis* isolated from wound and urine samples in the infection group and one *E. faecium* isolates in the colonization group were not found *vanA*/*B* resistance gene (Table 2).

The distribution of virulence genes found to be clinically significant in *vanA*-type resistant 150 *E. faecium* isolates. The *esp* was the most frequently detected gene, 55.8% (n=48) and 56.3% (n=36) in the infection and colonization groups, respectively.

The second most common virulence gene was *hyl* , 51.1% and 35.9%, respectively The difference between these two groups is probably statistically significant.($p < 0.05$).

The *esp* gene was detected in the infection group as 10.46% and in the colonization group as 18.75%. However, the *esp* gene coexisted with other virulence genes; e.g., with *hyl*+*gelE* (10.46%) and *hyl*+*asaI* (10.46%) in the infection group, and with *gelE* (9.37%) and *asaI* (9.37%) in the colonization group. The *gelE*+*cylA* genes were observed at the lowest rate in the virulence genes/gene combination in both groups (3.33 and 1.56%) (Table 4).

When the virulence genes of the strains resistant to VanB were examined, there were *hyl*+*gelE* and hyl genes in the two *E. faecalis* isolated from the infection group and *hyl*+*esp*+*gelE* and *gelE* gene in one *E. faecium* from the colonization group. The *esp*+*hyl*+*gelE* and *hyl*+*asaI* genes were determined in two non-VanA-VanB-resistant *E. faecalis* isolates in the infection group. One *E. faecium* isolated from the colonization group contained the *hyl*+*cylA* genes.

Table 2. Glycopeptide resistance, *van* resistance gene and clinical samples of *Enterococcus* spp.

Group		vanA/B gene			Glycopeptide resistance				Clinical samples						
					Vancomycin		Teicoplanin								
		VanA gene	VanB gene	Non-VanA-B gene	S	R	S	R	Blood	Urine	Wound swab	CSF	Rectal swab	Peritoneal fluid	Sputum
Infection (n=90)	*E.faecalis*	-	2	2	-	4	4	-	1	2	1	-	-	-	-
	E.faecium	*86*	-	-	-	86	-	86	23	39	2	6	-	9	7
Colonization (n=66)	*E.faecium*	64	1	1	-	66	2	64	-	-	-	-	66	-	-

Table 3. Distribution of virulence genes in infection and colonization groups (esp: enterococal surface protein, hyl: hyaluronidase, gelE: gelatinase, cylA: cytolysin A, asaI: aggregation substance)

Virulence genes	Colonization (64 isolates)	Infection (86 isolates)	Total (150 isolates)
esp	36(%56.25)	48 (%55.8)	84 (%56)
hyl	23 (%35.9)	44(%51.1)	67 (%44.6)
gelE	19 (%29.6)	30(%34.9)	49 (%32.6)
cylA	13 (%20.3)	20(%23.25)	33 (%22)
asaI	13 (%20.3)	18 (%20.9)	31 (%20.6)

Table 4. The combination of virulence genes in infection and colonization groups.

	Colonization isolates (64)		Infection isolates (86)		Total (150 isolates)	
	Number	%	Number	%	Number	%
esp+hyl+gelE	4	6.25	9	10.46	13	8.6
esp+hyl+asaI	3	4.68	9	10.46	12	8
esp+hyl	4	6.25	7	8.13	11	7.3
esp+cylA	3	4.68	5	5.81	8	5.3
gelE+cylA	1	1.56	3	3.48	4	2.6
hyl+gelE	4	6.25	7	8.13	11	7.3
esp+gelE	6	9.37	5	5.81	11	7.3
esp+asaI	4	6.25	4	4.65	8	5.3
hyl+cylA	4	6.25	7	8.13	11	7.3
esp	12	18.75	9	10.46	21	14
hyl	4	6.25	5	5.81	9	6
gelE	4	6.25	6	6.97	10	6.66
asaI	6	9.37	5	5.81	11	7.3
cylA	5	7.81	5	5.81	10	6.66

Based on the similarity value of ≥80% in PFGE analysis, the 150 *E. faecium* isolates carrying *vanA* resistance genes were divided into 24 main clusters (A-Y). Three isolates (3.7%) from the infection group formed single-member clusters (I, Q and X), and ten isolates (15.6%) from the colonization group were detected in single-member clusters (H, M, O, P, R, S, T, U, V and W). The remaining 137 isolates were divided into 11 clusters comprising 36 sub-clusters (Table 5).

