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Determination of virulence and multidrug resistance genes with polymerase chain reaction method in vancomycin-sensitive and -resistant enterococci isolated from clinical samples*

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Background/aim: Enterococci play an important role in nosocomial infections. Therefore, this study investigates multidrug resistance (MDR)1 gene areas in the pathogenicity of enterococci and virulence genes in both vancomycin-sensitive enterococci (VSE) and vancomycin-resistant enterococci (VRE) strains.

Materials and methods: Virulence genes and MDR genes of enterococci were investigated by polymerase chain reaction (PCR).

Results: We evaluated a total of 116 isolates, 93 being VRE and 23 being VSE. In this study, 95.6% of VRE (n = 93) were *Enterococcus faecium* (n = 89) and 4.3% were *E. faecalis* (n = 4), while 17.4% of VSE (n = 23) were *E. faecium* (n = 4) and 82.6% were *E. faecalis* (n = 19). The *vanA* MDR1 gene was detected in all VRE isolates. Among virulence genes, *esp* and *hyl* were detected in *E. faecium*, an enterococcus with the highest resistance to vancomycin, and *gelE* was detected in *E. faecalis*, an enterococcus with the highest sensitivity to vancomycin. Three or more virulence genes were identified only in VSE strains. We consider that it is a significant result that VSE had more virulence genes than VRE. Only *esp* was seen in VRE *E. faecium* strains.

Conclusion: This study includes experimental results on the association of virulence characteristics in VRE and VSE strains.

Key words: *Enterococcus faecium*, *Enterococcus faecalis*, vancomycin multidrug resistance genes, virulence genes

1. Introduction

Enterococci are the natural members of the gastrointestinal tract, mouth, urethra, and vaginal flora and may result in serious nosocomial infections despite their low virulence characteristics. Enterococci are often isolated particularly from patients in intensive care units with suppressed immune systems, hematological malignancies, catheter and prosthesis existence, prolonged hospitalization duration, and usage of broad-spectrum antibiotics (1).

The first vancomycin-resistant *Enterococcus* strain in the world was reported by Uttley et al. (2) in the UK in 1988. In Turkey, the first vancomycin-resistant *Enterococcus* strain was reported in a pediatric patient by Vural et al. (3) from Akdeniz University in 1998.

Resistance to vancomycin develops with the *vanA* and *vanB* genes coded by plasmids and *vanC*, *vanD*, and *vanG* coded by chromosomes. The most common multidrug resistance (MDR)1 genes among them are *vanA* and *vanB*. It is important for the pathogenesis of illnesses to know if enterococci have certain virulence genes other than resistance to antibiotics.

Attachment to epithelial tissue, formation of biofilm, the spread of bacteria over connective tissue, the breakdown of collagen, and hemolysis occur through proteins coded from *esp*, *hyl*, *gelE*, *asa1*, and *cyl* gene areas (4,5).

Enterococci, ranking first among causes of nosocomial infections, have epidemiologically become more important with the presence of both resistance and virulence factors. Studies are limited that investigate the presence of enterococci with these two characteristics together in nosocomial infections.

Our objective was to investigate both resistance and virulence gene areas in patient isolates with vancomycin-resistant enterococci and vancomycin-sensitive enterococci

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(VRE and VSE, respectively). We think that the obtained data will be epidemiologically useful in understanding the pathogenesis of enterococci.

2. Materials and methods

A total of 116 *Enterococcus* strains isolated from patients in various clinics of the Ankara and Adana hospitals of the Başkent University Faculty of Medicine were included in the study. If more than one sample was available from the same patient, only one sample was included in the study.

2.1. Identification of bacteria

Strains were kept in glycerol bouillon at –86 °C and then transferred for culture to 5% sheep blood agar media (Becton Dickinson, USA) and incubated at 37 °C for 24 h. In gram-positive coccus morphology, catalase-negative strains were applied for the PYR test with the BBL DrySlide PYR kit (Becton Dickinson). Isolates were also cultured in esculin medium and 6.5% NaCl medium. Strains with positive PYR test results, producing black colonies in the esculin medium and growing in 6.5% NaCl, were identified to be *Enterococcus* spp., and the species were further identified by Rapid ID 32 Strep kits (BioMérieux, France). Identifications of species were verified by the MALDI-TOF system (BioMérieux).

2.2. Determination of sensitivity of bacteria to antibiotics

2.2.1. Determination of sensitivity to vancomycin

2.2.1.1. Kirby–Bauer disk diffusion method

Sensitivity to vancomycin was assessed using 30-µg vancomycin disks in Mueller Hinton agar (Becton Dickinson) medium in accordance with recommendations of the Clinical and Laboratory Standards Institute (6).

2.2.1.2. Determination of minimal inhibitor concentration (MIC) with broth microdilution method

Vancomycin resistance/sensitivity was verified by the broth microdilution method. For determination of MICs, the broth microdilution method, Mueller Hinton II broth medium (Becton Dickinson), and standard powder of vancomycin (Sigma, Germany) were used (6). All tests were repeated twice. *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were used as quality control strains for each study. For all VRE strains, vancomycin MIC values were ≥128 µg/mL.

2.2.2. Determination of sensitivity to teicoplanin

The MIC value of teicoplanin was determined by gradient diffusion method. E-test strips (BioMérieux) were used for MIC determination. *E. faecalis* ATCC 29212 was used as a quality control strain. For all VRE, teicoplanin MIC values were ≥256 µg/mL.

2.3. Investigation of multidrug resistance and virulence genes with polymerase chain reaction (PCR)

DNAs of strains were extracted via gram-positive bacteria DNA extraction kit (Genoks, Turkey) and kept at –20 °C until DNAs were amplified. Primers described by Dutka-Malen et al. (7) were used to investigate MDR genes (Table 1). In investigation of MDR genes, *E. faecalis* ATCC 51559 was used as a *vanA*-positive control strain, *E. faecalis* ATCC 51299 was used as a *vanB*-positive control strain, and *E. faecalis* ATCC 29212 was used as a negative control strain. Primers described by Vankerckhoven et al. (8) were used for investigation of virulence genes (Table 2). In investigation of virulence genes, *E. faecalis* MMH594 (for *esp*, *cyl*, *asa1*, and *gelE*) and *E. faecium* C68 (for *hyl*) were used as positive control strains.

vanA and *vanB*, *esp* and *cyl*, and *asa1* and *gelE* were investigated as pairs by multiplex PCR. In the thermal cycle (Biometra, Germany), the following phases were carried out: at 94 °C for 2 min; then at 94 °C for 45 s, 58 °C for 45 s, and 72 °C for 45 s for 35 cycles; and a final cycle at 72 °C for 10 min.

Next, 10 µL of each amplification product was mixed with the loading dye and loaded in the tank. A 50-bp DNA ladder (O'RangeRuler, Fermentas, Lithuania) was used as a molecular standard marker. Electrophoresis was carried out at 120 V for 30 min. Bands were imaged with a UV transilluminator (TFX 20M, Vilber Lourmat, France).

2.4. Statistical analysis

Data were analyzed by Pearson chi-square test, likelihood ratio test, Fisher exact test, and z test depending on the frequency of tables and the number of cells in tables. $P < 0.05$ was considered to be statistically significant. SPSS 17.0 was used for data analyses (SPSS Inc., Chicago IL, USA).

Table 1. Multidrug resistance gene primer sequences (7).

Gene	Primer name	Oligonucleotide sequence (5' to 3')	Product size (bp)
vanA	A1	5' GGGAAAACGACAATTGC 3'	732
	A2	5' GTACAATGCGGCCGTTA 3'	
vanB	B1	5'ATGGGAAGCCGATAGTC3'	635
	B2	5'GATTTCGTTCCTCGACC3'	

Table 2. Virulence genes and factors (8).

