http://www.medscidiscovery.com

OPEN ACCESS JOURNAL ISSN: 2148-6832

MSD Medical Science and Discovery
2019; 6(12):310-5

Research Article Doi: 10.36472/msd.v6i12.327

Is there any association between antibiotic resistance and virulence genes in *Enterococcus* isolates?

Nergis Aşgın[1]*, Emre Taşkın[2]

Abstract

Objective: In this study, we aim to determine the frequency of antibiotic resistance and five virulence genes in *Enterococcus* species and the relationship between antibiotic resistance and virulence genes.

Material and Methods: A total of 86 *Enterococcus* strains isolated from inpatients between 2015 and 2016 were included. Identification and antibiotic susceptibilities of strains were determined using a BD Phoenix fully automated system. The presence of virulence-associated genes (*esp, gel E, asa1, hyl, and cyl*) were investigated by using PCR method.

Results: Of the 86 *Enterococcus* strains, 53 (61.6%) and 33 (38.4%) were *Enterococcus faecium* and *Enterococcus faecalis*, respectively. Vancomycin and high-level gentamicin resistance (HLGR) in *E. faecalis* strains were 0.6% and 60.6%, respectively. Furthermore, 52 of the 53 *E. faecium* strains were both vancomycin-resistant and HLGR. The frequency of *esp, gel E, asa1, cyl, and hyl* was 91.9%, 60.5%, 54.7%, 43%, and 26.7%, respectively. The *asa 1, cyl, and gel E* genes were detected at high frequencies in vancomycin-susceptible and non-HLGR strains, whereas hyl gene was detected at high frequencies in vancomycin-resistant and HLGR strains.

Conclusion: Virulence genes were more frequent in vancomycin-susceptible and non-HLGR *Enterococcus* strains than in the resistant strains. Although infections caused by multidrug-resistant strains are difficult to treat, it should be considered that susceptible strains have more virulence genes. This may reduce the in vivo efficacy of drugs and lead to treatment failures. Therefore, in addition to the in vitro susceptibilities of drugs, clinical efficacy should be monitored.

Key words: Antibiotic resistance, *Enterococcus faecalis*, *Enterococcus faecium*, virulence gene.

Introduction

Enterococci are Gram–positive, catalase-negative, no spore-forming facultative anaerobic bacteria. They can survive in a wide range of temperature (5°C–65°C) and pH (4.5–10) as well as in the presence of 6.5% NaCl (1,2). Enterococci are part of the human gut microbiota. After gastrointestinal colonization, they can cause blood-stream infections, endocarditis, and intra-abdominal and pelvic infections in critically-ill patients (2,3) In recently, enterococci are the second most common cause of nosocomial wound and urinary tract infections and the third most common cause of nosocomial bacteremia. (1,4,5) The *Enterococcus faecalis* and *E. faecium* are most prevalent species in human diseases (1-4).

Enterococci are intrinsically resistant to cephalosporins, sulfonamides, and low concentrations of aminoglycosides (6-8). In addition, acquired vancomycin and high-level aminoglycoside resistance limit the treatment options.

However, enterococci have various virulence factors such as enterococcus surface protein and aggregation substance that enable adhesion to host, colonization, and biofilm formation (1-3,9). Furthermore, hyaluronidase, cytolysin, and gelatinase enzymes secreted by enterococci contribute to invasion by causing damage to the host tissue (4,8,9).

Some of the genes encoding virulence factors are chromosomal origin, whereas others are subsequently acquired via plasmids (3,9). In this study, we aim to determine the frequency of antibiotic resistance and virulence genes in *Enterococcus* species and the relationship between antibiotic resistance and virulence factors.

Received 16-11-2019 **Accepted** 22-12-2019 **Available Online** 25-12-2019 **Published** 30-12-2019
1 Karabuk University School of Medicine, Dept of Medical Microbiology, Karabuk, TR
2 Karabuk University School of Medicine, Dept of Medical Biology and Genetics, Karabuk, TR
*** Corresponding Author:** Nergis Aşgın **E-mail:** drnasgin@gmail.com

Material and Methods

We included 86 *Enterococcus* strains isolated from rectal swabs and clinical samples of inpatients at Karabuk Training and Research Hospital between January 2015 and December 2016. The strains were stored in tryptic soy broth containing 10% glycerol at −25°C until molecular tests were performed. Only one strain from each patient was included. The ethical approval was obtained from the Non-interventional Clinical Research Ethics Board of Karabuk University (2016 13/3).

