Virulence Genes and Antibiotic Susceptibility of Enterococcus spp. in Bandar Abbas City, Iran

ABSTRACT

Backgrounds: In recent years, Enterococcus species have emerged as a leading cause of nosocomial infections worldwide. The aim of this study was to determine the virulence biomarkers and antibiotic resistance profiles of Enterococcus spp. collected from a main tertiary teaching hospital in Bandar Abbas, Iran.

Materials & Methods: A total of 71 Enterococcus were isolated from clinical specimens of patients in different wards of a hospital. Enterococcus spp. were verified by detecting dld gene using PCR-based method. Virulence-encoding genes including gelE and cylA were detected using PCR. Antibiotic resistance was assessed using the disk diffusion assay, and vancomycin resistance was identified using the E-test method.

Findings: Among Enterococcus isolates, 50 and 21 isolates were identified as E. faecalis and E. faecium, respectively. Most of the Enterococcus species were isolated from urine, followed by wound samples. The most prevalent virulence genes among E. faecalis isolates were cylA (60%) and gelE (30%); and 19 and 14% of E. faecium isolates were positive for cylA and gelE genes, respectively. Many isolates of E. faecalis (84%) and E. faecium (76%) were resistant to one or more antibiotics and showed high resistance to gentamicin and ciprofloxacin.

Conclusion: This study revealed a high prevalence of ciprofloxacin and gentamicin resistance and a high frequency of virulence genes among E. faecalis isolates. Due to the high prevalence of MDR Enterococcus strains, control measures are necessary to prevent the emergence and transmission of these strains in different hospital wards.

Keywords: Enterococcus faecalis, Enterococcus faecium, Antimicrobial Resistance, Virulence factors

How to cite this article
Dehghani T, Karmostaji A., Alizade H. Virulence Genes and Antibiotic Susceptibility of Enterococcus spp. in Bandar Abbas City, Iran. Infection Epidemiology and Microbiology. 2022;8(2):129-137

Article History
Received: January 31, 2022
Accepted: June 02, 2021
Published: June 22, 2022

Copyright@ 2022, TMU Press. This open-access article is published under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License which permits Share (copy and redistribute the material in any medium or format) and Adapt (remix, transform, and build upon the material) under the Attribution-NonCommercial terms.
Introduction

*Enterococcus* species are considered as a major part of the gastrointestinal tract normal flora, the third leading cause of bacterial infections, the fourth leading cause of nosocomial infections, and the second leading cause of urinary tract infections worldwide \[^1\]. *Enterococcus* spp. are important causes of nosocomial infections, especially in patients with prolonged hospital stays, immunocompromised patients, or those previously treated with broad-spectrum antibiotics. These isolates are the causative agents of multiple infections such as bacteremia, surgical site infections, urinary tract infections, and endocarditis \[^2\]. *Enterococcus faecium* (*E. faecium*) and *Enterococcus faecalis* (*E. faecalis*) species are the most common causes of healthcare-associated and nosocomial infections. *E. faecalis* is responsible for 80% of all *Enterococcus* infections. However, a recent study reported that the prevalence of *E. faecium* increased during 2012 to 2019, while the prevalence of *E. faecalis* remained stable for 10 years \[^3, 4\].

The traditional method used to detect *Enterococcus* spp. is bacterial growth on a culture medium, while this method takes more than 24-48 hrs. Moreover, after antimicrobial therapy, the number of bacteria is significantly reduced. Polymerase chain reaction (PCR)-based techniques are applied to detect microorganisms because these methods are sensitive and specific \[^5\].