Gene	Virulence factor	Primer name	Oligonucleotide sequence (5' to 3')	Product size (bp)
esp	Enterococcal surface protein	ESP 14F	AGATTTCATCTTTGATTCTTGG	510
		ESP 12R	AATTGATTCTTTAGCATCTGG	
cyl	Cytolysin	CYLI	ACTCGGGGATTGATAGGC	688
		CYLIIb	GCTGCTAAAGCTGCGCTT	
asa1	Aggregation substance	ASA11	GCACGC TATTACGAACTATGA	375
		ASA12	TAAGAAAGAACATCACCACGA	
gelE	Gelatinase	GEL11	TATGACAATGCTTTTTGGGAT	213
		GEL12	AGATGCACCCGAAATAATATA	
hyl	Hyaluronidase	HYL n1	ACAGAAGAGCTGCAGGAAATG	276
		HYL n2	GACTGACGTCCAAGTTTCCAA	

3. Results

3.1. Bacterial identification and sensitivity results

Of 116 isolates, 93 (80.2%) were identified to be VRE and 23 (19.8%) were identified to be VSE. Of the VRE (n = 93), 95.7% (n = 89) were identified as *E. faecium* and 4.3% (n = 4) were identified as *E. faecalis*. The most common species isolated in this study was *E. faecium*. Distribution of species by clinics for VRE and distribution of species by clinical samples for VRE and VSE are presented in Tables 3 and 4, respectively. Among VSE (n = 23), the most commonly isolated species was *E. faecalis* at 82.6% (n = 19), followed by *E. faecium* at 17.4% (n = 4).

Table 3. Distribution of species by clinics for VRE and VSE.

Clinics \ Bacteria		VRE				VSE			
		E. faecium	*E. faecalis*	Total	P	*E. faecium*	*E. faecalis*	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Surgery clinics*	Positive	20 (95.2)	1 (4.8)	21 (100)	0.201	0	2 (100)	2 (100)	0.999
	Negative	69 (95.8)	3 (4.2)	72 (100)		4 (19)	17 (81)	21 (100)	
Surgery polyclinics**	Positive	-	-	-	-	2 (20)	8 (80)	10 (100)	0.999
	Negative	-	-	-		2 (15.4)	11 (84.6)	13 (100)	
Internal polyclinics#	Positive	-	-	-	-	0	5 (100)	5 (100)	0.539
	Negative	-	-	-		4 (22.3)	14 (77.7)	18 (100)	
Intensive care units	Positive	18 (90)	2 (10)	20 (100)	0.999	0	3 (100)	3 (100)	0.999
	Negative	71 (97.3)	2 (2.7)	73 (100)		4 (20)	16 (80)	20 (100)	
Other clinics ##	Positive	16 (100)	0	16 (100)	0.999	-	-	-	-
	Negative	73 (94.8)	4 (5.2)	77 (100)		-	-	-	
Pediatrics	Positive	13 (100)	0	13 (100)	0.999	1 (50)	1 (50)	2 (100)	0.324
	Negative	76 (95)	4 (5)	80 (100)		1 (100)	0	1 (100)	
Nephrology	Positive	10 (90.9)	1 (9.1)	11 (100)	0.401	-	-	-	-
	Negative	79 (96.3)	3 (3.7)	82 (100)		-	-	-	
Burn units	Positive	7 (100)	0	7 (100)	0.999	-	-	-	-
	Negative	82 (95.3)	4 (4.7)	86 (100)		-	-	-	
Hematology	Positive	5 (100)	0	5 (100)	0.999	1 (100)	0	1 (100)	0.174
	Negative	84 (95.5)	4 (4.5)	88 (100)		3 (13.7)	19 (86.3)	22 (100)	

*General surgery, orthopedics.

**Urology, general surgery, orthopedics, obstetrics.

#Pediatrics, rheumatology, dermatology, endocrinology, infectious diseases.

##Neurology, internal medicine, cardiology, geriatrics, physical therapy and rehabilitation, gastroenterology, and chest disease services.

Table 4. Distribution of species by clinical samples for VRE and VSE.

Clinics \ Bacteria		VRE				VSE			
		E. faecium	*E. faecalis*	Total	P	*E. faecium*	*E. faecalis*	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Urine	Positive	32 (91.4)	3 (8.6)	35 (100)	0.147	2 (18.2)	9 (81.8)	11 (100)	
	Negative	57 (98.3)	1 (1.7)	58 (100)		2 (16.7)	10 (83.3)	12 (100)	0.999
Wounds	Positive	20 (100)	0	20 (100)	0.573	1 (12.5)	7 (87.5)	8 (100)	
	Negative	69 (94.5)	4 (4.5)	73 (100)		3 (20)	12 (80)	15 (100)	0.999
Blood	Positive	18 (100)	0	18 (100)	0.999	1 (50)	1 (50)	2 (100)	
	Negative	71 (94.6)	4 (5.4)	75 (100)		3 (14.3)	18 (85.7)	21 (100)	0.324
Rectal swabs	Positive	8 (100)	0	8 (100)	0.999	-	-	-	
	Negative	81 (95.2)	4 (4.8)	85 (100)		-	-	-	-
Sterile bodily fluids	Positive	6 (100)	0	6 (100)	0.999	0	1 (100)	1 (100)	
	Negative	83 (95.4)	4 (4.4)	87 (100)		4 (18.2)	18 (81.8)	22 (100)	0.999
Catheters	Positive	2 (66.6)	1 (33.4)	3 (100)	0.124	-	-	-	
	Negative	87 (96.6)	3 (0.4)	91 (100)		-	-	-	-
Tracheal aspirates	Positive	1 (100)	0	1 (100)	0.999	-	-	-	
	Negative	88 (95.6)	4 (4.4)	92 (100)		-	-	-	-
Sputum	Positive	2 (100)	0	2 (100)	0.999	-	-	-	
	Negative	87 (95.6)	4 (4.4)	93 (100)		-	-	-	-
Drainage tubes	Positive	-	-	-	-	0	1 (100)	1 (100)	
	Negative	-	-	-		4 (18.2)	18 (81.8)	22 (100)	0.999

3.2. Results of molecular analyses

vanA and *vanB* were initially investigated in all strains by PCR. *vanA* was detected in all 93 VRE strains (Figure 1). PCR images of virulence genes are shown in Figures 2–6.

The most common virulence factor was *esp* with a rate of 78.4% (n = 91) among all enterococci (n = 116). *esp* was positive in 75 strains (80.6%) out of 93 VRE strains. It was followed by *hyl* (15.1%, n = 14) and *gelE* (3.2%, n = 3) in VRE strains (n = 93), respectively. Multiple virulence factors were found in VRE (n = 93) strains: *esp+hyl* (12.4%) and *esp+gelE* (2.2%). Three virulence factors were not found together in any VRE strains. *Asa1* and *cyl*

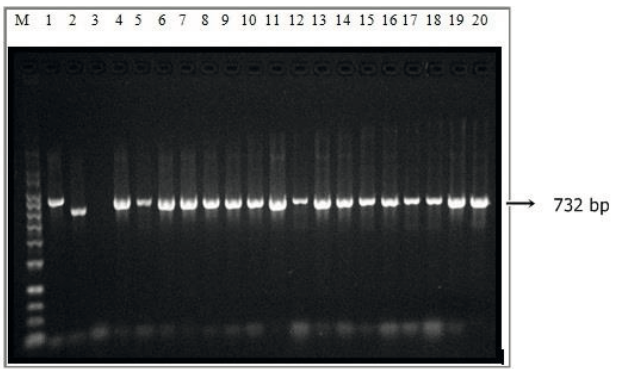

Figure 1. PCR image of 16 strains with *vanA* genotype: the first line (M) is the marker, line 1 is *E. faecalis* ATCC 51559 (732 bp) as a *vanA*-positive control strain, line 2 is *E. faecalis* ATCC 51299 (635 bp) as a *vanB*-positive control strain, line 3 is the negative control, and lines 4–20 are amplification products in specific 732 bp for the *vanA* gene in DNAs of strains.

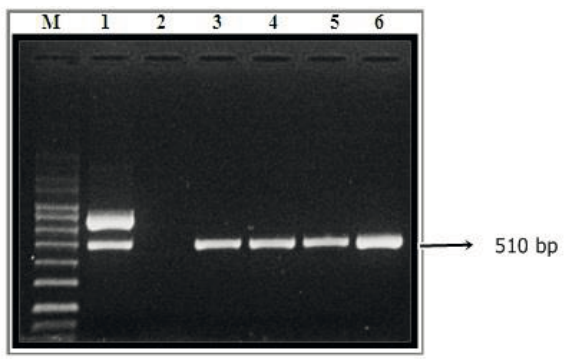

Figure 2. PCR image of VRE *esp* and *cyl* VRE: the first line (M) is the marker, line 1 is MMH594 as a positive control (*esp* and *cyl*), line 2 is the negative control, and lines 3, 4, 5, and 6 are amplification products in specific 510 bp for the *esp* gene in DNAs of strains.