Identification and antimicrobial susceptibility testing: Clinical samples sent to the microbiology laboratory were inoculated on Columbia agar supplemented with 5% sheep blood, eosin-methylene blue (EMB) agar, and chocolate agar, whereas rectal swabs were cultured only on Enterococcosel agar. [all, Becton Dickinson (BD) and Company Franklin Lakes, NJ, USA)]. Blood samples were collected in BD BACTEC Plus vials and incubated in Bactec FX 40 (BD, MD, USA) fully automated blood culture system for seven days. The vials that were positive for bacterial growth were cultured on blood agar, EMB agar, and chocolate agar. After incubation for 24–48 h, Gram staining was performed on catalase−negative, pyrrolidinyl aminopeptidase−positive colonies. Colonies that were found to be Gram−positive cocci on microscopic examination were further analyzed for identification and antibiotic susceptibility using a BD Phoenix™ (Becton Dickinson and Company BD, Sparks, MD, USA) fully automated system. Vancomycin resistance was also confirmed using gradient minimum inhibitory concentration (MIC) method with E-test strips (bioMérieux, Marcy-l'Étoile, France). Antibiotic susceptibility test results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (10). *Enterococcus faecalis* ATCC 29212 was used as the quality control strain.

PCR detection of virulence genes: The stock cultures of *Enterococcus* strains were inoculated on 5% sheep blood agar. The pure bacterial culture was obtained after 24 hours of incubation. PCR analysis of each virulence gene was separately performed. The genomic DNA was isolated using the GF-1 Bacterial DNA extraction kit (Vivantis, Malaysia). Previously defined primers were used for the detection of virulence genes and shown in Table 1 (11-13).

The Bioer TC 96 thermal cycler (Hangzhou Bioer, Zhejiang, China) was used for amplification. The amplification conditions were as follows: Initial denaturation for 5 min at 95°C; followed by 40 cycles of denaturation for 1 min at 95°C, annealing for 20 sec at 56°C, extension for 20 sec at 72°C, and final extension for 10 min at 72°C.

The amplified products were separated by electrophoresis for1 h at 100 V in a 1.5 % agarose gel, on 1.5% agarose gel, using an EV231 (Consort, Belgium) device.

Ethidium bromide-stained products were imaged using Syngene™ IG3 (Syngene, Cambridge, UK) gel DOC instrument under UV light. A 100-bp DNA ladder (New England Biolab, Ipswich, MA, USA) was used to compare the band size of each gene. *Enterococcus faecalis* MMH 594, and *Enterococcus faecium* C68 were used as positive control strains [*E. faecalis* MMH 594 *(esp, cyl, asa1* and *gel E* (+) ,*E. faecium C68 hyl* (+)].

Statistical analysis: The data were analyzed using the Minitab 17 (Minitab, Inc., PA, USA) statistical software program. The Anderson Darling test was performed to determine whether the data were normally distributed. Descriptive statistics were shown as numbers and percentages. The correlation between virulence genes and antibiotic resistance of strains was calculated using Chi-square test. A *P*- value ≤ .05 was considered statistically significant.

Results

The 86 *Enterococcus* species were obtained from rectal swab (n=41), blood (n=22), urine(n=19) and wound (n=4) samples. The 53 (61.6%) strains were *E. faecium* and, 33 (38.4%) strains were *E. faecalis.* Moreover, 54 (62.8%) and 72 (83.7%) strains were resistant to vancomycin and high-levels of gentamicin, respectively.

The frequency of vancomycin resistance and HLGR in *E. faecalis* were 0.6% (2/33) and 60.6% (20/33), respectively; 52 (98.1%) of *53 E. faecium* were vancomycin-resistant and HLGR. Of the 86 strains, 91.9 % (n=79) carried *esp* gene. This was followed by 60.5% *gel E* (n=52), 54.7% *asa1* (n=47), 43% *cyl* (n=37) , and 26.7% *hyl* (n= 23).

