

# Antibiotic Stewardship Related to Delayed Diagnosis and Poor Prognosis of Critically Ill Patients with Vancomycin-Resistant Enterococcal Bacteremia: A Retrospective Cohort Study

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**Purpose:** Patients with septicemia caused by vancomycin-resistant *Enterococcus* (VRE) bacteremia have higher mortality rates than patients infected by VSE. Vancomycin or teicoplanin is selected as the antibiotic stewardship intervention to cover methicillin-resistant *Staphylococcus aureus* infections before blood culture reveals VRE bacteremia in critically ill patients with Gram-positive cocci (GPC) bacteremia; this may require linezolid or daptomycin treatment instead. We thus evaluated antibiotic stewardship practices, such as appropriate timing of antibiotic use in GPC bacteremia, and clinical outcomes of critically ill patients with VRE infection.

**Patients and Methods:** This retrospective study enrolled 191 critically ill patients with enterococcal bacteremia at the Taipei Tzu Chi Hospital during January 1, 2019–December 31, 2020. Demographic and clinical characteristics, as well as disease outcomes and appropriate antibiotic use after GPC bacteremia diagnosis, were compared between the VRE and VSE groups.

**Results:** Of 191 patients, 55 had VRE bacteremia (case group) and 136 had VSE bacteremia (control group). The rate of antibiotic change after initial antibiotic use for GPC bacteremia was higher in the VRE bacteremia group (100% vs 10.3%;  $p<0.001$ ). The time to appropriate antibiotic administration after GPC bacteremia diagnosis was longer in the VRE bacteremia group ( $3.3\pm 2.1$  vs  $1.5\pm 1.8$  days;  $p<0.001$ ). Patients with VRE bacteremia had higher 28-day mortality rates (relative risk, 1.997; 95% confidence interval [CI], 1.041–3.83). Multivariate Cox regression analysis showed that delayed appropriate antibiotic administration of  $>3$  days after GPC bacteremia diagnosis increased the risks of 28-day all-cause mortality (adjusted hazard ratio, 2.045; 95% CI, 1.089–3.84;  $p=0.026$ ) in patients with VRE infection.

**Conclusion:** Patients with VRE bacteremia with delayed appropriate antibiotic administration of  $>3$  days after GPC bacteremia diagnosis had increased 28-day mortality risks. New strategies for early VRE detection in GPC bacteremia may shorten the time to administer appropriate antibiotics and lower mortality rates.

**Keywords:** VRE bacteremia, sepsis, early detection, appropriate antibiotic use, GPC bacteremia

## Introduction

Enterococci, which are facultative anaerobic and Gram-positive catalase-negative bacteria, generally exhibit low levels of virulence owing to their presence as natural members of human intestinal flora. Although normally distributed in the human gut, they may cause various infections, including bacteremia or sepsis.<sup>1</sup> Owing to their ability to survive in harsh environments (including high salt concentrations) and at wide temperature ranges (from 10 °C to  $>45$  °C), they easily spread through Caregiver hand contamination and Medical catheter-associated transmission.<sup>2</sup>

In the United States, *Enterococcus* species accounted for 11–13% of healthcare-associated infections during 2015–2017.<sup>3</sup> The emergence of vancomycin-resistant enterococci (VRE) has been alarming in the past two decades owing to high mortality rates.<sup>4</sup> Based on the National Nosocomial Infections Surveillance System Report from Taiwan in 2004, among patients admitted to intensive care units (ICUs), the resistance rate was around 20–30% for nosocomial VRE.<sup>5</sup> Moreover, longitudinal surveillance (1996–2009) of annual rates at National Taiwan University Hospital revealed a gradual rise in the prevalence of VRE (from 1.2% to 25.1%). These rates included all enterococcal isolates from patients with healthcare-associated infections.<sup>6</sup> The causes of multidrug-resistant enterococci emergence may include intrinsic resistance to several antimicrobial agents. Another cause may be acquired resistance through mobile elements, such as transposons and plasmids against glycopeptides. Furthermore, VRE can transfer genetic material to other Gram-positive pathogens, thus further inducing antibiotic resistance.<sup>7–9</sup>

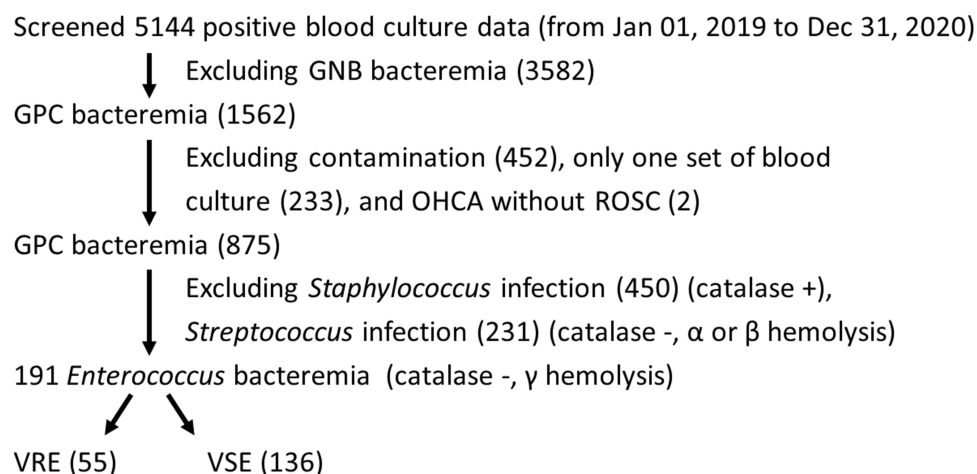
Patients with septicemia caused by VRE have a higher mortality rate than those with vancomycin-sensitive *Enterococcus* (VSE) bacteremia.<sup>10</sup> This is the case even in the era of effective VRE therapy.<sup>4</sup> Some studies indicate that VRE increases length of hospital stay and mortality.<sup>4,10–12</sup> Therefore, it is important to identify the risk factors that could be applied in critical care to prevent disastrous outcomes. Additionally, the appropriateness of antimicrobial therapy has a prognostic impact in patients with VRE bacteremia.<sup>13–16</sup> A vast majority of enterococcal bacteremia studies include different groups of patients such as patients who are immunosuppressed,<sup>17</sup> with cancer,<sup>18</sup> with VRE colonization,<sup>19,20</sup> or patients admitted in the ICU.<sup>21,22</sup> Linezolid and daptomycin are suitable treatments for confirmed VRE bacteremia. However, before blood culture reveals VRE bacteremia in critically ill patients with GPC bacteremia, vancomycin or teicoplanin is administered under antibiotic stewardship to cover methicillin-resistant *Staphylococcus aureus* (MRSA) infections. This delayed reporting of VRE leads to inappropriate antibiotic treatment, as linezolid or daptomycin treatment is required for VRE bacteremia instead. Therefore, this retrospective study aimed to determine clinical risk factors in patients with VRE bacteremia related to antibiotic stewardship application, such as appropriate timing of antibiotic administration, as well as determining clinical outcomes of patients with *Enterococcus* bacteremia.