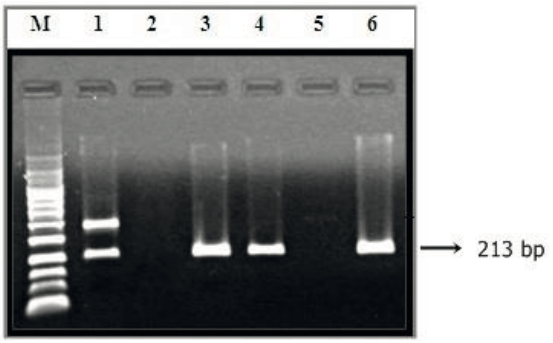

Figure 3. PCR image of VRE *asa1* and *gelE*: the first line (M) is the marker, line 1 is MMH594 as a positive control (*asa1* and *gelE*), line 2 is the negative control, and lines 3, 4, and 6 are amplification products in specific 213 bp for the *gelE* gene in DNAs of strains. Line 5 is the product without *asa1* or *gelE* genes.

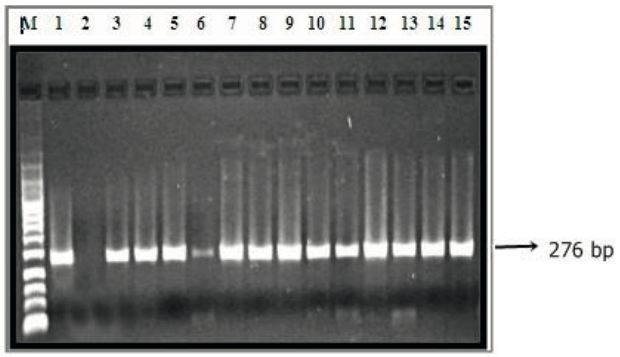

Figure 4. PCR image of the VRE *hyl* virulence gene: the first line (M) is the marker, line 1 is *E. faecium* C68 (*hyl* positive control), line 2 is the negative control, and all other lines are amplification products in specific 276 bp for the *hyl* gene in DNAs of strains.

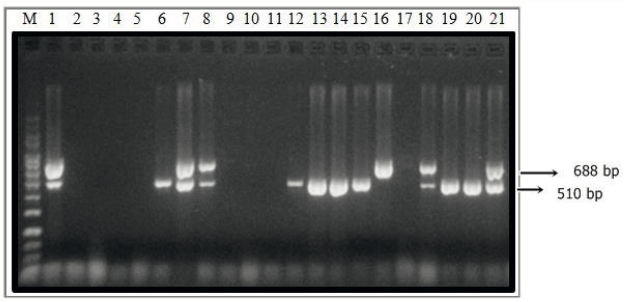

Figure 5. PCR image of VSE *esp* and *cyl*: the first line (M) is the marker, line 1 is MMH594 as a positive control (*esp* and *cyl*), and line 2 is the negative control. Lines 6, 7, 8, 12, 13, 14, 15, 18, 19, 20, and 21 are amplification products in specific 510 bp for the *esp* gene in DNAs of strains. Lines 7, 8, 16, 18, and 21 are amplification products in specific 688 bp for the *cyl* gene in DNAs of strains.

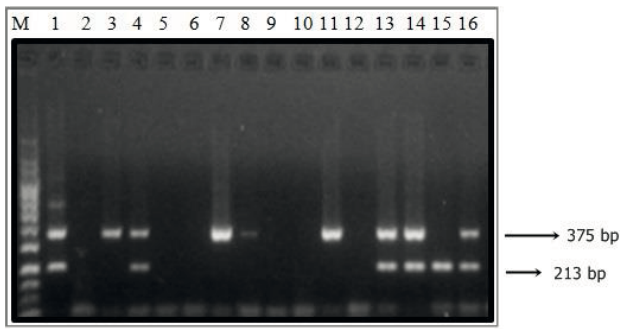

Figure 6. PCR image of VSE *asa1* and *gelE*: the first line (M) is the marker, line 1 is MMH594 as a positive control (*asa1* and *gelE*), and line 2 is the negative control. Lines 4, 13, 14,15, and 16 are amplification products in specific 213 bp for the *gelE* gene in DNAs of strains. Lines 3, 4, 7, 11, 13, 14, and 16 are amplification products in specific 375 bp for the *asa1* gene in DNAs of strains.

were not identified in any VRE strains. The rates of *esp*, *hyl*, and *gelE* were 79.8%/100%, 15.7%/0%, and 2.2%/25%, respectively, in VRE *E. faecium* (VREfm)/VRE *E. faecalis* (VREfs) (Table 5).

At least one virulence factor was found in all VSE strains. Of a total of 23 VSE strains, *esp* was identified in 16 (69.6%), *asa1* was identified in 13 (56.5%), *gelE* was identified in 12 (52.2%), *cyl* was identified in 8 (34.8%), and *hyl* was identified in 1 (4.3%). Two virulence factors were found together in 6 (26.1%) VSE (n = 23) strains, and the most common association (n = 4) was *esp* and *gelE*. Three or more virulence factors were found in a total of 9 (39.13%) samples of isolated VSE (n = 23) strains (Table 5).

The *esp* rate in the VRE clinical samples obtained from pediatrics patients was statistically significantly higher ($P < 0.01$). The *esp* rates in isolates obtained from other clinics (neurology, internal medicine, cardiology, geriatrics, physical therapy and rehabilitation, gastroenterology, and chest disease services) were statistically significant ($P < 0.05$) (Table 6).

The rates of *esp+hyl* and *esp+gelE* were higher in samples obtained from other clinics (neurology, internal medicine, cardiology, geriatrics, physical therapy and rehabilitation, gastroenterology, and chest disease service), intensive care units, and surgery clinics (urology, general surgery, orthopedics, and obstetrics). *Hyl* was the highest in pediatrics and intensive care units. The rates of *hyl* and *hyl+esp* were significantly highest in sputum samples ($P < 0.05$) among all VRE strains obtained from clinical samples (Table 7).

The dominant species was *E. faecium* (89/93) with a rate of 95.7% in VRE isolates, whereas it was *E. faecalis* with a rate of 82.6% (19/23) in VSE species. *esp* was found in VSE and VRE strains of both species but was more common in VRE isolates. However, this higher rate was not statistically significant. VREfm had the highest *hyl* rate

Table 5. Distribution of virulence genes by VRE and VSE.

	Resistant			Sensitive		
	E. faecium (n = 89)	*E. faecalis* (n = 4)	P	*E. faecium* (n = 4)	*E. faecalis* (n = 19)	P
	n (%)	n (%)		n (%)	n (%)	
Total *esp* (78.4%, n = 91)	71 (79.8)	4 (100)	0.999	3 (75.0)	13 (68.4)	0.999
Total *hyl* (12.9%, n = 15)	14 (15.7)	0 (0)	0.999	0 (0)	1 (5.3)	0.999
Total *gelE* (12.9%, n = 15)	2 (2.2)	1 (25)	0.125	1 (25)	11 (57.9)	0.317
Total *asa1* (11.2%, n = 13)	0 (0)	0 (0)	-	2 (50)	11 (57.9)	0.999
Total *cyl* (6.9%, n = 8)	0 (0)	0 (0)	-	2 (50)	6 (31.6)	0.589
esp alone (67.7%, n = 63)	61 (68.5)	0 (0)	0.999	1 (25)	1 (5.2)	0.324
hyl alone (3.2%, n = 3)	3 (3.3)	0 (0)	0.999	0 (0)	0 (0)	-
gelE alone (1%, n = 1)	0 (0)	0 (0)	-	0 (0)	1 (5.2)	0.999
asa1 alone (2.1%, n = 2)	0 (0)	0 (0)	-	0 (0)	2 (10.5)	0.999
cyl alone (n = 0)	0 (0)	0 (0)	-	0 (0)	0 (0)	-
esp+hyl (n = 12)	11 (12.4)	0 (0)	0.999	0 (0)	1 (5.3)	0.999
esp+gelE (n = 15)	11 (12.4)	0 (0)	0.125	0 (0)	4 (21.1)	0.999
asa1+gelE (n = 1)	0 (0)	0 (0)	-	0 (0)	1 (5.3)	0.999
asa1+gelE+cyl (n = 2)	0 (0)	0 (0)	-	1 (25)	1 (5.3)	0.324
esp+cyl+asa1 (n = 3)	0 (0)	0 (0)	-	1 (25)	2 (10.5)	0.453
asa1+gelE+esp (n = 2)	0 (0)	0 (0)	-	0 (0)	2 (10.5)	0.999
asa1+gelE+cyl+esp (n = 2)	0 (0)	0 (0)	-	0 (0)	2 (10.5)	0.999