The pathogenesis of *E. faecalis* and *E. faecium* species isolated from hospitalized patients is attributed to an array of genes encoding virulence biomarkers, including hyaluronidase (*hyl*), gelatinase (*gelE*), aggregation substance (*asa1*), enterococcal surface protein (*esp*), cytolysin (*cylA*), and collagen-binding-protein (*ace*) \[^6\]. Gelatinase hydrolyzes gelatin and collagen, causing damages to host tissues and facilitating bacterial spread, colonization, and biofilm formation. Cytolysin production by hemolytic strains significantly contributes to the exacerbation of enterococcal infections. Cytolysin-encoding genes (*cyl*) are integrated into a chromosome or carried on a plasmid \[^7, 8\].

*Enterococcus* spp. are increasingly resistant to two or three groups of antimicrobial agents, known as multiple-drug resistant (MDR) strains. These strains show resistance to aminoglycosides, fluoroquinolones, penicillin, and glycopeptides \[^9, 10\]. Also, the emergence of vancomycin-resistant enterococci (VRE) with high levels of resistance to aminoglycoside and vancomycin poses great challenges for controlling enterococci infections \[^11\]. Teicoplanin and vancomycin-resistant strains are of great concern due to the extensive therapeutic use of these antibiotics against MDR enterococci infections. *Enterococcus* spp. are intrinsically resistant to many antibiotics. Intrinsic resistance of *E. faecium* to many antimicrobial agents, especially glycopeptides, as well as *E. faecalis* to quinupristin/dalfopristin and clindamycin has been reported \[^12\].

Objectives: This study was designed to determine the prevalence, virulence genes, and antibiotic resistance profiles of *E. faecalis* and *E. faecium* isolates collected from a main tertiary teaching hospital in Bandar Abbas, southern Iran.

Methods and materials

Clinical samples: In this study, 71 *Enterococcus* isolates were collected from different wards of a main tertiary teaching hospital in Bandar Abbas located in the south of Iran (Payambar-e-Azam therapeutic center) during 2017-2018, including outpatient department (OPD), internal, neurology, cardiac care unit (CCU), intensive care unit (ICU), ear-nose and throat (ENT), gastroenterology, burn, urology, and surgery rooms. *Enterococcus* isolates were
retrieved from various clinical samples, including urine (n=51), wound (n=9), blood (n=3), abdominal drainage aspirate (n=3), bronchoalveolar lavage (n=2), abscess (n=2), and central venous catheter (n=1). Clinical samples were collected from 41 females and 30 males. They belonged to different age groups, including ≤15 years (n=4), 15 to 30 years (n=14), 30 to 45 years (n=24), and 45 to 85 years (n=29). All specimens were cultured on blood agar (Merck, Germany). Then in order to confirm Enterococcus isolates, standard biochemical and bacteriological tests were used according to the standard protocols [13].

**DNA extraction:** Enterococcus isolates genomic DNA was extracted by CinnapureTM DNA extraction kit (Cinnagen, Iran).

**Enterococcus spp. isolation:** To verify E. faecium and E. faecalis isolates, the ddl gene was detected by PCR-based method as described previously [11] (Table 1). Confirmed E. faecium and E. faecalis isolates were stored at -70 °C for further study.

**Virulence genes:** Multiplex PCR was performed to determine the presence of enterococcal virulence genes (cylA, and gelE) as described previously [14] (Table 1).

**Antimicrobial susceptibility testing:** Antibiotic susceptibility testing of Enterococcus isolates was performed by disk diffusion method following the Clinical Laboratory Standards Institute guidelines using commercial antimicrobial disks (Mast. Co., UK), including ciprofloxacin (5 μg), ampicillin (10 μg), vancomycin (30 μg), gentamicin (10 μg), teicoplanin (30 μg), linezolid (30 μg), and tigecycline (15 μg). The minimum inhibitory concentration (MICs) of vancomycin was determined using the E-test method based on the CLSI guidelines (2016). MIC breakpoints to determine vancomycin susceptibility were as follows: MIC values ≤4 were considered as sensitive, between 4-32 as intermediate resistant, and ≥32 as resistant [15].

