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Correlation between Infective Factors and Antibiotic Resistance in *Enterococci* Clinical Isolates in West of Iran

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The present study was done to scrutinize the possible relation between infective genes and antimicrobial resistance in *Enterococcus faecalis* and *Enterococcus faecium*. Considering the fact that the presence of recognized infective determinants among clinical isolates may promote the emergence of infections and persistence of *Enterococci* in hospital settings, which can lead to an increase in antimicrobial resistance. 175 *E. faecalis* and 67 *E. faecium* isolated from clinical specimens were used. The isolates were identified, and then antibiotic susceptibility testing was performed. The MIC of vancomycin and teicoplanin were determined by broth microdilution method. The presence of infective genes *esp*, *hyl* and asa_1 was scrutinized using PCR. Of the 280 *enterococcal* isolates, 175 (62.5%) isolates were identified as *E. faecalis*, 67 (24%) as *E. faecium* and 38 (13.5%) as *Enterococcus* spp. The results of the antibiotic susceptibility testing showed resistance rates of 5% and 73% to vancomycin and teicoplanin in *E. faecalis* and *E. faecium* isolates, respectively. The statistical analysis showed that the *esp* infective gene has significant associations with ciprofloxacin, erythromycin and tetracycline in *E. faecium* and with chloramphenicol in *E. faecalis* strains; the *hyl* with teicoplanin and vancomycin in *E. faecium* strains; and also asa_1 with vancomycin in *E. faecium* and with ampicillin and chloramphenicol in *E. faecalis* strains. Regarding the relationships between virulence genes and antibiotic resistance in strains of *E. faecalis* and *E. faecium*, detection of infective factors associated with invasive diseases has become a major issue of concern.

Key Words: *Enterococcus; Anti-Bacterial Agents; Virulence*

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INTRODUCTION

Enterococci bacteria are facultative anaerobic gram-positive cocci, which are considered part of the normal flora in humans and animals. However, these microorganisms may be the cause of several serious systematic infections too.[1-3]

The two most common *Enterococcus* species, *E. faecalis* and *E. faecium* are responsible for 80-90% and 5-10% of human *enterococcal* infections, respectively.[2] *E. faecalis* is the most common isolate of nosocomial infections, but newly, due to increasing resistance to some antimicrobial agents, especially vancomycin, *E. faecium* isolates are also being considered.[4] It has been shown that separate lineages of *E. faecalis* and *E. faecium* are leading causes of the large number of the multidrug-resistant *enterococcal* infections. According to several investigations, CC_{17} and closely related strains are the main agents of most hospital-acquired infections association with *E. faecium*.[5] Although these organisms lack strong virulence factors, they may have an innate resistance/tolerance to many important antibacterial agents such as, cephalosporins, cotrimoxazole, and low levels of penicillin and aminoglycosides, polymyxin, lincosamide, trimethroprim-sulfamethoxazole, monobactams, streptogramin. They are also able to acquire resistance to penicillin, chloramphenicol, tetracyclines, aminoglycosides and vancomycin.[3,6-8]

Pathogenicity and increased risk of acquisition of *enter-*

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ococcal infections is linked to antimicrobial resistance and expression of virulence factors including, adhesion factors, translocation, and immune evasion. In hospital settings, infective potential of enterococci can be due to selective the advantages conferred by their antibiotic resistance. Furthermore, there is great concern about infectious diseases because of spreading antimicrobial resistance genes. The most important infective agents of *enterococci* which have been identified, include: aggregation substances (asa_1), cytolysin (*cyl*), hyaluronidase (*hyl*), the *enterococcal* surface protein (*esp*) and gelatinase (*gelE*).[9,10] It has been found that some of virulence factors such as, *agg*, *esp* and *cyl* genes, located on 153-kb pathogenicity island.[11] Persistence of enterococci in the hospital setting may be associated with their virulence factors.[11] Based on several studies, the virulence factors of gelatinase, aggregation substance and cytolysin have not been found in *E. faecium* in contrast to *E. faecalis*. On the other hand, *esp* and *hyl* have been found in both *E. faecalis* and *E. faecium*.[12]

Furthermore, clinical isolates of enterococci have virulence determinants that may result in promoting the emergence of infections and the persistence of these organisms in hospital settings which consequently can lead to increased resistance.[13] This study was designed to scrutinize some virulence genes including asa_1 (aggregation substance), *esp* (*enterococcal* surface protein), *gelE* (gelatinase), *hyl* (hyaluronidase) in clinical isolates of *E. faecalis* and *E. faecium* and to investigate possible correlations between virulence and antibiotic resistance.

