Comparison of Enterococcus faecium Bacteremic Isolates from Hematologic and Non-hematologic Patients: Differences in Antimicrobial Resistance and Molecular Characteristics

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Background: Enterococcus faecium, especially vancomycin-resistant E. faecium (VREfm), is a major concern for patients with hematologic diseases. Exposure to antibiotics including fluoroquinolone, which is used as a routine prophylaxis for patients with hematologic (MH) diseases, has been reported to be a risk factor for infection with vancomycin-resistant enterococci. We compared the characteristics of E. faecium isolates according to their vancomycin susceptibility and patient group (MH vs non-MH patients).

Methods: A total of 120 E. faecium bacteremic isolates (84 from MH and 36 from non-MH patients) were collected consecutively, and their characteristics (susceptibility, multilocus sequence type [MLST], Tn1546 type, and the presence of virulence genes and plasmids) were determined.

Results: Among the vancomycin-susceptible E. faecium (VSEfm) isolates, resistance to ampicillin (97.6% vs 61.1%) and high-level gentamicin (71.4% vs 38.9%) was significantly higher in isolates from MH patients than in those from non-MH patients. Notably, hyl, esp, and pEF1071 were present only in isolates with ampicillin resistance. Among the VREfm isolates, ST230 (33.3%) and ST17 (26.2%) were predominant in MH patients, while ST17 (61.1%) was predominant in non-MH patients. Plasmid pLG1 was more prevalent in E. faecium isolates from MH patients than in those from non-MH patients, regardless of vancomycin resistance. Transposon analysis revealed five types across all VREfm isolates.

Conclusions: The antimicrobial resistance profiles and molecular characteristics of E. faecium isolates differed according to the underlying diseases of patients within the same hospital. We hypothesize that the prophylactic use of fluoroquinolone might have an effect on these differences.

Key Words: Ampicillin, Fluoroquinolone, Enterococcus, Multilocus sequence typing, pEF1071, pLG1, ST230

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INTRODUCTION

Enterococci have emerged as major nosocomial pathogens that cause healthcare-associated infections as well as prolonged colonization in patients with comorbidities [1]. They are the third most common cause of bacteremia at the Catholic Blood and Marrow Transplantation (BMT) Center of Seoul St. Mary’s Hospital, Seoul, Korea. Additionally, vancomycin-resistant enterococci (VRE) account for 28% of all enterococcal bacteremia in hematologic [MH] patients [2, 3] compared with approximately 15% in non-MH patients in this hospital. Recent history of antibiotic use, especially fluoroquinolone or broad-spectrum beta-lactam agents, has been reported as the major risk factor for VRE infection [4, 5]. Fluoroquinolone is commonly used as a prophylaxis during chemotherapy or stem cell transplantation (SCT) for MH patients.

One of the clinical concerns regarding enterococcal infection stems from its intrinsic resistance to broad-spectrum antibiotics, which can lead to a delay in appropriate antimicrobial therapy. Other important issues regarding VRE include the poor outcomes of infected patients and the possibility of horizontal transfer of vancomycin resistance [6, 7]. Vancomycin-resistant Enterococcus faecium (VREfm) has recently been recognized as an endemic nosocomial pathogen in many hospitals worldwide [8, 9].

As only a limited number of reports have investigated the molecular characteristics of E. faecium bacteremic isolates according to different host factors and antibiotic resistance, we compared the molecular characteristics of (1) vancomycin-susceptible E. faecium (VSEfm) and VREfm bacteremic isolates in general, (2) VSEfm from MH and non-MH patients, and (3) VREfm from MH and non-MH patients.

METHODS

1. Bacterial isolates
A total of 120 E. faecium bacteremic isolates were collected from January 2012 to December 2013 at Seoul St. Mary’s Hospital. An equal number of VSEfm and VREfm isolates were sequentially obtained from adult MH and non-MH patients: 42 VSEfm and 42 VREfm from MH patients, and 18 VSEfm and 18 VREfm from non-MH patients. For this study, E. faecium bacteremia was defined as isolation of E. faecium species from one or more blood cultures using an automated blood culture system (Bactec FX, Becton Dickinson, Sparks, MD, USA) [10]. If E. faecium was repeatedly isolated from a single patient, only the first bacteremic isolate was used for molecular analysis. This study was approved by the Institutional Review Board (IRB) of Seoul St. Mary’s Hospital at the Catholic University of Korea with an informed consent waiver because the study utilized previously collected bacterial isolates without any individualized patient information (IRB number: KC14SISI0696).