Table 5. The clonal relationship of the isolates by PFGE

Cluster	**Subcluster**	**Colonization 64 isolates**		**Infection 86 isolates**		**Total 150 isolates**
A	a1	1	esp+hyl+gelE	6	esp+hyl+asaI hyl+gelE (2) esp+gelE hyl gelE	7
	a2	2	esp+hyl+asaI hyl	6	esp+hyl+asaI esp+hyl(2) gelE asaI(2)	8
	a3	3	esp+asaI esp+gelE asaI	1	esp+gelE	4
	a4	1	asaI	0	-	1
B	b1	1	asaI	3	asaI (2) esp+asaI	4
	b2	1	esp+asaI	1	esp+asaI	2
C	c1	2	hyl+gelE cylA	2	hyl(2)	4
	c2	1	esp	3	hyl+gelE esp(2)	4
	c3	1	hyl	1	esp	2
	c4	1	cylA	1	hyl+cylA	2
D	d1	3	cylA hyl hyl+cylA	7	esp+hyl+gelE hyl+cylA esp+cylA esp hyl cylA(2)	10
	d2	6	esp+cylA hyl+gelE hyl+cylA esp hyl gelE	9	esp+hyl+gelE esp+cylA hyl+gelE(2) gelE+cylA hyl+cylA esp hyl gelE	15
	d3	4	esp+hyl+asaI hyl+gelE esp asaI	6	esp+hyl+asaI(2) hyl+gelE esp+asaI esp asaI	10
	d4	8	esp+hyl esp+asaI gelE+cylA hyl+cylA esp gelE(2)	9	esp+hyl+gelE esp+hyl+asaI esp+hyl(2) hyl+gelE esp+asaI gelE+cylA	17

			asaI		hyl+cylA gelE	
	d5	3	esp+hyl+gelE esp+gelE esp	6	esp+hyl+gelE(3) esp+gelE(2) esp	9
	d6	2	esp+cylA esp	3	esp+cylA(2) cylA	5
	d7	2	esp esp+hyl	4	esp+hyl(2) esp+hyl+gelE esp+hyl+asaI	6
	d8	1	esp	1	esp+cylA	2
E	e1	1	hyl+cylA	1	hyl+cylA	2
	e2	0	-	2	hyl+cylA cylA	2
F	f1	1	esp+hyl	0	-	1
	f2	0	-	1	esp+hyl+gelE	1
G	g1	2	esp+gelE gelE	1	gelE	3
	g2	1	esp+gelE	0	-	1
H	H	1	esp	0	-	1
I	İ	0	-	1	esp+hyl	1
J	j1	1	cylA	0	-	1
	j2	0	-	2	hyl+cylA cylA	2
K	k1	1	esp+hyl+asaI	1	esp+hyl+asaI	2
	k2	0	-	1	esp+hyl+asaI	1
L	l1	0	-	1	esp+hyl+gelE	1
	l2	1	esp+hyl	0	-	1
M	M	1	hyl+gelE	0	-	1
N	n1	2	esp+hyl+gelE esp+gelE	2	esp+gelE gelE	4
	n2	1	esp+asaI	2	esp+hyl+asaI esp	3
O	O	1	esp+hyl+gelE	0	-	1
P	P	1	esp	0	-	1
Q	Q	0		1	esp	1
R	R	1	cylA	0	-	1
S	S	1	esp+cylA	0	-	1
T	T	1	esp	0	-	1
U	U	1	esp	0	-	1
V	V	1	esp+gelE	0	-	1
W	W	1	asaI	0	-	1
X	X	0		1	gelE+cylA	1

The largest cluster was cluster D containing eight sub-clusters (d1-d8) and 74 members. Thirteen isolates (15.1%) of the infection group and seven isolates (11%) of the colonization group were located in cluster A. However, although 12 infection isolates (92.3%) were included in sub-clusters a1 and a2, only three isolates (32.8%) from the colonization group were in this sub-cluster. The isolates of infection (45 isolates, 52%) and colonization (29 isolates, 45%) were distributed with a similarity rate in cluster D.

DISCUSSION

In this study, we determined that vanA-type resistance was frequently detected in infection and colonization isolates. Similarly, in a previous study, 48 VRE strains isolated 417 rectal swab samples showed vanA-dependent resistance[13]. Another study determined vanA type resistance in three out of 189 (98.4%) VRE isolates[14]. The *vanA* gene was detected in 21 (65%) of 32 VRE isolated from clinical

samples[15]. We obtained low-level of vanB-dependent resistance among infection and colonization isolates (2.2% and 1.5%, respectively). Similarly, it was reported that the rate of vanB-type resistance was 1.6% in VRE strains isolated from the rectal swap samples of 162 patients and evaluated in a colonization group[16].