**Findings**

**Bacteria:** A total of 71 Enterococcus isolates, comprising 50 (70%) E. faecalis and 21 (30%) E. faecium, were isolated. Most Enterococcus species were isolated from patients in the age range of 30-45 and 45-85 years. E. faecalis strains were mostly isolated from urine (n=35; 70%), followed by wound (n=6; 12%), blood (n=3; 6%), abdominal drainage aspirate, bronchoalveolar lavage, and abscess (n=2; 4% for each of them) samples; also, 21 E. faecium strains were isolated from urine (n=16; 76%), wound (n=3; 14%), abdominal drainage aspirate, and central venous catheter (n=1; 4% for each of them) samples (Table 2). The clinical departments from which Enterococcus spp. were isolated (Table 2) included: OPD (n=26), internal (n=18), surgery (n=9), ICU (n=6), burn (n=4), urology (n=3), CCU (n=2), neurology (n=1), ENT (n=1), and gastroenterology (n=1).

**Table 1** Primers used for identification of Enterococcus species and virulence genes in this study

| Target | Sequence (5’ to 3’) | Ref |
|--------|----------------------|-----|
| *ddl* (E. faecalis) | ATCAAGTACAGT TAGTCTTTATTAG ACGATTCAAAAGCTAACCAGT | 8 |
| *ddl* (E. faecium) | TTGAGGGCAGACCAGATGACGATGACGATGACGATGACG | 8 |
| *gelE* | CGAAGTTGAAAAGGAGGC GGTGAAAGATTTCTC | 11 |
| *cylA* | ACTCAGGGGATGATGACG GCTGCTAAAGCTGCGCT | 11 |
Table 2) Characteristics of *E. faecium* and *E. faecalis* isolates

| Ward*         | Species | Sample** | Resistance Pattern*** | MIC (µg/mL) | Virulence Genes |
|---------------|---------|----------|-----------------------|-------------|-----------------|
| OPD           | *faecium* | Urine    | VA, CIP, GM, TEC      | -           | -               |
| *faecium*     | Urine    | VA, CIP, GM, TEC | 0.75           | -           |
| *faecium*     | Urine    | VA, CIP, AP, TEC | 1.5            | -           |
| *faecium*     | Urine    | VA, CIP, GM, TEC | 0.5            | -           |
| *faecium*     | Urine    | CIP, AP, GM | -               | -           |
| *faecalis*    | Urine    | CIP, GM   | -               | gelE        |
| *faecalis*    | Urine    | CIP, AP   | -               | cylA        |
| *faecalis*    | BAL      | CIP, GM   | -               | cylA        |
| *faecalis*    | Urine    | CIP, GM   | -               | cylA        |
| *faecalis*    | Urine    | CIP, GM   | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | -           |
| *faecalis*    | Urine    | GM        | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | gelE        |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | -           |
| *faecalis*    | Urine    | GM        | -               | gelE        |
| *faecalis*    | Urine    | GM        | -               | -           |
| *faecalis*    | Urine    | CIP       | -               | cylA, gelE  |
| *faecalis*    | Urine    | GM        | -               | gelE        |
| *faecalis*    | Wound    | CIP       | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | gelE        |
| *faecalis*    | Wound    | GM        | -               | cylA        |
| *faecalis*    | Urine    | GM        | -               | cylA        |
| *faecalis*    | Urine    | -         | -               | gelE        |
| *faecalis*    | Urine    | -         | -               | -           |

*Virulence and Antibiotic Susceptibility of Enterococcus*
PCR detection of virulence genes among *Enterococcus* isolates: Among *E. faecalis* isolates, *cylA* was the most prevalent gene (n=30; 60%), followed by *gelE* (n=15; 30%). Seven *E. faecalis* isolates were positive for both *gelE* and *cylA* genes. Among *E. faecium* isolates, four (19%) isolates possessed *cylA* gene, whereas three (14%) isolates showed the genetic marker *gelE*. Only one *E. faecium* isolate possessed both *gelE* and *cylA* genes (Table 2).