## Patients and Methods

This single-center, retrospective cohort study was approved by the Institutional Review Board (IRB) of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (Protocol No.: 10-XD-097) and was conducted in accordance with the guidelines of the amended Declaration of Helsinki. An informed consent waiver was received from the IRB, and patient privacy rights, including any individual person's data in any form (such as individual details, images, or videos), have been observed.

## Study Population and Design

This retrospective cohort study enrolled in-patients with *Enterococcus* bacteremia at the Taipei Tzu Chi Hospital from January 1, 2019 to December 31, 2020 (Figure 1). In these two years, 5144 positive blood cultures were screened. The study excluded patients with Gram-negative bacilli bacteremia, contamination, only having one set of blood cultures, and Gram-positive cocci (GPC) bacteremia excluding *enterococcus* species. All patients with *Enterococcus* bacteremia had at least two sets of blood cultures and underwent rectal swab examinations on admission. Records of patients with *Enterococcus* bacteremia were reviewed. Demographic and clinical characteristics were compared between the two groups (VRE and VSE bacteremia) according to variables defined as risk factors previously.<sup>15–26</sup> These possible risk factors included antibiotic exposure, catheter use, or comorbidities. In addition, clinical outcomes were also compared between the two groups (VRE and VSE bacteremia). Four subgroups of antibiotics were categorized according to VRE or VSE infection. The timing related to appropriate antibiotic administration after GPC bacteremia diagnosis was also considered during subgrouping. If risk factors were present in critically ill patients, special consideration would be provided regarding antibiotic selection for MRSA or VRE for patients with GPC bacteremia. As part of our hospital antibiotic stewardship program, vancomycin and teicoplanin are the first treatment choices for MRSA bacteremia.<sup>27</sup> However, linezolid or daptomycin is selected after confirmed VRE bacteremia.<sup>28</sup> Furthermore, the maintenance vancomycin dose was adjusted according to the trough level (target 15 to 20 mcg/mL), which was measured before the fourth dose following the most recent dose adjustment, within 30 min before infusion, to ensure the effectiveness of the treatment.



**Figure 1** Flow chart of patients enrolled in this study. We screened 5144 cases with blood culture data and excluded 3582 cases of GNB bacteremia. After further exclusion of 452 contaminated cultures, 233 single set blood cultures, and 2 cases of out-of-hospital cardiac arrest (OHCA) without return of spontaneous circulation (ROSC), 875 cases of GPC bacteremia remained. Furthermore, 191 *Enterococcus* bacteremia cases were analyzed after excluding 450 *Staphylococcus* and 231 *Streptococcus* cases. A total of 191 patients with enterococcal bacteremia (55 patients with VRE bacteremia [case group] and 136 patients with VSE bacteremia [control group]) were included in the analysis.

**Abbreviations:** GPC, Gram-positive cocci; GNB, Gram-negative bacteria; VRE, vancomycin-resistant *Enterococcus*; VSE, vancomycin-sensitive *Enterococcus*.

## Study Setting

Clinical information of patients in both groups (VRE and VSE bacteremia) was recorded in Excel spreadsheets. Demographic and clinical data of each patient included age, sex, vital signs, catheter use, comorbid diseases, Charlson comorbidity index,<sup>29</sup> laboratory data, antibiotic experiences, disease severity index, and infection events (other sites of *Enterococcus* infection and other bacteremia coinfections). Further data collected included initial antibiotic use with confirmed GPC bacteremia, antibiotic change rate after initial antibiotic use after GPC bacteremia diagnosis, VRE rectal swab, residence in a long-term care facility, antacid use, probiotic use, history of surgery within 6 months, and time to appropriate antibiotic administration after enterococcal bacteremia. The clinical outcome analysis after admission included in-hospital mortality, 28-day mortality, length of hospital stay, ICU admission rate, septic shock, use of mechanical ventilation, and ventilator days.

Disease severity indexes included sepsis-related organ failure assessment scores, Acute Physiology and Chronic Health Evaluation scores,<sup>30</sup> and oxygenation use. Comorbidities included malignancy, diabetes mellitus, renal insufficiency, chronic obstructive pulmonary disease, liver disease, heart disease, and history of transplantation. Antibiotic use was defined as exposure to vancomycin, glycopeptide, carbapenem, cephalosporin, penicillin, colimycin, fluoroquinolones, Baktar (trimethoprim-sulfamethoxazole), metronidazole, clindamycin, macrolides (erythromycin, azithromycin), aminoglycosides, or tetracycline for >3 days within 3 months before the bacteremic episode. VRE bacteremia was defined as a blood culture growing *Enterococcus* species with a vancomycin minimum inhibitory concentration of  $\geq 32$  mcg/mL. The antibiotic susceptibility, catalase, and hemolysis tests were all performed at the clinical laboratory department. The duration of appropriate antibiotic treatment was recorded from the time to GPC bacteremia diagnosis to the time to appropriate antibiotic administration, in which final cultured microorganisms were susceptible to the antibiotics used. The 28-day mortality rate was determined as the number of patients who died within 28 days after enterococcal bacteremia divided by the total number of hospitalized patients.

