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CLINICAL THERAPEUTICS

Prospective Observational Study of the Clinical Prognoses of Patients with Bloodstream Infections Caused by Ampicillin-Susceptible but Penicillin-Resistant *Enterococcus faecalis*

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ABSTRACT The purpose of this study was to evaluate the clinical impacts of ampicillin-susceptible but penicillin-resistant (ASPR) phenotypes of *Enterococcus faecalis* on clinical outcomes in patients with bloodstream infection (BSI). A total of 295 patients with an *E. faecalis* BSI from six sentinel hospitals during a 2-year period (from May 2016 to April 2018) were enrolled in this study. Putative risk factors, including host-, treatment-, and pathogen-related variables, were assessed to determine the associations with the 30-day mortality rate of patients with an *E. faecalis* BSI. The proportion of ASPR *E. faecalis* isolates was 22.7% (67/295). ASPR isolates (adjusted odds ratio, 2.27; 95% confidence interval, 1.01 to 5.02) exhibited a significant association with an increased 30-day mortality rate, and a significant difference in survival was identified in a group of patients treated with ampicillin- and/or piperacillin-based regimens who were stratified according to the penicillin susceptibility of the causative pathogen ($P = 0.011$ by a log rank test). ASPR *E. faecalis* BSIs resulted in a >2-fold-higher 30-day mortality rate (26.9%; 18/67) than for the BSIs caused by penicillin-susceptible strains (12.3%; 28/228). The differences in mortality rates of patients stratified by penicillin susceptibility were likely due to the treatment failures of ampicillin and/or piperacillin in patients with an ASPR *E. faecalis* BSI.

KEYWORDS CC28, *Enterococcus faecalis*, ampicillin, clinical outcome, penicillin

Enterococci are common Gram-positive pathogens that cause opportunistic infections, including bloodstream infections (BSIs), particularly in immunocompromised patients. *Enterococcus faecalis* was previously the dominant human pathogen among enterococci; however, *Enterococcus faecium* has recently become a more frequent human pathogen than *E. faecalis*, possibly due to the differing abilities of these species to acquire antimicrobial resistance determinants (1). The mortality rate of patients with an enterococcal BSI has been reported to range from 20% to 50%, and comorbidities of the patients and the antimicrobial resistance of the causative pathogens are considered major risk factors for early mortality (2–4).

Antimicrobial regimens that inhibit bacterial cell wall synthesis required for the treatment of enterococcal infections are determined according to *in vitro* susceptibil-

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ities of infection-causing pathogens to ampicillin and vancomycin (5). Ampicillin resistance in enterococci is associated with two different mechanisms: (i) penicillinase production, which is rarely observed in *E. faecalis* (6), and (ii) increased production of penicillin-binding proteins (PBPs) with a low affinity for beta-lactams, which is predominantly observed in *E. faecium* (7). Vancomycin resistance is mainly associated with the acquisition of the *vanA* or *vanB* gene cluster (8). For the antimicrobial treatment of patients with an *E. faecalis* BSI, the administration of ampicillin either alone or in combination with aminoglycosides is recommended because the species rarely acquires resistance to ampicillin and/or vancomycin (1).

Recently, ampicillin-susceptible but penicillin-resistant (ASPR) *E. faecalis* strains have been reported in many countries around the world, including Greece, Denmark, and Brazil (9–11). The resistance phenotype is associated with mutations in the *pbp4* gene and its promoter region, resulting in the overproduction of altered PBP4 with a low affinity for beta-lactam antimicrobials (12, 13). However, the clinical impacts of ASPR *E. faecalis* infections in humans have never been reported, and thus, a consensus regarding the proper antimicrobial treatment options for these infections is unavailable. Therefore, we conducted a comprehensive analysis by performing a prospective, multicenter, observational study to investigate the risk factors affecting the early mortality rate in patients with an *E. faecalis* BSI, focusing on the penicillin resistance of the pathogen.

RESULTS

Description of the patients with *E. faecalis* BSI. Among the 296 patients with an *E. faecalis* BSI, one patient was excluded due to death on the day of the initial blood culture. Ultimately, 295 patients were enrolled in this study (Table 1). The median age of the patients was 70.0 years (interquartile range [IQR], 58.0 to 79.0 years), and 61.7% (182/295) were male. Malignancy (28.8%; $n = 85$) was the most common underlying disease, followed by diabetes mellitus (23.1%; $n = 68$), end-stage renal diseases (19.0%; $n = 56$), cardiovascular diseases (18.0%; $n = 53$), cerebrovascular diseases (16.3%; $n = 48$), and liver cirrhosis (8.8%; $n = 26$). The median Charlson comorbidity index value was 2.0 (IQR, 1.0 to 3.0). More than half (59.3%, $n = 175$) of the patients acquired a hospital-originated (HO) infection, and 33.9% ($n = 100$) of the patients were hospitalized in intensive care units (ICUs). The median sequential organ failure assessment (SOFA) score was 4.0 (IQR, 1.0 to 6.0). Secondary BSI was identified by culture in 49 cases (16.6%), including 33 (11.2%) that originated from the urinary tract. Central line-associated BSI was identified in 5 cases (1.7%), and the primary site of infection was not identified 241 cases (81.7%). Only six patients (2.0%) were associated with infective endocarditis. Appropriate antimicrobial regimens were administered to 43.4% ($n = 128$) of patients as empirical treatment and to 62.4% ($n = 184$) of patients as definitive treatment. The 30-day all-cause mortality rate of patients with an *E. faecalis* BSI was 15.6% (46/295); however, all six patients associated with infective endocarditis survived during the 30-day follow-up.

