ANNALS OF LABORATORY MEDICINE

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

Sung-Yeon Cho, M.D.[1,4,5], Yeon-Joon Park, M.D.[2], Hanwool Cho, M.D.[2], Dong Jin Park, M.D.[2], Jin Kyung Yu, M.S.[2], Hayeon Caitlyn Oak[3], and Dong-Gun Lee, M.D.[1,4,5]

Division of Infectious Diseases[1], Department of Internal Medicine and Department of Laboratory Medicine[2], Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; Department of Brain and Cognitive Science[3], Massachusetts Institute of Technology, Massachusetts, United States of America; Vaccine Bio Research Institute[4] and Catholic Blood and Marrow Transplantation Center[5], Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

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 eneterocci. 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], Tn*1546* 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

Cho S-Y, et al.
Different *E. faecium* isolates from different hosts

ANNALS OF
LABORATORY
MEDICINE

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 consecutively 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, ddl, 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* (adhesin 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 Tn*1546* elements

For the structural analysis of Tn*1546* elements, overlapping internal regions of Tn*1546* 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). The ac-

quired nucleotide sequences were analyzed using the BLASTN tool from the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

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%)	5 (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*.

Table 2. Distribution of multilocus sequence types of *Enterococcus faecium* bacteremic isolates and Tn*1546* element characteristics

STs* (N)	VSEfm (N=60)			VREfm (N=60)		
	Multilocus Sequence Typing, N (%)†			Multilocus Sequence Typing, N (%): Tn1546 type (N)†		
	MH (N=42)	Non-MH (N=18)	P	MH (N=42)	Non-MH (N=18)	P
ST17 (44)	18 (42.9%)	4 (22.2%)	0.155	11 (26.2%): I (1), II (3), IV (2), V (5)	11 (61.1%): I (4), II (5), IV (2)	**0.010**
ST230 (23)	6 (14.3%)	2 (11.1%)	>0.999	14 (33.3%): II (10), IV (4)	1 (5.6%): II (1)	**0.025**
ST192 (17)	6 (14.3%)	3 (16.7%)	>0.999	6 (14.3%): II (2), IV (4)	2 (11.1%): II (1), V (1)	>0.999
ST78 (8)	4 (9.5%)	1 (5.6%)	>0.999	2 (4.8%): II (1), III (1)	1 (5.6%): I (1)	>0.999
ST262 (5)	1 (2.4%)	0 (0%)	>0.999	4 (9.5%): I (1), II (1), IV (2)	0 (0%)	0.306
ST18 (4)	3 (7.1%)	0 (0%)	0.547	0 (0%)	1 (5.6%): IV (1)	0.300
ST812 (4)	1 (2.4%)	3 (16.7%)	0.077	0 (0%)	0 (0%)	-
ST64 (2)	1 (2.4%)	0 (0%)	>0.999	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; VSEfm, vancomycin-susceptible *E. faecium*; VREfm, vancomycin-resistant *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 ST812 (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-
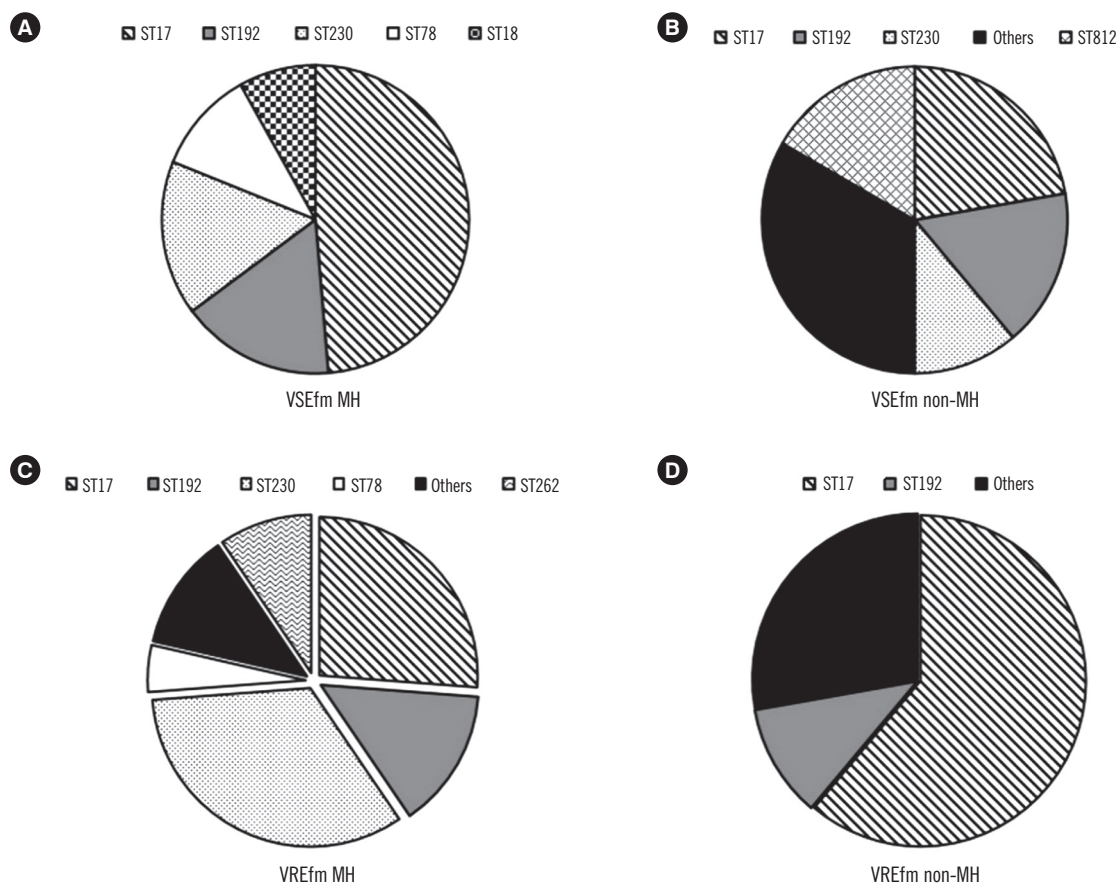