## Statistical Analyses

Data was analyzed using SPSS version 26.0 (IBM Corp, Armonk, NY, USA), and a P value of <0.05 indicated statistical significance. Categorical variables are expressed as frequencies and percentages and were compared using the chi-square test or Fisher's exact test, if the expected values were below 5. Continuous variables are expressed as mean  $\pm$  standard deviation and were analyzed using Student's *t*-test. A multivariate analysis was performed using logistic regression to determine risk factors for VRE bacteremia. All risk factors with a significance level of <0.05 in the univariate analysis

were included in the multiple logistic regression model. The Kaplan–Meier survival curve was used to analyze mortality in the VRE and VSE bacteremia subgroups. Multivariate analysis using Cox regression analysis was performed to determine the factors associated with all-cause 28-day mortality, after adjusting for other confounding factors.

## Results

### Characteristics and Laboratory Data of Participants

In our study, after the exclusion of patients with contaminated blood cultures, only one set of blood cultures, and OHCA without ROSC, 875 cases of GPC bacteremia were identified in the 2 study years (Figure 1). *Staphylococcus* bacteremia (positive catalase test) was the most common infection (51.4%), followed by *Enterococcus* (negative catalase test and  $\gamma$ -hemolysis) and *Streptococcus* (negative catalase test and  $\alpha$ - or  $\beta$ -hemolysis) bacteremia (21.8%).

A total of 191 patients with *Enterococcal* bacteremia (55 patients with VRE bacteremia [case group] and 136 patients with VSE bacteremia [control group]) were included in the analysis. The VRE bacteremia case group included 15 women and 40 men, with a mean age of 72.1 years ( $\pm 9.9$  years), while the VSE bacteremia control group included 53 women and 83 men with a mean age of 72.4 years ( $\pm 15.9$  years). Demographic characteristics and clinical data of patients are presented in Table 1 and Supplementary Table 1. Age, sex, and disease severity index were not significantly different between the case and control groups. Comorbidities were not significantly different between the two groups, except for hematological malignancy, gastrointestinal disease, and solid organ transplant. Laboratory data showed that hemoglobin, albumin, and lactate levels were lower while  $\text{HCO}_3^-$  and Na levels were higher in the VRE group than in the VSE group. Additionally, the VRE group had more patients with VRE rectal swabs, antacid use, history of surgery within 6 months, and mechanical ventilation use. Moreover, catheter use and antibiotic use were higher in the VRE group than in the VSE group. Prior use of glycopeptides, carbapenem, cephalosporin, penicillin, colimycin, fluoroquinolones, trimethoprim-sulfamethoxazole, metronidazole, and tetracycline was significantly higher in the VRE bacteremia group ( $p < 0.05$ ). The first line antibiotics to treat GPC bacteremia were selected for 7.3–10.3% of patients. Other initial antibiotic treatments included vancomycin and teicoplanin, where vancomycin was selected for 12.5–14.5% of patients and teicoplanin was selected for 77.2–78.2% of patients. Furthermore, the antibiotic change rate after initial antibiotic administration for GPC bacteremia was higher in the VRE bacteremia group (100% vs 10.3%;  $p < 0.001$ ; Table 1).

All significant univariate variables were included and adjusted for in the final multivariate model (Table 2; other insignificant variables are present in Supplementary Table 2). However, multivariate logistic regression showed that central venous catheter (CVC) use (odds ratio [OR], 3.116; 95% confidence interval [CI], 1.386–7.008), glycopeptide exposure (OR, 5.734; 95% CI, 2.297–14.312), and cephalosporin exposure (OR, 2.923; 95% CI, 1.346–6.345) were associated with VRE bacteremia after adjusting for other confounding factors. These findings suggest that CVC use, glycopeptide exposure, and cephalosporin exposure for  $>3$  days within 3 months are significant risk factors for VRE bacteremia in patients with *Enterococcus* bacteremia.

### Clinical Outcomes

Patients with VRE bacteremia had higher in-hospital mortality (relative risk [RR], 2.595; 95% CI, 1.366–4.933) and 28-day mortality (RR, 1.997; 95% CI, 1.041–3.83) rates than patients with VSE bacteremia (Table 3). The mean length of hospital stay after bacteremia was longer for patients with VRE bacteremia than for those with VSE bacteremia ( $25.1 \pm 26.8$  days vs  $15.0 \pm 14.5$  days). Moreover, the days of ventilation usage were longer in the VRE group ( $p < 0.05$ ).

### Time to Appropriate Antibiotic Use

The time to appropriate antibiotic use after confirmed GPC bacteremia was longer in the VRE bacteremia group ( $3.3 \pm 2.1$ ) than in the VSE bacteremia group ( $1.5 \pm 1.8$ ) ( $p < 0.001$ ; Table 1). The median time to appropriate antibiotic administration after confirmed GPC bacteremia in patients with VRE bacteremia was 3 days. The four subgroups were categorized according to VRE or VSE infection and appropriate antibiotic use of  $\leq 3$  days or  $>3$  days after GPC bacteremia diagnosis and were analyzed for all-cause 28-day mortality (Table 3). The VRE group with appropriate antibiotic administration of  $>3$  days after GPC bacteremia diagnosis had the highest mortality rate (68.2%) ( $p < 0.05$ ).

