

Clinical and microbiological characteristics of vancomycin-resistant *Enterococcus faecium* bloodstream infection in Central Taiwan

Chang-Hua Chen, MD, HSc, PhD^{a,b,c,*}, Li-Chen Lin, MT^a, Yu-Jun Chang, PhD^d, Chih-Yen Chang, MD^e

Abstract

Bloodstream infections (BSIs) due to vancomycin-resistant *Enterococcus faecium* (VRE_{fae}) remain a therapeutic challenge. This study aimed to evaluate mortality from BSIs due to VRE_{fae} in Central Taiwan.

We retrospectively analyzed patients with significant VRE_{fae} BSIs in the Changhua Christian Hospital System between January 1, 2010 and December 31, 2014.

Of the 152 patients with *Enterococcal* BSI, 56 patients (36.8%) were admitted to intensive care units (ICUs) at the onset of BSI and 20 (13.2%) patients were associated with polymicrobial bacteremia. VRE_{fae} BSI was observed in 36 (23.7%) patients. *Van A* (100%) is the prevalence genotype, and ST 17 (41.7%) is the predominant ST type among 36 VRE_{fae} isolates during the study period. The 30-day mortality rate was 13.2% (20/152). The multivariate logistic regression analysis showed that the onset of VRE_{fae} BSI in the ICU (odds ratio [OR]=4.2, 95% confidence interval [CI]=1.7–10.0, *P*=.002) was a significant risk factor for 30-day mortality, whereas an appropriate antimicrobial therapy was a protective factor for 30-day mortality (OR=0.33, 95% CI=0.14–0.79, *P*=.013).

Our results underscore the need to assist patients who are admitted to ICUs with VRE_{fae} BSIs. We emphasize the use of an appropriate antimicrobial therapy for VRE_{fae} BSI with the aim to treat more patients with these infections.

Abbreviations: BSI = bloodstream infections, CC = clonal complex, CCH = Changhua Christian Hospital, CCHS = Changhua Christian Hospital System, CI = confidence interval, ICUs = intensive care units, MIC = minimum inhibitory concentration, MLST = multilocus sequence typing, OR = odds ratio, PCR = polymerase chain reaction, VRE = vancomycin-resistant enterococci, VRE_{fae} = vancomycin-resistant *Enterococcus faecium*.

Keywords: bloodstream infection, *Enterococcus faecium*, mortality, multilocus sequence typing, vancomycin-resistant enterococci

1. Introduction

The management of bloodstream infections (BSIs) due to *Enterococcus faecium*, particularly those due to vancomycin-

resistant enterococci (VRE), has become a therapeutic challenge. BSIs due to vancomycin-resistant *E faecium* (VRE_{fae}) were first reported in 1988, and a short time later, they were reported in the USA and European countries.^[1] The first VRE BSI in Taiwan was reported in 1996.^[2] In the USA, VRE has become an important nosocomial pathogen, and their ratio among all nosocomial pathogens has increased from 0.3% in 1989 to nearly 30% in 2003 according to data from the National Nosocomial Infections Surveillance System.^[3] In addition, VRE accounts for 14% of the enterococcal isolates among patients in intensive care units (ICUs).^[3] Although VRE represented <2% of the enterococci identified in an antimicrobial resistance surveillance program in Taiwan in 2000,^[4] a rapid increase in vancomycin resistance from 12.4% in 2007 to 42.0% in 2016 among nosocomial enterococcal isolates in ICU was reported by the Taiwan Nosocomial Infection Surveillance System.^[5] The persistence of *E faecium* is a cause for concern because the treatment options and infection control measures are limited, and there is a low clinical awareness. Therefore, BSIs due to VRE_{fae} are an important clinical issue.

Most studies on BSI due to enterococcal infections focus on the general infection, geography of the infection, and vancomycin resistance.^[6,7] In addition, the majority of studies published to date have been conducted in the USA, where the epidemiological conditions differ from those occurring in Asia.^[8] The risk factors associated with in-hospital mortality from BSIs due to *E faecium* have been reported,^[9] but most of these factors are indicators in basic medical sciences, and the molecular biomarkers are not useful to physicians. Furthermore, information regarding BSIs due to VRE_{fae} in Central Taiwan is particularly scarce.^[9–11] An

Editor: Duane R. Hopenhthal.

Funding: Changhua Christian Hospital (grant 104-CCH-IPR-001 and 105-CCH-IPR-001).

Ethical approval: The study was approved by the institutional review board of Changhua Christian Hospital (CCH IRB No. 140304).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

^a Center of Infection Prevention and Control, ^b Division of Infectious Diseases, Department of Internal Medicine, Yuanlin Christian Hospital, Changhua, ^c Department of Nursing, College of Medicine & Nursing, Hung Kuang University, Sha-lu District, Taichung, ^d Epidemiology and Biostatistics Center, Changhua Christian Hospital, ^e Department of Internal Medicine, Yuanlin Christian Hospital, Changhua, Taiwan.

* Correspondence: Chang-Hua Chen, Division of Infectious Diseases, Department of Internal Medicine, Changhua Christian Hospital, Changhua, Taiwan (e-mail: chenchanghuachad@gmail.com)

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

Medicine (2017) 96:49(e9000)

Received: 7 February 2017 / Received in final form: 8 November 2017 /

Accepted: 9 November 2017

<http://dx.doi.org/10.1097/MD.0000000000009000>

increasing resistance rate to vancomycin among enterococcal isolates has been documented globally.^[12] The clonal spread of certain epidemic *VREfae* strains belonging to clonal complex 17 (CC17) contributed to this increase.^[13]

Therefore, the aim of the present study was to analyze the clinical features and microbiological characteristics of BSIs due to *VREfae* in Central Taiwan.