MATERIALS AND METHODS

1. Identification of *enterococcal* isolates

One hundred and seventy-five *E. faecalis* and sixty-seven *E. faecium* strains were collected from discrete clinical samples submitted to three teaching hospitals (including Beheshti, Besat and Farshchian Hospitals) located in Hamedan, Iran, from December 2012 to May 2014. The origins of the isolates were as follows: urine 200 (82.6%), endotracheal aspirate 23 (9.5%), blood 8 (3.3%), Skin soft tissue 6 (2.5%), and body fluids 5 (2.1%). The isolates were pinpointed using routine microbiological methods.[14] Then, PCR targeting D-alanine- D-alanine ligases for *E. faecalis* (*ddl E. faecalis*) and *E. faecium* (*ddl E. faecium*) was used to confirm phenotypic speciation.[15]

2. Detection of *E. faecalis* and *E. faecium* species by PCR assay

Firstly, enterococcal DNA was extracted by boiling.[16] Then, a mastermix PCR Kit [(PCR 2X Taq premix Mastermix), Ariatous Biotec Co.] was used to perform the PCR reaction. PCRs were performed with specific primers for each gene (Table 1) with some modifications to Kariyama's protocol[15] using Eppendorf and Biorad thermocycler in a final volume of 20 μL. The thermal cycle program was performed by initial denaturation at 95°C for 5 min, followed by amplification in 30 cycles of denaturation at 95°C for 30 s, annealing at 52.5°C for 30 s and elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. *E. faecalis* ATCC 29212 and *E. faecium* BM4147 were used as quality control strains.

3. Antibiotic susceptibility testing

The antimicrobial susceptibilities of 175 *E. faecalis* and 67 *E. faecium* strains were examined by using the disk agar diffusion (DAD) method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.[17,18] Erythromycin (15 μg), Tetracycline (30 μg), Ciprofloxacin (5 μg), Vancomycin (30 μg), Teicoplanin (30 μg), Norfloxacin (10 μg), Nitrofurantoin (300 μg), Quinopristin-Dalfopristin [Synercid (15 μg)] (Mast Co., UK), Chloramphenicol (30 μg), Gentamicin (10 μg), Linezolid (30 μg), and Ampicillin (10 μg) (HiMedia Mumbai Co., India) were used for antimicrobial susceptibility testing (AST).

In addition, minimum inhibitory concentrations (MIC) of the glycopeptide antibiotics i.e. vancomycin and teicoplanin (Sigma-Aldrich, Poole, Co., UK) against the *E. faecalis* and *E. faecium* isolates were determined using the microdilution broth method.[17,18] *E. faecalis* ATCC 29212 (Vancomycin sensitive), *E. faecalis* ATCC 51299 (*vanB* positive), *E. faecalis* E206 (*vanA* positive) were used as quality control.

TABLE 1. Primers used in this study

Gene targets	Primer sequences (5' to 3')	Amplicon/product size (bp)	References
asa_1	F: GCACGCTATTACGAACTATGA R: TAAGAAAGAACATCACCACGA	375	13
hyl	F: ACAGAAGAGCTGCAGGAAATG R: GACTGACGTCCAAGTTTCCAA	276	19
esp	F: AGATTTCATCTTTGATTCTTGG R: AATTGATTCTTTAGCATCTGG	510	13
ddl E. faecalis	F: ATCAAGTACAGTTAGTCTTTATTAG R: ACGATTCAAAGCTAACTGAATCAGT	941	13
ddl E. faecium	F: TTGAGGCAGACCAGATTGACG R: TATGACAGCGACTCCGATTCC	658	13

