Antimicrobial resistance pattern, virulence determinants and molecular analysis of Enterococcus faecium isolated from children infections in Iran

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Abstract

Background: Enterococcus species continues to be an important cause of hospital-acquired infection worldwide. This study was designed to determine the antibiotic resistance profiles, virulence genes and molecular characteristics of Enterococcus faecium strains isolated from an Iranian children hospital in a four-years period.

Results: A total 189 Enterococcus strains, comprising 108 (57%) E. faecium, 67 (35%) E. faecalis and 14 (7%) isolates of other spp. were isolated during the collection period. More than 92% of E. faecium isolates were resistant to ampicillin (92.5%), ciprofloxacin (96%), erythromycin (100%) and clindamycin (96%). A high frequency of resistance to clindamycin (100%), erythromycin (98.5%) and ciprofloxacin (80.5%) was observed among E. faecalis isolates, while resistance to ampicillin (7%) was less frequent. The prevalence of vanA gene among vancomycin resistant E. faecium and vancomycin resistant E. faecalis was 95 and 50%, respectively. The analysis of 108 E. faecium isolates revealed 34 variable number tandem repeat (VNTR) patterns and 27 Multi Locus VNTR Analysis (MLVA) types (MTs).

Conclusions: The results show a shift from E. faecalis to E. faecium as the dominant enterococcal species among patients at the children Hospital. Our data revealed that the majority of E. faecium isolates (66%) belonged to three common MTs and these types were isolated from different wards in children hospital.

Keywords: Enterococcus, Virulence factors, Antimicrobial resistance, MLVA

Background

Enterococcus continues to be an important cause of hospital-acquired infection worldwide [1]. Two species (Enterococcus faecalis and Enterococcus faecium) are responsible for the majority of enterococcal infections in humans and these species have become resistant to multiple antimicrobial agents such as vancomycin (vancomycin resistant enterococci; VRE), aminoglycosides (the high-level gentamicin resistant; HLGR), macrolides, and tetracyclines [2, 3]. The glycopeptide resistance in enterococci is mediated by nine (vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM, vanN) mobile gene clusters [4]. Among them, vanA genotype is the most common type of enterococcal vancomycin resistance in several countries [5]. The presence of aac (6’)-le-aph(2’)-la gene, which is carried on transposon is the main cause of HLGR emergence [6, 7]. In addition to the increasing antibiotic resistance, some virulence determinants described to be associated with pathogenesis in E. faecium including, collagen-binding adhesin of E. faecium (Acm), aggregation substance (Asa1), cytolysin (CylA), enterococcal surface protein (Esp), gelatinase (GelE) [4, 8]. Recently, several reports have described Multilocus variable-number of tandem repeat analysis based on PCR-amplification of variable number tandem repeat (VNTR) located on chromosome, is a suitable tool for learning the genetic relationships of important bacterial pathogens, including E. faecium [3, 9]. Despite the high incidence rate of resistant enterococci in Iran, especially VRE and HLGR [10, 11], there is limited information on enterococcal strains isolated from children infections. This study was
designed to determine the antibiotic resistance profiles, virulence genes and the prevalence of different VNTR patterns among E. faecium strains isolated from an Iranian children hospital in a four-years period.

**Results**

A total 189 Enterococcus strains, comprising 108 (57%) E. faecium, 67 (35%) E. faecalis and 14 (7%) isolates of other spp. were isolated during the collection period. Distribution of E. faecium and E. faecalis isolates based on isolation time (Fig. 1) was showed that during 2015, the prevalence of E. faecium were significantly higher than E. faecalis ($P=0.0001$). Most of the E. faecium strains (74%) were isolated from urine, followed by blood (11%), body fluids (7%) and wound (2%). The majority proportion of E. faecium isolates were obtained from urology hospitalized patients (14%) and outpatients (13%).