In this study, the most frequently detected virulence gene was *esp* (56%) in both infection and colonization strains. Many studies indicate that this gene facilitates colonization and invasion. It was found that the *esp* gene had an important role in persistence and intestinal colonization of *E. faecalis* and *E. faecium* in mouse models[17]. *esp* proteins are responsible especially urinary tract colonization and biofilm formation. Furthermore, *esp* gene deletion mutants significantly decrease cell adhesion and biofilm forming ability[18]. Consistent with the literature, we determined that the *esp*-positive *E. faecium* strains were at a higher rate among the colonization isolates[19,20,21]. The second most common virulence gene was *hyl*. The difference between these two groups is probably statistically significant (51.1% and 35.9%, respectively) ($p < 0.05$). In a study, similarly, it was determined that *esp* and *hyl* to be the most common genes in rectal swab samples[21]. In another study, the isolates containing or transconjugating the *hyl* gene were found to have increased ability of colonization[22]. In the current study, the rate of the *hyl* gene in infection isolates were significantly higher than the colonization isolates ($p < 0.05$). In another study, it was also reported that the presence of *hyl* gene was higher in clinical isolates (27%) than fecal isolates (14%)[23]. These findings suggest that the *esp* and *hyl* genes are important factors for the development of colonization and infection.

The clonal relationships between infection and colonization were also evaluated by the PFGE method. The clonal relationship of infection and colonization isolates located in cluster D had similarities. Thus, it was thought that some colonization strains might have higher genetic potential for infection.

However, the number of infection isolates was statistically higher than colonization isolates in sub-clusters a1 and a2. Therefore, some strains exhibited greater tendency to infect than colonize. It was observed that the colonization isolates had more orphan clusters. In that case, Colonization isolates was more heterogeneous population than the infection isolates.

Interestingly, the infection and colonization isolates determined to have the same PFGE pattern had different virulence genes/gene combination. The PFGE patterns of 24 VR *E. faecalis* strains isolated from colonized and infected patients had 100% similarity, but virulence profiles were different[20]. This finding thought that different virulence genes can be transported via mobile genetic elements. Also, virulence genes carried by mobile genetic elements may not be sufficient to change the size of the polymorphic DNA band in PFGE analysis.

When the virulence genes and combinations of infection and colonization isolates in PFGE clusters were examined, it was determined that the cluster distribution was not statistically significant in the two groups, but the *hyl* gene was more common in infection isolates. This can be explained by the fact that the number of infection and colonization isolates in the same PFGE cluster may be insufficient for statistical analysis.

As a result, in this study, the distribution of virulence genes and clusters was similar in both groups, except for minor differences in the number of isolates. Some studies have shown that resistance and virulence genes spread between the strains on the gastrointestinal tract or the hospital flora[24,25]. Colonization isolates which gain virulence and resistance genes may have the potential for infection and cause infections when they found the opportunity. In our study, it can be said that colonization isolates containing similar virulence factors in the same PFGE cluster as the infection isolates may be a potential endogenous and exogenous infectious agents.

Yazar Katkıları: Çalışma konsepti/Tasarımı: FK; Veri toplama: TGG, BA, TN, BK, MM; Veri analizi ve yorumlama: TGG, FK; Yazı taslağı: TGG, FK; İçeriğin eleştirel incelenmesi: TGG; Son onay ve sorumluluk: TGG, TN, BA, BK, MM, FK; Teknik ve malzeme desteği: FK; Süpervizyon: FK, TGG; Fon sağlama (mevcut ise): yok.
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REFERENCES

1. Eliopoulos GM, Gold HS. Vancomycin-resistant enterococci: mechanisms and clinical observations. Clin Infect Dis. 2001;33:210-19.