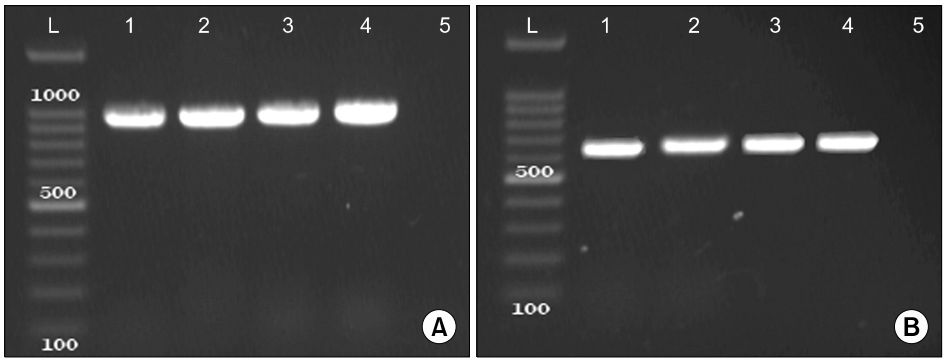

FIG. 1. PCR amplification of *ddl E. faecalis*, *ddl E. faecium* genes. (A) PCR products *ddl E. faecalis* gene (941 bp). (B) PCR products *ddl E. faecium* genes (658 bp). L: molecular size marker 100 bp, 1: positive control, 2-4: samples, 5: negative control.

4. Detection of infective genes *esp*, *hyl*, and *asa1* by PCR

Multiplex PCR and single PCR were used for the identification of *esp*, *asa1* and *hyl* virulence determinants using specific primers for each gene with some modifications on Vankerckhoven's protocol (Table 1).[13,19] Briefly, the final volume multiplex PCR reaction for genes *esp* and *asa1*, was 25 μL and for *hyl* gene was 20 μL. The PCR reactions were done for both mixtures on a Eppendorf and Biorad thermocycler (ASTEC Co., Japan) with an initial denaturation at 95°C for 10 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min.[13] The *E. faecalis* ATCC 29212 (*asa1* positive), *E. faecium* C68 (*hyl* and *esp* positive) were used as quality control.

5. Statistical analysis

Correlation between antibiotic susceptibility patterns and occurring virulence genes was analyzed statistically using the Chi-Square test; the differences were considered significant for $p < 0.0012$ using the Bonferroni correction based on several primary comparisons. In addition, significant differences for simultaneous occurrance of virulence genes in enterococci strains was determined using Fisher's Exact test with a significance level of $p < 0.05$. All tests were performed using SPSS software (version 19).

RESULTS

1. Isolation of *enterococci* strains

Using biochemical methods, of the 280 enterococcal isolates, 190 (67.8%), 75 (26.8%) and 15 (5.4%) isolates were recognized as *E. faecalis*, *E. faecium* and *Enterococcus* spp., respectively. Among the determined presumptive *E. faecalis*, *E. faecium* isolates, 175 (62.5%) *E. faecalis* and 67 (24%) *E. faecium* strains were confirmed using the PCR method (Fig. 1). Therefore, a total of 38 strains (13.5%) remained as a part of the *Enterococcus* genus and were excluded from the current study. Urine samples were the highest isolation source of *E. faecalis* and *E. faecium* strains. Among 175 *E. faecalis* strains, 153 isolates and among 67 *E. faecium* strains, 48 isolates were isolated from urine samples; followed by tracheal sample with 17 isolates (11 strains were determined as *E. faecalis* and 6 strains as *E. faecium*).

TABLE 2. Distribution of *E. faecalis* and *E. faecium* strains isolated from Hamadan hospitals based on the sample source

Site of isolation	*E. faecalis*	*E. faecium*
Urine	152	48
Tracheal	11	6
Blood	5	3
Wound	2	4
Body fluids	3	2
Other organs	2	4
Total	175	67

The highest prevalence of *E. faecium* strains was observed in wounds and other organs samples (abscess and pulmonary secretions); among 12 strains isolated from wounds and other organs, 8 strains belonged to *E. faecium* (Table 2).

2. Antimicrobial susceptibility testing

Table 3 shows the susceptibility patterns of 67 *E. faecium* and 175 *E. faecalis* strains to 12 commonly used antibiotics. Resistance to the majority of antibiotics except for chloramphenicol, tetracycline, and quinopristin-dalfopristin was higher in *E. faecium* isolates compared to *E. faecalis* isolates. However, they both showed good rates of sensitivity to linezolid (100%), nitrofurantoin and chloramphenicol (74.6%). In fact, no resistance to the linezolid antibiotic was observed among *E. faecalis* and *E. faecium* strains; and all isolates of *E. faecalis* were susceptible to nitrofurantoin. Among 175 *E. faecalis* strains, the greatest resistance was observed for Synercid antibiotic with frequency of 167 (95.4%) isolates; followed by tetracycline with 154 (88%), erythromycin with 109 (62.3%), ciprofloxacin with 69 (39.4%), gentamicin with 63 (36%), Norfloxacin with 58 (33%), chloramphenicol with 57 (32.6%), vancomycin and teicoplanin with 9 (5%) and ampicillin with 6 (3.4%) isolates. Among 67 *E. faecium* isolates, the greatest resistance was observed for gentamicin with a frequency of 62 (92.5%) strains, followed by erythromycin with 58 (86.6%), norfloxacin with 56 (83%), ciprofloxacin with 54 (80.6%), vancomycin with 51 (76%), tetracycline and teicoplanin with 49 (73%), Synercid with 47 (70%), ampicillin with 42 (62.7%), nitrofurantoin with 17 (25.4%),