Table 6. According to clinics, virulence genes in isolated distribution of VRE.

		esp				*hyl*				*gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Intensive care unit	Positive	14 (70)	6 (30)	20 (100)	0.205	4 (20)	16 (80)	20 (100)	0.491	1 (5)	19 (95)	20 (100)	0.521
	Negative	61 (83.6)	12 (16.4)	73 (100)		10 (13.7)	63 (86.3)	73 (100)		2 (2.7)	71 (97.3)	73 (100)	
Surgery clinics***	Positive	19 (90.5)	2 (9.5)	21 (100)	0.345	3 (14.3)	18 (85.7)	21 (100)	0.999	2 (9.5)	19 (90.5)	21 (100)	0.127
	Negative	56 (77.8)	16 (22.2)	72 (100)		11 (15.5)	61 (84.7)	77 (100)		1 (1.4)	71 (98.6)	72 (100)	
Burn units	Positive	6 (85.7)	1 (14.3)	7 (100)	0.999	11 (14.3)	6 (85.7)	17 (100)	0.999	0	7 (100)	7 (100)	0.999
	Negative	69 (80.2)	17 (19.8)	86 (100)		13 (15.1)	73 (84.9)	86 (100)		3 (3.5)	83 (96.5)	86 (100)	
Nephrology	Positive	9 (81.8)	2 (18.2)	11 (100)	0.999	1 (9.1)	10 (90.9)	11 (100)	0.999	0	11 (100)	11 (100)	0.999
	Negative	66 (80.5)	16 (19.5)	82 (100)		13 (15.9)	69 (84.1)	82 (100)		3 (3.7)	79 (96.3)	82 (100)	
Hematology	Positive	5 (100)	0	5 (100)	0.579	0	5 (100)	5 (100)	0.999	0	5 (100)	5 (100)	0.999
	Negative	70 (79.5)	18 (20.5)	88 (100)		14 (15.9)	74 (81.1)	88 (100)		3 (3.4)	85 (94.4)	88 (100)	
Pediatrics	Positive	6 (46.2)	7 (53.8)	13 (100)	0.003**	3 (23.1)	10 (76.9)	13 (100)	0.407	0	13 (100)	13 (100)	0.999
	Negative	69 (86.3)	11 (13.8)	80 (100)		11 (13.8)	69 (86.3)	80 (100)		3 (3.8)	77 (96.3)	80 (100)	
Other clinics***	Positive	16 (100)	0	16 (100)	0.035*	2 (12.5)	14 (87.5)	16 (100)	0.999	0	16 (100)	16 (100)	0.999
	Negative	59 (76.6)	18 (23.4)	77 (100)		12 (15.6)	65 (84.4)	77 (100)		3 (3.9)	74 (96.1)	77 (100)	

*P < 0.05; **P < 0.01.

***Orthopedics, general surgery.

****Neurology, internal medicine, cardiology, geriatrics, physical therapy and rehabilitation, gastroenterology, and chest disease services.

Table 6. (Continued).

		esp+hyl				*esp+gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Intensive care unit	Positive	4 (20)	16 (80)	20 (100)	0.242	1 (5)	19 (95)	20 (100)	0.521
	Negative	7 (9.6)	66 (90.4)	73 (100)		2 (2.7)	71 (97.3)	73 (100)	
Surgery clinics***	Positive	3 (14.3)	18 (85.7)	21 (100)	0.707	2 (9.5)	19 (90.5)	21 (100)	0.127
	Negative	8 (11.1)	64 (88.99	72 (100)		1 (1.4)	71 (98.6)	72 (100)	
Burn units	Positive	1 (14.3)	6 (85.7)	7 (100)	0.999	0	7 (100)	7 (100)	0.999
	Negative	10 (11.6)	76 (88.4)	86 (100)		3 (3.5)	83 (96.5)	86 (100)	
Nephrology	Positive	0	11 (100)	11 (100)	0.350	0	11 (100)	11 (100)	0.999
	Negative	11 (13.4)	71 (86.6)	82 (100)		3 (3.7)	79 (96.3)	82 (100)	
Hematology	Positive	0	5 (100)	5 (100)	0.999	0	5 (100)	5 (100)	0.999
	Negative	11 (12.5)	77 (87.5)	88 (100)		3 (3.4)	85 (96.6)	88 (100)	
Pediatrics	Positive	1 (7.7)	12 (92.3)	13 (100)	0.999	0	13 (100)	13 (100)	0.999
	Negative	10 (12.5)	70 (87.5)	80 (100)		3 (3.8)	77 (96.3)	80 (100)	
Other clinics****	Positive	2 (12.5)	14 (87.7)	16 (100)	0.999	0	16 (100)	16 (100)	0.999
	Negative	98 (11.7)	68 (88.3)	77 (100)		3 (3.9)	74 (96.1)	77 (100)	

Table 7. The distribution of virulence genes in VRE isolated from clinical samples.

		esp				*hyl*				*gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Urine	Positive	24 (72.7)	9 (27.3)	33 (100)	0.177	2 (6.1)	31 (93.9)	33 (100)	0.127	2 (6.1)	31 (93.9)	33 (100)	0.286
	Negative	51 (85)	9 (15)	60 (100)		12 (20)	48 (80)	60 (100)		1 (1.7)	59 (98.3)	60 (100)	
Blood	Positive	14 (77.8)	4 (22.2)	18 (100)	0.745	5 (27.8)	13 (72.2)	18 (100)	0.136	0	18 (100)	18 (100)	0.999
	Negative	61 (81.3)	14 (18.7)	75 (100)		9 (12)	66 (88)	75 (100)		3 (4)	72 (96)	75 (100)	
Rectal swabs	Positive	8 (100)	0	8 (100)	0.347	1 (12.5)	7 (87.5)	8 (100)	0.999	0	8 (10.09)	8 (100)	0.999
	Negative	67 (78.8)	18 (21.2)	85 (100)		13 (15.3)	72 (84.7)	85 (100)		3 (3.5)	82 (96.5)	85 (100)	
Wounds	Positive	18 (90)	2 (10)	20 (100)	0.343	2 (10)	18 (90)	20 (100)	0.726	1 (59)	19 (95)	20 (100)	0.521
	Negative	57 (78.1)	16 (21.9)	73 (100)		12 (16.4)	61 (83.6)	73 (100)		2 (2.79)	71 (97.39)	73 (100)	
Sterile bodily fluids	Positive	4 (66.7)	2 (33.3)	6 (100)	0.328	0	6 (100)	6 (100)	0.586	0	6 (100)	6 (100)	0.999
	Negative	71 (81.6)	16 (18.4)	87 (100)		14 (16.1)	73 (83.9)	87 (100)		3 (3.4)	84 (96.6)	87 (100)	
Catheters	Positive	3 (100)	0	3 (100)	0.999	1 (33.3)	2 (66.7)	3 (100)	0.391	0	3 (10.09)	3 (100)	0.999
	Negative	72 (80)	18 (20)	90 (100)		13 (14.4)	77 (85.6)	90 (100)		3 (3.3)	87 (96.7)	90 (100)	
Sputum	Positive	2 (100)	0	2 (100)	0.999	2 (100)	0	2 (100)	0.021*	0	2 (100)	2 (100)	0.999
	Negative	73 (80.2)	18 (19.8)	91 (100)		12 (13.2)	79 (86.8)	91 (100)		3 (3.3)	88 (96.7)	91 (100)	
Drainage tubes	Positive	1 (100)	0	1 (100)	0.999	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999
	Negative	74 (80.4)	18 (19.6)	92 (100)		14 (15.2)	78 (84.8)	91 (100)		3 (3.3)	89 (96.7)	92 (100)	

*P < 0.05.
**P < 0.01.