**Antimicrobial susceptibility**

More than 92% of E. faecium isolates were resistant to ampicillin (92.5%), ciprofloxacin (96%), erythromycin (100%) and clindamycin (96%). A high frequency of resistance to clindamycin (100%), erythromycin (98.5%) and ciprofloxacin (80.5%) was observed among E. faecalis isolates, while resistance to ampicillin (7%) was less frequent. HLGR was found in 75 and 49% of E. faecium and E. faecalis strains, respectively. Inducible resistance to clindamycin was 7% among E. faecium isolates, but not in E. faecalis strains. Vancomycin resistance were detected in 70% of E. faecium and 9% of E. faecalis isolates. The MIC values of Vancomycin Resistant E. faecium (VREfm) and Vancomycin Resistant E. faecalis (VREfs) were $\geq 128$ μg/ml and $\geq 128$ μg/ml respectively. The prevalence of vanA gene among VREfm and VREfs isolates was 95 and 50%, respectively. The presence of $aac(6')-le-aph(2')-Ia$ gene among HLGR isolates of E. faecium and E. faecalis was 48 and 67%, respectively.

**Prevalence of virulence genes among E. faecium isolates**

The acm was the most commonly detected gene (81%), followed by esp (17.5%), gelE (16%), and ace (6%). Only two (2%) isolates carried asa1 gene and cylA was not seen in any of the isolates. The presence of the esp gene was significantly higher ($P=0.011$) among VREfm isolates than vancomycin sensitive E. faecium isolates.

**Molecular analysis of E. faecium**

The results of MLVA typing of E. faecium isolates are presented in Table 1. The analysis of 108 E. faecium strains revealed 34 VNTR patterns and 27 MTs. Forty-three isolates (40%) were identified as MT1, 15 (13.8%) as MT2 and 14 (12.9%) as MT3. MT1 was isolated from different wards of the hospital, while MT2 and MT3 were not found in outpatients who were referred to this center. By comparing antibiotic resistance genes in three common types (MT1-MT3), $aac(6')-le-aph(2')-Ia$ was significantly higher in MT3 than MT1 ($P=0.0046$). Also, virulence gene esp had more frequency in MT3 than MT1 ($P=0.0003$). The most prevalent pattern of antibiotic resistance in common types (MT1-MT3) was related to pattern gentamicin, ampicillin, ciprofloxacin, erythromycin, and clindamycin. Moreover, the results of the antibiotic resistance genes pattern in common types indicated that pattern vanA+ $aac(6')-le-aph(2')-Ia$ in MT3 was significantly more frequent than MT1 ($P=0.013$).

**Discussion**

In the current study, the majority (57%) of the isolates was E. faecium. This observation is similar to reports.
Table 1 Characteristics of *E. faecium* isolates