TABLE 3. Antibiotic resistance behavior of *Enterococci* isolates using disk diffusion method

Antimicrobial agent	*E. faecalis*=175			*E. faecium*=67			Total=242		
	S	I	R	S	I	R	S	I	R
Vancomycin	166	0	9	16	0	51	182	0	60
Teicoplanin	166	0	9	18	0	49	184	0	58
Ampicillin	169	-	6	25	-	42	194	-	48
Tetracycline	17	4	154	14	4	49	31	8	203
Ciprofloxacin	64	42	69	0	13	54	64	55	123
Norfloxacin	110	7	58	0	11	56	110	18	114
Erythromycin	46	20	109	0	9	58	46	29	167
Synercid	8	0	167	16	4	47	24	4	214
Chloramphenicol	95	23	57	50	10	7	145	33	64
Gentamicin	98	14	63	1	4	62	99	18	125
Nitrofurantoin	175	0	0	50	0	17	225	0	17
Linezolid	175	0	0	67	0	0	242	0	0

TABLE 4. The prevalence of virulence determinants among *E. faecalis* and *E. faecium* clinical isolates

Clinical samples	*E. faecalis* virulence genes (N=175)			*E. faecium* virulence genes (N=67)		
	esp	*hyl*	asa_1	*esp*	*hyl*	asa_1
Urine	126	93	152	41	37	48
Treacheal	4	2	8	3	3	6
Blood	4	3	5	2	2	3
Wound	1	1	2	4	3	4
Body fluids	1	0	2	1	1	2
Other organs	1	0	1	4	2	4
Total (%)	137 (78.3)	99 (56.6)	170 (97)	55 (82)	48 (71.6)	67 (100)

and chloramphenicol with 7 (10.4%).

Of 175 *E. faecalis* isolates, resistances of 9 (5%) and sensitivity of 166 (95%) isolates to vancomycin and teicoplanin were confirmed by the microdilution broth method. Of 67 *E. faecium* isolates, 51 strains (76%) were resistant to vancomycin using the disk diffusion method, but resistance of 49 strains (73%) to vancomycin was confirmed by the Microdilution Broth method. 2 strains (3%) of *E. faecium* that were determined as resistant strains by disk diffusion using the Microdilution Broth method were identified as intermediate strains. In addition, resistance of 49 (73%) and sensitivity of 18 (27%) *E. faecium* isolates to teicoplanin using disk diffusion method were confirmed by the microdilution broth method. All 9 vancomycin-resistant *E. faecalis* (VRE_{fs}) and 49 vancomycin-resistant *Enterococcus faecium* (VRE_{fm}) strains had high-level resistances to vancomycin and teicoplanin, concurrently. The MIC value among 9 VRE_{fs} strains and VRE_{fm} strains was $\leq$512, 512.

3. Presence of infective genes

The distribution of infective factors among *E. faecalis* and *E. faecium* strains are presented in Table 4 and (Fig. 2 and 3). Among the *E. faecalis* strains, the asa_1 gene was the most prevalent factor (97%), followed by the *esp* (78.3%) and *hyl* genes (56.6%); additionally, in *E. faecium* strains, the asa_1 gene had the highest prevalence (100%) and *hyl* gene has the lowest frequency (71.6%), followed by the *esp* gene (82%).

In a total of 242 *Enterococci* strains, the asa_1 gene was the most prevalent factor (98%), followed by the *esp* (79.3%) and *hyl* genes (60.7%). In the current study, the *esp* gene was found in 75% of the strains isolated from blood samples and 83% of strains isolated from wound infections.

Using statistical analysis, there was no significant difference for simultaneous occurrence of virulence genes in studied enterococci strains ($p > 0.05$).