2. Antimicrobial susceptibility test
The susceptibility to ampicillin, vancomycin, teicoplanin, tigecycline, linezolid, and quinupristin/dalfopristin and high-level resistance to gentamicin (HLGR) and streptomycin were evaluated using the Vitek AST-P600 and Vitek II systems (bioMérieux, Hazelwood, MO, USA), according to the CLSI guidelines [11]. The presence of vanA and vanB was determined using the Seeplex VRE ACE Detection kit (Seegene, Seoul, Korea).

3. Molecular typing, plasmid analysis, and virulence gene profiling
Multilocus sequence typing (MLST) of E. faecium isolates was conducted as previously described, based on seven housekeeping genes (adk, atpA, ddi, gdh, gyd, purK, and pstS) [12]. Different sequences at a given locus were assigned an allele number based on the E. faecium MLST database (http://efaecium.mlst.net), and each unique combination of alleles (the allelic profile) was designated as an ST.

Eight enterococcal plasmids (pIP501, pRE25, PEF1071, pRI, pRUM, pEF418, pMG1, and pLG1) were sequenced by PCR-based typing as previously described [7, 13]. The presence of the virulence genes hyl (glucoside hydrolase), cylA (cytolysin), gelE (gelatinase), esp (enterococcal surface protein), acm (adhesive of collagen from E. faecium), scm (second collagen adhesion of E. faecium, fms10), sgrA (serine-glutamate repeat containing protein A), ecbA (E. faecium collagen binding protein, fms18), asa1 and agg (aggregation substances), pilA and pilB (pilus-like structures), fms11, fms14, and fms15 (E. faecium surface proteins) was evaluated by PCR as previously described [13, 14].

4. Structural analysis of Tn1546 elements
For the structural analysis of Tn1546 elements, overlapping internal regions of Tn1546 were amplified using PCR as previously described [15]. Representative isolates in each ST exhibiting PCR fragments longer or shorter than those of the BM4147 prototype vanA gene cluster 7 were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Purified PCR products were directly sequenced using an ABI Prism 3700 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).
5. Statistical analysis

Statistical analysis was performed using SPSS software version 24.0 (SPSS Korea, Seoul, Korea). Chi-square analysis and Student’s t-test were used to compare categorical variables and continuous variables, respectively. If any of the cells of a contingency table were below five, Fisher’s exact test was used to compare categorical variables. Mann-Whitney U test was used to compare nonparametric continuous variables. A two-tailed P value ≤0.05 was considered statistically significant.

RESULTS

1. Antimicrobial resistance

The results of resistance to eight antibiotics are listed in Table 1. Ampicillin resistance was observed in 86.7% (52 of 60) of VSEfm isolates and was higher in VSEfm from MH patients than in VSEfm from non-MH patients (97.6% vs 61.1%, P = 0.001). HLGR was also higher in VSEfm from MH patients than in VSEfm from non-MH patients (71.4% vs 38.9%, P = 0.023). The resistance rate for the other antibiotics was similar in E. faecium isolates from

Table 1. In vitro resistance rate of Enterococcus faecium bacteremic isolates

|                      | VSEfm (N = 60) | VREfm (N = 60) |
|----------------------|---------------|---------------|
|                      | MH (N = 42)   | Non-MH (N = 18) | P     | MH (N = 42) | Non-MH (N = 18) | P     |
| Ampicillin           | 41 (97.6%)    | 11 (61.1%)    | 0.001 | 42 (100%)   | 18 (100%)       | -     |
| High level gentamicin resistance | 30 (71.4%)    | 7 (38.9%)     | 0.023 | 26 (61.9%)  | 11 (61.1%)      | > 0.999 |
| High level streptomycin resistance | 10 (23.8%)    | 3 (16.7%)     | 0.736 | 5 (11.9%)   | 7 (27.8%)       | 0.256 |
| Vancomycin           | 0 (0%)        | 0 (0%)        | -     | 42 (100%)   | 18 (100%)       | -     |
| Teicoplanin          | 0 (0%)        | 0 (0%)        | -     | 41 (97.6%)  | 18 (100%)       | 0.303 |
| Linezolid            | 0 (0%)        | 0 (0%)        | -     | 2 (4.8%)    | 0 (0%)          | > 0.999 |
| Quinupristin-dalfopristin | 1 (2.4%)†    | 0 (0%)        | > 0.999 | 1 (2.4%)†  | 1 (5.6%)†       | 0.514 |
| Tigecycline          | 0 (0%)        | 0 (0%)        | -     | 0 (0%)      | 0 (0%)          | -     |