| Warda | Isolate | Time of isolation | Sample | Resistance pattern | Resistance genes | Virulence genes | MLVA type (MT) |
|-------|---------|-------------------|--------|-------------------|-----------------|----------------|----------------|
| Out patient | 1 | 1/2012 | Urine | GM, AP, CIP, E, CD | vanA | acm, esp | 1 |
| | 2 | 1/2012 | Urine | AP, E, CD | – | acm | 10 |
| | 3 | 6/2012 | Urine | GM, AP, CIP, E, CD | aac(6′)-le-aph(2″)-la | acm, ace | 1 |
| | 4 | 6/2012 | Urine | GM, AP, CIP, E, CD | aac(6′)-le-aph(2″)-la | acm | 1 |
| | 5 | 6/2012 | Urine | AP, CIP, E, CD | – | acm | 1 |
| | 6 | 6/2012 | Urine | GM, AP, CIP, E, CD | – | acm | 13 |
| | 7 | 9/2012 | Urine | GM, AP, CIP, E, CD | vanA | acm | 11 |
| | 8 | 10/2012 | Urine | AP, CIP, E, CD | – | – | 9 |
| | 9 | 6/2013 | Urine | GM, AP, CIP, E, CD | – | – | 17 |
| | 10 | 7/2013 | Urine | AP, CIP, E, CD | vanA | acm | 1 |
| | 11 | 9/2013 | Urine | AP, CIP, E, CD | vanA | acm | 1 |
| | 12 | 9/2013 | Blood | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm | 1 |
| | 13 | 11/2013 | Urine | GM, AP, CIP, E, CD | vanA | acm | 1 |
| | 14 | 4/2014 | Urine | GM, AP, CIP, E, CD | vanA | acm | 1 |
| Urology | 15 | 5/2012 | Urine | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm | 20 |
| | 16 | 5/2012 | Urine | GM, AP, CIP, E, CD | vanA | acm, ace | 25 |
| | 17 | 5/2012 | Urine | AP, CIP, E, CD | – | acm, ace | 6 |
| | 18 | 6/2012 | Urine | GM, AP, CIP, E, CD | aac(6′)-le-aph(2″)-la | acm | 7 |
| | 19 | 6/2012 | Urine | AP, CIP, E, CD | – | – | 11 |
| | 20 | 6/2012 | Urine | GM, AP, CIP, E, CD | – | acm | 8 |
| | 21 | 7/2012 | Urine | AP, CIP, E, CD | – | – | 23 |
| | 22 | 8/2012 | Urine | AP, CIP, E, CD | – | – | 4 |
| | 23 | 9/2012 | Urine | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm, esp | 3 |
| | 24 | 10/2012 | Urine | GM, AP, CIP, E, CD | – | acm, esp | 2 |
| | 25 | 6/2013 | Urine | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm, gelE | 1 |
| | 26 | 11/2013 | Urine | GM, AP, CIP, E, CD | vanA | acm, esp | 3 |
| | 27 | 2/2015 | Blood | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm | 2 |
| | 28 | 5/2015 | Urine | GM, AP, CIP, E, CD | vanA | acm | 3 |
| | 29 | 5/2015 | Urine | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm, esp | 1 |
| Surgery | 30 | 5/2012 | CSF | AP, CIP, E, CD | vanA | acm | 1 |
| | 31 | 5/2012 | CSF | AP, CIP, E, CD | vanA | acm, esp | 1 |
| | 32 | 8/2012 | Urine | AP, CIP, E, CD | vanA | acm, esp | 1 |
| | 33 | 5/2013 | Urine | CIP, E | – | acm | 27 |
| | 34 | 6/2013 | Urine | GM, AP, CIP, E, CD | vanA | acm | 1 |
| | 35 | 11/2013 | Urine | GM, CIP, E, CD | aac(6′)-le-aph(2″)-la | asa1, gelE | ace | 5 |
| | 36 | 4/2014 | Blood | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm, esp, gelE | 3 |
| | 37 | 1/2015 | Urine | GM, AP, CIP, E, CD | vanA | acm, esp | 2 |
| | 38 | 4/2015 | Urine | AP, CIP, E, CD | vanA | acm, gelE | 1 |
| | 39 | 12/2015 | Urine | GM, AP, CIP, E, CD | vanA | acm | 1 |
| NICU | 40 | 10/2012 | Urine | GM, AP, CIP, E, CD | – | – | 10 |
| | 41 | 8/2013 | Tracheal aspirate | GM, AP, CIP, E, CD | vanA | acm | 1 |
| | 42 | 1/2015 | Urine | GM, AP, CIP, E, CD | vanA | acm | 4 |
| | 43 | 1/2015 | Urine | GM, AP, CIP, E, CD | vanA, aac(6′)-le-aph(2″)-la | acm | 1 |
| Ward* | Isolate | Time of isolation (m/y)<sup>b</sup> | Sample | Resistance pattern<sup>‡</sup> | Resistance genes | Virulence genes | MLVA type (MT) |
|-------|---------|-------------------------------|--------|-------------------------------|-----------------|----------------|----------------|
|       |         |                               |        |                               |                 |                |                |
| 44    | 1/2015  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm, esp |                | 2              |
| 45    | 2/2015  | Urine                         | GM, AP, CIP, E, CD | vanA aac(6’)-le-aph(2”)-la | acm, esp |                | 2              |
| 46    | 3/2015  | Blood                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm, esp |                | 1              |
| 47    | 8/2015  | Blood                         | GM, AP, CIP, E, CD |aac(6’)-le-aph(2”)-la | acm ace |                | 1              |