**Table I** Comparisons of Demographic Characteristics Between Critically Ill Patients with VRE Bacteremia and Those with VSE Bacteremia

Demographic Characteristics	VRE Bacteremia (N=55)	VSE Bacteremia (N=136)	p value
<b>Sex</b>			NS
Female	15 (27.3%)	53 (39%)	
Male	40 (72.7%)	83 (61%)	
<b>Age</b>	72.1±9.9	72.4±15.9	NS
<b>Comorbidities</b>			
Charlson comorbidity index	6.9±2.5	6.3±3.2	NS
Any cancer	22 (40%)	37 (27.2%)	NS
Solid organ tumor	15 (27.3%)	37 (27.2%)	NS
Hematological malignancy	7 (12.7%)	0 (0%)	<0.001 <sup>a</sup>
DM	17 (30.9%)	57 (41.9%)	NS
COPD	7 (12.7%)	16 (11.8%)	NS
Chronic renal failure	24 (43.6%)	54 (39.7%)	NS
Heart disease	30 (54.5%)	62 (45.6%)	NS
Liver cirrhosis	7 (12.7%)	7 (5.1%)	NS
Gastrointestinal disease	29 (52.7%)	40 (29.4%)	0.002 <sup>a</sup>
Hepatobiliary disease	13 (23.6%)	29 (21.3%)	NS
Bone marrow transplant	0 (0%)	0 (0%)	NS
Solid organ transplant	5 (9.1%)	1 (0.7%)	0.003 <sup>a</sup>
<b>Catheter</b>			
Catheter total number	2.4±1.8	1.0±1.3	<0.001 <sup>a</sup>
Central venous catheter	29 (52.7%)	22 (16.2%)	<0.001 <sup>a</sup>
Urinary catheter	30 (54.5%)	38 (27.9%)	0.001 <sup>a</sup>
NG tube	31 (56.4%)	36 (26.5%)	<0.001 <sup>a</sup>
Drainage tube	11 (20%)	6 (4.4%)	0.001 <sup>a</sup>
Endo tube	16 (29.1%)	9 (6.6%)	<0.001 <sup>a</sup>
Tracheostomy	2 (3.6%)	6 (4.4%)	NS
Double lumen (or perm)	15 (27.3%)	19 (14%)	0.03 <sup>a</sup>
<b>Antibiotic experiences</b>			
Antibiotic exposure within 3 months	46 (83.6%)	64 (47.1%)	<0.001 <sup>a</sup>
Vancomycin	5 (9.1%)	5 (3.7%)	NS
Glycopeptide	27 (49.1%)	10 (7.4%)	<0.001 <sup>a</sup>
Carbapenem	26 (47.3%)	17 (12.5%)	<0.001 <sup>a</sup>

(Continued)

**Table 1** (Continued).

Demographic Characteristics	VRE Bacteremia (N=55)	VSE Bacteremia (N=136)	p value
Cephalosporin	40 (72.7%)	49 (36%)	<0.001 <sup>a</sup>
Penicillin	30 (54.5%)	27 (19.9%)	<0.001 <sup>a</sup>
Colimycin	9 (16.4%)	5 (3.7%)	0.002 <sup>a</sup>
Fluoroquinolones	23 (41.8%)	28 (20.6%)	0.003 <sup>a</sup>
Baktar	6 (10.9%)	2 (1.5%)	0.003 <sup>a</sup>
Metronidazole	4 (7.3%)	2 (1.5%)	0.037 <sup>a</sup>
Clindamycin	0 (0%)	2 (1.5%)	NS
Macrolides	0 (0%)	0 (0%)	NS
Aminoglycosides	2 (3.6%)	4 (2.9%)	NS
Tetracycline	4 (7.3%)	2 (1.5%)	0.037 <sup>a</sup>
<b>Infection events</b>			
Other sites of <i>Enterococcus</i> infection	17 (30.9%)	43 (31.6%)	NS
Other bacteremia coinfections	35 (63.6%)	74 (54.4%)	NS
<b>Others</b>			
Rectal swab test result positive for VRE	14 (25.5%)	6 (4.4%)	<0.001 <sup>a</sup>
Antacid use	34 (61.8%)	52 (38.2%)	0.003 <sup>a</sup>
Probiotic use	1 (1.8%)	7 (5.1%)	NS
History of surgery within 6 months	24 (43.6%)	30 (22.1%)	0.003 <sup>a</sup>
From long-term care facility	5 (9.1%)	19 (14%)	NS
Septic shock	8 (14.5%)	13 (9.6%)	NS
Mechanical ventilation use	27 (49.1%)	37 (27.2%)	0.004 <sup>a</sup>
<b>Initial antibiotic during confirmed GPC bacteremia</b>			
First line antibiotics	4 (7.3%)	14 (10.3%)	NS
Vancomycin	8 (14.5%)	17 (12.5%)	NS
Teicoplanin	43 (78.2%)	105 (77.2%)	NS
Linezolid	0	0	NS
Daptomycin	0	0	NS
Tigecycline	0	0	NS
<b>Antibiotic change rate</b>	55 (100%)	14 (10.3%)	<0.001 <sup>a</sup>
<b>Time to appropriate antibiotic administration after GPC bacteremia diagnosis</b>	3.3±2.1	1.5±1.8	<0.001 <sup>a</sup>

Notes: <sup>a</sup>Statistically significant, p<0.05.

**Abbreviations:** VRE, vancomycin-resistant *Enterococcus*; VSE, vancomycin-sensitive *Enterococcus*; NG, nasogastric; DM, diabetes mellitus; COPD, chronic obstructive pulmonary disease; GPC, Gram-positive cocci; NS, non-significant.