Table 7. (Continued).

		esp+hyl				*esp+gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Urine	Positive	2 (6.1)	31 (93.9)	33 (100)	0.317	2 (6.1)	31 (93.9)	33 (100)	0.286
	Negative	9 (15)	51 (85)	60 (100)		1 (1.7)	59 (98.3)	60 (100)	
Blood	Positive	3 (16.7)	15 (83.3)	18 (199)	0.440	0	18 (100)	18 (100)	0.999
	Negative	8 (10.7)	67 (89.3)	75 (100)		3 (4)	72 (96)	75 (100)	
Rectal swabs	Positive	1 (12.5)	7 (87.5)	8 (100)	0.999	0	8 (100)	8 (100)	0.999
	Negative	10 (11.8)	75 (88.2)	85 (100)		3 (3.5)	82 (96.5)	85 (100)	
Wounds	Positive	2 (10)	18 (90)	20 (100)	0.999	1 (5)	19 (95.5)	20 (100)	0.521
	Negative	9 (12.3)	64 (87.7)	73 (100)		2 (2.7)	71 (97.3)	73 (100)	
Sterile bodily fluids	Positive	0	6 (100)	6 (100)	0.999	0	6 (100)	6 (100)	0.999
	Negative	11 (12.6)	76 (87.4)	87 (100)		3 (3.4)	84 (96.6)	87 (100)	
Catheters	Positive	1 (33.3)	2 (66.79)	3 (100)	0.318	0	3 (100)	3 (100)	0.999
	Negative	10 (11.1)	80 (88.99)	90 (100)		3 (3.3)	87 (96.79)	90 (100)	
Sputum	Positive	2 (100)	0	2 (100)	0.013**	0	2 (100)	2 (100)	0.999
	Negative	9 (9.9)	82 (90.1)	91 (100)		3 (3.3)	88 (96.7)	91 (100)	
Drainage tubes	Positive	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999
	Negative	11 (12)	81 (88)	92 (100)		3 (3.3)	89 (96.7)	92 (100)	

of 15.7%. With a rate of 57.9%, *gelE* was the highest in the VSEfs strains. *Asa1* and *cyl* were not found in VRE strains but were detected in VSEfs strains in particular among VSE strains. The distributions of virulence genes in VRE and VSE according to species are presented in Table 5.

4. Discussion

Enterococci have become important as isolation rates have recently increased in community-acquired and nosocomial infections and they have developed resistance to many antibiotics, including glycopeptides. Enterococci are very common in the world and play important roles in nosocomial infections, as the second most common nosocomial pathogens after *S. aureus* in the United States (9).

According to data from the National Nosocomial Infections Surveillance System, the number of hospitals with VRE isolated is increasingly growing in Turkey. This rate was 5.4%, 6.1%, 11.2%, and 17.7% respectively for 2008, 2009, 2010, and 2012 (10,11).

Risk factors for VRE include malignancy, neutropenia, intraabdominal surgery, prolonged hospitalization duration, transplantation, staying in hematology-oncology and intensive care units, antineoplastic therapy, and use of vancomycin and 2nd and 3rd generation cephalosporin.

In a study from Turkey, Aygün et al. (12) reported that out of 467 rectal swab samples the VRE rate was 1.9% (n = 9). Aral et al. (13) reported that 158 *Enterococcus* strains were isolated from inpatients at various clinics, particularly 34% from pediatrics, 27% from surgery, and 20% from intensive care units in 4 years (13). Of VRE strains in our study, 22.6% were isolated from samples of surgery clinics, 21.5% were isolated from samples of intensive care units, and 17.2% were isolated from samples of other clinics.

Aral et al. (13) sorted 158 *Enterococcus* strains according to clinical samples in 4 years and reported that the urine samples (58%) took first place, which was followed by blood (16%) and wound (17%) samples. A Greek study (14) indicated that 37.2% of identified VRE samples were isolated from urine samples, followed by 21.5% from blood samples and 19.7% from wound samples. Of isolates in our study, 37% were from urine, 21.5% wounds, 19.4% blood, 8.6% rectal swabs, and 6.5% sterile bodily fluids.

In the distribution of *Enterococcus* spp., the most common species are *E. faecalis* and *E. faecium* among enterococci leading to infection in humans. Despite the common species being *E. faecalis*, the rate of *E. faecium* has significantly increased in resistant enterococci in particular over the past years. SENTRY data show that VREfm strains increased from 40% to 62% from 1997 to 2002. In the same period, VREfs decreased from 4% to 3%. Regardless of the geographic region, currently many VRE clinical isolates are *E. faecium* (15). Wang et al. (16) analyzed data from their surveillance study of 8 years and

reported that the rate of *E. faecium* increased from 12.4% (in 2002) to 27.3% (in 2010). In the same surveillance study vancomycin resistance rates were also increased.

In the surveillance study (17) of the Hacettepe University Faculty of Medicine conducted in 2008, 12 VRE were isolated and all of these isolates were *E. faecium*. Yiş et al. (18) evaluated 123 rectal swab samples taken from different services and 18 (14.6%) were identified as VRE.

As reported above, the most common species appear to be *E. faecium* and *E. faecalis*. Of 93 VRE isolates in our study, 95.7% were *E. faecium* (89/93) and 4.3% were *E. faecalis* (4/93). On the other hand, of VSE strains, 82.6% were *E. faecalis* (19/23) and 17.4% were *E. faecium* (4/23).

The most significant characteristic of enterococci that increases their importance is resistance to glycopeptides. The most common MDR1 gene is *vanA*, which is followed by *vanB*. In *vanA* type resistance, there is a high level of resistance to both vancomycin and teicoplanin. In *vanB* type resistance, there are various degrees of resistance to vancomycin, and sensitivity to teicoplanin. Both of the resistance types can be induced. The *vanA* resistance phenotype is more common in the United States and European countries as compared to others. A study by Protonotariou et al. (14) conducted in 2010 with 2123 *Enterococcus* isolates investigated MDR1 gene areas and identified 79.1% as *vanA* and 20.9% as *vanB* MDR1 genes. The results of a surveillance study (19) performed in Canada between 1999 and 2009 revealed that 81 of 128 VRE samples causing bacteremia were *E. faecium*, and 90.1% of them had *vanA* while 9.9% had *vanB* MDR1 gene areas (19). Çakırlar et al. (20) reported that all VRE strains included in their study harbored the *vanA* gene.

In the present study, 19.8% of total strains (23/116) were sensitive to vancomycin and teicoplanin, while 93 (80.17%) were resistant to both antibiotics. The *vanA* resistance type was present in all of the VRE strains. It was expected to identify the *vanA* resistance type because a large part of enterococci in this study were *E. faecium*.

In addition to MDR1 gene areas, the presence of virulence genes in enterococci is important in colonization of bacteria and occurrence of infections. We investigated the presence of virulence gene areas in a total of 116 *Enterococcus* strains, 93 being VRE and 23 being VSE.

The esp enterococcal surface protein, expressed on the surface of bacteria, can be transferred to other enterococci through conjugation. A high amount of *esp* is found in isolates causing bacteremia and endocarditis despite its low amount in enterococci isolated from stool samples (1). *esp* is thought to protect bacteria from the immune system of the host and is known to increase colonization in the urinary system, contributing to persistence (5). Expression of *esp* is correlated with formation of biofilm. Although *esp* is common in *E. faecalis* isolates, it is more common in hospital-acquired *E. faecium* isolates in particular (4). The rate of *esp* expression in enterococcal isolates is 49.5%–77% (21–23) in Asian countries, 33%–65% (8,24,25) in European countries, and 33%–76% in continental America (26–28). A study (29) conducted in Turkey reported that this rate was 25.6%. The *esp* virulence gene had the highest rate at 78.4% in our study. In Asian studies, the *esp* gene rates of species were 71%–87.5% for *E. faecium*, whereas they varied between 0% and 100% for *E. faecalis* (21,30,31).

We found *esp* gene rates of species to be 79.8% in VRE *E. faecium* and 100% in VRE *E. faecalis*, 75% in VSE *E. faecium*, and 68.49% in VSE *E. faecalis*, and no statistical correlation was present between the virulence gene and species (Table 5).