2. Materials and methods

2.1. Clinical setting and study population

The population living in the rural areas of Central Taiwan is mostly served by the Changhua Christian Hospital System (CCHS), which consists of 4000 beds. The Changhua Christian Hospital (CCH) is the largest hospital among 9 branch hospitals in the CCHS and is an 1800-bed tertiary referral medical center situated in Central Taiwan. This study was conducted in the CCHS and was approved by the institutional review board of CCH. Cases of BSIs due to *VREfae* were analyzed by reviewing the medical records, and a cross-sectional retrospective study was conducted between January 1, 2010 and December 31, 2014 in the CCHS.

2.2. Patient identification, patient analysis, and definition

The occurrence of symptomatic enterococcal BSI was defined as >2 blood cultures positive for *Enterococcus* sp. or a single blood culture positive for *Enterococcus* sp. combined with a significant source of infection. Patients with BSI due to *VREfae* were identified from the microbiological databases and medical records according to the ninth edition of the International Classification of Diseases, with some clinical modifications (ICD-9-CM) in the CCHS in Central Taiwan. We used computerized indices to identify cases with the following ICD-9-CM codes: V09.8, 041.04, 790.7, 038, and 038.9. All the patients documented with BSI due to *VREfae* were enrolled during the study period. The exclusion criteria included age <18 years, inadequate clinical data, inconsistency between the data from the ICD-9 code and the microbiological dataset, and misinterpretation of the microbiological examinations. A standardized case report form was used to collect the data contained in the medical records regarding the medical diagnoses, medical treatment, and other key information. The medical records of all cases involving BSI due to *VREfae* were manually reviewed by the primary investigator (CCH) to confirm the diagnosis (using CCHS resources). Clinically equivocal cases were discussed and decisions were made by the primary and the secondary investigator (CCY). Only the first episode of BSI due to *VREfae* in each patient during the study period was included in the statistical analysis.

The date of the onset of BSI was defined as the date on which the culture from the first blood sample was positive. The case group was defined as BSIs due to vancomycin-resistant *E faecium* (*VREfae*), and the control group was defined as enterococcal BSIs other than *VREfae*. Polymicrobial bacteremia was defined as the presence of >1 microorganism from the same blood culture specimen. The underlying severity was classified according to the McCabe and Jackson criteria.^[14] During the initial 24 hours from the onset of BSI due to *VREfae*, the systemic inflammatory response syndrome, sepsis, severe sepsis, and septic shock were defined as previously described.^[15] The source of infection was defined as the site of infection where it was microbiologically and

clinically documented.^[15] The outcome was evaluated 30 days after the onset of BSI due to *VREfae* in the CCHS. Death was considered associated with BSI when the patient died <2 weeks after the onset of BSI, and no other cause of death was identified. The all-cause 30-day mortality group was defined as patients with BSI due to *VREfae* who died within 30 days of diagnosis. BSI due to *VREfae* was classified as healthcare-onset or community-acquired according to a modified version of Horan's description.^[16] An appropriate antimicrobial therapy was defined as patients who received effective antibiotics as evaluated by susceptibility tests during the period of effective empirical therapy.

2.3. Microbiological identification, susceptibility test, and molecular analysis

Between January 1, 2010 and December 31, 2014, all *VREfae* isolates cultured from blood samples were considered significant findings, and 36 clinical isolates were identified as VRE in the CCHS in Central Taiwan. Only the initial isolate from each patient was analyzed. The laboratory identification of *Enterococcus* spp. was performed using the 6.5% NaCl/bile-esculin agar test and confirmed with the Vitek-2 gram-positive identification system (bioMérieux, Marcy l'Etoile, France) at CCHS. Then, those isolates were sent to a reference laboratory (Research Laboratory, Division of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital) for re-confirmation. The susceptibility to various antimicrobial agents by determination of MICs was performed with the Vitek-2 system (bioMérieux, Marcy l'Etoile, France).^[17] The following drugs were tested: ampicillin, ciprofloxacin, erythromycin, linezolid, minocycline, penicillin, rifampin, teicoplanin, tigecycline, and vancomycin. The interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) were used to determine the isolate susceptibility.^[17] Vancomycin resistance was defined as a minimum inhibitory concentration (MIC) ≥ 32 $\mu\text{g/mL}$.

The genotypes of *VREfae* were determined using polymerase chain reaction (PCR)-based methods as described previously.^[18] Multilocus sequence typing (MLST) for *VREfae* was also performed as previously reported.^[19]

2.4. Statistical analysis

Continuous variables were expressed as the mean \pm standard deviation or the median and range if the distribution was not normal and were compared using Student's *t*-test or Mann-Whitney U test. Continuous data were expressed as the mean \pm standard deviation or as the median and range. Univariate and multivariate analyses were performed to determine the independent risk factors for the all-cause 30-day mortality using logistic regression models. A *P*-value <.05 was considered statistically significant. All tests were performed using SPSS V.17.0 for Windows (SPSS Inc, Chicago, IL).