Antimicrobial susceptibilities of *E. faecalis* blood isolates. All 295 *E. faecalis* blood isolates were susceptible to ampicillin, while 22.4% ($n = 66$) of them were not susceptible to either penicillin or imipenem, and one isolate was resistant to penicillin but susceptible to imipenem; i.e., 22.7% ($n = 67$) of *E. faecalis* blood isolates exhibited ASPR phenotypes. Beta-lactamase was not produced in any of the ASPR isolates, according to the Cefinase test. High-level aminoglycoside resistance was observed in 39.3% ($n = 116$) of the isolates for gentamicin and 14.9% ($n = 44$) for streptomycin. Only one isolate exhibiting resistance to both vancomycin and teicoplanin harbored the *vanA* gene. Antimicrobial susceptibilities of the isolates stratified according to strain type are summarized in Table S1 in the supplemental material.

Risk factors for 30-day mortality. Among the 295 patients enrolled in this study, 6 patients were censored before 30 days and were excluded from the logistic regression analysis. In the univariable analyses stratified by host factor variables (Table 1), an increased Charlson comorbidity index (odds ratio [OR], 1.26; 95% confidence interval

TABLE 1 Patient-, treatment-, and pathogen-related variables associated with 30-day mortality in patients with *E. faecalis* BSIs[c]

Variable	Value for group			Univariable analysis[a]		Multivariable analysis[a]	
	Total (*n* = 295; 100%)	Nonsurvivors (*n* = 46; 15.6%)	Survivors (*n* = 249; 84.4%)	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
Patients							
Mean age (yr) (range)	70.0 (58.0–79.0)	76.0 (62.0–81.0)	69.0 (57.0–79.0)	1.02 (0.99–1.04)	0.083		
No. (%) of males	182 (61.7)	27 (58.7)	155 (62.2)	0.85 (0.45–1.63)	0.622		
No. (%) with comorbidity							
Malignancy	85 (28.8)	18 (39.1)	67 (26.9)	1.69 (0.87–3.23)	0.117		
Diabetes mellitus	68 (23.1)	18 (39.1)	50 (20.1)	2.48 (1.26–4.82)	0.008		
Cardiovascular disease	**53 (18.0)**	**16 (34.8)**	**37 (14.9)**	**2.97 (1.45–5.94)**	**0.002**	**2.98 (1.29–6.83)**	**0.010**
Cerebrovascular disease	48 (16.3)	7 (15.2)	41 (16.5)	0.88 (0.34–2.01)	0.782		
Liver cirrhosis	26 (8.8)	7 (15.2)	19 (7.6)	2.12 (0.78–5.18)	0.115		
End-stage renal disease	56 (19.0)	13 (28.3)	43 (17.3)	1.89 (0.89–3.82)	0.086		
Mean Charlson comorbidity index (IQR)	**2.0 (1.0–3.0)**	**3.0 (2.0–4.0)**	**2.0 (1.0–3.0)**	**1.26 (1.10–1.44)**	**0.001**	**1.21 (1.02–1.43)**	**0.025**
No. (%) with host factor							
ICU admission	100 (33.9)	25 (54.3)	75 (30.1)	2.77 (1.46–5.31)	0.002		
Hospital-originated infection	175 (59.3)	31 (67.4)	144 (57.8)	1.47 (0.77–2.93)	0.258		
Polymicrobial infection	67 (22.7)	15 (32.6)	52 (20.9)	1.78 (0.87–3.50	0.102		
Primary site of infection of:							
Urinary tract	33 (11.2)	5 (10.9)	28 (11.2)	0.94 (0.30–2.38)	0.898		
Central line	5 (1.7)	0 (0)	5 (2.0)				
Others	16 (5.4)	3 (6.5)	13 (5.2)				
Unknown	241 (81.7)	38 (82.6)	203 (81.5)				
Concurrent infective endocarditis	6 (2.0)	0 (0)	6 (2.4)				
Mean SOFA score (IQR)	**4.0 (1.0–6.0)**	**7.0 (5.0–12.0)**	**3.0 (1.0–5.0)**	**1.34 (1.23–1.47)**	**<0.001**	**1.33 (1.21–1.47)**	**<0.001**
No. (%) of patients with adequate antimicrobial treatment							
Empirical	128 (43.4)	28 (60.9)	100 (40.2)	2.32 (1.23–4.48)	0.011	1.84 (0.87–3.94)	0.111
Definitive	184 (62.4)	29 (63.0)	155 (62.2)	1.03 (0.54–2.02)	0.919		
Pathogens							
No. (%) of isolates of strain type							
CC16	97 (32.9)	15 (32.6)	82 (32.0)	0.99 (0.49–1.90)	0.967		
CC28[b]	67 (22.7)	17 (37.0)	50 (20.1)	2.38 (1.19–4.66)	0.012		
CC507	23 (7.8)	2 (4.3)	21 (8.4)	0.48 (0.08–1.72)	0.334		
Other STs	108 (36.6)	12 (26.1)	96 (38.6)	0.56 (0.27–1.11)	0.107		
No (%) of isolates nonsusceptible to:							
Ampicillin	0 (0)	0 (0)	0 (0)				
Penicillin	**67 (22.7)**	**18 (39.1)**	**49 (19.7)**	**2.68 (1.35–5.23)**	**0.004**	**2.27 (1.01–5.02)**	**0.045**
Imipenem[b]	66 (22.4)	17 (37.0)	49 (19.7)	2.44 (1.22–4.78)	0.010		
Ciprofloxacin	174 (59.0)	31 (67.4)	143 (57.4)	1.50 (0.78–2.98)	0.237		
High-level gentamicin	116 (39.3)	21 (45.7)	95 (38.2)	1.35 (0.71–2.56)	0.349		
High-level streptomycin	44 (14.9)	9 (19.6)	35 (14.1)	1.50 (0.63–3.27)	0.333		
Vancomycin	1 (0.3)	0 (0)	1 (0.4)				
Teicoplanin	1 (0.3)	0 (0)	1 (0.4)				
Tetracycline	217 (73.6)	38 (82.6)	179 (71.9)	1.85 (0.86–4.44)	0.139		
Linezolid	19 (6.4)	3 (6.5)	16 (6.4)				
Tigecycline	6 (2.0)	0 (0)	6 (2.4)				
No. (%) of isolates with MDR phenotype	92 (31.2)	21 (45.7)	71 (28.5)	2.12 (1.11–4.03)	0.022		