In a previous study *esp* gene rates for VRE and VSE were 85.7% and 44.2%, respectively (32). In another study, out of 135 strains, 73 were VRE and 62 were VSE, and *esp* gene rates were 79.5% and 54.8%, respectively (33). In our study the *esp* gene rates for VRE and VSE were 79.8% and 75%, respectively (Table 5).

For VRE, the clinic where *esp* was most often isolated was hematology, and other clinics were 100%, while surgery clinics were 90.5%, burn clinics were 85.7%, nephrology was 81.8%, intensive care units were 7%, and pediatrics was 46.2%. The rate of the *esp* gene in strains isolated from pediatrics and other clinics was statistically significantly higher ($P < 0.005$) (Table 6). Most of our isolates were enterococci grown in urine samples, which was followed by wound and blood samples: 72.7% of urine samples had the *esp* gene (Table 7).

It was an interesting finding that the presence of the *esp* virulence gene area was higher than 40% in enterococci isolated from clinics other than intensive care units (Table 6).

The *esp* gene was detected from internal disease polyclinic patients at a rate of 100%, surgery polyclinic patients at a rate of 50%, and internal medicine intensive care units at 33.3%. Additionally, 100% of enterococci isolated from blood samples, 75% from sterile bodily fluids and wound samples, and 63.6% from urine samples had the *esp* virulence gene area (Tables 8 and 9). The existence of studies reporting that *esp* is thought to protect bacteria from the immune system of the host and is known to increase colonization in infections explains why the *esp* gene was mostly found in blood, sterile body fluids, wound, and urine samples.

With regard to the presence of the *hyl* virulence gene area, the rate of *hyl* was 4%–7% in European countries (8,24,34,35), 17.2%–100% in continental America (27,36,37), and 53% in Saudi Arabia (38) in VSE strains. We found that 12.9% of all isolates had the *hyl* gene in this study. A Chinese study (30) conducted in 2012 with VRE reported that the rate of *hyl* was 31.3% in *E. faecium* and no *hyl* was found in *E. faecalis*.

Table 8. According to clinics, virulence genes in isolated distribution of VSE.

		esp				*hyl*				*gelE*				*asa1*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Surgery polyclinics*	Positive	5 (50)	5 (50)	10 (100)	0.169	0	10 (100)	10 (100)	0.999	7 (70)	3 (30)	10 (100)	0.214	7 (70)	3 (30)	10 (100)	0.402
	Negative	11 (84.6)	2 (15.4)	13 (100)		1 (7.7)	12 (92.3)	13 (100)		5 (38.5)	8 (61.5)	13 (100)		6 (46.2)	7 (53.8)	13 (100)	
Internal polyclinics#	Positive	5 (100)	0	5 (100)	0.242	1 (20)	4 (80)	5 (100)	0.217	2 (40)	3 (60)	5 (100)	0.640	2 (40)	3 (60)	5 (100)	0.618
	Negative	11 (61.1)	7 (38.9)	18 (100)		0	18 (100)	18 (100)		10 (55.6)	8 (44.4)	18 (100)		11 (61.1)	7 (31.9)	18 (100)	
Internal intensive care units	Positive	1 (33.3)	2 (66.7)	3 (100)	0.209	0	3 (100)	3 (100)	0.999	1 (33.3)	2 (66.7)	3 (100)	0.590	2 (66.7)	1 (33.3)	3 (100)	0.999
	Negative	15 (75)	5 (25)	20 (100)		1 (5)	19 (95)	20 (100)		11 (55.9)	9 (45)	20 (100)		11 (55)	9 (45)	18 (100)	
Surgery clinics**	Positive	2 (100)	0	2 (100)	0.999	0	2 (100)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.999
	Negative	14 (66.7)	7 (33.3)	21 (100)		1 (4.8)	20 (95.2)	21 (100)		11 (52.4)	10 (47.6)	21 (100)		12 (57.1)	9 (42.99)	21 (100)	
Pediatrics	Positive	2 (100)	0	2 (100)	0.999	0	2 (100)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.999
	Negative	14 (66.7)	7 (33.3)	21 (100)		1 (4.8)	20 (95.2)	21 (100)		11 (52.4)	10 (47.6)	21 (100)		12 (57.1)	9 (42.99)	21 (100)	
Hematology	Positive	1 (100)	0	1 (100)	0.999	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.478	0	1 (100)	1 (100)	0.435
	Negative	15 (68.2)	7 (31.8)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		12 (54.5)	10 (45.5)	22 (100)		13 (59.1)	9 (40.9)	22 (100)	

*Urology, general surgery, orthopedics, obstetrics.
#Pediatrics, rheumatology, dermatology, endocrinology, infectious diseases.
**General surgery, orthopedics.

Table 8. (Continued).

		esp				*hyl*				*gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Surgery polyclinics*	Positive	2 (20)	8 (80)	10 (100)	0.405	0	10 (100)	10 (100)	0.999	1 (10)	9 (90)	10 (100)	0.604
	Negative	5 (38.5)	8 (61.5)	13 (100)		1 (7.7)	12 (92.3)	13 (100)		3 (23.1)	10 (76.9)	13 (100)	
Internal polyclinics#	Positive	1 (20)	4 (80)	5 (100)	0.999	1 (20)	4 (80)	5 (100)	0.217	1 (20)	4 (80)	5 (100)	0.999
	Negative	6 (33.3)	1 (66.7)	18 (100)		0	18 (100)	18 (100)		3 (16.7)	15 (83.3)	18 (100)	
Internal intensive care units	Positive	2 (66.7)	1 (33.3)	3 (100)	0.209	0	3 (100)	3 (100)	0.999	0	3 (100)	3 (100)	0.999
	Negative	5 (25)	15 (75)	20 (100)		1 (5)	19 (95)	20 (100)		4 (20)	16 (80)	20 (100)	
Surgery clinics**	Positive	1 (50)	1 (50)	2 (100)	0.526	0	2 (100)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.324
	Negative	6 (28.6)	15 (71.4)	21 (100)		1 (4.8)	20 (95.2)	21 (100)		3 (14.3)	18 (85.79)	21 (100)	
Pediatrics	Positive	1 (50)	1 (50)	2 (100)	0.526	0	2 (100)	2 (100)	0.999	1 (50)	1 (50)	2 (100)	0.324
	Negative	6 (28.6)	15 (71.4)	21 (100)		1 (4.8)	20 (95.2)	2 (100)		3 (14.3)	18 (85.79	21 (100)	
Hematology	Positive	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999
	Negative	78 (31.8)	15 (68.2)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		4 (18.2)	18 (81.8)	22 (100)	

In this study, the rate of *hyl* was 15.5% and 4.3% respectively in *E. faecium* and *E. faecalis* in all isolates, whereas this rate was 15.7% in VREfm isolates but 0% in VREfs isolates. The *hyl* gene is a virulence factor that facilitates the spread of bacteria and toxins over tissues. The results of our study are consistent with the results of European and American studies for the *hyl* gene area. We found *hyl* to be the most commonly found virulence factor after *esp* in VRE strains. This factor was only found in one sample of VSEfs strains.

hyl was mostly isolated from pediatrics (23.1%), intensive care units (20%), and other clinic patients (17.6%) in VRE isolates and was mostly found in enterococci isolated from sputum (100%), catheter (33.3%), and blood (27.8%) samples. The association of *hyl* and *hyl* plus *esp* was statistically significant in enterococci isolated from sputum samples ($P < 0.021$, $P < 0.013$).

The rate of *gelE* was 12.9% in all isolates. The incidence rates of *gelE* were 27%–65.9% (21,22,39) in Asia, 60% in continental America (28), and 74.3% in Europe (40). Our rate was very low as compared to the rates of other studies. This can be considered a state specific to Turkey.

The *gelE* rates were 2% and 25% respectively in VREfm and VREfs, whereas it was 25% and 57.9% in VSEfm and VSEfs.