**Table 2** Univariate and Multivariate Logistic Regression Analyses to Identify Variables Associated with Vancomycin-Resistant *Enterococcus* (VRE) Bacteremia in Critically Ill Patients with *Enterococcus* Bacteremia

	Univariate		Multivariate	
	OR (95% CI)	p	Adjusted OR (95% CI)	p
Gastrointestinal disease	2.677 (1.404–5.103)	0.003 <sup>a</sup>		
Solid organ transplant	13.5 (1.539–118.404)	0.019 <sup>a</sup>		
Total bilirubin	1.085 (1.004–1.171)	0.038 <sup>a</sup>		
Albumin	0.496 (0.306–0.802)	0.004 <sup>a</sup>		
Lactate (mmol/L)	0.784 (0.632–0.974)	0.028 <sup>a</sup>		
HCO <sub>3</sub>	1.088 (1.006–1.177)	0.036 <sup>a</sup>		
A-a DO <sub>2</sub>	1.002 (1–1.004)	0.03 <sup>a</sup>		
Overall catheter use	1.801 (1.437–2.256)	<0.001 <sup>a</sup>		
Central venous catheter	5.78 (2.873–11.627)	<0.001 <sup>a</sup>	3.116 (1.386–7.008)	0.006 <sup>a</sup>
Urinary catheter	3.095 (1.616–5.926)	0.001 <sup>a</sup>		
NG tube	3.588 (1.864–6.908)	<0.001 <sup>a</sup>		
Drainage tube	5.417 (1.892–15.507)	0.002 <sup>a</sup>		
Endo tube	5.789 (2.373–14.126)	<0.001 <sup>a</sup>		
Double lumen (or perm)	2.309 (1.073–4.969)	0.032 <sup>a</sup>		
Antibiotic exposure within 3 months	5.575 (2.61–12.666)	<0.001 <sup>a</sup>		
Glycopeptide	12.150 (5.281–27.951)	<0.001 <sup>a</sup>	5.734 (2.297–14.312)	<0.001 <sup>a</sup>
Carbapenem	6.276 (3.013–13.072)	<0.001 <sup>a</sup>		
Cephalosporin	4.735 (2.377–9.43)	<0.001 <sup>a</sup>	2.923 (1.346–6.345)	0.007 <sup>a</sup>
Penicillin	4.844 (2.46–9.54)	<0.001 <sup>a</sup>		
Colimycin	5.126 (1.633–16.087)	0.005 <sup>a</sup>		
Fluoroquinolones	2.772 (1.407–5.462)	0.003 <sup>a</sup>		
Baktar	8.204 (1.602–42.016)	0.012 <sup>a</sup>		
Antacid use	2.615 (1.373–4.983)	0.003 <sup>a</sup>		
History of surgery within 6 months	2.735 (1.400–5.343)	0.003 <sup>a</sup>		
Rectal swab test result positive for VRE	7.398 (2.671–20.492)	<0.001 <sup>a</sup>		
Mechanical ventilation use	3.314 (1.604–6.850)	0.001 <sup>a</sup>		

**Notes:** Dependent variable: VRE bacteremia. <sup>a</sup>Statistically significant,  $p < 0.05$ . All significant univariate variables were included and adjusted for in the final multivariate model.

**Abbreviations:** OR, odds ratio; CI, confidence interval; NS, non-significant.

The survival analysis was performed using the Kaplan–Meier method (Figure 2) (Log rank test,  $p = 0.033$ ). VRE with delayed appropriate antibiotic administration ( $> 3$  days) was a significant risk factor for all-cause 28-day mortality. Moreover, because CVC use, glycopeptide, and cephalosporin were not associated with all-cause 28-day mortality, they were excluded from the final multivariate Cox regression analysis (Table 4). The significant univariate variables, such as Acute Physiologic Assessment and Chronic Health Evaluation II score, Sequential Organ Failure Assessment score, and enterococcal bacteremia with appropriate antibiotic use (AAU), were included in the final multivariate

**Table 3** Comparisons of Clinical Outcomes Between Patients with VRE Bacteremia and Those with VSE Bacteremia

	VRE Bacteremia (N=55)	VSE Bacteremia (N=136)	RR (95% CI)	p value
All-cause hospital mortality	30 (54.5%)	43 (31.6%)	2.595 (1.366–4.933)	0.004 <sup>a</sup>
All-cause 28-day mortality	24 (43.6%)	38 (27.9%)	1.997 (1.041–3.830)	0.037 <sup>a</sup>
AAU ≤3 D	9/33 (27.3%)	29/107 (27.1%)	0.991 (0.413–2.382)	0.985
AAU >3 D	15/22 (68.2%)	9/29 (31%)	4.762 (1.444–15.703)	0.01 <sup>a</sup>
Length of stay in hospital	25.1±26.8	15.0±14.5	1.025 (1.009–1.041)	0.002 <sup>a</sup>
Ventilator use days	29.8±29.1	15.9±20.2	1.024 (1.001–1.047)	0.04 <sup>a</sup>

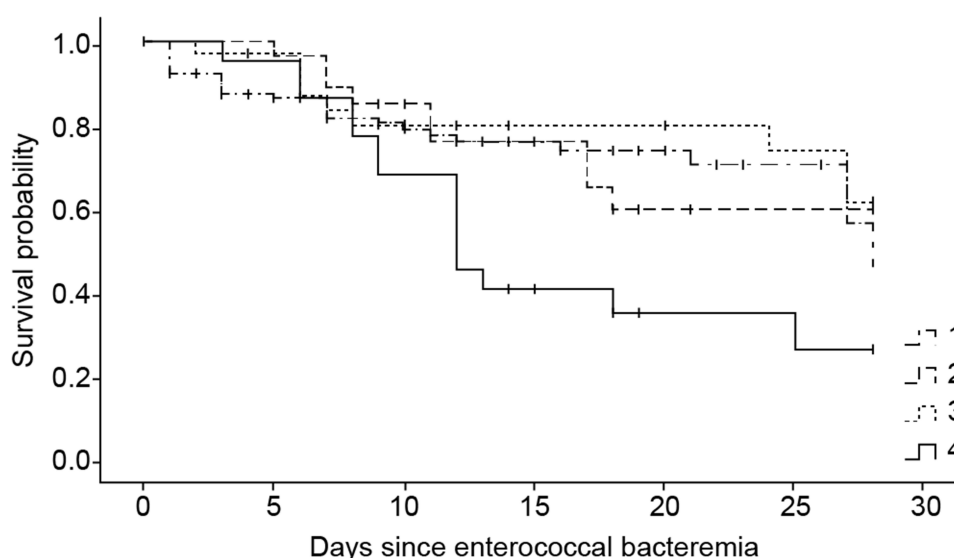
**Notes:** <sup>a</sup>Statistically significant,  $p < 0.05$ .