[a]Six patients censored before 30 days were excluded from the univariate and multivariate logistic regression analyses.

[b]CC28 (VIF = 15.1) and nonsusceptibility to imipenem (VIF = 6,496,558) were excluded from multivariate analysis due to multicollinearity with nonsusceptibility to penicillin.

[c]Boldface type indicates variables significantly associated with 30-day mortality in the multivariable analysis. Abbreviations: CC, clonal complex; CI, confidence interval; ICU, intensive care unit; IQR, interquartile range; MDR, multidrug resistant; OR, odds ratio; aOR, adjusted odds ratio; SOFA, sequential organ failure assessment; ST, sequence type.

[CI], 1.10 to 1.44), diabetes mellitus (OR, 2.48; 95% CI, 1.26 to 4.82), cardiovascular diseases (OR, 2.97; 95% CI, 1.45 to 5.94), admission to ICUs (OR, 2.77; 95% CI, 1.46 to 5.31), and an increased SOFA score (OR, 1.34; 95% CI, 1.23 to 1.47) were significantly correlated with an increased 30-day mortality rate in patients with an *E. faecalis* BSI. Nonsusceptibilities of the causative pathogens to penicillin (OR, 2.68; 95% CI, 1.35 to 5.23) and imipenem (OR, 2.44; 95% CI, 1.22 to 4.78) were associated with an increased 30-day mortality rate. When isolates were stratified by strain type, clonal complex 28 (CC28) (OR, 2.38; 95% CI, 1.19 to 4.66) was significantly associated with an increased 30-day mortality rate compared to non-CC28 isolates. Both the variables nonsusceptibility to imipenem (variance inflation factor [VIF] = 6,496,558) and CC28 (VIF = 15.1) were excluded from the multivariate analyses due to multicollinearity with nonsusceptibility to penicillin.

The forward stepwise multivariate logistic regression analyses revealed that three host factor variables, an increased Charlson comorbidity index (adjusted OR [aOR], 1.21; 95% CI, 1.02 to 1.43), cardiovascular diseases (aOR, 2.98; 95% CI, 1.29 to 6.83), and an increased SOFA score (aOR, 1.33; 95% CI, 1.21 to 1.47), were risk factors for an increased 30-day mortality rate. In addition, a pathogenic factor variable, nonsusceptibility to penicillin (aOR, 2.27; 95% CI, 1.01 to 5.02), was also an independent risk factor.

ASPR *E. faecalis* BSI. The majority (97.0%; 65/67) of ASPR *E. faecalis* isolates were identified as belonging to CC28, including 62 isolates of sequence type 28 (ST28) (*gdh-gyd-pstS-gki-aroE-xpt-yqiL*, 4-4-8-3-8-1-3) and 1 isolate each of ST207 (4-4-46-3-2-1-3), ST867 (4-4-8-3-8-7-3), and ST882 (4-4-8-99-8-1-3). The ASPR *E. faecalis* isolates mainly (98.5%; 66/67) exhibited multidrug-resistant (MDR) phenotypes (Fig. 1). ASPR *E. faecalis* BSIs resulted in a >2-fold-higher 30-day mortality rate (26.9%; 18/67) than BSIs caused by penicillin-susceptible strains (12.3%; 28/228). A log rank test showed a significant difference in survival ($P = 0.002$) in patients stratified according to the penicillin susceptibility of the causative *E. faecalis* pathogens (Fig. 2A). The impacts of the penicillin susceptibility of BSI-causing *E. faecalis* on patients' clinical outcomes were evaluated in groups stratified by the antimicrobial treatment regimen administered. A statistically significant difference in survival was observed in patients stratified according to the penicillin susceptibility of BSI-causative pathogens using the log rank test in a group of patients treated with ampicillin- and/or piperacillin-based regimens ($P = 0.011$) (Fig. 2B) but not in another group of patients treated with glycopeptide-based regimens ($P = 0.290$) (Fig. 2C).