**Antibiotic resistance profiles:** In the disk diffusion assay, 42 (84%) *E. faecalis* isolates were resistant to one or more antibiotics, and the highest resistance was shown against gentamicin (n=39; 78%), ciprofloxacin (n=23; 46%), vancomycin and ampicillin (n=6; 12% for each of them), teicoplanin (n=5; 10%), linezolid (n=2; 4%), and tigecycline (n=1; 2%). Out of 21 *E. faecium* strains isolated, 16 isolates (76%) showed resistance to at least one antibiotic. The highest resistance was observed against gentamicin (n=14; 67%), followed by vancomycin (n=9; 43%).

**Table 2** Characteristics of *E. faecium* and *E. faecalis* isolates

| Ward* | Species | Sample** | Resistance Pattern*** | MIC (µg/mL) | Virulence Genes |
|-------|---------|----------|-----------------------|-------------|-----------------|
| Surgery | faecalis | Urine | VA, CIP, AP, GM, TEC | 0.75 | *cylA* |
| faecalis | Wound | VA, CIP, GM, TEC | 0.5 | - |
| faecium | Wound | VA, GM, TEC | 0.75 | - |
| faecalis | Wound | VA, CIP, GM | 1.5 | *cylA* |
| faecalis | Abdominal | CIP, AP | - | *cylA* |
| faecalis | Abdominal | GM | - | *gelE* |
| faecalis | Abscess | GM | - | *gelE* |
| faecalis | Urine | GM | - | - |
| faecium | Urine | GM | - | - |
| ICU | faecalis | Urine | GM, LZD | - | - |
| faecium | Catheter | VA, TEC | - | - |
| faecalis | Blood | GM, TGC | - | *cylA* |
| faecalis | Urine | CIP, GM | - | - |
| faecalis | Urine | CIP, GM | - | - |
| faecalis | Urine | CIP, GM | - | *cylA* |
| Burn | faecalis | BAL | AP, GM | - | *cylA* |
| faecalis | Wound | GM | - | *cylA* |
| faecium | Wound | GM | - | *cylA* |
| faecalis | Wound | - | - | *cylA* |
| Urology | faecalis | Urine | CIP, GM | - | *cylA* |
| faecalis | Wound | GM | - | *cylA* |
| faecalis | Urine | GM | - | *cylA* |
| faecalis | Urine | GM | - | *cylA*, *gelE* |
| CCU | faecalis | Urine | CIP, GM | - | - |
| faecium | Urine | - | - | - |
| Digestive | faecalis | Blood | CIP, GM | - | *gelE* |
| Neurology | faecalis | Urine | GM | - | *cylA* |
| ENT | faecalis | Urine | GM | - | *cylA*, *gelE* |

*OPD: outpatient department
**BAL: broncoalveolar lavage, abdominal: abdominal drainage aspirate, catheter: central venous catheter
***CIP: ciprofloxacin, AP: ampicillin, VA: vancomycin, GM: gentamicin, TEC: teicoplanin, LZD: linezolid, TGC: tigecycline
ciprofloxacin (n=8; 38%), teicoplanin (n=7; 33%), ampicillin (n=6; 28%), and tigecycline (n=1; 5%). In addition, eight E. faecalis and five E. faecium isolates were sensitive to all of the antibiotics surveyed in this study. The antimicrobial resistance profile of Enterococcus isolates is presented in Table 2. Designation of MIC levels showed that 11 Enterococcus isolates were susceptible to vancomycin with MIC values in the range of 0.5 to 2 µg/mL (Table 2).