The distribution of virulence genes in *Enterococcus* species has been shown in Table 2. The *esp* gene was detected in >90% strains in both species (*P* = .88).

However, *asa1*, *cyl*, and *gel E* virulence genes were significantly more frequent in *E. faecalis* strains than in *E. faecium* strains (*P* < .001). Furthermore, 90.9% of *E. faecalis* strains carried *asa*1, whereas this rate was 32% for *E. faecium* strains (*P* < .001). Similarly, *gel E* was more frequent in *E. faecalis* strains, and the rates were 84.8% and 45.3% in *E. faecalis* and *E. faecium*, respectively. In addition, *cyl* gene was detected in 78.8% of *E. faecalis* strains and 20.8% of *E. faecium* strains (*P* < .001). In contrast, *hyl* gene was significantly more frequent in *E. faecium* strains than in *E. faecalis* strains (39.6% vs. 6%, *P* < .001).

The distribution of virulence genes in HLGR and non-HLGR strains has been shown in Table 4. The hyl gene was significantly more frequent in HLGR strains, whereas *asa1*, *cyl*, and *gel E* genes were significantly more frequent in non-HLGR strains.

Table 1. Primer sequences used for the amplification of the target genes.

Virulence factor	Target gene	Primer sequences (5'-3')	Amplicon size (bp)	Reference
Aggregation substance	***asa1***	GCACGCTATTACGAACTATGA	375	[11]
		TAAGAAAGAACATCACCACGA		
Gelatinase	***gel E***	CGA AGT TGG AAA AGG AGG C	372	[13]
		GGT GAA GAA GTT ACT CTG A		
Enterococcal surface protein	***esp***	AGATTTCATCTTTGATTCTTGG	510	[11]
		AATTGATTCTTTAGCATCTGG		
Hyaluronidase	***hyl***	CCCTGGACACATGAAATGCG	605	[12]
		AGCATCGGCCGTTGATAGAC		
Cytolysin	***cyl***	ACTCGGGGATTGATAGGC	688	[11]
		GCTGCTAAAGCTGCGCTT		

Table 2: Distribution of virulence genes among *Enterococcus* species (n %)

Virulence gene	*E. faecalis* (n=33)	*E. faecium* (n=53)	*P*-value
asa1	30 (90.9)	17 (32.1)	< .001
cyl	26 (78.8)	11 (20.8)	< .001
esp	30 (90.9)	49 (92.5)	.88
gel E	28 (84.8)	24 (45.3)	< .001
hyl	2(6.0)	21 (39.6)	< .001

Table 3: Distribution of virulence genes among *VRE and **VSE enteroccal strains (n %)

Virulence gene	VRE (n=54)	VSE (n=32)	*P*-value
	n %	n %	
asa1	17 (32.1)	30 (90.9)	< .001
cyl	11 (20.8)	26 (78.8)	< .001
esp	50 (94.3)	29 (87.8)	.74
gel E	24 (45.3)	28 (84.8)	< .001
hyl	21 (39.6)	2 (6.0)	< .001

*VRE: Vancomycin-resistant enterococcus. **VSE:Vancomycin-sensitive enterococcus.

Table 4: Distribution of virulence genes among *HLGR and non HLGR enterococcal strains (n %)

Virulence gene	HLGR (n=72)	non HLGR (n=14)	*P*-value
	n %	n %	
asa1	34 (47.2)	13 (91.6)	.002
cyl	24 (33.3)	13 (91.6)	<.001
esp	66 (91.7)	13 (91.6)	.88
gel E	40 (55.5)	12 (85.7)	.02
hyl	22 (30.6)	1 (7.1)	.04