The effects of the penicillin, ampicillin, and imipenem MICs for causative *E. faecalis* isolates on the 30-day mortality rate of BSI patients treated with an ampicillin- and/or a piperacillin-based regimen as a definitive treatment were evaluated using Pearson's correlation coefficients. The penicillin MIC exhibited a strong and positive linear correlation (correlation coefficients [*r*], 0.960) with the 30-day mortality rate: at an MIC of ≤4 μg/ml, the 30-day mortality rate was 13.0% (6/46); at an MIC of 8 μg/ml, the rate was 20.0% (4/20); and at an MIC of ≥16 μg/ml, the rate was 41.2% (7/17) (Fig. 3A). Furthermore, MICs of both imipenem ($r = 0.902$) and ampicillin ($r = 0.988$) also showed strong positive linear correlations with the 30-day mortality rate (Fig. 3B and C).

Characteristics of ASPR isolates. Penicillin MICs for the 295 *E. faecalis* blood isolates ranged from ≤1 to 64 μg/ml (MIC_{50} = 4 μg/ml; MIC_{90} = 32 μg/ml), and those for the 67 ASPR isolates ranged from 16 to 64 μg/ml (MIC_{50} = 32 μg/ml; MIC_{90} = 32 μg/ml) (Table 2). All ASPR isolates were identified as belonging to CC28 ($n = 65$), except for two isolates identified as belonging to CC507. Nucleotide deletion of a single A residue in a string of seven A residues upstream of a putative −35 region for the *pbp4* gene, a variant identical to the one described previously by Rice et al., was identified in all the 65 ASPR isolates of CC28 but not in other isolates (13). Eight amino acid substitutions in PBP4, including seven in the penicillin-binding domain (PBD) (A369V, T418A, L475M, F499I, P520S, M652L, and D666P) and one in the non-penicillin-binding domain (nPBD) (T50I), were identified compared to the reference *E. faecalis* strain ATCC