DISCUSSION

In the present study, we assessed antimicrobial resistance and three infective factors in clinically isolated *E. faecalis* and *E. faecium* and further analysis was conducted to scrutinize the relationship between the presence of virulence factors and antimicrobial resistances. In this study, there was a slight discrepancy between biochemical and PCR results for species identification, which is in accordance with other studies.[20,21] The main reason for this discrepancy is the similarities between *Enterococcus* species and the high phenotypic variation within individual species. PCR is a more accurate technique in comparison

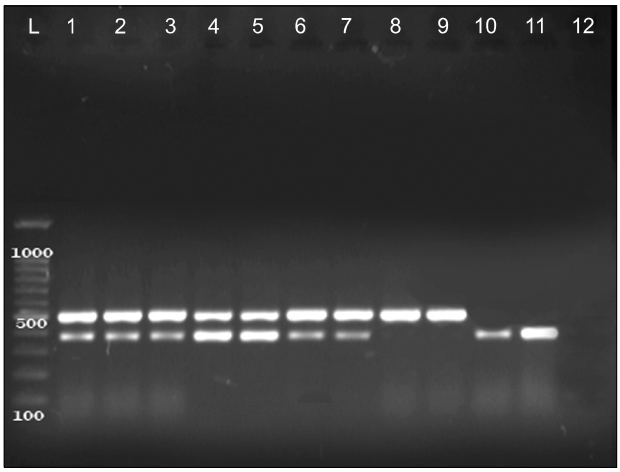

FIG. 2. PCR amplification of *esp*, *asa1* genes. PCR products *esp*, *asa1* virulence genes is 510 bp and 375 bp respectively. L: molecular size marker 100 bp, 1: positive control, 2-11: samples, 12: negative control.

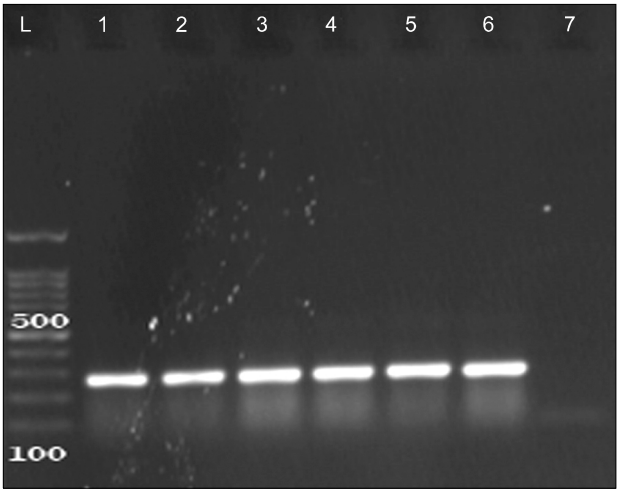

FIG. 3. PCR amplification of *hyl* gene. PCR product *hyl* virulence gene is 275 bp. L: molecular size marker 100 bp, 1: positive control, 2-6: samples, 7: negative control.

to the biochemical approach, so PCR results were preferred for the strains with discrepant identification.[22] In this study, the *esp* gene was detected in 78.3% of *E. faecalis* and 82% of *E. faecium* isolates, this finding is similar to the results of other studies, which identified the *esp* gene in 47.1%,[23] 73%,[24] 68.4%[7] of *E. faecalis*, and 80%,[25,26] 65%,[27] 66%,[23] 71%,[24] 75%[12] of *E. faecium* strains. However, this is in contrast to the findings of Shankar et al.[28] and Channaiah et al.[29] that reported the absence of *esp* in *E. faecium*. Although, as illustrated by Shankar et al.[28] the *esp* gene was detected only in *E. faecalis* strains and other available studies have demonstrated a higher prevalence of the *esp* gene in *E. faecalis*,[16] however, a study on the food and medical isolates indicated an increasing incidence of esp in clinical *E. faecium* isolates compared to *E. faecalis*.[25]