*HLGR: High level gentamicin resistant

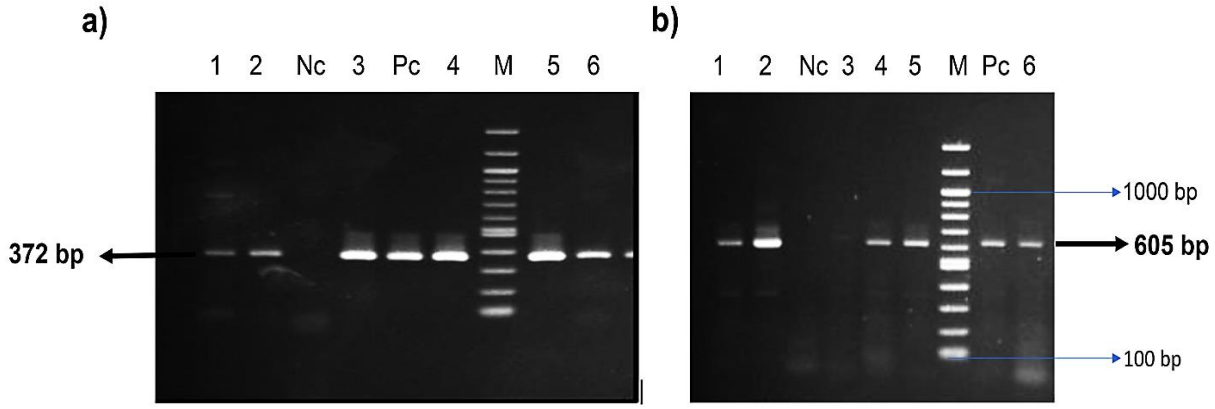

Figure 1. a) Agarose gel electrophoresis images of ***gel E*** (372 bp) in *Enterococcus* strains. Note: Nc: Negative control (ultrapure H_2O). Pc: Positive control (*E. faecalis* MMH 594) M:100 bp DNA Ladder (New England Biolabs, USA) **b)** Agarose gel electrophoresis images of ***hyl*** (605 bp) in *Enterococcus* strains. Note: Nc: Negative control (ultrapure H_2O). Pc: Positive control (*E. faecium* C68) M:100 bp DNA Ladder (Product code :N3231S, New England Biolabs, USA)

http://dx.doi.org/10.36472/msd.v6i12.327

Discussion

Although enterococci are members of the normal human intestinal flora, they cause severe infections such as sepsis, endocarditis, and intra-abdominal abscess following gastrointestinal colonization in patients hospitalized for a long time, exposed to invasive intervention, and those using broad-spectrum antibiotics (2,3,6,7). Enterococcus faecalis is the most frequently isolated species in clinical specimens, and a high level of antibiotic resistance is common in E. faecium strains (2,7,14)

In the present study, 53 (61.6%) and 33 (38.4%) of the 86 Enterococcus strains were identified as E. faecium and E. faecalis, respectively. The majority of clinical isolates of E. faecalis and E. faecium strains were vancomycin-resistant that were isolated from the rectal swabs (n = 41). Vancomycin resistance and HLGR were 62.7% and 83.7% in E. faecium strains and 0.6% and 60.6% in E. faecalis strains, respectively. Consistent with our results, several studies from Turkey have also reported higher rates of vancomycin resistance and HLGR in E. faecium strains (15-17).

The esp was the most frequently virulence gene encoding the enterococcal surface protein. In 1999, the enterococcal surface protein was first identified in gentamicin resistant E. faecalis species (18). The esp gene secreted from the pathogenicity islands of both E. faecalis and E. faecium (1,3,18). It is associated with adhesion, invasion, and evasion (9,19). In addition, it contributes to biofilm formation. Biofilm formation is closely-associated with the development of endocarditis and urinary tract infection (1,9,19).

In the present study, esp was detected in 90.9% and 92.5% of E. faecalis and E. faecium strains, respectively (P = 0.8). Studies conducted in Turkey have reported esp frequencies of 25.6%–87% (15-17,20-22). The high frequency of esp, might be due to regional differences and the collection date of the strains. In enterococci isolates, the acquisition of virulence genes increases in time parallel to the increase in antibiotic resistance. The prevalence of esp gene reported in other countries were 46.6% in Iran, 76% in Brazil, 81.5% in Australia, and 100% in Trinidad (23-26). Vankerckoven et al. reported that the frequency of esp gene was 65% in 271 E. faecium isolates collected from European countries (11). Udo et al. reported a lower rate (31.5%) in 466 E. faecalis isolates collected from eight hospitals in Kuwait (19). Oancea et al. reported that esp gene is transferred among the enterococcal species via the conjugative plasmid in vitro (27). We detected esp gene in >90% strains in both species, supporting this finding.