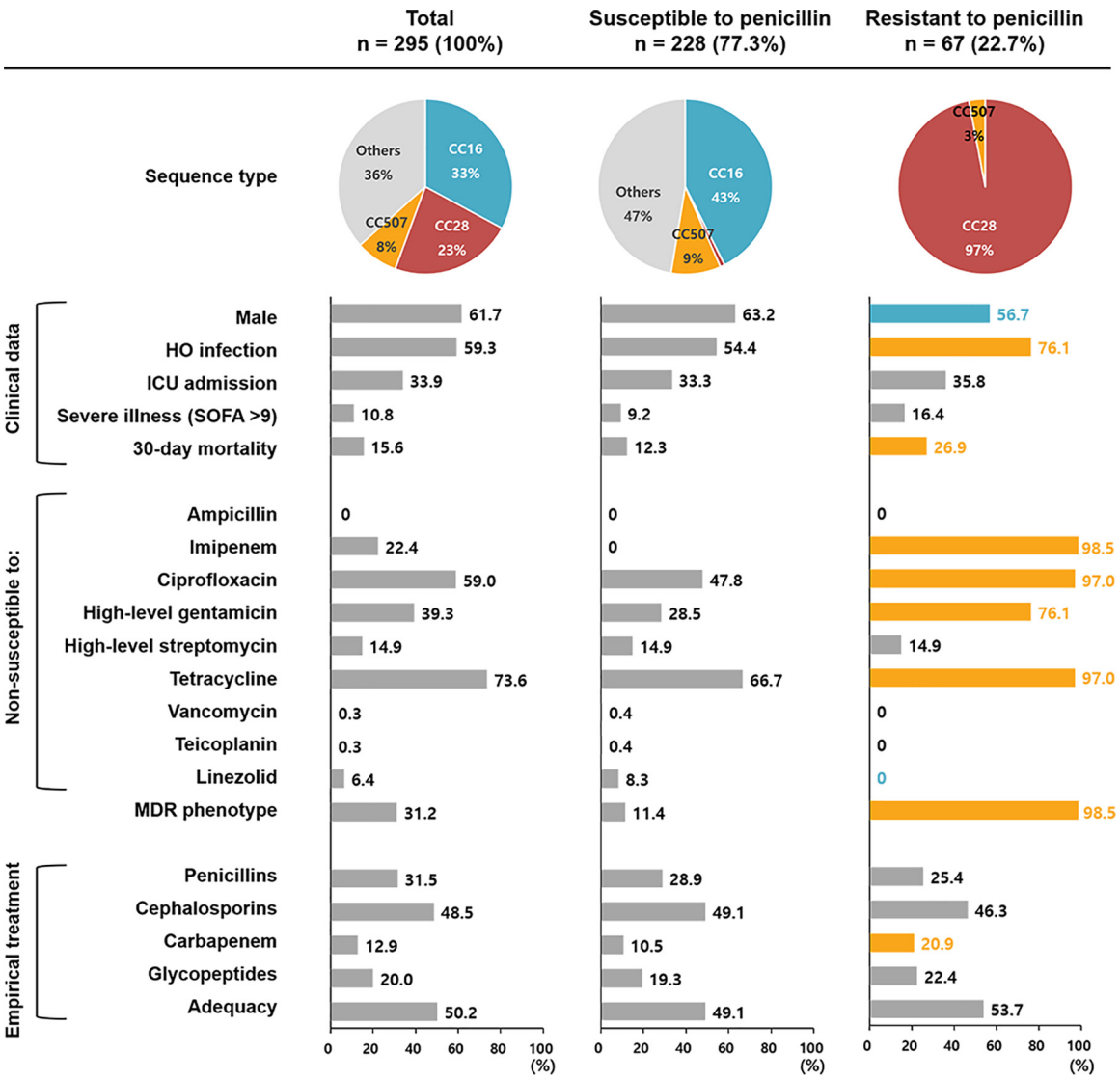


FIG 1 Comparison of characteristics of patients with *E. faecalis* BSIs and their causative pathogens stratified according to penicillin susceptibility. The pie charts indicate the proportions of major sequence types, and the proportions of each variable are indicated in the bar charts. Statistical significance ($P < 0.05$), assessed using the chi-square test, is presented for the penicillin-resistant group with colored bars and numbers (orange, more prevalent; blue, less prevalent).

29212. All 67 CC28 isolates, including 65 ASPR isolates and 2 penicillin-susceptible isolates, shared both substitutions T50I in the nPBD and A369V in the PBD, and the 65 ASPR isolates of CC28 carried 2 to 4 additional substitutions in the PBD along with a nucleotide deletion in the promoter region of the *pbp4* gene. All 23 CC507 isolates shared the A369V and P520S substitutions in the PBD; however, two CC507 isolates exhibiting ASPR phenotypes did not show any additional substitution in PBP4, nor did they show a nucleotide deletion in the promoter region of the *pbp4* gene. Other amino acid substitutions in PBP4 observed in penicillin-resistant isolates in previous studies, i.e., L218N and V231I in the nPBD and D573E, A617T, and D632E in the PBD, were not identified in this study (12, 13).

DISCUSSION

Clinical isolates of *E. faecalis* are mostly susceptible to ampicillin *in vitro*. MICs of penicillin and ampicillin show good concordance, and most *E. faecalis* clinical isolates are regularly cross-susceptible to penicillin and imipenem (14). Therefore, antimicrobial susceptibility testing for either ampicillin or penicillin has been recommended as a

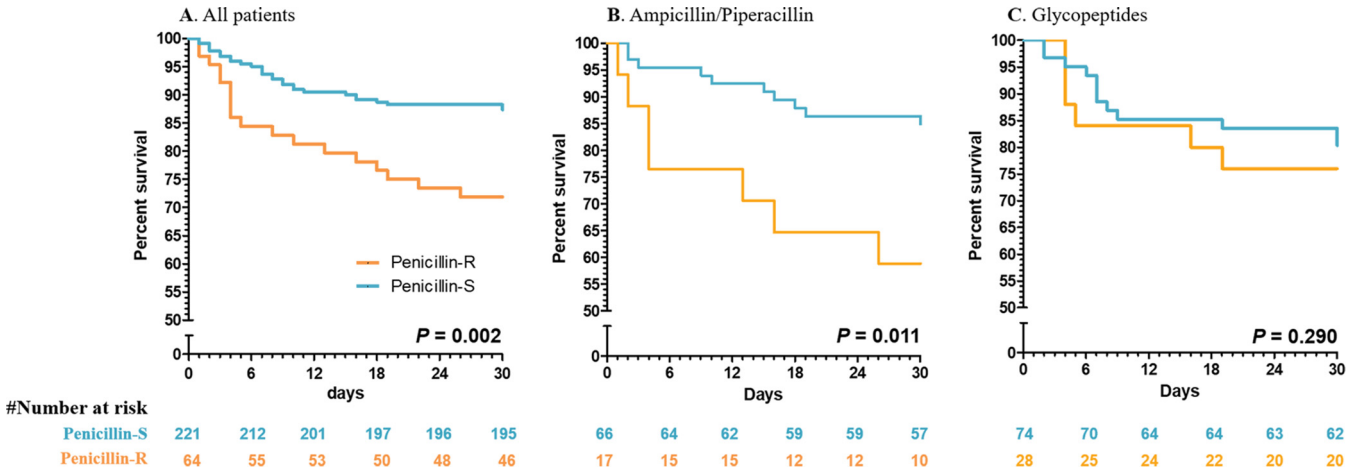


FIG 2 Survival analysis. Kaplan-Meier curves were constructed for three groups stratified by penicillin susceptibility: all patients (A), patients treated with an ampicillin- and/or a piperacillin-based regimen as a definitive treatment (B), and patients treated with a glycopeptide-based regimen as a definitive treatment (C). Statistical significance was determined using the log rank test.

critical assessment to determine an antimicrobial regimen for bacterial cell wall inhibition in patients with an enterococcal BSI (5). Recently, ASPR *E. faecalis* clinical strains have been identified worldwide (9–11); however, to the best of our knowledge, the adequacy of the cell wall-inhibiting beta-lactam regimens as a treatment for ASPR *E. faecalis* BSIs has not yet been evaluated.