2. Sujatha S, Praharaj I. Glycopeptide resistance in gram-positive cocci: a review. Interdis Persp Infect Dis. 2012;781679: 10.
3. Mundy LM, Sahm DF, Gilmore. Relationships between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev. 2000;13:513–22.
4. Çetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev. 2000;13:686-707.
5. Leclercg R, Courvalin P. Resistance to glycopeptides in enterococci. Clin. Infect Dis. 1997;24:545-56.
6. Van der Heijden KM, Van der Heijden IM, Galvao FH, Lopes CG, Costa SF, Abdala E et al. Intestinal translocation of clinical isolates of vancomycin resistant enterococcus faecalis and esbl-producing escherichia coli in a rat model of bacterial colonization and liver ischemia/reperfusion injury. PLOS One. 2014;9:e108453.
7. Higuita NIA, Huycke MM. Enterococcal Disease, Epidemiology, and Implications for Treatment. In: Gilmore MS, Clewell DB, Ike Y, et al. editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Boston: Massachusetts Eye and Ear Infirmary. 2014
8. European Commitee on Antimicrobial Susceptibility Testing. Breakpoint Tables for Interpretation of MICs and Zone Diameters Versions3.1. Available from: http://www.eucast.org/ast_of_bacteria/previous_versions_of_documents/ (accessed Oct 2014).
9. Meier A, Heifets L, Wallace RJ, Zhang Y, Brown BA, Sander P et al. Molecular mechanisms of clarithromycin resistance in mycobacterium avium: observation of multiple 238 rdna mutations in a clonal population. J Infect Dis. 1996;174:354-60
10. Terkuran M, Erginkaya Z, Ünal E, Güran M, Kızılyıldırım S, Ugur G et al. The relationship between virulence factors and vancomycin resistance among enterococci collected from food and human samples in southern Turkey. Ankara Üniv Vet Fak Derg. 2014;61:133-140.
11. Togay OS, Keskin AÇ, Açık L, Temiz A. Virulence genes, antibiotic resistance and plasmid profiles of enterococcus faecalis and enterococcus faecium from naturally fermented turkish foods. J Appl. Microbiol. 2010;109:1084-92.
12. Garcia-Migura L, Pleydell E, Barnes S, Davies RH, Liebana E. Characterization of vancomycin-resistant enterococcus faecium isolates from broiler poultry and pig farms in England and Wales. J Clin Microbiol. 2005;43:3283–89.
13. Karakeçe E, Çiftçi IH, Aşık G. Vankomisin dirençli enterokoklarda direncin moleküler yöntemlerle araştırılması. Ankem Derg. 2013;27:135-39.
14. Corso AC, Gagetti PS, Rodrı´Guez MM, Melano RG, Ceriana PR, Faccone DF et al. Molecular epidemiology of vancomycin-resistant enterococcus faecium in Argentina. Inter J Inf Dis. 2007;11:69-75.
15. Mirzaei B, Farivar TN, Juhari P, Mehr MA, Babaei R. Investigation of the prevalence of vana and vanb genes in vancomycin resistant enterococcus (VRE) by TaqMan realtime PCR assay. J Mic Inf Dis. 2013;3:192-8.
16. Domingo MC, Huletsky A, Giroux R, Boissinot K, Picard FJ, Lebel P et al. High prevalence of glycopeptide resistance genes vanB, vanD, and vanG not associated with enterococci in human fecal flora. Antimicrob Agents Chemother. 2005;49:4784–86.
17. Rice LB, Lakticová V, Carias LL, Rudin S, Hutton R, Marshall SH. Transferable capacity for gastrointestinal colonization in enterococcus faecium in a mouse model. J Infect Dis. 2009;199:342-49.
18. Leendertse M, Willems RJL, Oei GA, Florquin S, Bonten MJM., Van der Poll M. Intestinal enterococcus faecium colonization improves host defense during polymicrobial peritonitis. J Infect Dis. 2009;200:735-44.
19. Diani M, Esiyok OG, Ariafar MN, Yuksel FN, Altuntas EG, Akcelik F. The interactions between esp, fsr, gelE genes and biofilm formation and PFGE analysis of clinical enterococcus faecium strains. Afr J Microbiol Res. 2014;8:129-37.
20. Camargo ILBC, Gilmore MS, Darini ALC. Multilocus sequence typing and analysis of putative virulence factors in vancomycin-resistant and vancomycin-sensitive Enterococcus faecium strains isolated in Brazil. Clin Microbiol Infect. 2006;11:1130.
21. Çopur ŞS, Şahin F, Göçmen JS. Determination of virulence and multidrug resistance genes with polymerase chain reaction method in vancomycin sensitive and resistant enterococci isolated from clinical samples. Turk J Med Sci. 2016;46:877-91.
22. Freitas AR, Tedim AP, Novais C, Ruiz-Garbajosa P, Werner G, Laverde-Gomez JA et al. Global spread of the hyl(efm) colonization-virulence gene in megaplasmids of the enterococcus faecium CC17 polyclonal subcluster. Antimicrob Agents Chemother. 2010;54:2660-65.
23. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R et al. Development of a multiplex PCR for the detection of asa1, gele, cyla, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of enterococcus faecium. J Clin Microbiol. 2004;42:4473-79.
24. Huddleston JR. Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect Drug Resist. 2014;7:167-76.
25. Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and Enterococcus faecium. Clin Microbiol Infect. 2010;16:541-54.