| 48    | 10/2015 | Ascites                       | GM, AP, CIP, E, CD |vanA | – | 2 |
| 49    | 10/2015 | Ascites                       | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 2 |
| CICU  | 50      | 1/2012                         | Ascites | GM, AP, CIP, E, CD | vanA |                | 1              |
|       |         |                               |        |                               |                 |                |                |
| 51    | 9/2013  | Urine                         | GM, AP, CIP, E, CD | vanA | – | 1 |
| 52    | 6/2013  | Urine                         | CIP, E, CD | vanA | acm,esp, gelE | 2 |
| 53    | 1/2015  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 2 |
| 54    | 3/2015  | Ascites                       | GM, AP, CIP, E, CD | – | – | 1 |
| 55    | 5/2015  | Urine                         | GM, AP, CIP, E, CD | vanA | acm | 14 |
| 56    | 6/2015  | Blood                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm ace | 3 |
| 57    | 6/2015  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm ace | 1 |
| PICU  | 58      | 12/2012                        | Wound  | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm, esp, gelE | 3 |
| 59    | 4/2013  | Tracheal aspirate             | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 1 |
| 60    | 1/2014  | Wound                         | GM, AP, CIP, E, CD | vanA | acm | 22 |
| 61    | 3/2014  | Blood                         | GM, AP, CIP, E, CD | vanA | acm, esp | 2 |
| 62    | 11/2014 | Ascites                       | GM, AP, CIP, E, CD | vanA | acm | 2 |
| 63    | 2/2015  | Urine                         | GM, AP, CIP, E, CD | vanA | acm | 1 |
| 64    | 3/2015  | Blood                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 2 |
| 65    | 6/2015  | Dialysis fluid               | GM, AP, CIP, E | vanA | acm | 1 |
| Dialysis center | 66 | 5/2012                        | Urine | AP, CIP, E, CD | – | acm | 1 |
|       |         |                               |        |                               |                 |                |                |
| 67    | 5/2012  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 1 |
| 68    | 10/2012 | Urine                         | E, CD | – | asa1 | 5 |
| 69    | 1/2013  | Catheter                      | GM, AP, CIP, E, CD | vanA | acm, esp | 3 |
| 70    | 1/2013  | Urine                         | CIP, E, CD | – | – | 6 |
| 71    | 6/2013  | Urine                         | AP, CIP, E, CD | – | acm | 1 |
| 72    | 3/2014  | Urine                         | GM, AP, CIP, E, CD | vanA | acm, gelE | 4 |
| Neonatal | 73 | 12/2011                       | Urine | GM, AP, CIP, E, CD | vanA | acm | 7 |
|       |         |                               |        |                               |                 |                |                |
| 74    | 5/2012  | Urine                         | GM, AP, CIP, E, CD | vanA | acm | 1 |
| 75    | 6/2012  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 3 |
| 76    | 9/2012  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm | 3 |
| 77    | 9/2012  | Urine                         | GM, AP, CIP, E, CD | vanA | acm | 13 |
| 78    | 12/2013 | Urine                         | GM, AP, CIP, E, CD | vanA | gelE | 18 |
| Emergancy | 79 | 1/2012                        | Urine | E, CD | – | acm | 1 |
|       |         |                               |        |                               |                 |                |                |
| 80    | 1/2012  | CSF                            | GM, AP, CIP, E | aac(6’)-le-aph(2”)-la | acm | 1 |
| 81    | 1/2012  | Urine                         | GM, AP, CIP, E, CD | – | acm | 19 |
| 82    | 2/2013  | Blood                         | GM, AP, CIP, E, CD | vanA | acm, gelE | 1 |
| 83    | 2/2013  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm, gelE | 1 |
| 84    | 2/2013  | Urine                         | GM, AP, CIP, E, CD | vanA, aac(6’)-le-aph(2”)-la | acm, gelE | 2 |