**Abbreviations:** VRE, vancomycin-resistant *Enterococcus*; VSE, vancomycin-sensitive *Enterococcus*; RR, relative risk; ICU, intensive care unit; NS, non-significant; AAU, appropriate antibiotic use.

model analysis. The multivariate Cox regression analysis (Table 4) showed that patients with VRE and delayed AAU of >3 days (adjusted hazard ratio, 2.045; 95% CI: 1.089–3.84,  $p = 0.026$ ) had increased risks of all-cause 28-day mortality.

## Discussion

In this single-center, retrospective study, we found that CVC use, glycopeptide exposure, and cephalosporin exposure were significant risk factors for VRE bacteremia in patients with *Enterococcus* bacteremia after adjusting for other confounding factors. CVC use is an important risk factor for VRE bacteremia, and the association is well established.<sup>23,31</sup> Our findings further suggest that VRE is spread from environmental surfaces to the CVC and then into the bloodstream, therefore emphasizing the importance of infection control practices. Glycopeptide exposure for >3 days within 3 months was a significant risk factor for VRE bacteremia ( $p < 0.001$ ) in our study. However, prior vancomycin exposure was not a significant risk factor for VRE bacteremia, which differs from the study findings of Furtado et al.<sup>16</sup> For the prevention of adverse events (skin rash and red man syndrome), teicoplanin was used more frequently than vancomycin in our study,



**Figure 2** Survival curves according to VRE or VSE bacteremia and the time to appropriate antibiotic administration after GPC bacteremia diagnosis (four groups). The Kaplan–Meier survival analysis was adopted for patients with (1) VSE bacteremia with time to appropriate antibiotic administration ≤3 days, (2) VSE bacteremia with time to appropriate antibiotic administration >3 days, (3) VRE bacteremia with time to appropriate antibiotic administration ≤3 days, and (4) VRE bacteremia with time to appropriate antibiotic administration >3 days during their hospital stay (cut-off point: 28 days). Log rank test:  $p < 0.05$ .



**Table 4** Cox Regression Model to Determine Factors Associated with All-Cause 28-Day Mortality

	Univariate		Multivariate	
	HR (95% CI)	p value	Adjusted HR (95% CI)	p value
VSE with AAU ≤3 D	Reference	0.047	Reference	0.048
VSE with AAU >3 D	0.948 (0.449–2.005)	NS	1.053 (0.493–2.249)	NS
VRE with AAU ≤3 D	0.744 (0.35–1.58)	NS	0.679 (0.308–1.498)	NS
VRE with AAU >3 D	2.096 (1.123–3.913)	0.02 <sup>a</sup>	2.045 (1.089–3.84)	0.026 <sup>a</sup>
APACHE II	1.04 (1.009–1.072)	0.011 <sup>a</sup>	1.040 (1.009–1.072)	0.011 <sup>a</sup>
SOFA score	1.083 (1.014–1.158)	0.018 <sup>a</sup>	1.057 (0.962–1.16)	NS
Central venous catheter use	1.452 (0.872–2.418)	NS		
Antibiotic exposure for 3 days within 3 months	1.474 (0.839–2.589)	NS		
Glycopeptide	0.89 (0.496–1.595)	NS		
Cephalosporin	1.299 (0.78–2.164)	NS		
Septic shock	1.995 (1.062–3.748)	0.032	1.249 (0.604–2.585)	NS
Mechanical ventilation use	1.207 (0.700–2.083)	NS	0.796 (0.400–1.585)	NS

**Notes:** Events: all-cause 28-day mortality. Time: days since enterococcal bacteremia diagnosis to 28 days. <sup>a</sup>Statistically significant,  $p < 0.05$ .

**Abbreviations:** AAU, appropriate antibiotic use; HR, hazard ratio; CI, confidence interval; NS, non-significant.

and for that reason, only 10 patients had prior exposure to vancomycin (Table 1). Regarding cephalosporin, as demonstrated in the current study, several previous studies also showed that prior use of cephalosporins for >3 days within 3 months was a significant risk factor for VRE bacteremia.<sup>19,32</sup> In our study, CVC use, previous glycopeptide treatment, and previous cephalosporin treatment were associated with VRE bacteremia (Table 2). However, these risk factors for VRE bacteremia were the same as those for MRSA bacteremia.<sup>33,34</sup> This result, however, does not offer any benefit for antibiotic stewardship.

Comorbidities and laboratory data were not significant risk factors for VRE bacteremia after adjusting for other confounding factors in our study. However, Lautenbach et al<sup>15</sup> found that renal insufficiency and neutropenia were independent risk factors for VRE bacteremia in 72 patients with VRE bacteremia and 188 patients with VSE bacteremia. Moreover, Peel et al<sup>23</sup> observed that neutropenia, allogeneic bone marrow transplantation, and hypoalbuminemia were independent risk factors for VRE bacteremia after adjusting for other confounding factors in 80 patients with VRE bacteremia and 80 matched control patients. Furthermore, Johnstone et al<sup>25</sup> demonstrated that bone marrow transplantation, solid organ transplantation, cancer, and heart disease were independent risk factors for VRE bacteremia after adjusting for other confounding factors in 217 patients with VRE bacteremia and 651 matched control patients. As our study population was relatively small, further multicenter studies with longer follow-up periods are needed to confirm the present findings.