Data are presented as n (%).

*The minimal inhibitory concentrations (MICs) for two linezolid non-susceptible isolates were ≥8 mg/dL; †The MICs for three quinupristin-dalfopristin non-susceptible isolates were 2 mg/dL.

Abbreviations: MH, hematologic patients; non-MH, non-hematologic patients; VREfm, vancomycin-resistant E. faecium; VSEfm, vancomycin-susceptible E. faecium.

2. Distribution of multilocus sequence types of Enterococcus faecium bacteremic isolates and Tn1546 element characteristics

| STs* (N) | VSEfm (N = 60) | VREfm (N = 60) |
|----------|----------------|---------------|
| ST17 (44) | 18 (42.9%) | 11 (61.1%): I (1), II (3), IV (2), V (5) | 11 (61.1%): I (4), II (5), IV (2) | 0.010 |
| ST230 (23) | 6 (14.3%) | 14 (33.3%): II (10), IV (4) | 1 (5.6%): II (1) | 0.025 |
| ST192 (17) | 6 (14.3%) | 6 (14.3%): II (2), IV (4) | 2 (11.1%): II (1), V (1) | > 0.999 |
| ST78 (8) | 4 (9.5%) | 2 (4.8%): II (1), III (1) | 1 (5.6%): I (1) | > 0.999 |
| ST262 (5) | 1 (2.4%) | 4 (9.5%): I (1), II (1), IV (2) | 0 (0%) | 0.306 |
| ST18 (4) | 3 (7.1%) | 0 (0%) | 1 (5.6%): IV (1) | 0.300 |
| ST812 (4) | 1 (2.4%) | 0 (0%) | 0 (0%) | - |
| ST64 (2) | 1 (2.4%) | 1 (2.4%): II (1) | 0 (0%) | > 0.999 |

Data are presented as n (%).

*Other STs not presented in the table are singletons (ST66, ST80, ST117, ST178, ST202, ST203, ST233, ST389, ST7850, ST994, ST995, ST996, and ST997); Chi-square analysis was used to compare categorical variables. If any of the cells of a contingency table are below five, Fisher’s exact test was used.

Abbreviations: MH, hematologic patients; non-MH, non-hematologic patients; VREfm, vancomycin-resistant E. faecium; VSEfm, vancomycin-susceptible E. faecium; Tn, transposon.
MH and non-MH patients. All 60 VREfm isolates carried the vanA gene.

2. MLST
The predominant ST among the 120 E. faecium isolates tested was ST17 (36.7%, 44 of 120), followed by ST230 (19.2%, 23 of 120), ST192 (14.2%, 17 of 120), ST78 (6.7%, 8 of 120), and ST262 (4.2%, 5 of 120). These five major clones represented 80.8% (97 of 120) of E. faecium bacteremic isolates. Table 2 shows the MLST results segregated by vancomycin resistance and a comparison between MH and non-MH patients.

No significant differences were observed in the overall distribution of STs between VSEfm and VREfm. However, the ST distribution of VSEfm and VREfm could be distinguished according to the patient group. Among the 42 VSEfm isolates from MH patients (VSEfm MH), ST17 was predominant (42.9%), followed by ST192 and ST230 (14.3%) (Fig. 1A). In comparison, no predominant clone was observed among the 18 VSEfm isolated from non-MH patients (VSEfm non-MH); identified STs included ST17 (22.2%), ST192, and ST18 (16.7%) (Fig. 1B). ST230 was the most common (33.3%) ST among the VREfm isolates from MH patients (VREfm MH), followed by ST17 (26.2%) (Fig. 1C), while ST17 was predominant (61.1%) in VREfm isolates from non-MH patients (VREfm non-MH) (Fig. 1D). A population snapshot obtained using eBURST analysis (http://eburst.mlst.net) is shown in Fig. 2.