3. Results

3.1. Study population

Two hundred seventy-nine patients at CCHS were found to have a positive blood culture for enterococci. Among them, 177 patients were at CCH, and the other 102 patients were at other branches of CCHS. Symptomatic BSI was identified in 194

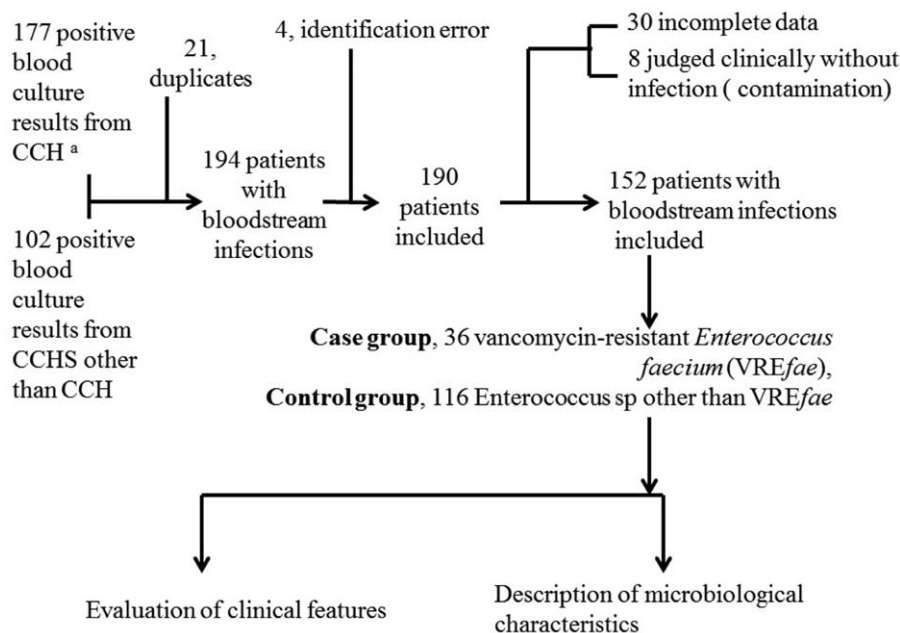


Figure 1. Concept frame of this study. CCH=Changhua Christian Hospital, CCHS=Changhua Christian Hospital System, VRE*fae*=vancomycin-resistant *Enterococcus faecium*. ^aPartial data were reported at Rev Chilena Infectol (Chang-Hua C, Li-Chen L, Yu-Jun C, Chih-Yen C.[Mortality analysis of *Enterococcus faecium* bloodstream infection in central Taiwan]. *Rev Chilena Infectol* 2016 Aug;33(4):395–402.[Article in Spanish]).

patients after excluding duplicates from 21 patients. Thirty-eight patients were excluded because 30 patients had incomplete data, and 8 patients were clinically determined to be without significant evidence of an infection; therefore, 152 patients were analyzed (Fig. 1).

3.2. Clinical features of the case group (BSI due to VRE*fae*) and control group (BSI other than VRE*fae*)

The epidemiological characteristics and underlying conditions of the 152 patients are shown in Table 1. The distribution of all analyzed characteristics was not significantly different between the case group (36/152, 23.7%) and the control group (116/152, 76.3%). Thirty-nine patients (51.8%) were men, and the median age was 59.1 years (range, 21–89 years). The most common underlying disease was biliary disease (75.0%), followed by solid cancer (44.7%). According to the McCabe and Jackson criteria, the underlying diseases were nonfatal in 82 patients (53.9%), ultimately fatal in 68 patients (44.7%), and rapidly fatal in 2 patients (1.3%). Forty-six (30.3%) patients had a history of hospital admissions, and 29 patients (19.1%) had biliary drainage catheters in place. Thirty-nine (51.8%) BSI episodes occurred in the hospital with a median admission period of 9.0 days (standard deviation, 6.8 days; range, 3–89 days) before the onset of BSI. Fifty-two patients (36.8%) were in ICUs at the onset of BSI. Of the enterococcal isolates recovered from the 152 patients, 36 (23.7%) were VRE*fae*. Twenty (13.2%) patients had polymicrobial bacteremia with microorganisms, including *Escherichia coli* (8 patients), *Klebsiella pneumoniae* (4 patients), *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Citrobacter freundii*, and *Acinetobacter baumannii* (2 patients each). Of the 20 patients with polymicrobial bacteremia, 6 (30%) involved VRE*fae* (30.0%). Of the 152 patients evaluated, only 6 patients received parenteral vancomycin before developing BSI due to

VRE*fae*, and none received oral vancomycin. The 30-day mortality rate was 13.2% (20 of 152 patients). There were significant statistical differences between VRE*fae* BSI and enterococcal BSI other than VRE*fae* BSI upon admission at the medical ICU in terms of the onset of BSI ($P=.035$) and the appropriate antimicrobial therapy ($P=.025$). The multivariate logistic regression analysis, including a univariate analysis of variables with $P < .05$, showed that the onset of VRE*fae* BSI in ICUs (odds ratio [OR]=4.2, 95% confidence interval [CI]=1.7–10, $P=.002$) was the only significant risk factor for 30-day mortality. An appropriate antimicrobial therapy was a protective factor for 30-day mortality (OR=0.33, 95% CI=0.14–0.79, $P=.013$).