In the present study, gel E gene was the second most common virulence gene (60.5%). The gel E is of chromosomal origin and encodes gelatinase, also known as a metalloprotease that hydrolyze small peptides such as collagen, elastin, and casein. Thus, it provides nutrients to the bacteria and contributes to invasion (1-3). In this study, the frequency of gel E gene was 84.8% in E. faecalis and 45.3% in E. faecium strains (P: 0.000). Similarly, Al Taib et al. detected gel E in 76.5% and 66.7% of E. faecalis and E. faecium strains, respectively, isolated in Malaysia (13). The frequency of gel E gene is between 0% and 22.8% in studies conducted in Turkey (15-17,21). However, a higher frequency (0%–78%) is reported in studies around the world (13,19,24,25). The *gel E* gene among representative strains is shown in Figure 1.

In the current study, asa1 gene, encoding the aggregation substance, was the third most common virulence gene with a frequency of 54.7%. Aggregation substance is a glycoprotein expressed on the surface of *E. faecalis*. It provides contact between cells during adherence to eukaryotic cells and conjugation. It increases the adhesion of *enterococci* to the surfaces of neutrophils, endocardial and, renal epithelial cells (2,3,9). Besides, it increases the hydrophobicity of the enterococcal surface and enables escape from the immune system by preventing the fusion of lysosomal vesicle and phagosome (3,11,19).

The asa1 gene was found to be significantly more frequent in *E. faecalis* strains than in *E. faecium* strains (90.9% vs. 32.1%, respectively, P = 0.000). Similarly, Baylan et al. (20) and Coşkun (16) from Turkey also detected asa1 gene in *E. faecalis* strains at significantly higher rates. In a study conducted in Iran, Hasani et al. reported the prevalence of asa1 as 92.4% in *E. faecalis* and 7.6% in *E. faecium* (28). In the present study, *asa1* was significantly more frequent in vancomycin-susceptible strains than vancomycin-resistant strains (90.9% vs. 32%, respectively, P = 0.00). This can be attributed to the fact that vancomycin-susceptible strains are mostly *E. faecalis* and vancomycin-resistant strains are mostly *E. faecium*. Mete et al. did not detect asa1 in vancomycin-resistant strains (15). The *asa1* was significantly more frequent in non-HLGR strains than in HLGR strains (91.6% vs. 47.2%, respectively, P = 0.002). In contrast, Al Hasan et al. found *asa1* at similar rates in HLGR (54.1%) and non-HLGR strains (52.8%) in 220 enterococcal isolates in Iran (28). The frequency of *asa1* in enterococcal strains of human origin is between 11.2% and 45% in Turkey (15-17,20). In other studies, *asa1* frequency was reported to be 38% in Brazil, 54.3% in Iran, 79% in Sweden, and 100% in China (4,24,29,30). In a study conducted in Australia, Worth et al. did not detect asa1 gene in vancomycin-resistant *E. faecium* strains (25).

Cytolysin is a toxin whose expression is under the control of eight genes defined as cyl (15,19). Cytolysin is hemolysis in the human, rabbit, and horse erythrocytes. However, it is not active against to sheep erythrocytes (1,3,15). Moreover, it is a bacteriocin that shows bactericidal effect against many Gram-positive bacteria. *Cyl* has a lytic effect in retinal and intestinal cells as well as macrophages and neutrophils (1,3). The *cyl* genes are transmitted via plasmids (3). However, cyl genes can also be found in the pathogenicity islands integrated into the bacterial chromosome (3,15). In the present study, the frequency of *cyl* gene was 78.8% in *E. faecalis* strains and only 20.8% in E. faecium strains (P = 0.000). Additionally, the frequency of *cyl* gene was extremely high in vancomycin-susceptible and non-HLGR strains. The frequency of *cyl* gene was reported to be 6.9%–33.2% in Turkey (15-17), 13% in Sweden (29), and 30.4% in Iran (4).