3. Prevalence of the virulence genes and plasmids
Of the 15 virulence genes tested, several were rarely identified or not identified in the 120 E. faecium isolates; these included cylA (n=1), gelE (n=0), asa1 (n=1), and agg (n=0). In contrast, acm, which encodes a factor related to adhesion of colla-
Fig. 2. Population snapshot by eBURST analysis (http://eburst.mlst.net) showing clusters of linked and unlinked sequence types (STs) identified in this study. The boxed numbers represent sequence types.

different E. faecium isolates from different hosts

Table 3. Virulence factors and plasmids observed in E. faecium isolates

| Virulence factor* | VSEfm (N = 60) | VREfm (N = 60) | P     |
|------------------|----------------|----------------|-------|
| hyl              | 45 (76.7%)     | 55 (91.7%)     | 0.024 |
| esp              | 45 (75.0%)     | 52 (86.7%)     | 0.104 |
| scm              | 45 (75.0%)     | 46 (76.7%)     | >0.999|
| sgrA             | 51 (85.0%)     | 60 (100%)      | 0.003 |
| ecbA             | 34 (56.7%)     | 43 (71.7%)     | 0.087 |
| pilA             | 41 (68.3%)     | 39 (65.0%)     | 0.699 |
| pilB             | 40 (66.7%)     | 41 (68.3%)     | 0.845 |
| fms11            | 49 (81.7%)     | 51 (85.0%)     | 0.654 |
| fms15            | 23 (38.3%)     | 19 (31.7%)     | 0.444 |
| fms15*           | 40 (66.7%)     | 49 (81.7%)     | 0.061 |

Plasmid

| Plasmid | VSEfm (N = 60) | VREfm (N = 60) | P     |
|---------|----------------|----------------|-------|
| pIP501  | 3 (5.0%)       | 6 (10.0%)      | 0.491 |
| pRE25   | 48 (80.0%)     | 52 (86.7%)     | 0.327 |
| PEF1071 | 42 (70.0%)     | 54 (90.0%)     | 0.006 |
| pRI     | 50 (83.3%)     | 56 (93.3%)     | 0.153 |
| pRUM    | 12 (20.0%)     | 8 (13.3%)      | 0.327 |
| pEF418  | 45 (75.0%)     | 42 (70.0%)     | 0.540 |
| pMG1    | 8 (13.3%)      | 16 (26.7%)     | 0.068 |
| pLG1    | 44 (73.3%)     | 50 (83.3%)     | 0.184 |

*Of the 15 virulence genes tested, several virulence genes were rarely identified or not found in the 120 E. faecium isolates; cydA (n=1), geEl (n=0), asaI (n=1), agg (n=0). acm was found in all of the isolates tested in this study (n=120).

Abbreviations: VSEfm, vancomycin-susceptible E. faecium; VREfm, vancomycin-resistant E. faecium.
Furthermore, *E. faecium* harboring pEF1071 more frequently harbored hyl (93.8% vs 45.8%, *P* <0.001), esp (91.7% vs 37.5%, *P* <0.001), sgra (99.0% vs 66.7%, *P* <0.001), ecbA (71.9% vs 33.3%, *P* <0.001), pilB (76.0% vs 33.3%, *P* <0.001), and fms15 (80.2% vs 50.0%, *P* =0.002).

pLG1 was more frequent in ampicillin-resistant *E. faecium* isolates than in ampicillin-susceptible isolates (81.3% vs 37.5%, *P* =0.012). pLG1 was also more prevalent in both VSEfm (81.0% vs 55.6%, *P* =0.041) and VREfm (90.5% vs 66.7%, *P* =0.023) from MH patients than those from non-MH patients. This prevalence was significantly related to the presence of esp, sgra, pilB, and fms15; *E. faecium* harboring pLG1 also harbored esp (85.1% vs 65.4%, *P* =0.024), sgra (95.7% vs 80.8%, *P* =0.022), pilB (73.4% vs 46.2%, *P* =0.009), and fms15 (79.8% vs 53.8%, *P* =0.007) more frequently than did *E. faecium* without pLG1.