3.3. Microbiological characteristics of 36 VRE*fae*

The antibiotic susceptibility of the 36 VRE*fae* isolates is shown in Table 2. The genotypic testing for 36 VRE*fae* showed that all isolates carried the *van A* gene. The results of the MLST and their relationships with antibiotic susceptibilities for the 36 VRE*fae* isolates are shown in Table 2. Overall, all isolates were susceptible to linezolid. All 36 VRE*fae* isolates were resistant to ampicillin, penicillin, ciprofloxacin, and vancomycin. All isolates were resistant to erythromycin except for 4 ST-17 isolates. Two isolates (5.6%) were susceptible to teicoplanin, and both were of the *van A* genotype. The resistance rates to minocycline and tigecycline varied with different STs. Among the VRE*fae* isolates, there were 2 major STs as follows: ST-17 and ST-78. Both ST-17 and ST-78 accounted for 59.6% of all VRE*fae* isolates tested. Before 2010, ST-414 and ST-18 were the 2 predominant STs, accounting for 79.6% of isolates (Appendix 1, <http://links.lww.com/MD/B990>). However, ST-17 (41.7%) and ST-78 (18.0%) were the predominant STs during this study period (Fig. 2).

Table 1**Clinical features of patients in the case group (VRE*fae* BSI) and control group (enterococcal BSI other than VRE*fae*).**

Characteristic	Control, n = 116 (%)	Case, n = 36 (%)	All, n = 152 (%)	P
Male sex	62 (53.4)	16 (44.4)	101 (51.3)	.672
Mean age, years (\pm standard deviation) (range)	62.1 \pm 9.9 (21–89)	58.1 \pm 19.8 (40–77)	59.1 \pm 17.8 (21–89)	.149
<i>Underlying diseases*</i>				
Biliary disease	88 (75.9)	26 (72.2)	114 (75)	.792
Solid tumor cancer	56 (48.2)	12 (33.3)	68 (44.7)	.689
Hypertension	16 (13.8)	8 (22.2)	24 (15.8)	.412
Diabetes mellitus	14 (12.1)	8 (22.2)	22 (14.5)	.517
Liver cirrhosis	6 (5.2)	2 (5.6)	8 (5.3)	.289
Neurologic disease	0	4 (11.1)	4 (2.6)	–
Multiple trauma	2 (1.7)	0	2 (1.3)	–
Haematological malignancy	2 (1.7)	0	2 (1.3)	–
<i>McCabe and Jackson criteria</i>				
Nonfatal disease	54 (46.6)	28 (77.8)	82 (53.9)	.165
Ultimately fatal disease	60 (51.7)	10 (27.8)	70 (44.7)	.412
Rapidly fatal disease	2 (1.7)	0	2 (1.3)	–
<i>Risk factors*</i>				
Biliary drainage catheter	21 (21.6)	4 (11.1)	35 (19.1)	.415
Prior hospital admission within 6 months	34 (29.3)	12 (33.3)	46 (30.3)	.75
Leukopenia [†]	16 (13.8)	2 (5.6)	18 (13.2)	.83
Central venous catheter	6 (5.2)	8 (22.2)	14 (9.2)	.075
Indwelling urinary catheter	2 (1.7)	8 (22.2)	8 (6.6)	.066
Prior intensive care unit care [‡]	2 (1.7)	8 (22.2)	10 (6.6)	.066
Recent surgery [‡]	2 (1.7)	4 (11.1)	6 (3.9)	.897
Mechanical ventilation	2 (1.7)	4 (11.1)	6 (3.9)	.79
Immunosuppressive therapy [§]	8 (6.9)	0	8 (5.3)	–
Bleeding in prior 2 weeks	2 (1.7)	0	2 (1.3)	–
Cancer chemotherapy [‡]	2 (1.7)	0	2 (1.3)	–
<i>Place of bacteremia acquisition</i>				
Hospital	62 (53.4)	16 (44.4)	78 (51.3)	.064
Community	54 (46.5)	20 (55.6)	74 (48.7)	.076
<i>Ward at the time of bacteremia</i>				
Medical ward	60 (51.7)	16 (44.4)	76 (50)	.059
Emergency room	40 (34.5)	10 (27.8)	50 (31.6)	.067
Surgical ward	14 (12.1)	4 (11.1)	18 (11.9)	.78
Medical intensive care unit	40 (34.5)	18 (50)	44 (28.9)	.035
Surgical intensive care unit	2 (1.7)	0	2 (1.3)	–
<i>Length of hospital stay before bacteremia</i>				
median days (range)	10.0 (3–46)	8.0 (3–89)	9.0 (3–89)	.875
Appropriate antimicrobial therapy	106 (91.4)	8 (22.2)	114 (88.2)	.025
<i>Initial manifestation within 24 hours</i>				
Bacteremia without SIRS	20 (17.2)	10 (27.8)	30 (19.7)	.418
Sepsis	80 (68.9)	22 (61.1)	102 (65.8)	.68
Severe sepsis	12 (10.3)	2 (5.5)	14 (9.2)	.79
Septic shock	6 (5.2)	2 (5.5)	8 (5.3)	.789
Polymicrobial bacteremia	16 (13.8)	4 (11.1)	20 (13.2)	.429
<i>Source</i>				
Biliary tract source	21 (21.6)	4 (11.1)	29 (19.1)	.412
Center catheter source	5 (4.3)	4 (11.1)	9 (5.9)	.076
Urinary tract source	5 (4.3)	4 (11.1)	9 (5.9)	.067
Respiratory tract source	4 (3.4)	2 (5.6)	6 (3.9)	.059
Wound source, skin and soft tissue source	4 (3.4)	2 (5.6)	6 (3.9)	.064
Unknown source	79 (68.1)	20 (55.6)	99 (65.1)	.689
<i>Outcome</i>				
All-cause 30-day mortality [¶]	16/116 (13.8)	4/36 (11.1)	20/152 (13.2)	

Data is reported in number (%) of patients, unless otherwise indicated.

BSI = bloodstream infections, SIRS = systemic inflammatory response syndrome, VRE*fae* = vancomycin-resistant *Enterococcus faecium*.