ASPR phenotypes were identified in a considerable proportion (22.7%) of *E. faecalis* blood isolates collected in this study, ranging from 14.0% to 35.2% by sentinel hospital (Fig. 4). The rate of resistance to penicillin in *E. faecalis* clinical isolates has rarely been reported so far, although a high frequency of ASPR *E. faecalis* isolates, at 30% of the total, was described in Brazil in 2006 (11). Multivariable analyses using putative risk factors, including host-, treatment-, and pathogen-related variables, showed that the penicillin resistance of the causative pathogen was an independent risk factor for 30-day mortality. In addition, a significant difference in survival was observed in a group of patients treated with ampicillin and/or piperacillin who were stratified according to the penicillin susceptibility of the causative pathogen. Based on these observations, this study provides the first clinical evidence that the administration of ampicillin or piperacillin to patients with an ASPR *E. faecalis* BSI might lead to a treatment failure, resulting in a high early mortality rate for patients even though they exhibited *in vitro* susceptibility to ampicillin.

The Clinical and Laboratory Standards Institute (CLSI) recommends clinical break-

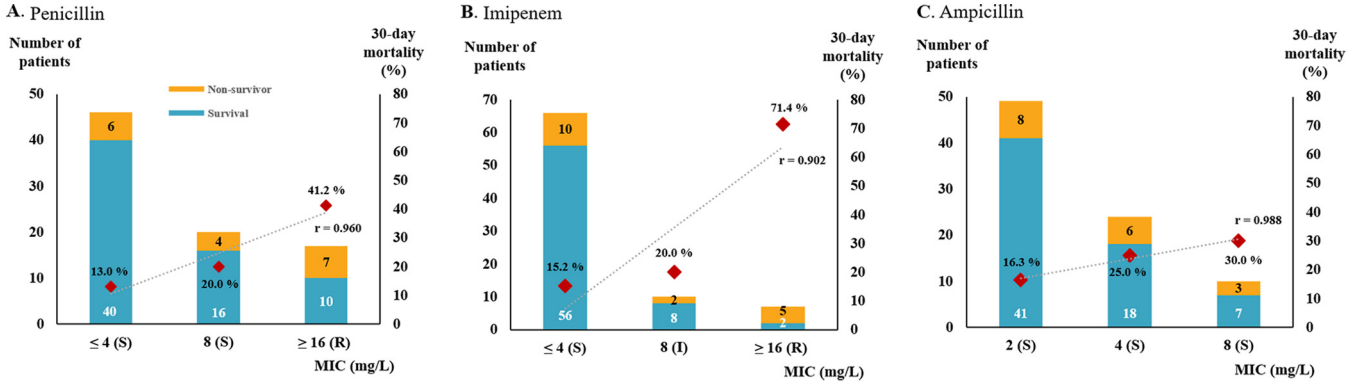


FIG 3 Thirty-day mortality rates in patients treated with an ampicillin- and/or a piperacillin-based regimen stratified according to the MICs of penicillin, imipenem, and ampicillin. Bar charts indicate numbers of patients stratified by the MIC of each antimicrobial. Red rhombi indicate 30-day mortality rates. As MICs of each antimicrobial increase, trends in the 30-day mortality rates are plotted with dotted lines obtained from Pearson's correlation coefficients.

TABLE 2 Amino acid substitutions in PBP4 and a nucleotide deletion in the promoter region of the *pbp4* gene compared with MICs of penicillin, imipenem, and ampicillin[d]

Study (reference) and strain type	No. of isolates	Nucleotide deletion in promoter region[a]	Alteration in PBP4[b]																No. of isolates with MICs (μg/ml) of antimicrobial[c]														
			nPBD			PBD													Penicillin							Imipenem						Ampicillin				
			T50I	L218N	V223I	A369V	T418A	L475M	F499I	P520S	A536T	D573E	A617T	D632E	M652L	D666P	≤1	2	4	8	16	32	64	≤1	2	4	8	16	32	≤1	2	4	8	16
This study																																	
CC28	63	O	O			O	O	O	O							O					19	43	1				**25**	34	4		1	26	36	
	1	O	O			O	O	O								O					1					1						1	
	1	O	O			O									O	O					1						**1**					1	
	2		O			O												2							1	1				1	1		
CC16	51					O				O								1	31	19					12	39				7	32	12	
	46					O											1	4	36	5				4	18	24				16	23	7	
CC507	23					O				O								1	15	5	2			2	15	4	**2**			7	10	6	
Others	106					O												14	63	29				9	50	47				33	58	14	1
	2					O				O									2							2				1	1		
Conceição et al. (12)																																	
CC9	4	NE										O									1	2	1				**1**	1	2		1	1	2	
	1	NE									O	O										1							1				1	
	1	NE										O		O								1							1				1	
NE	10	NE										O									8	2					**2**	3	5			6	4	
	1	NE		O								O									1								1			1	
Rice et al. (13)																																	
NE	1	O			O								O								1							1					1

[a]Deletion of a single A residue in a string of seven A residues upstream of a putative −35 region for the *pbp4* gene described previously by Rice et al. (13).

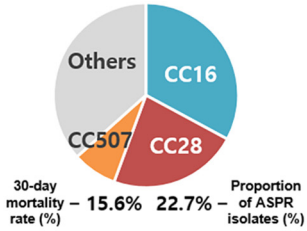
[b]The amino acid sequences of PBP4 of *E. faecalis* isolates were compared with that of the reference *E. faecalis* strain ATCC 29212.

[c]MICs designating intermediate resistance and resistance are indicated by boldface type and gray-shaded areas, respectively.