The rate of the *asa1* gene was 11.1% in the present study. The percentage of the *asa1* gene was 50% in VSEfm and 57.9% in VSEfs. The rate of the *asa1* gene was 23%–53.6% (21,22,39) in Asian studies and 63.5% (40) in a European study. These rates varied between 26.7% and 40% in Turkish studies (29,41). This gene was not found in our VRE strains. The *asa1* virulence gene is a virulence factor that allows bacteria to attach to eukaryotic surfaces, facilitating development of systemic infections. This result of our study is highly interesting. Although we did not detect this gene area in VRE strains, it is important that it was found in VSE strains.

The rate of the *cyl* gene was 6.8% in all isolates. Many studies did not find *cyl* in VRE isolates (8,36,42,43). We did not find *cyl* in VRE but did find it in VSE isolates with a rate of 6.9%. It is important to detect the cyl protein with cytolytic activity in VSE for nosocomial bacteremia caused by VSE strains.

In this study, all 15 strains without virulence factors were VRE. Although we detected one to four virulence genes in all of our VSE, we found a maximum of two virulence genes in VRE strains.

The association of *esp* and *hyl* was mostly present in VRE strains at 12.4%, whereas this rate was 5.3% in VSE strains. The rate of isolates with *esp* plus *gelE* was 6% in this study. These rates were 21.1%, 25%, and 2.2% respectively in VSEfs, VREfs, and VREfm.

Isolates with three or more virulence genes were found in urine and wound samples isolated from surgery and internal medicine polyclinics.

VRE are primary agents to consider in clinical approaches, and there is a common opinion that measures should be taken for nosocomial infections. However, virulence factors of bacteria are important in the spreading of infections caused by enterococci over tissues and organs. We consider that it is a significant result that VSE had more virulence genes than VRE. Based on these findings, it is

Table 9. The distribution of virulence genes in VSE isolated from clinical samples.

		esp				*hyl*				*gelE*				*asa1*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Urine	Positive	7 (63.6)	4 (36.4)	11 (100)	0.667	1 (9.1)	10 (90.9)	11 (100)	0.478	5 (45.5)	6 (54.5)	11 (100)	0.684	7 (63.6)	4 (36.4)	11 (100)	0.680
	Negative	9 (75)	3 (75)	12 (100)		0	12 (100)	12 (100)		7 (58.3)	5 (41.7)	12 (100)		6 (50)	6 (50)	12 (100)	
Blood	Positive	2 (100)	0	2 (100)	0.999	0	2 (100)	2 (100)	0.999	0	2 (100)	2 (100)	0.217	1 (50)	1 (50)	2 (100)	0.999
	Negative	14 (66.7)	7 (33.3)	21 (100)		1 (4.8)	20 (95.2)	21 (100)		12 (57.1)	9 (42.2)	21 (100)		12 (57.1)	9 (42.9)	21 (100)	
Wounds	Positive	6 (75)	2 (25)	8 (100)	0.999	0	8 (100)	8 (100)	0.999	6 (75)	2 (25)	8 (100)	0.193	4 (50)	4 (50)	8 (100)	0.685
	Negative	10 (66.7)	5 (33.3)	15 (100)		1 (6.7)	14 (93.3)	15 (100)		6 (40)	9 (60)	15 (100)		9 (60)	6 (40)	15 (100)	
Sterile bodily fluids	Positive	1 (100)	0	1 (100)	0.999	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.478	1 (100)	0	1 (100)	0.999
	Negative	15 (68.2)	7 (31.8)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		12 (54.5)	10 (45.5)	22 (100)		12 (54.5)	10 (45.5)	22 (100)	
Drainage tubes	Positive	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999	1 (100)	0	1 (100)	0.999	0	1 (100)	1 (100)	0.435
	Negative	16 (72.7)	6 (27.3)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		11 (50)	11 (50)	22 (100)		13 (59.1)	9 (40.9)	22 (100)	

Table 9. (Continued).

		esp				*hyl*				*gelE*			
		Positive	Negative	Total	P	Positive	Negative	Total	P	Positive	Negative	Total	P
		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Urine	Positive	3 (27.3)	8 (72.7)	11 (100)	0.999	1 (9.1)	10 (90.9)	11 (100)	0.478	1 (9.1)	10 (90.9)	11 (100)	0.590
	Negative	4 (33.3)	8 (66.79)	12 (100)		0	12 (100)	12 (100)		3 (25)	9 (75)	12 (100)	
Blood	Positive	1 (50)	1 (50)	2 (100)	0.526	0	2 (100)	2 (100)	0.999	0	2 (100)	2 (100)	0.999
	Negative	6 (28.6)	15 (71.4)	21 (100)		1 (4.8)	20 (95.2)	21 (100)		4 (19)	17 (81)	21 (100)	
Wounds	Positive	2 (25)	6 (75)	8 (100)	0.999	0	8 (100)	8 (100)	0.999	3 (37.5)	5 (62.5)	8 (100)	0.103
	Negative	5 (33.3)	10 (66.7)	15 (100)		1 (6.7)	14 (93.3)	15 (100)		1 (6.7)	14 (93.3)	15 (100)	
Sterile bodily fluids	Positive	1 (100)	0	1 (100)	0.304	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999
	Negative	6 (27.3)	16 (72.7)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		4 (18.2)	18 (81.8)	22 (100)	
Drainage tubes	Positive	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999	0	1 (100)	1 (100)	0.999
	Negative	7 (31.8)	15 (68.2)	22 (100)		1 (4.5)	21 (95.5)	22 (100)		4 (18.2)	18 (81.8)	22 (100)	

important and necessary to investigate MDR1 gene areas in the pathogenicity of enterococci as well as to investigate virulence genes in both VRE and VRE strains.

This study includes in vitro results of the association of virulence characteristics in VRE and VSE strains. VRE are important causes of difficult-to-treat infections, especially in hospitalized patients. According to our results, VSE are also important microorganisms due to their virulence factors. Vancomycin resistance virulence factors should also be taken into consideration. In conclusion, we think that not only VRE but also VSE may cause serious infections. In our opinion, new studies extended with clinical findings, hospitalization duration, severity of infection, and treatment protocols would help further clarify the subject.

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References

1. Gültekin M. Enterococci: microbiology, epidemiology and pathogenesis. In: Ulusoy S, Usluer G, Ünal S, editors. Gram-Positive Bacterial Infections. 2nd ed. Ankara, Turkey: Scientific Medical Publisher; 2012 (in Turkish).
2. Uttley AH, Collins CH, Naidoo J, George RC. Vancomycin-resistant enterococci. Lancet 1988; 1: 57-58.
3. Vural T, Şekercioğlu AO, Ögünç D, Gültekin M, Çolak D, Yeşilipek A, Ünal S, Kocagöz S, Mutlu G. Vankomisine dirençli *Enterococcus faecium* suşu. ANKEM Derg 1999; 13: 1-4 (in Turkish).
4. Sava IG, Heikens E, Huebner J. Pathogenesis and immunity in enterococcal infections. Clin Microbiol Infect 2010; 16: 533-540.
5. Kayaoğlu G, Orstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. Crit Rev Oral Biol Med 2004; 15: 308-320.
6. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplements. CLSI Document M100- S19. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2012.
7. 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.
8. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H. 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: 4473-4479.
9. Panesso D, Reyes J, Rincon S, Diaz L, Galloway-Pena J, Zurita J, Carrillo C, Merentes A, Guzman M, Adachi JA et al. Molecular epidemiology of vancomycin-resistant *Enterococcus faecium*: a prospective, multicenter study in South American hospitals. J Clin Microbiol 2010; 48: 1562-1569.
10. Turkish Ministry of Health. Antimicrobial Resistance Rates. National Nosocomial Infections Surveillance Study: 2011-2012 Statistics. Ankara, Turkey: Ministry of Health; 2013. Available online at http//www.uhes.saglik.gov.tr.

11. Turkish Ministry of Health. Antimicrobial Resistance Rates. National Nosocomial Infections Surveillance Study: 2008-2009-2010 Statistics. Ankara, Turkey: Ministry of Health; 2013. Available online at http//www.uhes.saglik.gov.tr.

12. Aygün H, Memikoğlu OK, Tekeli A, Azap A, Yörük F. Hastanede yatan riskli hasta gruplarında vankomisin dirençli enterokok kolonizasyonunun surveyansı. Turk J Anaesth Reanim 2008; 36: 168-173 (in Turkish).