The increase in the prevalence of *E. faecium* ascites and hydrothorax was changed in the favour of *E.* species derived from clinical samples from other countries in which the distribution of *E. faecium* isolates was 70 and 9%, relatively. The occurrence of VRE varies in different countries, with a high frequency of VREfm and VREfs [5, 19, 20]. A possible explanation for this variation is probably related to the presence of other resistance gene such as *vanB* or presence of other resistance mechanism including thicker cell wall production [21–23]. Similar to previous finding in Iran, 75% of *E. faecium* and 49% of *E. faecalis* isolates were HLGR [11, 24]. In the current study, 48 and 61% of HLGR in *E. faecium* and *E. faecalis* strains carried the *aac(6\')-Ie-aph(2\')-la* gene. This finding was similar with previous studies in which have been shown that the *aac(6\')-Ie-aph(2\')-la* gene is the predominant gene responsible for HLGR. [5, 11, 25–27]. In this study, inducible resistance to clindamycin was observed in only 7% of *E. faecium* strains. Since the resistance to erythromycin and clindamycin antibiotics depends on the use of these agents in the clinic, inducible resistance to clindamycin between *E. faecium* strains might be attributed to the wide intake of these antibiotics in our study center [28]. Our result showed that the *acm* gene was most prevalent virulence gene in *E. faecium* strains. Similar findings were observed in other studies [8, 25, 29]. It seems that the *acm*
gene have a role in the improved ability of members of the hospital-associated *E. faecium* to cause disease [30]. Similar to previous report, the prevalence of *ace* and *gelE* genes was 6 and 16% [25]. The *cytA* gene was not detected in any of the 108 *E. faecium* isolates which is in line with the results stated by other investigators who also tested *E. faecium* strains for the presence of *cytA* or more of virulence genes [27, 31]. The rates of *esp* and *asa1* genes were 17.5 and 2%. In some studies, these genes were reported in higher prevalence but in our study and some other studies these genes were identified in lower prevalence among *E. faecium* isolates [32, 33]. Similar to former studies, the presence of the *esp* gene was significant among VRE isolates [34, 35]. Recently, a variant of *esp* gene in VREfm clones has been reported. Also, *esp* gene has been found to be more common in clinical isolates than fecal isolates, which shows the role of *esp* gene in pathogens of enterococci [34, 36]. The MLVA typing of 108 *E. faecium* isolates produced 34 VNTR patterns and 27 MTs. In a study conducted by Top et al. MLVA of 392 *E. faecium* isolates revealed 127 different MTs [9]. In a study piloted by Gawryszewska et al. MLVA of 112 invasive *E. faecium* isolates showed 12 different MTs [3]. Unlike MT1 strains that were isolated from all wards in the 4 years period; two MT2 and MT3 were only found in hospitalized patients in the 4 years of study. Differences in the number of types between the present study and previous studies are probably due to different naming patterns for MTs and the term “VNTR pattern” in the present study is equivalent to MT in two other studies. Three common types (MT1, MT2 and MT3) were resistant to gentamicin, ampicillin, ciprofloxacin, erythromycin, clindamycin and had *acm* and ampicillin resistance, which is more prevalent in nosocomial strains [2], had high frequency in isolates of three common types. This probably indicates the presence of a multi-drug resistant clone that is compatible with the treatment center and the infection control strategies appear to be ineffective so the organism is stable and spreading to patients in different departments and outpatients referring to this center. On the other hand, MT2 and MT3 strains were likely to mutate in order to adapt to the hospital setting. For example the resistance gene pattern *vanA*+*aac(6′)-le-aph(2″)-Ia* and *esp* virulence gene in MT3 were significantly more abundant than MT1. Since the *esp* gene in isolates of *E. faecium* is a marker of a pathogenic island that can be transmitted through conjugation to other isolates and *vanA* and *aac(6′)-le-aph(2″)-Ia* genes are often found on plasmids [7, 37], identification of these isolates is necessary in order to review the infection control strategies to prevent the release of resistance genes, *vanA* and *aac(6′)-le-aph(2″)-Ia*, and the virulence gene, *esp*, to other cells.