[d]"O" indicates that the specified sequence variant was identified. Abbreviations: CC, clonal complex; nPBD, non-penicillin-binding domain; NE, not evaluated; PBD, penicillin-binding domain.

Total

Beds	5,194
Patients subjected to blood culture	131,024
Patients with *E. faecalis* BSIs	296 (0.23%)
Excluded patients	1
Enrolled patients	295



Sentinel hospital A

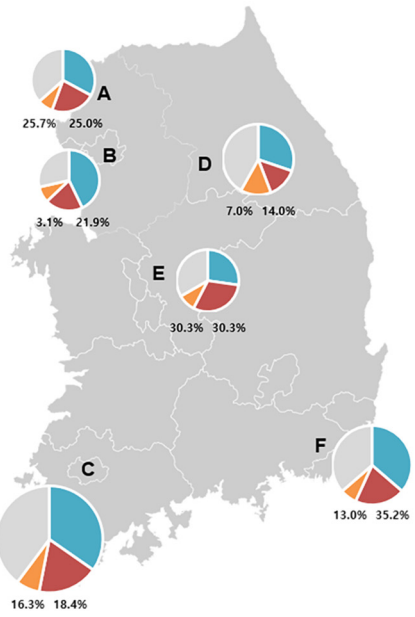
Beds	818
Patients subjected to blood culture	19,197
Patients with *E. faecalis* BSIs	35 (0.18%)
Excluded patients	0
Enrolled patients	35

Sentinel hospital B

Beds	815
Patients subjected to blood culture	18,568
Patients with *E. faecalis* BSIs	33 (0.18%)
Excluded patients	1
Enrolled patients	32

Sentinel hospital C

Beds	1,050
Patients subjected to blood culture	30,691
Patients with *E. faecalis* BSIs	98 (0.32%)
Excluded patients	0
Enrolled patients	98



Sentinel hospital D

Beds	851
Patients subjected to blood culture	26,778
Patients with *E. faecalis* BSIs	43 (0.16%)
Excluded patients	0
Enrolled patients	43

Sentinel hospital E

Beds	823
Patients subjected to blood culture	20,863
Patients with *E. faecalis* BSIs	33 (0.16%)
Excluded patients	0
Enrolled patients	33

Sentinel hospital F

Beds	837
Patients subjected to blood culture	14,927
Patients with *E. faecalis* BSIs	54 (0.36%)
Excluded patients	0
Enrolled patients	54

FIG 4 Geographic distribution of sentinel hospitals (hospitals A to F) in the Korean peninsula. The numbers of beds and *E. faecalis* bloodstream infection cases in each hospital are indicated in the tables. The pie charts indicate the proportions of major sequence types of causative *E. faecalis* strains, and the percentages below the pie charts indicate the 30-day mortality rates for patients with *E. faecalis* BSIs and the proportions of ASPR strains. The relative sizes of the pie charts indicate the number of *E. faecalis* cases in each hospital.

points of resistance determination as MICs of ≥16 μg/ml for both ampicillin and penicillin for enterococci (15). However, all isolates in the present study had 1- to 3-fold-lower MICs for ampicillin than for penicillin (MIC_{50} of 2 μg/ml and MIC_{90} of 8 μg/ml for ampicillin; MIC_{50} of 4 μg/ml and MIC_{90} of 32 μg/ml for penicillin), consistent with data from previous reports (12, 14). The emergence of ASPR *E. faecalis* might be due to the breakpoint. From this perspective, penicillin is a better surrogate marker than ampicillin in tests of the antimicrobial susceptibilities of *E. faecalis* clinical isolates to antienterococcal beta-lactam agents to avoid reporting substantial very major errors in the results. Furthermore, additional MIC determinations for penicillin should be performed for *E. faecalis* isolates exhibiting MICs of 4 or 8 μg/ml for ampicillin to exclude the ASPR phenotypes.

Among the 8 amino acid substitutions in PBP4 identified in this study, both T50I and A369V were reported to be irrelevant to the beta-lactam resistance of bacterial hosts, consistent with our findings (16). P520S was suggested to be a key substitution required for the development of high-level resistance to beta-lactams in *E. faecalis* isolates (16); however, this substitution was identified in both penicillin-susceptible and -resistant isolates in this study, indicating that P520S might be irrelevant to the beta-lactam resistance of bacterial hosts. The remaining 5 amino acid substitutions in PBP4, T418A, L475M, F499I, M652L, and D666P, were novel, and they were exclusively identified in 65 ASPR *E. faecalis* isolates of CC28. Additional studies are needed to investigate the roles of these substitutions in elevating the MICs of penicillin for bacterial hosts. In addition, the nucleotide deletion of a single A residue in the

promoter region of the *pbp4* gene, identical to that described previously by Rice et al., was also identified in these 65 ASPR isolates, which might result in overexpression of the altered PBP4 protein (13). However, two ASPR *E. faecalis* isolates of CC507 did not display any relevant amino acid substitutions in PBP4, nor did they show any nucleotide deletions in the promoter region, suggesting that the resistance phenotypes might be related to alterations in another PBP.