* Some patients had >1 underlying disease (or risk factors).

[†] Leukocyte count <4000/mm³.

[‡] Within the past month.

[§] Receipt of steroid therapy for >10 days or use of other immunosuppressant (tacrolimus, mycophenolate mofetil, azathioprine, cyclosporine A, or anti-CD3 monoclonal antibody) for 11 weeks within the previous 1 month.

^{||} The analysis of hospital-acquired cases only included those acquired at Changhua Christian Hospital System.

[¶] Death from all causes within 30 days.

Table 2

Microbiological characteristics of the 36 VREfae isolates.

Code	Isolated time	Polymerase chain reaction														Minimum inhibitory concentration										
		<i>vanaA</i> / <i>vanB</i>	<i>atpA</i>	<i>ddl</i>	<i>gdh</i>	<i>purK</i>	<i>gyd</i>	<i>psfS</i>	<i>adk</i>	MLST	Ampicillin	Ciprofloxacin	Erythromycin	Linezolid	Minocycline	Penicillin	Rifampin	Teicoplanin	Tigecycline	Vancomycin						
1	M0633	20100202	A	15	1	1	1	1	1	1	1	1	1	1	203	>128	64	16	16	32	0.06	>128	16	32	0.06	>128
2	S001	20100404	A	7	1	1	1	1	1	1	1	1	1	1	17	>128	64	0.25	16	32	0.125	>128	16	32	0.125	>128
3	M0634	20100621	A	7	1	1	1	1	5	1	1	1	1	18	>128	>128	16	16	64	0.125	>128	4	64	0.125	>128	
4	M0631	20100814	A	1	5	1	1	1	1	20	1	1	1	479	>128	128	16	2	16	16	0.06	>128	2	16	0.06	128
5	M0625	20110126	A	7	1	1	1	1	5	7	1	1	1	262	>128	>128	16	2	32	0.25	>128	16	32	0.25	>128	
6	M0628	20110414	A	1	1	1	1	1	1	1	1	1	1	17	>128	>128	16	2	16	0.125	>128	16	64	0.125	>128	
7	M0629	20110430	A	1	1	1	1	1	1	1	1	1	1	17	>128	>128	16	2	16	0.125	>128	16	32	0.125	>128	
8	M0630	20110606	A	1	3	1	1	1	1	1	1	1	1	280	>128	64	16	2	16	0.125	>128	16	64	0.125	>128	
9	M0626	20111012	A	1	1	1	1	1	1	1	1	1	1	17	>128	>128	16	2	32	0.5	>128	128	128	0.5	>128	
10	M0615	20120106	A	7	1	1	1	1	5	1	1	1	1	18	>128	>128	16	2	16	0.25	>128	16	32	0.25	>128	
12	M0620	20120325	A	7	1	1	1	1	5	1	1	1	1	18	>128	>128	16	2	16	0.25	>128	16	32	0.25	>128	
13	S002	20120404	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	0.25	16	32	0.125	>128	16	32	0.125	>128	
14	M0621	20120419	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	16	0.125	>128	16	16	0.125	128	
15	M0622	20120616	A	15	1	1	1	1	1	1	1	1	1	78	>128	64	16	2	16	0.06	>128	16	32	0.06	>128	
16	M0623	20120619	A	15	1	1	1	1	1	1	1	1	1	78	>128	64	16	2	16	0.125	>128	32	64	0.125	>128	
17	M0624	20120705	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	128	0.25	>128	4	128	0.25	>128	
18	M0618	20121108	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	2	2	128	0.25	>128	4	128	0.25	>128	
19	M0616	20121110	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	16	2	32	0.125	>128	4	128	0.125	>128	
20	M0617	20121112	A	7	1	1	1	1	5	1	1	1	1	18	>128	>128	16	2	32	0.125	>128	8	32	0.125	>128	
21	M0619	20121211	A	7	1	1	1	1	5	7	1	1	1	262	>128	>128	16	2	32	0.125	>128	16	32	0.125	>128	
22	M0609	20130313	A	15	5	1	1	1	1	1	1	1	1	341	>128	64	16	2	64	0.06	>128	4	64	0.06	>128	
23	M0610	20130429	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	128	0.125	>128	4	128	0.125	>128	
24	M0613	20130918	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	64	0.125	>128	4	64	0.125	>128	
25	M0611	20131017	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	16	2	128	0.125	>128	4	128	0.125	>128	
26	M0612	20131130	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	64	0.125	>128	4	64	0.125	>128	
27	M0608	20131206	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	64	0.125	>128	4	64	0.125	>128	
28	M0614	20131222	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	32	0.125	>128	4	32	0.125	>128	
29	M0601	20140404	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	2	2	128	0.125	>128	32	128	0.125	>128	
30	M0602	20140503	A	15	1	1	1	1	1	1	1	1	1	78	>128	32	16	16	64	0.06	>128	16	64	0.06	>128	
31	S003	20140504	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	0.25	16	32	0.125	>128	16	32	0.125	>128	
32	M0603	20140616	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	0.25	16	16	0.125	>128	16	32	0.125	>128	
33	M0604	20140626	A	15	1	1	1	1	1	1	1	1	1	78	>128	>128	16	2	16	0.06	>128	0.25	16	0.06	>128	
34	M0605	20140724	A	1	1	1	1	1	1	1	1	1	1	17	>128	64	16	16	16	0.125	>128	16	32	0.125	>128	
35	M0606	20141105	A	15	5	1	1	1	1	1	1	1	1	341	>128	64	16	2	128	0.125	>128	4	128	0.125	>128	
36	M0607	20141112	A	15	5	1	1	1	1	1	1	1	1	341	>128	64	16	2	64	0.125	>128	4	64	0.125	>128	

vanaA, *vanB*, *atpA*, *ddl*, *gdh*, *purK*, *gyd*, *psfS*, and *adk* are the target gene names.
MLST = multilocus sequence typing, VAEfae=vancomycin-resistant *Enterococcus faecium*.