Despite the availability of effective VRE therapy, VRE bacteremia remains an important risk factor for in-hospital mortality and length of hospital stay when compared with VSE bacteremia.<sup>4</sup> In our cohort, 30 (54.5%) patients with VRE bacteremia died in the hospital. The in-hospital mortality rate (RR, 2.595; CI 95%, 1.366–4.933) and the 28-day mortality rate (RR, 1.997; CI 95%, 1.041–3.83) were evaluated for VRE- and VSE-infected patients. The results show that the rates were higher for patients with VRE bacteremia than for patients with VSE bacteremia. This might be owing to inappropriate initial antimicrobial coverage.<sup>13,14</sup> Moreover, in critical care, when GPC bacteremia is combined with shock or critical illness, MRSA may be considered, and vancomycin or teicoplanin is administered. On the other hand, not only pathogen and antibiotic susceptibility but also antibiotic dosing and appropriate time of administration need to be considered in critically ill patients. For instance, for the effectiveness of the treatment, appropriate vancomycin dosing

requires consideration of the type and severity of infection, patient weight, kidney function, and even pharmacokinetic/pharmacodynamic efficacy.<sup>35–37</sup> However, VRE is often ignored in these instances. In reality, appropriate antibiotic treatment of VRE is always delayed until VRE confirmation. Notably, a large dataset cohort study showed that delay in initial antibiotic administration was associated with increased in-hospital mortality.<sup>38</sup> This study also showed a linear increase in the risk of mortality for each hour of delay in antibiotic administration. Another retrospective study conducted in Italy<sup>39</sup> found that time (since blood culture collection) to appropriate antibiotic therapy was an independent predictor of 30-day mortality in patients with carbapenemase-producing *Klebsiella pneumoniae* bacteremia. Another single-center retrospective study<sup>14</sup> revealed that receiving appropriate therapy within the first 48 hours (from blood culture to receiving the first dose of appropriate therapy) was associated with reduced mortality in patients with hospital-onset *Enterococcus* bacteremia. In our study, patients with VRE bacteremia with delayed appropriate antibiotic administration (>3 days) after GPC bacteremia diagnosis had an increased risk of 28-day mortality. This is the first study to investigate antibiotic stewardship application in patients with VRE bacteremia in Taiwan. CVC use, previous glycopeptide treatment, and previous cephalosporin treatment were associated with VRE bacteremia but not with all-cause 28-day mortality (Tables 2 and 4). Because risk factors for VRE bacteremia were the same as for MRSA bacteremia,<sup>33,34</sup> these results do not offer any benefit for antibiotic stewardship. Therefore, early detection of VRE bacteremia and timely use of appropriate antibiotics may be more important in antibiotic stewardship practices.

Recently, film array and matrix-assisted laser desorption/ionization-time of flight mass spectrometry may help with the rapid detection of VRE bacteremia within hours.<sup>40–43</sup> Hence, our findings demonstrate that the only effective factor in improving appropriate antibiotic administration in VRE bacteremia and improving clinical outcomes is the rapid VRE detection in GPC bacteremia.

Our study had some limitations. Because this is a single-center retrospective study, it is important to evaluate the study validity carefully before making any decision or changing clinical practice. Moreover, selection bias may be a concern in the subgroup analyses (VRE or VSE bacteremia and the time to appropriate antibiotic administration after GPC bacteremia diagnosis) and the sample size may be insufficient. However, we are still glad to share our study result and to offer the issue of delayed appropriate antibiotic treatment for patients with VRE bacteremia. Further prospective studies are needed to evaluate early detection of VRE and clinical outcomes.

## Conclusion

In this retrospective cohort study, patients with VRE bacteremia with delayed treatment (antibiotic administration >3 days after GPC bacteremia diagnosis) had higher risks of all-cause 28-day mortality. Our findings suggest that early detection of VRE bacteremia in GPC bacteremia may shorten the duration to appropriately administer antibiotics and therefore reduce mortality rates. Further prospective studies are needed to validate these present findings.

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## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Raza T, Ullah SR, Mehmood K, Andleeb S. Vancomycin resistant enterococci: a brief review. *J Pak Med Assoc.* 2018;68(5):768–772.
2. Arias CA, Murray BE. The rise of the Enterococcus: beyond vancomycin resistance. *Nat Rev Microbiol.* 2012;10(5):266–278. doi:10.1038/nrmicro2761
3. Weiner-Lastinger LM, Abner S, Edwards JR, et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. *Infect Control Hosp Epidemiol.* 2020;41(1):1–18. doi:10.1017/ice.2019.296
4. Prematunge C, MacDougall C, Johnstone J, et al. VRE and VSE bacteremia outcomes in the era of effective VRE therapy: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol.* 2015;37(1):26–35. doi:10.1017/ice.2015.228