Discussion
In the current study, virulence genes and antibiotic susceptibility profiles of Enterococcus spp. isolated from clinical samples were investigated. The isolation rate of E. faecalis (70%) was higher than that of E. faecium (30%). This finding contradicts the findings of other studies in which the prevalence of Enterococcus species isolated from clinical specimens has been reported in favor of E. faecium [16-18]. Haghi et al. (2019) in northwestern Iran reported that E. faecalis isolates were the predominant enterococci isolated from urine samples, which is similar to the results of the current study [14]. Another study in Iran indicated that E. faecalis isolates were more prevalent among enterococci derived from various clinical samples [3]. These results show that the prevalence of Enterococcus species varies in different clinical samples and geographical regions.

MDR enterococci as the main pathogens have become a serious problem in nosocomial infections [14]. In this study, 82% of Enterococcus isolates were resistant to one or more antibiotics. The prevalence of antibiotic resistance among E. faecalis isolates was more than in E. faecium isolates; also, the results showed a high prevalence of MDR Enterococcus isolates in urine specimens. Most Enterococcus isolates were sensitive to linezolid and tigecyclin (97% for each). Previous studies in Iran have shown a high frequency of antibiotic resistance among Enterococcus spp., except linezolid, tigecycline, and fosfomycin [11, 14]. A study in China reported a high prevalence of resistance to rifampicin, penicillin, ampicillin, fosfomycin, ciprofloxacin, chloramphenicol, levofloxacin, erythromycin, quinupristin/dalfopristin, minocycline, and tetracycline among Enterococcus spp., while the prevalence of resistance to vancomycin, teicoplanin, and linezolid was low in E. faecium and E. faecalis isolates [19]. Screening of antimicrobial resistance indicated that 75% of the isolates were resistant to gentamycin, which is similar to the results of recent studies indicating that the prevalence of gentamycin resistance ranges from 50 to 65% [20, 21]. In the current study, resistance to vancomycin in E. faecium and E. faecalis isolates was 43 and 12%, respectively. Another study in Iran showed that the prevalence of vancomycin resistance in E. faecium isolates was more than in E. faecalis isolates [3]. In a study in Turkey, Çopur et al. (2016) reported a high frequency of vancomycin resistance among E. faecium isolates (95.6%) compared to E. faecalis isolates (4.3%), and most VRE strains were isolated from specimens of surgery clinics and intensive care units [22]. A higher prevalence rate of VR among E. faecium isolates was also reported in a study in Saudi Arabia (62.3%) [23]. In this study, 8% of VRE isolates were isolated from clinical samples of the internal ward, and 6% were isolated from samples of OPD and surgery rooms. A previous study reported that the high prevalence of antibiotic resistance detected in ICU and burn hospital wards may be attributed to immunodeficiency, long-term antibiotic use, and patients’ critical illness [19].

In this study, the gelE gene was detected in 30% of E. faecalis and 14% of E. faecium
isolates, this finding is consistent with the finding of a previous Indian study documenting a high frequency of gelE among E. faecalis compared to E. faecium [7]. In another study in Iran, most Enterococcus spp. (79.7%) isolated from clinical samples carried the gelE gene; also, 82% out of 128 E. faecalis isolates and 60% out of 15 E. faecium isolates harbored gelE [24]. Banerjee and Anupurba (2015) reported that among enterococci strains isolated from clinical samples, the gelE gene was detected in 9.6 and 8.3% of E. faecalis and E. faecium isolates, respectively; also, most virulence factors were associated with biofilm formation [25]. For invasive Enterococcus isolates (infective endocarditis), virulence biomarkers may be more relevant to other traits than adherence, such as collagen and gelatin degradation (by gelE gene) which may be relevant to dissemination and invasion [26].