A limitation of the present study is that the sentinel hospitals are geographically restricted to a single country, South Korea. Therefore, ethnic or racial diversities were not considered in this study, and the high proportion of ASPR *E. faecalis* isolates observed in this study might be limited to South Korea. However, previous studies have reported the emergence of ASPR strains in other countries (9–11). In addition, ASPR *E. faecalis* isolates were identified in all sentinel hospitals in this study, although they are located in different provinces. The lack of a functional evaluation of amino acid substitutions in PBP4 represents another potential limitation of the study. Further studies should be performed to confirm the possible changes in the beta-lactam affinities of PBP4 induced by the amino acid substitutions.

In conclusion, in the present prospective, multicenter, observational study, ASPR phenotypes in *E. faecalis* resulted in an inadequate choice of antimicrobial treatment regimens for patients with BSIs, ultimately resulting in fatal clinical outcomes. ASPR *E. faecalis* should be considered clinically resistant to all antienterococcal beta-lactams, including ampicillin and piperacillin, when choosing antimicrobial regimens for the definitive treatment of BSIs.

MATERIALS AND METHODS

Study design. This prospective observational study was performed with all patients with an *E. faecalis* BSI during the 2-year period between May 2016 and April 2018 in six general hospitals participating in the Global Antimicrobial Surveillance System (GLASS) in South Korea (Kor-GLASS) (17). The six hospitals are located in different districts of the Korean peninsula, and the number of beds ranges from 815 to 1,050 per hospital (Fig. 4). A total of 131,024 patients were subjected to blood culture for suspected BSIs, and 296 patients (0.2%) were diagnosed with an *E. faecalis* BSI. Clinical information about the demographic conditions, underlying diseases, and antimicrobial treatments were retrieved from the electronic medical records of each hospital. The Charlson comorbidity index and the sequential organ failure assessment (SOFA) score were assessed at the time of the initial blood culture (18, 19). Clinical outcomes were assessed by calculating the 30-day mortality rate. Only the first isolate from each patient was collected for microbiological evaluation, and sequential isolates were discarded. All the isolates collected at each hospital were transferred to the analysis center. The requirement for informed consent from the participants was waived by all local ethical committees of the six sentinel hospitals.

Definition. A hospital-originated (HO) infection was defined when an initial blood culture was performed after ≥2 calendar days of hospitalization. The primary infection site was determined when a site-specific infection with an *E. faecalis* isolate was identified at another body site by bacterial culture (20). The multidrug-resistant (MDR) phenotype of the causative pathogen was determined as an *in vitro* lack of susceptibility to ≥3 drug classes, as described previously by Magiorakos et al. (21). Empirical antimicrobial treatment was defined as an initial blind antimicrobial treatment without *in vitro* antimicrobial susceptibility information for the responsible pathogen, and definitive treatment was defined as a revised antimicrobial treatment based on the *in vitro* susceptibility of the responsible pathogen within 72 h from the initial blood culture. An ampicillin- or piperacillin-based regimen was defined as an administration of ampicillin or piperacillin with or without any combination with drugs of other classes except glycopeptides, and a glycopeptide-based regimen was defined as the administration of vancomycin or teicoplanin with or without any combination with drugs of other classes. An appropriate antimicrobial treatment was defined as the use of *in vitro*-susceptible cell wall-inhibiting antimicrobials, irrespective of their combination with drugs of other classes.

Microbiological analysis. Bacterial species were identified with a MALDI Biotyper (Bruker Daltonik GmbH, Bremen, Germany) and using 16S rRNA gene sequencing. MICs of ampicillin, penicillin, imipenem, vancomycin, teicoplanin, gentamicin (high level), and streptomycin (high level) were determined with the broth microdilution test using Mueller-Hinton broth (Difco Laboratories, Detroit, MI) according to CLSI guidelines (15). In addition, antimicrobial susceptibilities to ciprofloxacin and tetracycline were tested using the disc diffusion method on cation-adjusted Mueller-Hinton agar (Difco Laboratories). For penicillin-nonsusceptible isolates, beta-lactamase production was assessed using the nitrocefin disc test. Nucleotide sequences of the *pbp4* gene and its promoter region for all the *E. faecalis* isolates were determined by PCR and direct sequencing using primer sets, as previously described (12, 16), and were compared to those of the *E. faecalis* reference strain ATCC 29212. The *vanA*, *vanB*, and *vanM* genes were detected in vancomycin-nonsusceptible isolates using PCR (22). For strain typing, multilocus sequence typing (MLST) was performed by comparing partial sequences of seven housekeeping genes,

gdh, *gyd*, *pstS*, *gki*, *aroE*, *xpt*, and *yqiL*, to the *E. faecalis* MLST database (https://pubmlst.org/efaecalis/) to determine the allelic types and the sequence types (STs) (23).

Statistical analysis. Analyses were performed using R software version 3.4.3 (R Development Core Team 2017 [http://www.R-project.org/]). Differences between groups were analyzed using the Mann-Whitney U test and Fisher's exact test for continuous variables and categorical variables, respectively. A univariable logistic regression analysis was performed to calculate odds ratio (OR) of each variable to 30-day mortality. Adjusted ORs (aORs) were calculated for variables with P values of <0.05 using a forward stepwise multivariable logistic regression analysis. Kaplan-Meier curves were constructed, and log rank tests were performed to compare the mortality dynamics between groups. The results of the statistical analyses were considered significant when the P values were <0.05.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00291-19.

SUPPLEMENTAL FILE 1, PDF file, 0.05 MB.

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We do not have conflicts of interest to declare.

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