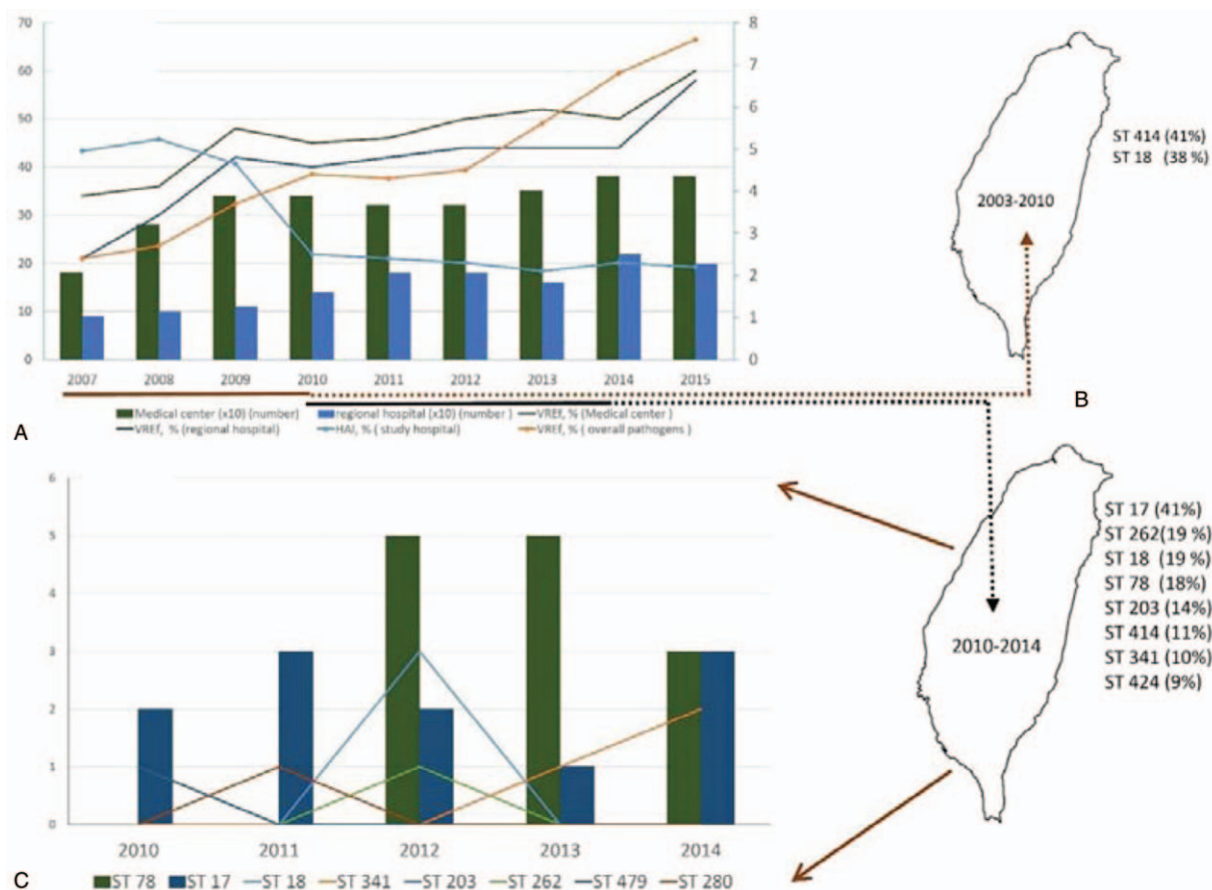


Figure 2. Nine-year trend of the rate of vancomycin-resistant *Enterococcus faecium* isolates. (A) A rapid increase in vancomycin resistance from 12.4% in 2007 to 39.9% in 2015 among enterococcal isolates was reported in Taiwan Nosocomial Infection Surveillance System (TNIS), and an increase of *Enterococcus faecium* from 2.4% in 2007 to 8.6% in 2015 among Enterococcal isolates was noted in TNIS (data from Taiwan Centers for Disease Control). (B) Before 2010, ST-414 and ST-18 of vancomycin-resistant *Enterococcus faecium* isolates were the 2 predominant STs, accounting for 79.6% of the isolates (data from Appendix 1, <http://links.lww.com/MD/B990>). (C) ST-17 (41.7%) and ST-78 (18.0%) vancomycin-resistant *Enterococcus faecium* isolates were the predominant STs during the study period (data from this study). HAI=healthcare-associated infection, TNIS=Taiwan Nosocomial Infection Surveillance System, VRE_{fae}=vancomycin-resistant *Enterococcus faecium*.