Willems demonstrated that the esp gene is a marker of the high prevalence of *Enterococcus* strains resistant to vancomycin in hospitalized patients,[30] but according to the study of Woodford et al.,[31] Sauer et al.,[32] and Jahangiri et al.[12] the *esp* gene also was identified in sensitive-vancomycin strains. In some studies, the prevalence of gene *esp* is reversed among VRE_{fm} and VSE_{fm} strains. Whereas in other studies, the prevalence of gene *esp* was identical in VRE_{fm} and VSE_{fm} strains. In accordance with the majority of these investigations, in the present study, the gene *esp* also was identified in a large number of VRE_{fm} strains (61%) compared with in VSE_{fm} (21%). Camargo et al.[33] demonstrated that *esp* was restricted to VRE_{fm} (56%) and not found in VSE_{fm}. Vankerckhoven et al.[10] surveyed virulence genes in eight European hospitals and found higher prevalences of *esp* in the clinical VRE_{fm} isolates (77%). Worth et al.[34] and Sharifi et al.[13] also found a higher incidence of *esp* in 80.5% and 71.05% in the clinical VRE_{fm} isolates, respectively. In the study by Terkuran et al.[35] the *esp* gene was detected in 15.7% of VRE_{fm} isolates and the *hyl* gene in 28.6% of VRE_{fm} strains, but these genes were identified in 2.9% of *Enterococcus* sensitive and intermediate strains. In the study by Sauer et al.[32] the *esp* gene was identified in 62.9% and 46.3% of *VRE* and *VSE* strains, respectively. Also, in the study by Jahangiri et al.[12] *esp* gene was identified in 82% of clinical VRE_{fm} isolates and 53% of VSE_{fm} isolates. The *esp* and *hyl* genes have significantly a higher prevalence among ampicillin-resistant VRE_{fm} isolates (53.7%, 37.3%) than ampicillin-susceptible VRE_{fm} isolates (19.4%, 22.4%); which is similar to the results of other studies.[33-37] The high frequency of esp gene, which was shown in the present study and most of the analogous studies, could be due to the fact that strains containing this gene can obtain antibiotic resistant genes and antibiotic resistant bacteria have long term stability in the body.[23,27]

In the present study, the *asa1* gene, (which encodes aggregation substance), was found in high frequency among *E. faecalis* (97%) and *E. faecium* strains (100%). A high incidence of this gene in *E. faecalis* was reported in previous studies. Results of studies on clinical *E. faecium* isolates are contradictory. In some studies, *asa1* was not found in *E. faecium* but in contrast, in some studies this gene was detected in lower frequency and in our study and some other studies this gene was identified in higher prevalence among *E. faecium* isolates (Comerlato et al.[38] and Kowalska-Krochmal et al.[39]), were detected it among 5%, 65% of VRE_{fm} and 2.7%, 60% of VRE_{fs} strains, respectively. Jahangiri et al.[12] were not found *asa1* gene in either 49 of VRE_{fm} strains or 17 of VSE_{fm} strains. Sharifi et al.[13] were detected *asa1* gene in 80% of VRE_{fs} and 7.89% of VRE_{fm} strains. Hällgren et al.[24] also were reported prevalence of *asa1* in 79% of *E. faecium* strains. In studies by Huyckl & Gilmore, the *asa1* gene was detected in 100% of blood isolates and 32% of non-blood isolates of *Enterococcus*. In studies by Elsner et al, Eaton & Gasson, and Archimbaud et al, the *asa1* gene was detected in 40%-78% of clinical isolates of *Enterococci*.[24] Hyaluronidase, coded by the chromosomal

gene *hyl*, that influence the hyaluronic acid (hyaluronate, HA).[35] Hyaluronidase in *Enterococcus*, indicates some homology to the hyaluronidases in other bacteria such as, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.[9] We found the *hyl* gene among 49.3% of VRE_{fm} and 22.4% of VSE_{fm} isolates, which is in accordance to findings of Rice et al.[40] who detected the *hyl* gene among 71% of the United Kingdom VRE_{fm} isolates. But it was in contrast to the study by Jahangiri et al.[12] which detected *hyl* gene among 80% of VSE_{fm} and in 28.5% of VRE_{fm} isolates.

The reasons for the diversity in frequencies of *hyl* and asa_1 can be as follows; *Entrococcus* strains are genetically different from each other based on their geographical origins. Moreover, the media which were used as sampling sources were varied between studies. In other words, some studies took their samples from blood, while others used urine, foods, or sewage.[3,8,26,35,41] As demonstrated by Billström et al.[19] and Wardal et al.[42] the *esp* and *hyl* genes are linked with ampicillin and ciprofloxacin-resistant *enterococci*, particularly in CC_{17} which is an especially virulent, hospital adapted clone found globally. In the present study, it was shown that the *esp* virulence gene has a significant association with ciprofloxacin (p=0.001), erythromycin (p=0.001) and tetracycline (p=0.001) susceptibility patterns in *E. faecium* and with chloramphenicol (p=0.001) in *E. faecalis* strains; the *hyl* with teicoplanin (p=0.001) and vancomycin (p=0.001) in *E. faecium* strains; and also asa_1 with vancomycin (p=0.001) in *E. faecium* and with ampicillin (p= 0.001) and chloramphenicol (p=0.001) in *E. faecalis* strains (Table 5).