Cytolysin, encoded by cylA, is a bacteriocin-type exotoxin with hemolytic activity towards eukaryotic cells. This exotoxin exhibits toxic properties against erythrocytes, leukocytes, and macrophages and bactericidal properties towards Gram-negative bacteria. Cytolysin-encoding sequences (cyl) have been detected in Enterococcus strains isolated from both invasive and non-invasive infections [27]. Arshadi et al. (2018) in Iran reported that 7.1, 6.2, and 0% of E. faecium intestinal isolates, clinical isolates, and environmental isolates possessed hemolysin gene, respectively [28]. In the present study, the frequency of cylA gene among Enterococcus strains isolated from clinical specimens was 48% (60 and 19% among E. faecalis and E. faecium strains, respectively). A study in Brazil reported the presence of cyl genes in 54.4% of clinical enterococcal strains [29].

Conclusion
This study indicates that E. faecalis isolates carry more virulence genes than E. faecium. Thus, we are faced with MDR E. faecalis strains with virulence and antimicrobial resistance genes which enable them to adapt and survive in hospital settings and cause severe infections. Infections caused by VRE and MDR isolates could be associated with high mortality in patients. Given that most of the isolates were sensitive to linezolid and tigecycline, it is suggested that therapeutic strategies be reviewed according to new antimicrobial resistance patterns.

Acknowledgments
The authors acknowledge Hormozgan University of Medical Sciences for its financial support of this research.

Ethical permission: This study was ethically endorsed by the Ethics Committee of Hormozgan University of Medical Sciences with number IR.HUMS.REC.1395.103.

Conflict of Interest: There is no conflict of interest between the authors.

Authors’ Contribution: Conceptualization: KA, AH, DT; data curation: KA, DT; formal analysis: KA, DT; funding acquisition: DT; investigation: KA, AH, DT; methodology: KA, AH, DT; project administration: KA; resources: DT; software: DT; supervision: KA, AH; writing of the original draft: KA, AH; writing-review and editing: KA, AH, DT.

Fundings: The present study was financially supported by the Infectious and Tropical Diseases Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Consent to participate: Written informed consents were obtained from participants.