**Conclusions**

This study has demonstrated changes over time in species distribution in enterococci isolated from an Iranian children’s hospital. The results show a shift from *E. faecalis* to *E. faecium* as the dominant enterococcal species among patients at the children Hospital. Our data revealed that the majority of *E. faecium* isolates (66%) belonged to three common MTs and these types were isolated from different wards in children hospital. Moreover, the results of this study shows that there is a significant difference in the prevalence rate of antimicrobial resistance and virulence genes among common MTs.

**Methods**

**Bacterial isolates**

One hundred and eighty-nine non-repetitive isolates of *Enterococcus* spp. were collected during December 2011 to November 2015 from various clinical samples of children admitted to a children hospital in Tehran, Iran. Enterococcal isolates were initially re-identified in the microbiology laboratory of Tehran university of Medical Science based on a series of conventional microbiological tests [38]. To confirm the identity of isolate as *E. faecium* and *E. faecalis* the *ddl* gene was amplified by a Polymerase Chain reaction (PCR)-based method as described previously [39]. Isolates identified as *E. faecium* were studied further. The study was approved by the Ethics Committee of Tehran University of Medical Sciences.

**Antimicrobial susceptibility testing**

Antibiotic susceptibility testing was performed by disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines [40] with the following antimicrobial disks (Mast Group Ltd., Merseyside, UK): ampicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg). HLGR isolates were also determined by disk diffusion method by using 120 μg gentamicin disk. Inducible clindamycin resistance was determined by D-test [40]. The minimum inhibitory concentrations (MICs) of vancomycin was determined by the agar dilution method. *E. faecalis* ATCC29212 and *S. aureus* ATCC25923 were used as controls [40].

**Antimicrobial resistance and virulence genes detection**

Bacterial genomic DNA was extracted from overnight grown colonies by boiling method [19]. The genes encoding resistance to vancomycin (*vanA*) and aminoglycoside (*aac(6′)-le-aph(2″)-Ia*) among *E. faecium* and *E. faecalis* isolates and virulence factor genes (*cytA*, *gelE*, *esp*, *acm*, *ace*, *asa1*) among *E. faecium* were detected by a series of PCR assays [5, 25, 34, 41].
Molecular analysis
To examine the genotypic diversity of *E. faecium* isolates, MLVA was carried out by a modified Top method [9], as previously described. Briefly, 5 VNTR loci (VNTR-1, VNTR-7, VNTR-8, VNTR-9, VNTR-10) were targeted by PCR using the following steps: an initial denaturation at 95 °C for 5 min and final extension at 72 °C for 5 min. For VNTR-1, 30 cycles of 95 °C for 50 s, 54 °C for 50 s and 72 °C for 80 s were performed. For VNTR-7 a touchdown PCR was done that involved 30 cycles, comprising of 30 s at 95 °C, 30 s at 65 °C down to 55 °C and 1 min at 72 °C. For VNTR-8, VNTR-9, and VNTR-10, 50 s at 95 °C, 45 s at 59 °C and 1 min at 72 °C was prepared. Amplified products were separated by electrophoresis in 2% agarose gels with 0.5X TBE (Tris/Borate/EDTA) buffer. The amplicon bands were visualized with UV illumination after staining with KBC power load dye (GelRed Nucleic Acid Gel Stain, 10,000x in water, Kawsar Biotech Co., Tehran, Iran). MLVA type (MT) were assigned on the basis of one or more loci differences, congruence with a similarity index of approximately 80%. Therefore, MTs were defined as isolates sharing 80% or higher similarity.

Statistical analysis
The Fisher’s test was used to compare the frequency of antibiotic resistance, virulence factors and resistance genes in common MTs (95% confidence intervals and *P* value ≤ 0.05 considered significant). All results were rounded down if they were < 0.5, were presented as whole numbers if they were > 0.5 and were regarded 0.5 itself if they were = 0.5.

Abbreviations
Acm: Collagen-binding adhesin; AP: Ampicillin; Asa1: Aggregation substance; CD: Clindamycin; CICU: Coronary Intensive Care Unit; CIP: Ciprofloxacin; CLSI: Clinical and Laboratory Standards Institute; CSF: Cerebrospinal fluid; CyE: Cyotolysin; E: Enthymochyn; Exp: Enterococcal surface protein; GelE: Gelatinase; GM: Gentamicin; HLGR: High-level gentamicin resistant; m/y: month/year; MIC: Minimal inhibitory concentration; MTmt: MLVA Type E. faecium; NICU: Neonatal Intensive Care Unit; PCR: Polymerase Chain reaction; PICU: Paediatric Intensive Care Unit; VNTR: Variable number tandem repeat; VRE: Vancomycin resistant enterococci; VREfm: Vancomycin resistant enterococci; VREs: Vancomycin resistant enterococci

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Authors’ contributions
ME and FJ designed the experiments. AS and NNF conducted the experiments, AS drafted the manuscript. ME and RB revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Please contact author for data requests.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Tehran University of Medical Sciences. Consent to participate is not applicable for this study because the isolates included in the study were obtained from existing clinical collections routinely assembled as part of laboratory practices of university hospitals.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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