Fig. 1. Distribution of VSEfm and VREfm sequence types in MH and non-MH patients. (A) STs of VSEfm isolated from MH patients. (B) STs of VSEfm isolated from non-MH patients. (C) STs of VREfm isolated from MH patients. (D) STs of VREfm isolated from non-MH patients. "Others" comprises single individual STs.
Abbreviations: MH, hematologic patients; non-MH, non-hematologic patients; ST, sequence type; VREfm, vancomycin-resistant *E. faecium*; VSEfm, vancomycin-susceptible *E. faecium*.
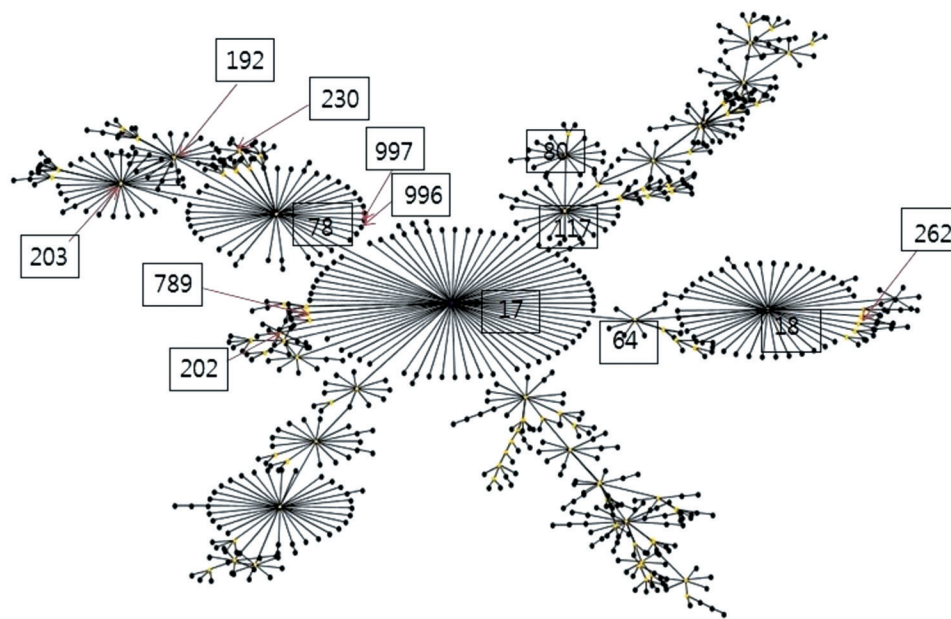
ANNALS OF
LABORATORY
MEDICINE