13. Aral M, Paköz NİE, Aral İ, Doğan S. Antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* strains isolated from various clinical samples. Turk Bull Hyg Exp Biol 2011; 68: 85-92 (in Turkish with English abstract).

14. Protonotariou E, Dimitroulia E, Pournaras S, Pitiriga V, Sofianou D, Tsakris A. Trends in antimicrobial resistance of clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium* in Greece between 2002 and 2007. J Hosp Infect 2010; 75: 225-227.

15. Mendes RE, Woosley LN, Farrell DJ, Sader HS, Jones RN. Oritavancin activity against vancomycin-susceptible and vancomycin-resistant enterococci with molecularly characterized glycopeptide resistance genes recovered from bacteremic patient, 2009-2010. Antimicrob Agents Chemother 2012; 56: 1639-1642.

16. Wang JT, Chang SC, Wang HY, Chen PC, Shiau YR, Lauderdale TL. High rates of multidrug resistance in *Enterococcus faecalis* and *E. faecium* isolated from inpatient and outpatient in Taiwan. Diagn Microbiol Infect Dis 2013; 75: 406-411.

17. Altun B, Cengiz AB, Kara A, Ceyhan M, Ünal S, Seçmeer G, Gür D. First vancomycin-resistant blood isolate of *Enterococcus faecium* in a children's hospital and molecular analysis of the mechanism of resistance. Turk J Pediatr 2008; 50: 554-558.

18. Yiş R, Aslan S, Çıtak Ç, Değirmenci S. Evaluation of vancomycin-resistant *Enterococcus* colonization at Gaziantep Children's Hospital, Turkey. Mikrobiyol Bul 2011; 45: 646-654 (in Turkish with English abstract).

19. McCracken M, Wong A, Mitchell R, Gravel D, Conly J, Embil J, Johnston L, Matlow A, Ormiston D, Simor AE et al. Molecular epidemiology of vancomycin-resistant enterococcal bacteraemia: results from the Canadian Nosocomial Infection Surveillance Program 1999-2009. J Antimicrob Chemother 2013; 68: 1505-1509.

20. Cakirlar FK, Samasti M, Baris I, Kavakli H, Karakullukcu A, Sirekbasan S, Bagdatli Y. The epidemiological and molecular characterization of vancomycin-resistant enterococci isolated from rectal swab samples of hospitalized patient in Turkey. Clin Lab 2014, 60: 1807-1812.

21. Hasani A, Sharifi Y, Ghotaslou R, Naghili B, Hasani A, Aghazadeh M, Milani M, Bazmai A. Molecular screening of virulence genes in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens in Northwest Iran. Indian J Med Microbiol 2012; 30: 175-181.

22. Sharifi Y, Hasani A, Ghotaslou R, Varshochi M, Hasani A, Aghazadeh M, Milani M. Survey of virulence determinants among vancomycin resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens of hospitalized patients of North west of Iran. Open Microbiol J 2012; 6: 34-39.

23. Ira P, Sujatha S, Chandra PS. Virulence factors in clinical and commensal isolates of *Enterococcus* species. Indian J Pathol Microbiol 2013; 56: 24-30.

24. Billström H, Lund B, Sullivan A, Nord CE. Virulence and antimicrobial resistance in clinical *Enterococcus faecium*. Int J Antimicrob Agents 2008; 32: 374-377.

25. Woodford N, Soltani M, Hardy KJ. Frequency of *esp* in *Enterococcus faecium* isolates. Lancet 2001; 358: 584.

26. Lopes-Salas P, Llaca-Diaz J, Morfin-Otero R, Tinoco JC, Rodriguez-Noriega E, Salcido-Gutierres L, Gonzales GM, Mendoza-Olazaran S, Garza-Gonzales E. Virulence and antibiotic resistance of *Enterococcus faecalis* clinical isolates recovered from three states of Mexico. Detection of linezolid resistance. Arch Med Res 2013; 44: 422-428.

27. Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, Klare I, Nallapareddy SR, Huang W, Murray BE. A potential virulence gene, hyl_{Efm}, predominates in *Enterococcus faecium* of clinical origin. J Infect Dis 2003; 187: 508-512.

28. Comerlato CB, Resende MC, Caierao J, d'Azevedo PA. Presence of virulence factors in *Enterococcus faecalis* and *Enterococcus faecium* susceptible and resistant to vancomycin. Mem Inst Oswaldo Cruz 2013; 108: 590-595.

29. Baylan O, Nazik H, Bektöre B, Çitil BE, Turan D, Öngen B, Özyurt M, Açıkel CH, Haznedaroğlu T. The relationship between antibiotic resistance and virulence factors in urinary *Enterococcus* isolates. Mikrobiyol Bul 2011; 45: 430-445 (in Turkish with English abstract).

30. Sun H, Wang H, Xu Y, Jones RN, Costello AJ, Liu Y, Li G, Chen M, Mendes RE. Molecular characterization of vancomycin-resistant *Enterococcus* spp. clinical isolates recovered from hospitalized patients among several medical institutions in China. Diagn Microbiol Infect Dis 2012; 74: 399-403.

31. Chang CM, Wang LR, Lee HC, Lee NY, Wu CJ, Ko WC. Characterisation of vancomycin-resistant enterococci from hospitalised patients at a tertiary centre over a seven-year period. J Hosp Infect 2010; 74: 377-384.

32. Al-Talib H, Zuraina N, Kamarudin B, Yean CY. Genotypic variations of virulent genes in *Enterococcus faecium* and *Enterococcus faecalis* isolated from three hospitals in Malaysia. Adv Clin Exp Med 2015; 24: 121-127.

33. Kang M, Xie Y, He C, Chen ZX, Guo L, Yang Q, Liu JY, Du Y, Ou QS, Wang LL. Molecular characteristics of vancomycin-resistant *Enterococcus faecium* from a tertiary care hospital in Chengdu, China: molecular characteristics of VRE in China. Eur J Clin Microbiol Infect Dis 2014; 33: 933-939.

34. Bourdon N, Fines-Guyon M, Thiolet JM, Maugat S, Coignard B, Leclercq R, Cattoir V. Changing trends in vancomycin-resistant enterococci in French hospitals, 2001-08. J Antimicrob Chemother 2011; 66: 713-721.

35. Thierfelder C, Keller PM, Kocher C, Gaudenz R, Hombach M, Bloemberg GV, Ruef C. Vancomycin-resistant *Enterococcus*. Swiss Med Wkly 2012; 142: 13540.

36. Camargo IL, Gilmore MS, Darini AL. Multilocus sequence typing and analysis of putative virulence factors in vancomycin-resistant and vancomycin-sensitive *Enterococcus faecium* isolates from Brazil. Clin Microbiol Infect 2006; 12: 1123-1130.

37. Palazzo IC, Pitondo-Silva A, Levy CE, da Costa Darini AL. Changes in vancomycin-resistant *Enterococcus faecium* causing outbreaks in Brazil. J Hosp Infect 2011; 79: 70-74.

38. Khan MA, van der Wal M, Farrell DJ, Cossins L, van Belkum A, Alaidan A, Hays JP. Analysis of *vanA* vancomycin-resistant *Enterococcus faecium* isolates from Saudi Arabian hospitals reveals the presence of clonal cluster 17 and two new Tn1546 lineage types. J Antimicrob Chemother 2008; 62: 279-283.

39. Udo EE, Al-Sweih N. Frequency of virulence-associated genes in *Enterococcus faecalis* isolated in Kuwait hospitals. Med Princ Pract 2011; 20: 259-264.

40. Creti R, Imperi M, Bertuccini L, Fabretti F, Orefici G, Di Rosa R, Baldassarri L. Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. J Med Microbiol 2004; 53: 13-20.

41. 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 2006; 30: 477-482.

42. Song JY, Cheong HJ, Seo YB, Kim IS, Heo JY, Noh JY, Choi WS, Kim WJ. Clinical and microbiological characteristics of vancomycin-resistant enterococci with the *vanD* phenotype and *vanA* genotype. Jnp J Infect Dis 2013; 66: 1-5.

43. Brilliantova AN, Kliasova GA, Mironova AV, Tishkov VI, Novichkova GA, Bobrynina VO, Sidorenko SV. Spread of vancomycin-resistant *Enterococcus faecium* in two haematological centres in Russia. Int J Antimicrob Agents 2010; 35: 177-181.