The *hyl* gene encoding the hyaluronidase enzyme is found especially in E. faecium species and causes tissue damage by degrading the hyaluronic acid (3). Furthermore, disaccharides formed as a result of hyaluronic acid degradation may be a source of food in bacteria. (3,12,19) In the present study, we detected hyl gene at a frequency of 26.7%. The *hyl* gene among representative strains has been shown in Figure 1. This frequency was 39.6% in *E. faecium* strains and 6% in E. faecalis strains (P = 0.000). Coşkun (16) and Çopur et al. (17) reported the frequency of *hyl* gene as 8.7% and 12.9% in Turkey. On the other hand, Gozalan et al. did not detect *hyl* gene in any of the 55 vancomycin-resistant *E. faecium* isolates (21). In other studies, *hyl* gene frequency was reported to be 2.4% in Australia (25), 27.5% in China (30), and 35.4% in Iran (12). Bilström et al. reported a frequency of 4% in 267 *E. faecium* isolates in Sweden (8).

There are some limitations of the study. The study was conducted at a single center and had a small sample size. Furthermore, vancomycin-resistance genes were not investigated using molecular methods.

Conclusion

We detected *esp* gene in >90% *Enterococcus* species. The *asa1, cyl, and gel E* genes were significantly more frequent in *E. faecalis* strains, whereas *hyl* gene was significantly more frequent in *E. faecium* strains. Vancomycin resistance and HLGR were lower in *E. faecalis* strains, but virulence genes (except *hyl*) were more common. All virulence genes (except *hyl*) studied in vancomycin-susceptible and non-HLGR strains were more frequent than those in vancomycin-resistant and HLGR strains. The limited of treatment options for multidrug-resistant enterococci infections is a great concern. Vancomycin -susceptible and non-HLGR strains have multiple virulence factors, which may reduce the *in vivo* efficacy of antibiotics, leading to treatment failures. Therefore, clinical efficacy as well as *in vitro* efficacy of antibiotics used in the treatment of enterococcal infections should be monitored.

Acknowledgements: The authors would like to thank Professor Baris Otlu for providing positive control strains (*E. faecalis* MMH 594 *and E. faecium* C68).

Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: NA, ET; Research concept and design, Research the literature, ET; Genetic analyses NA; preparation of the article, Revision of the article.

References

1. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. Microbiology. 2009;155(6):1749–57.

2. Cesar AA, Murray BE. Enterococcus species, Streptococcus gallolyticus Group, and Leuconostoc Species. In: Bennet JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia (PA): Elsevier Inc.; 2015.p. 2328–39.

3. Baylan O.Virulence Factors And Immunopathogenesis In Enterococcal Infections. Nobel Med. 2019; 15(2):5–16.

4. Heidari H, Emaneini M, Dabiri H, Jabalameli F. Virulence factors, antimicrobial resistance pattern and molecular analysis of enterococcal strains isolated from burn patients. Microb Pathog. 2016; 90:93–97

5. Guzman Prieto AM, van Schaik W, Rogers MRC, et al. Global emergence and dissemination of enterococci as nosocomial pathogens: Attack of the clones? Front Microbiol. 2016;7(5):1–15.

6. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. Virulence. 2012;3(5):421–33.

7. Cetinkaya Y, Falk P, Mayhall C. Vancomycin-Resistant Enterococci. Clin Microbiol Rev.2000;13(4):86–707.

8. Billström H,Lunda B, Sullivana A, Nord CE.Virulence and antimicrobial resistance in clinical Enterococcus faecium. Int J Antimicrob Agents. 2008; 32(5):374–77.

9. Fiore E, Van Tyne D, Gilmore M. Pathogenicity of Enterococci. Microbiol Spectr. 2019. 7(4):GPP3-0053-2018.

10. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 5.1, 2015. http://www.eucast.org. Accession date: 10 October 2019.

11. 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(10): 4473–79.