Cho S-Y, et al.
Different *E. faecium* isolates from different hosts

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.

gen, was detected in all bacteremic isolates (n=120). The distribution of virulence factors differed between VSEfm and VREfm; *hyl* (91.7% vs 76.7%, *P*=0.024) and *sgrA* (100% vs 85.0%, *P*=0.003) were more frequent in VREfm than in VSEfm (Table 3). In addition, *hyl* (85.7% vs 55.6%, *P*=0.011), *esp* (83.3% vs 55.6%, *P*=0.023), and *sgrA* (92.9% vs 66.7%, *P*=0.016) were more frequent in VSEfm MH than in VSEfm non-MH.

Furthermore, we compared the virulence factors correlated with resistance to antibiotics other than vancomycin. *Hyl* (90.2%, vs 0%, *P*<0.001), *esp* (86.6% vs 0%, *P*<0.001), *sgrA* (97.3% vs 25.0%, *P*<0.001), *ecbA* (67.9% vs 12.5%, *P*=0.003), *scm* (78.6% vs 37.5%, *P*=0.020), *pilB* (71.4% vs 12.5%, *P*=0.002), and *fms15* (78.6% vs 12.5%, *P*<0.001) were more frequently detected in ampicillin-resistant *E. faecium* than in ampicillin-susceptible *E. faecium*. Additionally, *hyl* (93.2% vs 68.9%, *P*<0.001), *esp* (87.8% vs 68.9%, *P*=0.011), *sgr* (98.6% vs 82.2%, *P*=0.002), *ecbA* (71.6% vs 51.1%, *P*=0.024), and *pilB* (74.3% vs 55.6%, *P*=0.034) were more frequent in *E. faecium* with HLGR than in *E. faecium* without HLGR.

Similar to the virulence factors, the plasmids harboring *rep* genes exhibited different patterns according to antimicrobial resistance and/or patient group. pEF1071 was more frequent in VREfm than in VSEfm (90.0% vs 70.0%, *P*=0.006; Table 3). In addition, the prevalence of pEF1071 was higher among ampicillin-resistant *E. faecium* isolates (85.7%), whereas it was not found in any of the ampicillin-susceptible *E. faecium* isolates.

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

	VSEfm (N=60)	VREfm (N=60)	*P*
Virulence factor*			
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
fms14	23 (38.3%)	19 (31.7%)	0.444
fms15	40 (66.7%)	49 (81.7%)	0.061
Plasmid			
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; *cylA* (n=1), *gelE* (n=0), *asa1* (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*.

Cho S-Y, et al.
Different *E. faecium* isolates from different hosts

ANNALS OF
LABORATORY
MEDICINE

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 Tn*1546*

Result of the structural analysis of the main Tn*1546* type for VREfm is presented in Table 2 and Fig. 3. None of the isolates harbored the prototype Tn*1546*. The isolates were classified into five (I–V) main Tn types according to the presence of *orf1* and insertion of IS*1542* and/or IS*1216*. *orf1* was detected in most (54 of 60, 90%) of the isolates. Insertion of IS*1542* in the *orf2-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 IS*1216V* 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 IS*1542* insertion. In other words, 88.3% (53 of 60) and 61.7% (37 of 60) of all the VREfm isolates had IS*1542* and IS*1216V* 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.