Taken together, the common virulence factors esp and sgra were more prevalent in *E. faecium* isolates from MH patients, ampicillin-resistant isolates, and isolates harboring pEF1071 or pLG1. In addition, of the seven virulence factors prevalent in ampicillin-resistant isolates, six were also more prevalent among isolates harboring pEF1071, as pEF1071 was found only in ampicillin-resistant isolates.

### 4. Structural analysis of Tn1546

Result of the structural analysis of the main Tn1546 type for VREfm is presented in Table 2 and Fig. 3. None of the isolates harbored the prototype Tn1546. The isolates were classified into five (I–V) main Tn types according to the presence of *orfI* and insertion of IS1542 and/or IS1216. *orfI* was detected in most (54 of 60, 90%) of the isolates. Insertion of IS1542 in the *orfI*–vanR region was observed in 85.7% (36 of 42) of VREfm MH and 94.4% (17 of 18) of VREfm non-MH, respectively. Insertion of IS1216V in the vanX–vanY intergenic region was found in 57.1% (24 of 42) of VREfm MH and 72.2% (13 of 18) of VREfm non-MH, and most of these isolates also harbored an IS1542 insertion. In other words, 88.3% (53 of 60) and 61.7% (37 of 60) of all the VREfm isolates had IS1542 and IS1216V insertions, respectively. Type II was the most common, accounting for 50% (30 of 60) of all VREfm isolates. Tn types exhibited a variable pattern of distribution, which was difficult to characterize according to ST or host specificity.

![Genetic maps of Tn1546 in vancomycin-resistant *Enterococcus faecium* bacteremic isolates. The positions of genes and open reading frames and the direction of transcription are marked by arrows. Inverted triangles represent insertion sequence (IS) elements. Dotted lines indicate deletions.](https://doi.org/10.3343/alm.2018.38.3.226)
DISCUSSION

In this study, we compared the molecular characteristics of *E. faecium* bacteremic isolates according to host factors (MH vs non-MH patients). Seoul St. Mary’s Hospital is a 1,300-bed, university-affiliated, tertiary care center in Seoul, South Korea; approximately 230 beds were allocated to MH patients. The Catholic BMT Center performs over 500 SCTs annually. Oral ciprofloxacin (500 mg twice daily) was used as a routine prophylaxis during chemotherapy or SCT throughout the study period. The initial empirical treatment for neutropenic fever in patients with hematological malignancy includes anti-pseudomonal cephalosporin (cefazidime or ceftazidime) and/or an aminoglycoside (isepamicin), excluding the initial use of glycopeptides [16, 17]. The medical illness severity of MH patients and prolonged antibiotic use might be related to the relatively higher rate of ampicillin-resistant *E. faecium* and VREfm isolates in MH patients than in non-MH patients. We found that ampicillin resistance is much higher in VSEfm MH than in VSEfm non-MH (97.6% vs 61.1%). We also found that ampicillin-resistant isolates harbored several virulence genes more frequently than ampicillin-susceptible isolates; hyl and esp were detected only in ampicillin-resistant isolates. Interestingly, there were also significant differences in ampicillin resistance rate among the non-MH isolates according to the type of ward: 94.1% in general ward (GW), 83.3% in intensive care unit (ICU), and 42.9% in emergency department (ER), respectively (P = 0.011). Similar trends were also observed for the prevalence of hyl (88.2% in GW, 83.3% in ICU, and 42.9% in ER, P = 0.038) and esp (82.4% in GW, 75.0% in ICU, and 42.9% in ER, P = 0.093). These differences may be due to previous exposure to antibiotics or to the bloodstream infection developed in a nosocomial or community setting. However, the scope of this study did not include investigating antibiotic exposure in the non-MH group.

pEF1071 was not detected in any of the ampicillin-susceptible isolates, although the result should be interpreted bearing in mind that the number of these isolates was low (n = 7). pEF1071 encodes enterocins 1071A and 1071B [18]; as enterocins possess antimicrobial activity against closely related species, a producer strain would have a selective advantage over other strains in the same ecological niche [19].