The correlation between infective genes and antibiotic resistance in *Enterococcus* may vary from country to country. Based on the study by Hanna Billström et al.[19] a significant relationship between imipenem, ampicillin, and ciprofloxacin resistance pattern and the esp_{fm} gene was found. Resistance to ciprofloxacin, imipenem, ampicillin antibiotics and the prevalence of the *esp* and *hyl* genes in these isolates were reported 90%, 80%, 77%, 56% and 4%, respectively. In the Study by Baylan et al.[43] (Turkey, 2008) on *E. faecalis* strains, a significant association between the carriage asa_1 gene and ciprofloxacin, norfloxacin and levofloxacin resistance pattern and between the *esp* gene and doxycycline resistance pattern were observed; in addition, a significant association between the *hyl* gene and nitrofurantoin resistance pattern in *E. faecium* strains was indicated. In the other study by Jankoska et al.[41] there was no significant relationship between virulence genes and antibiotic resistance patterns; the *esp* gene was detected in 76% of isolates and all strains were susceptible to vancomycin and nitrofurantoin, 24%, 34%, and 28% of strains were resistant to ampicillin, ciprofloxacin and ceftriaxone, respectively.

According to the results of studies by Jahangiri et al.[12] and Duprè,[44] it was found that most of *esp*-positive isolates were resistant to more than 3 antibiotics. Lund et al.[45] demonstrated that the existence of a strong correlation between the carriage of *esp* gene and antimicrobial resistance could be due to the higher conjugation frequencies in strains carrying the *esp* gene compared with strains lacking this gene. Sharifi et al.[13] showed that *E. faecium* strains carrying the *esp* gene were resistant to more than 90% of the tested antibiotics and 64% of them were resistant to vancomycin. Considering these results, it seems that the esp gene facilitates *E. faecium* isolates ability to acquire antibiotic resistance genes. Van Wamel et al.[46] showed that the expression level of the esp gene vary constantly between *E. faecium* strains depending on growth conditions and it is associated with bothe the initial connection and biofilm formation. Due to increasing resistance rates of enterococci to most common antibiotics, strict infection control measures are required. Antibiotic susceptibility testing is recommended for all patients before treatment for rational antibiotic use. There is a significant relationship between virulence genes and antibiotic resistance patterns. The virulence factors involve conjugative transfer of antibiotic resistance genes among enterococci strains and other species especially as the transfer of vancomycin resistance to *staphylococcus aureus* strains may occur.

TABLE 5. Statistical analysis for determining possible relationship between antibiotic resistance pattern and *esp*, *hyl*, asa_1 virulence genes in *E. faecium* and *E. faecalis* strains

Antimicrobial agents	*E. faecium*			*E. faecalis*		
	esp	*hyl*	asa_1	*esp*	*hyl*	asa_1
CIP	p=0.001	p=0.037	p>0.05	p>0.05	p>0.05	p>0.05
E	p=0.001	p=0.048	p>0.05	p>0.05	p>0.05	p>0.05
TEC	p=0.048	p=0.001	p=0.002	p>0.05	p>0.05	p>0.05
VAN	p=0.048	p=0.001	p=0.001	p=0.048	p>0.05	p>0.05
T	p=0.001	p=0.020	p>0.05	p>0.05	p>0.05	p>0.05
A	p=0.037	p=0.020	p>0.05	p>0.05	p>0.05	p=0.001
C	p>0.05	p>0.05	p>0.05	p=0.001	p>0.05	p=0.001
NOR	p>0.05	p>0.05	p>0.05	p=0.003	p>0.05	p>0.05

CIP: ciprofloxacin, E: erythromycin, TEC: teicoplanin, VAN: vancomycin, T: tetracycline, A: ampicillin, C: chloramphenicol, NOR: norfloxacin. p: probability value based on Chi-Square test.

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CONFLICT OF INTEREST STATEMENT

None declared.

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