Fig. 3. Genetic maps of Tn*1546* 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.

ANNALS OF LABORATORY MEDICINE

Cho S-Y, et al.
Different *E. faecium* isolates from different hosts

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 (ceftazidime or cefepime) 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 *hyl*$_{Efm}$ 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 Tn*1546* 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

within a hospital. In addition, diverse STs harbored Tn*1546* types II and IV. In cases of common STs, ST17 harbored Tn*1546* types I, II, IV, and V, and ST230 contained Tn*1546* 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.

Acknowledgments

REFERENCES

1. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
2. Cho SY, Lee DG, Choi SM, Kwon JC, Kim SH, Choi JK, et al. Impact of vancomycin resistance on mortality in neutropenic patients with enterococcal bloodstream infection: a retrospective study. BMC Infect Dis 2013;13:504.
3. Kwon JC, Kim SH, Choi JK, Cho SY, Park YJ, Park SH, et al. Epidemiology and clinical features of bloodstream infections in hematology wards: one year experience at the Catholic Blood and Marrow Transplantation Center. Infect Chemother 2013;45:51-61.
4. Vergis EN, Hayden MK, Chow JW, Snydman DR, Zervos MJ, Linden PK, et al. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. a prospective multicenter study. Ann Intern Med 2001;135:484-92.
5. Peel T, Cheng AC, Spelman T, Huysmans M, Spelman D. Differing risk factors for vancomycin-resistant and vancomycin-sensitive enterococcal bacteraemia. Clin Microbiol Infect 2012;18:388-94.
6. Bonten MJ, Willems R, Weinstein RA. Vancomycin-resistant *enterococci*: why are they here, and where do they come from? Lancet Infect Dis 2001;1:314-25.
7. Rosvoll TC, Pedersen T, Sletvold H, Johnsen PJ, Sollid JE, Simonsen GS, et al. PCR-based plasmid typing in *Enterococcus faecium* strains reveals widely distributed pRE25-, pRUM-, pIP501- and pHTbeta-related replicons associated with glycopeptide resistance and stabilizing toxin-antitoxin systems. FEMS Immunol Med Microbiol 2010;58:254-68.
8. Cattoir V and Leclercq R. Twenty-five years of shared life with vancomycin-resistant *enterococci*: is it time to divorce? J Antimicrob Chemother 2013;68:731-42.
9. De Angelis G, Cataldo MA, De Waure C, Venturiello S, La Torre G, Cauda R, et al. Infection control and prevention measures to reduce the spread of vancomycin-resistant *enterococci* in hospitalized patients: a systematic review and meta-analysis. J Antimicrob Chemother 2014;69:1185-92.
10. Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections. Am J Infect Control 1988;16:128-40.
11. CLSI. Performance standards for antimicrobial susceptibility testing. 24th ed. CLSI supplement, M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.
12. Homan WL, Tribe D, Poznanski S, Li M, Hogg G, Spalburg E, et al. Multilocus sequence typing scheme for *Enterococcus faecium*. J Clin Microbiol 2002;40:1963-71.
13. Rosvoll TC, Lindstad BL, Lunde TM, Hegstad K, Aasnaes B, Hammerum AM, et al. Increased high-level gentamicin resistance in invasive *Enterococcus faecium* is associated with aac(6')Ie-aph(2″)Ia-encoding transferable megaplasmids hosted by major hospital-adapted lineages. FEMS Immunol Med Microbiol 2012;66:166-76.
14. 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;42:4473-9.
15. Park SH, Park C, Choi SM, Lee DG, Kim SH, Kwon JC, et al. Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* bloodstream infections among patients with neutropenia over a 6-year period in South Korea. Microb Drug Resist 2011;17:59-65.
16. Lee DG, Kim SH, Kim SY, Kim CJ, Park WB, Song YG, et al. Evidence-based guidelines for empirical therapy of neutropenic fever in Korea. Korean J Intern Med 2011;26:220-52.
17. Weissinger F, Auner HW, Bertz H, Buchheidt D, Cornely OA, Egerer G, et al. Antimicrobial therapy of febrile complications after high-dose chemotherapy and autologous hematopoietic stem cell transplantation--guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol 2012;91:1161-74.
18. Balla E, Dicks LM, Du Toit M, van der Merwe MJ, Holzapfel WH. Char-