4. Discussion

This is the first study to describe the clinical features of BSI due to VRE_{fae} and identify the microbiological characteristics of VRE_{fae} in Central Taiwan. After comparing our results with those of other recent studies on BSI due to *Enterococcus* sp., we noted that the proportion (7.6%) of enterococcal BSI was similar to that of the total BSI episodes (5.0%–7.1%).^[20,21] The proportion (23.7%) of VRE_{fae} BSI was in good agreement with previous results.^[9,22,23] VRE_{fae} infections in food-producing animals derived from broiler production have also been reported.^[24]

We observed that the 30-day mortality rate (20/152, 13.2%) from VRE_{fae} BSI among our patients was lower than that observed in previous studies.^[20,25] The lower mortality rate observed may be due to multiple factors, including patient populations, variations in the portal of entry, serious underlying conditions, and lack of lethal clonal spread. However, further experiments are needed to elucidate these factors. The emergence of antimicrobial-resistant bacteria, including VRE_{fae}, poses a difficult task for physicians who have limited therapeutic options. Critically ill patients admitted to ICUs are

at a major risk of being infected with resistant bacteria that will have an adverse impact on mortality.^[26] Jung's study showed that VRE_{fae} colonization was associated with increased mortality,^[27] and the results of our study showed that the onset of VRE_{fae} BSI in the ICU was another significant risk factor for 30-day mortality (OR=4.2, 95% CI=1.7–10.0, $P=.002$). The length of ICU stay, which may reflect the severity of BSI, was also strongly associated with the odds of death from BSI due to VRE_{fae}.^[27] Certainly, critically ill patients admitted to the ICUs are at a major risk of mortality, and VRE_{fae} can play an important role as the causative agent. In addition, an appropriate antimicrobial therapy for VRE_{fae} BSI was a protective factor for 30-day mortality (OR=0.33, 95% CI=0.14–0.79, $P=.013$). The results of our study were similar to the results of other studies.^[27] Importantly, the results of the present study suggest that an appropriate antimicrobial therapy results in lower odds of all-cause 30-day mortality. The international guidelines for the management of severe sepsis and septic shock recommend the administration of broad-spectrum antimicrobials within 1 hour of the diagnosis of septic shock (1B) and severe sepsis without septic shock (1C) as the goal of therapy.^[28] Therefore, we highlight the need to address the patients admitted to the ICU with VRE_{fae} BSI and use

appropriate antimicrobial therapy for VRE*fae* BSI with the aim to treat more patients with these infections.

Among the VRE*fae* isolates, there were 2 major STs, that is, ST-17 and ST-78. These 2 STs accounted for 59.6% of all VRE*fae* isolates tested. Before 2010, ST-414 and ST-18 were the 2 predominant STs, accounting for 79.6% of the isolates (Appendix 1, <http://links.lww.com/MD/B990>). However, ST-17 (41.7%) and ST-78 (18.0%) were the predominant STs during the study period (Fig. 2).

An increasing resistance rate to vancomycin among enterococcal isolates has been documented globally.^[12] The clonal spread of certain epidemic VRE*fae* strains belonging to CC17 contributed to this increase.^[13] The 2 major STs among the VRE*fae* isolates tested in our study, ST-17 and ST-78, all belong to CC17, which might explain the same increasing trend of vancomycin resistance in Taiwan. A previous study demonstrated that ST-78 was the epidemic strain causing VRE infections in Taiwan in 2007.^[29,30] Our study showed that the VRE*fae* isolates of ST-18 and ST-414 were first noted before 2010. However, in 2010 and 2014, ST-17 and ST-78 became the predominant STs, which temporally correlated with the rapid increase in VRE*fae*. Because of the small sample size of individual STs in this study, an accurate correlation between STs and mortality rate cannot be calculated. Among the blood enterococcal isolates, 23.7% (36/152) were VRE*fae*, and all 36 VRE*fae* remained highly susceptible to linezolid and tigecycline. ST-17 and ST-78 were the 2 predominant STs during the study period. The pathogenicity and virulence of predominant VRE*fae* STs warrants further study. We assumed that no predominant clonal spread occurred in the study year due to the absence of outbreaks.

The present study has several strengths. Most importantly, we accumulated a complete longitudinal dataset over 4 years. We had access to excellent data to evaluate the demographic features of VRE*fae* BSI. In addition, the present findings provide invaluable epidemiological information about BSI due to VRE*fae* in Central Taiwan.

Our study has several limitations. The first limitation was that our facility was unable to perform the molecular typing of the isolates; therefore, the clonality and genotypes of the isolates could not be evaluated. Second, we could describe the correlation between ICU stay and VRE*fae* BSI for all-cause 30-day mortality, but the causality analysis between ICU stay and VRE*fae* BSI could not be clearly established due to retrospective analysis. We suggested each episode of BSI at ICU should serve patient aggressively and carefully. Third, our microbiological laboratory did not evaluate the microbial susceptibility to daptomycin. We assessed the adequacy of the antibiotic therapy using the in vitro breakpoint method reported by CLSI.^[17] Vancomycin therapy for strains with MIC \leq 32 μ g/mL was regarded as adequate. Lastly, some data suggest that resistance may be inducible in some strains.^[29] Therefore, vancomycin therapy against infection due to *vanC*-VRE has been regarded as inadequate. Furthermore, *vanC* genotype was not included in this study, but we can assume that a similar phenomenon has occurred in our institute, as previously reported.^[23]

In conclusion, our results suggest that onset of VRE*fae* BSI at ICU was the only significant risk factors for all-cause 30-day mortality. An appropriate antimicrobial therapy was a protective factor for 30-day mortality. Therefore, we highlight the need to use appropriate antimicrobial therapy in patients admitted to the ICUs with VRE*fae* BSIs with the aim to treat more patients with these infections.