It is interesting that hyl, esp, and sgrA were the virulence factors most frequently identified in isolates from MH patients, ampicillin-resistant isolates, and isolates harboring pEF1071. esp is known to be associated with resistance to ampicillin, imipenem, and ciprofloxacin [20, 21]. A recent study observed high prevalence and persistence of ampicillin-resistant *E. faecium* colonization in patients receiving levofloxacin prophylaxis [22]. We hypothesize that the relatively high ampicillin resistance rate in *E. faecium* from MH patients in our hospital might be associated with ciprofloxacin prophylaxis administered to the MH patients. Further study is needed to determine whether there is any link between ampicillin resistance, virulence factors, and pEF1071.

Regardless of patient group, hyl and sgrA were more frequent in VREfm than in VSEfm. This might be due to co-localization of vanA and hylEfm on the same plasmid [23]. sgrA, along with other virulence factors (esp, hyl, acm, scm, ecbA, pilA, and pilB), is known to play an important role in the emergence of ST78 VREfm in nosocomial infections [24]. Considering that hyl, esp, and sgrA are related to colonization of the gastrointestinal tract, primary surface attachment, and adhesion [25-27], these virulence factors could play a role in the development of enterococcal bacteremia originating from the gastrointestinal tracts of MH patients who frequently suffer from severe gut mucositis during prolonged, severe neutropenia. It is noteworthy that the prevalence of hyl and esp among the VREfm MH isolates increased from 49% to 88% and 68% to 86%, respectively, compared with a previous report from this BMT center [15]. esp plays a significant role in the prevalence of VREfm [27, 28].

In terms of plasmid replicon typing, the majority of *E. faecium* isolates from MH patients harbored pLG1, which is a newly sequenced, 280-kb, conjugative plasmid encoding VanA-type glycopeptide resistance, macrolide resistance, carbon uptake-utilization genes, and putative virulence genes including hyl and a pilin gene cluster [23]. Further study is needed to investigate the role of pLG1 in the acquisition and transmission of vancomycin resistance in *E. faecium*.

In this study, we also analyzed the molecular epidemiology of *E. faecium* bacteremic isolates. Most *E. faecium* isolates (80.8%) belonged to clonal complex 17 (CC17) and comprised various STs, including ST17, ST230, ST192, ST78, and ST262. Previous studies have shown that CC17 can be resolved into three different lineages, originating from ST17, ST18, and ST78. While lineages 17 and 18 were predominant from 1990 to 2004, lineage 78 has become predominant since 2005 [29, 30]. However, ST17 remained the most prevalent ST while ST230, a single locus variant of ST78, was predominant among MH patients at this hospital. In addition, five types of Tn1546 were identified, of which two (II and IV) accounted for >70% of the VREfm isolates. This finding indicates that both clonal spread and horizontal transfer played a role in the spread of vancomycin resistance.

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within a hospital. In addition, diverse STs harbored Tn1546 types II and IV. In cases of common STs, ST17 harbored Tn1546 types I, II, IV, and V, and ST230 contained Tn1546 types II and IV.

We wish to emphasize three findings. First, the rate of ampicillin resistance was higher in VSEfm MH than in VSEfm non-MH, and hyl, esp, and pEF1071 were detected only in isolates with ampicillin resistance. Based on these findings, we have decided to limit administration of ciprofloxacin prophylaxis to a select group of patients. Second, the prevalence of pLG1 was higher in E. faecium MH than in E. faecium non-MH, regardless of vancomycin resistance. Third, both clonal and horizontal transfers contributed to the transmission of VRE. Further study is, therefore, needed to investigate the genetic link between antimicrobial resistance and virulence factors.

In conclusion, antimicrobial resistance profiles and molecular characteristics, including the distribution of STs, virulence genes, and plasmids, were different and associated with the underlying diseases of patients within the same hospital. We presume that the prophylactic use of fluoroquinolone might have affected the antimicrobial resistance profiles and molecular characteristics of E. faecium and have thus decided to use fluoroquinolone more stringently in MH patients.

Authors’ Disclosures of Potential Conflicts of Interest

The authors declare that they have no conflicts of interest related to this study.

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