ANNALS OF
LABORATORY
MEDICINE

Cho S-Y, et al.
Different *E. faecium* isolates from different hosts

acterization and cloning of the genes encoding enterocin 1071A and enterocin 1071B, two antimicrobial peptides produced by *Enterococcus faecalis* BFE1071. Appl Environ Microbiol 2000;66:1298-304.

19. Balla E and Dicks LM. Molecular analysis of the gene cluster involved in the production and secretion of enterocins 1071A and 1071B and of the genes responsible for the replication and transfer of plasmid pEF1071. Int J Food Microbiol 2005;99:33-45.

20. Dupre I, Zanetti S, Schito AM, Fadda G, Sechi LA. Incidence of virulence determinants in clinical *Enteorococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). J Med Microbiol 2003;52:491-8.

21. Billstrom H, Ludn B, Sullivan A, Nord CE. Virulence and antimicrobial resistance in clinical *Enterococcus faecium*. Int J Antimicrob Agents 2008;32:374-7.

22. Sánchez-Díaz AM, Cuartero C, Rodríguez JD, Lozano S, Alonso JM, Rodríguez-Domínguez M, et al. The rise of ampicillin-resistant *Enterococcus faecium* high-risk clones as a frequent intestinal colonizer in onco-haematological neutropenic patients on levofloxacin prophylaxis: a risk for bacteraemia? Clin Microbiol Infect 2016;22:e1-8.

23. Laverde Gomez JA, van Schaik W, Freitas AR, Coque TM, Weaver KE, Francia MV, et al. A multiresistance megaplasmid pLG1 bearing a hylEfm genomic island in hospital *Enterococcus faecium* isolates. Int J Med Microbiol 2011;301:165-75.

24. Yang J, Jiang Y, Guo L, Ye L, Ma Y, Luo Y. Prevalence of diverse clones of vancomycin-resistant *Enterococcus faecium* ST78 in a Chinese hospital. Microb Drug Resist 2016;22:294-300.

25. Rice LB, Lakticová V, Carias LL, Rudin S, Hutton R, Marshall SH. Transferable capacity for gastrointestinal colonization in *Enterococcus faecium* in a mouse model. J Infect Dis 2009;199:342-9.

26. van Wamel WJ, Hendrickx AP, Bonten MJ, Top J, Posthuma G, Willems RJ. Growth condition-dependent Esp expression by *Enterococcus faecium* affects initial adherence and biofilm formation. Infect Immune 2007;75:924-31.

27. Sava IG, Heikens E, Huebner J. Pathogenesis and immunity in enterococcal infections. Clin Microbiol Infect 2010;16:533-40.

28. Hendrickx AP, van Schaik W, Willems RJ. The cell wall architecture of *Enterococcus faecium*: from resistance to pathogenesis. Future Microbiol 2013;8:993-1010.

29. Willems RJ, Top J, van Schaik W, Leavis H, Bonten M, Sirén J, et al. Restricted gene flow among hospital subpopulations of *Enterococcus faecium*. MBio 2012;3:e00151-12.

30. Galloway-Peña JR, Nallapareddy SR, Arias CA, Eliopoulos GM, Murray BE. Analysis of clonality and antibiotic resistance among early clinical isolates of *Enterococcus faecium* in the United States. J Infect Dis 2009;200:1566-73.