References
1. Masoumi Zavaryani S, Mirnejad R, Piranfar V, Moosazadeh Moghadam M, Sajjadi N, Saeedi S. Assessment of susceptibility to five common antibiotics and their resistance pattern in clinical Enterococcus isolates. Iran J Pathol. 2020;15(2):96-105.
2. Benamrouche N, Guettou, B, Henniche FZ, Assaous F, Laouar H, Ziane H, et al. Vancomycin-resistant Enterococcus faecium in Algeria: Phenotypic and
genotypic characterization of clinical isolates. J Infect Dev Ctries. 2021;15(1):95-101.
3. Arbabi L, Boustanshenas M, Rahbar M, Owlia P, Adabi M, Rasouli Koohi S, et al. Antibiotic susceptibility pattern and virulence genes in Enterococcus spp. isolated from clinical samples of Milad hospital of Tehran, Iran. Arch Clin Infect Dis. 2016;11(3):e36260.
4. Sumpradit N, Wongkongkathep S, Malatham K, Janejai N, Paveenkitporn W, Yingyong T, et al. Thailand's national strategic plan on antimicrobial resistance: progress and challenges. Bull World Health Organ. 2021;1;99(9):661-73.
5. Chen X, Ma K, Yi X, Xiao Z, Xiong L, Wang Y, et al. A novel detection of Enterococcus faecalis using multiple cross displacement amplification linked with gold nanoparticle lateral flow biosensor. Infect Drug Resist. 2019;12:3771-81.
6. Ferguson DM, Talavera GN, Hernández LAR, Weisberg SB, Ambrose RF, Jay JA. Virulence genes among Enterococcus faecalis and Enterococcus faecium isolated from coastal beaches and human and nonhuman sources in southern California and Puerto Rico. J Pathog. 2016;2016.
7. Kiruthiga A, Padmavathy K, Shabana P, Naveen Kumar V, Gnanadesikan S, Malaiyan J. Improved detection of esp, hyl, asa1, gelE, and cylA virulence genes among clinical isolates of enterococci. BMC Res Notes. 2020;13(1):1-7.
8. Vankerkhovens V, Augeardien TV, Vaes 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):4473-9.
9. Hemalatha G, Bhaskaran K, Sowmya M, Anusheela A, Sethumadhavan K. A study on virulence factors and antimicrobial resistance pattern among enterococci isolated from various clinical specimens from a tertiary care hospital. Int J Res Med Sci. 2017;5(7):2969-74.
10. Abedini P, Soleimani N. A review of designing new vaccines to prevent hospital-acquired antibiotic-resistant infections. Int Electron J Med. 2018;7(2):21-9.
11. Kafil HS, Asgharzadeh M. Vancomycin-resistant Enterococcus faecium and Enterococcus faecalis isolated from education hospital of Iran. Maedica. 2014;9(4):323-7.
12. Golob M, Pate M, Kušar D, Dermota U, Avberšek J, Papić B, et al. Antimicrobial resistance and virulence genes in Enterococcus faecium and Enterococcus faecalis from humans and retail red meat. Biomed Res Int. 2019;2019.
13. Church DL. Aerobic bacteria. In: Leber AL, editor. Clinical microbiology procedures handbook. 4th ed. USA, Washington DC: ASM Press; 2016.
14. Haghi F, Lohrasbi V, Zeighami H. High incidence of virulence determinants, aminoglycoside and vancomycin resistance in enterococci isolated from hospitalized patients in Northwest Iran. BMC Infect Dis. 2019;19(1):1-10.
15. Clinical and Laboratory Standards Institute. M100S: Performance standards for antimicrobial susceptibility testing. 26th Edition. Wayne: Clinical and Laboratory Standards Institute; 2016.
16. Sattari-Maraji A, Jabalameli F, Node Farahani N, Beigverdi R, Emaneini M. Antimicrobial resistance pattern, virulence determinants and molecular analysis of Enterococcus faecium isolated from children infections in Iran. BMC Microbiol. 2019;19(1):1-8.
17. Gawryszewska I, Zabicka D, Bojarska K, Malinowska K, Hryniewicz W, Sadowy E. Invasive enterococcal infections in Poland: The current epidemiological situation. Eur J Clin Microbiol Infect Dis. 2016;35(5):847-56.
18. Niu H, Yu H, Hu T, Tian G, Zhang L, Guo X, et al. The prevalence of aminoglycoside-modifying enzyme and virulence genes among enterococci with high-level aminoglycoside resistance in Inner Mongolia, China. Braz J Microbiol. 2016;47(3):691-6.
19. Jia W, Li G, Wang W. Prevalence and antimicrobial resistance of Enterococcus species: A hospital-based study in China. Int J Environ Res Public Health. 2014;11(3):3424-42.
20. Somily AM, Al-Mohizea MM, Absar MM, Fatani AJ, Ridha AM, Al-Ahdal MN, et al. Molecular epidemiology of vancomycin resistant enterococci in a tertiary care hospital in Saudi Arabia. Microb Pathog. 2016;97:79-83.
21. 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;90:937-.
25. Banerjee T, Anupurba S. Prevalence of virulence factors and drug resistance in clinical isolates of enterococci: A study from North India. J Pathog. 2015;2015.

26. Soares RO, Fedi AC, Reiter KC, Caierao J, d’Azevedo PA. Correlation between biofilm formation and gelE, esp, and agg genes in Enterococcus spp. clinical isolates. Virulence. 2014;5(5):634-7.

27. Chajecka-Wierczowska W, Zadernowska A, Laniewska-Trokenheim L. Virulence factors of Enterococcus spp. presented in food. LWT. 2017;75:670-6.

28. Arshadi M, Mahmoudi M, Motahar MS, 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.

29. Medeiros AW, Pereira RI, Oliveira DV, Martins PD, d’Azevedo PA, Van der Sand S, et al. Molecular detection of virulence factors among food and clinical Enterococcus faecalis strains in South Brazil. Braz J Microbiol. 2014;45(1):327-32.