12. Soheili S, Ghafourian S, Sekawi Z, Neela V, Sadeghifard N, Ramli R et al. Wide distribution of virulence genes among Enterococcus faecium and Enterococcus faecalis clinical isolates. The Scientific World Journal, 2014. http://dx.doi.org/10.1155/2014/623174

13. Al-Talib, 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(1):121–27.

14. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015; 8:217–230.

15. Mete E, Kaleli I, Cevahir N, Demir M, Akkaya Y, Kiris Satilmis O. Evaluation of Virulence Factors in Enterococcus Species. Microbiol Bul. 2017; 51(2):101–14.

16. Say Coskun US. Investigation of the relationship between virulence factors and antibiotic resistance of Enterococci isolates. Cell Mol Biol. 2019, 65(2):114–17.

17. Baylan O, Nazik H, Bektöre B, Çitil BE, Turan D, Öngen B et al. The relationship between antibiotic resistance and virulence factors in urinary Enterococcus isolates. Mikrobiyol Bul. 2011; 45(3): 430–45.

18. Shankar V, Baghdayan AS, Huycke MM, Lindahl G, Gilmore MS. Infection-derived Enterococcus faecalis strains are enriched in esp, a gene encoding a novel surface protein. Infect. Immun. 1999;67(1): 93–200.

19. Udo EE, Al-Sweih N. Frequency of virulence-associated genes in Enterococcus faecalis isolated in Kuwait hospitals. Med Princ Pract. 2011; 20(3): 259–64.

20. Copur SS, Sahin F, Gocmen 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(3): 877–91.

21. Gozalan A, Coskun-Ari FF, Ozdem B, Unaldi O, Celikbilek N, Kirca F et al. Molecular characterization of vancomycin-resistant Enterococcus faecium strains isolated from carriage and clinical samples in a tertiary hospital, Turkey. J Med Microbiol. 2015;64(7):759–66.

22. Cakirlar FK, Samasti M, Baris I, Kavakli H, Karakullukcu A, Sirekbasan S. et al. The epidemiological and molecular characterization of vancomycin-resistant enterococci isolated from rectal swab samples of hospitalized patient in Turkey. Clin Lab. 2014; 60(11):1807–12.

23. Arshadi M, Mahmoudi M, Motahar MS, Saber Soltani S, Pourmand MR. Virulence Determinants and Antimicrobial Resistance Patterns of Vancomycin-resistant Enterococcus faecium Isolated from Different Sources in Southwest Iran. Iran J Public Health. 2018;47(2):264–72.

24. 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(5): 590–95.

25. Worth LJ, Slavin MA, Vankerckhoven V, Goossens H, Grabsch EA, Thursky KA. Virulence determinants in vancomycin-resistant Enterococcus faecium vanB: clonal distribution, prevalence and significance of esp and hyl in Australian patients with haematological disorders. J Hosp Infect 2008; 62(2):137–44.

26. Akpaka P, Kissoon S, Jayaratne P. Molecular Analysis of Vancomycin-Resistant Enterococci Isolated from Regional Hospitals in Trinidad and Tobago. Advances in Medicine. 2016, 1-8. http://dx.doi.org/10.1155/2016/876269.

27. Oancea C, Klare I, Witte W,Werner G. Conjugative transfer of the virulence gene, esp, among isolates of Enterococcus faecium and Enterococcus faecalis. J Antimicrob Chemother. 2004;45(1):232–35.

28. Hasani A, Sharifi Y, Ghotaslou R, Naghili B, Hasani A, Aghazadeh M, et al. 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;30:175–81.

29. Hällgren A, Claesson C, Saeedi B, Monstein HJ, Hanberger H, Nilsson LE. Molecular detection of aggregation substance, enterococcal surface protein, and cytolysin genes and in vitro adhesion to urinary catheters of Enterococcus faecalis and Enterococcus faecium of clinical origin. Int J Med Microbiol. 2009;299(5): 323–32.

30. Yang JX, Li T, Ning YZ, Shao DH, Liu J, Wang S, Liang GW. Molecular characterization of resistance, virulence and clonality in vancomycin-resistant Enterococcus faecium and Enterococcus faecalis: A hospital-based study in Beijing, China. Infect Genet Evol. 2015;33:253–60.