Acknowledgments

All authors thank Doctor Jann-Tay Wang and his research assistant Duckling Chen for their help in performing drug susceptibilities testing and molecular studies. All authors thank the assistant of the Clinical Microbiology Laboratory of Changhua Christian Hospital. This research project would not have been possible without the support of many people. The authors wish to express their gratitude to staffs of Division of Infectious Diseases and Division of Critical Care Medicine of Changhua Christian Hospital who were offered patient care and invaluable support.

References

- Uttley AH, Collins CH, Naidoo J, et al. Vancomycin-resistant enterococci. *Lancet* 1988;103:57–8.
- Ben RJ, Lu JJ, Young TG, et al. Clinical isolation of vancomycin-resistant enterococcus faecalis in Taiwan. *J Formos Med Assoc* 1996;95:946–9.
- National Nosocomial Infections Surveillance System. National nosocomial infections surveillance (NNIS) system report data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004;32:470–85.
- McDonald LC, Lauderdale TL, Shiao YR, et al. TSAR Participating Hospitals. The status of antimicrobial resistance in Taiwan among gram-positive pathogens: the Taiwan surveillance of antimicrobial resistance (TSAR) programme, 2000. *Int J Antimicrob Agents* 2004;23:362–70.
- Taiwan Centers for Disease Control. Statistics of communicable diseases and surveillance report in Taiwan 2015. Taiwan, 2016. [in English].
- Conde-Estévez D, Grau S, Albanell J, et al. Clinical characteristics and outcomes of patients with vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* bacteremia in cancer patients. *Eur J Clin Microbiol Infect Dis* 2011;30:103–8.
- McBride SJ, Upton A, Roberts SA. Characteristics and outcomes of patients with vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* bacteraemia—a five-year retrospective review. *Eur J Clin Microbiol Infect Dis* 2010;29:107–14.
- Arias CA, Murray BE. The rise of the enterococcus: beyond vancomycin resistance. *Nat Rev Microbiol* 2012;10:266–78.
- Chang CM, Wang LR, Lee HC, et al. Characterisation of vancomycin-resistant enterococci from hospitalised patients at a tertiary centre over a seven-year period. *J Hosp Infect* 2010;74:377–84.
- Wang J, Chen YC, Chang SC, et al. Control of vancomycin-resistant enterococci in a hospital: a five-year experience in a Taiwanese teaching hospital. *J Hosp Infect* 2004;58:97–103.
- Tan C, Lai CC, Wang JY, et al. Bacteremia caused by non-faecalis and non-faecium enterococcus species at a medical center in Taiwan, 2000 to 2008. *J Infect* 2010;61:34–43.
- Werner G, Coque TM, Hammerum AM, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill* 2008;13:pii:19046.
- Willems RJ, Top J, van Santen M, et al. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* 2005;11:821–8.
- McCabe WR, Jackson G. Gram-negative bacteremia. I. etiology and ecology. *Arch Intern Med* 1962;110:847–55.
- Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. *Chest* 1992;101:1481–3.
- Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;36:309–32.
- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth Informational Supplement M100-S26. Wayne, PA, USA, 2016.
- Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995;33:1434.
- Homan WL, Homan WL, Tribe D, et al. Multilocus sequence typing scheme for *Enterococcus faecium*. *J Clin Microbiol* 2002;40:1963–71.
- Caballero-Granado FJ, Becerril B, Cuberos L, et al. Attributable mortality rate and duration of hospital stay associated with *Enterococcal bacteraemia*. *Clin Infect Dis* 2001;32:587–94.
- Peset V, Tallón P, Sola C, et al. Epidemiological, microbiological, clinical, and prognostic factors of bacteremia caused by high-level vancomycin-resistant enterococcus species. *Eur J Clin Microbiol Infect Dis* 2000;19:742–9.

- [22] Zhao C, Sun H, Wang H, et al. Antimicrobial resistance trends among 5608 clinical Gram-positive isolates in china: results from the Gram-positive cocci resistance surveillance program (2005–2010). *Diagn Microbiol Infect Dis* 2012;73:174–81.
- [23] Lu CL, Chuang YC, Chang HC, et al. Microbiological and clinical characteristics of vancomycin-resistant *Enterococcus faecium* bacteraemia in Taiwan: Implication of sequence type for prognosis. *J Antimicrob Chemother* 2012;67:2243–9.
- [24] Nilsson O. Ncomycin resistant enterococci in farm animals—occurrence and importance. *Infect Ecol Epidemiol* 2012;20:3402.
- [25] Suppli M, Aabenhus R, Harboe ZB, et al. Mortality in Enterococcal bloodstream infections increases with inappropriate antimicrobial therapy. *Clin Microbiol Infect* 2011;17:1078–83.
- [26] Cornejo-Juárez P, Vilar-Compte D, Pérez-Jiménez C, et al. The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit. *Int J Infect Dis* 2015;31:31–4.
- [27] Cheah AL, Spelman T, Liew D, et al. Enterococcal bacteraemia: factors influencing mortality, length of stay and costs of hospitalization. *Clin Microbiol Infect* 2013;19:e181–189.
- [28] Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign Guidelines Committee including The Pediatric Subgroup. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013;39:165–228.
- [29] Hsieh YC, Lee WS, Ou TY, et al. Clonal spread of CC17 vancomycin-resistant *Enterococcus faecium* with multilocus sequence type 78 (ST78) and a novel ST444 in Taiwan. *Eur J Clin Microbiol Infect Dis* 2010;29:25–30.
- [30] Hsueh PR, Teng LJ, Pan HJ, et al. Emergence of vancomycin-resistant enterococci at a university hospital in Taiwan: persistence of multiple species and multiple clones. *Infect Control Hosp Epidemiol* 1999;20:828–33.