5. 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(8):470–485. doi:10.1016/j.ajic.2004.10.001
6. Jean SS, Hsueh PR. Antimicrobial drug resistance in Taiwan. *J Formos Med Assoc*. 2011;110(1):4–13. doi:10.1016/S0929-6646(11)60002-8
7. Murray BE, Wood AJJ. Vancomycin-resistant enterococcal infections. *N Engl J Med*. 2000;342(10):710–721. doi:10.1056/NEJM200003093421007
8. Mundy LM, Sahn DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. *Clin Microbiol Rev*. 2000;13(4):513–522. doi:10.1128/CMR.13.4.513
9. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett*. 1992;72:195–198. doi:10.1111/j.1574-6968.1992.tb05089.x
10. DiazGranados CA, Zimmer SM, Klein M, Jernigan JA. Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: a meta-analysis. *Clin Infect Dis*. 2005;41(3):327–333. doi:10.1086/430909
11. Su L-H, Chen IL, Tang Y-F, Lee J-S, Liu J-W. Increased financial burdens and lengths of stay in patients with healthcare-associated infections due to multidrug-resistant bacteria in intensive care units: a propensity-matched case-control study. *PLoS One*. 2020;15(5):e0233265. doi:10.1371/journal.pone.0233265
12. Salgado CD, Farr BM. Outcomes associated with vancomycin-resistant enterococci: a meta-analysis. *Infect Control Hosp Epidemiol*. 2003;24(9):690–698. doi:10.1086/502271
13. Chou CH, Lee NY, Lee HC, Chang CM, Lee CC, Ko WC. Emergence of vancomycin-resistant *Enterococcus* bloodstream infections in southern Taiwan. *J Microbiol Immunol Infect*. 2012;45(3):221–227. doi:10.1016/j.jmii.2011.11.005
14. Zasowski EJ, Claeys KC, Lagnf AM, Davis SL, Rybak MJ. Time is of the essence: the impact of delayed antibiotic therapy on patient outcomes in hospital-onset enterococcal bloodstream infections. *Clin Infect Dis*. 2016;62(10):1242–1250. doi:10.1093/cid/ciw110
15. Lautenbach E, Bilker WB, Brennan PJ. Enterococcal bacteremia: risk factors for vancomycin resistance and predictors of mortality. *Infect Control Hosp Epidemiol*. 1999;20(05):318–323. doi:10.1086/501624
16. Furtado GH, Mendes RE, Pignatari AC, Wey SB, Medeiros EA. Risk factors for vancomycin-resistant *Enterococcus faecalis* bacteremia in hospitalized patients: an analysis of two case-control studies. *Am J Infect Control*. 2006;34(7):447–451. doi:10.1016/j.ajic.2005.08.015
17. Kang Y, Vicente M, Parsad S, et al. Evaluation of risk factors for vancomycin-resistant *Enterococcus* bacteremia among previously colonized hematopoietic stem cell transplant patients. *Transpl Infect Dis*. 2013;15(5):466–473. doi:10.1111/tid.12120
18. Ghanem G, Hachem R, Jiang Y, Chemaly RF, Raad I. Outcomes for and risk factors associated with vancomycin-resistant *Enterococcus faecalis* and vancomycin-resistant *Enterococcus faecium* bacteremia in cancer patients. *Infect Control Hosp Epidemiol*. 2007;28:1054–1059. doi:10.1086/519932
19. Kaya A, Kaya SY, Balkan II, et al. Risk factors for development of vancomycin-resistant enterococcal bacteremia among VRE colonizers: a retrospective case control study. *Wien Klin Wochenschr*. 2020;133(9–10):478–483. doi:10.1007/s00508-020-01733-7
20. Olivier CN, Blake RK, Steed LL, Salgado CD. Risk of vancomycin-resistant *Enterococcus* (VRE) bloodstream infection among patients colonized with VRE. *Infect Control Hosp Epidemiol*. 2008;29:404–409. doi:10.1086/587647
21. Se YB, Chun HJ, Yi HJ, Kim DW, Ko Y, Oh SJ. Incidence and risk factors of infection caused by vancomycin-resistant enterococcus colonization in neurosurgical intensive care unit patients. *J Korean Neurosurg Soc*. 2009;46(2):123–129. doi:10.3340/jkns.2009.46.2.123
22. Pan S-C, Wang J-T, Chen Y-C, Chang -Y-Y, Chen M-L, Chang S-C. Incidence of and risk factors for infection or colonization of vancomycin-resistant enterococci in patients in the intensive care unit. *PLoS One*. 2012;7(10):e47297. doi:10.1371/journal.pone.0047297
23. 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(4):388–394. doi:10.1111/j.1469-0691.2011.03591.x
24. Gouliouris T, Warne B, Cartwright EJP, et al. Duration of exposure to multiple antibiotics is associated with increased risk of VRE bacteraemia: a nested case-control study. *J Antimicrob Chemother*. 2018;73(6):1692–1699. doi:10.1093/jac/dky075
25. Johnstone J, Chen C, Rosella L, et al. Patient- and hospital-level predictors of vancomycin-resistant *Enterococcus* (VRE) bacteremia in Ontario, Canada. *Am J Infect Control*. 2018;46(11):1266–1271. doi:10.1016/j.ajic.2018.05.003
26. Ye JJ, Shie SS, Cheng CW, et al. Clinical characteristics and treatment outcomes of vancomycin-resistant *Enterococcus faecium* bacteremia. *J Microbiol Immunol Infect*. 2018;51(6):705–716. doi:10.1016/j.jmii.2017.08.025
27. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis*. 2011;52(3):285–292. doi:10.1093/cid/cir034
28. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2009;49(1):1–45. doi:10.1086/599376
29. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373–383. doi:10.1016/0021-9681(87)90171-8
30. Knaus WA, Zimmerman JE, Wagner DP, Draper EA, Lawrence DE. APACHE-acute physiology and chronic health evaluation: a physiologically based classification system. *Crit Care Med*. 1981;9(8):591–597. doi:10.1097/00003246-198108000-00008
31. Lucas GM, Lechtzin N, Puryear DW, Yau LL, Flexner CW, Moore RD. Vancomycin-resistant and vancomycin-susceptible enterococcal bacteremia: comparison of clinical features and outcomes. *Clin Infect Dis*. 1998;26(5):1127–1133. doi:10.1086/520311
32. McNeil SA, Malani PN, Chenoweth CE, et al. Vancomycin-resistant enterococcal colonization and infection in liver transplant candidates and recipients: a prospective surveillance study. *Clin Infect Dis*. 2006;42(2):195–203. doi:10.1086/498903
33. Pujol M, Pena C, Pallares R, Ayats J, Ariza J, Gudiol F. Risk factors for nosocomial bacteremia due to methicillin-resistant *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis*. 1994;13(1):96–102. doi:10.1007/BF02026134
34. Rezende NA, Blumberg HM, Ray SM, Metzger BS, Larsen NM, McGowan JE. Risk factors for methicillin-resistance among patients with *Staphylococcus aureus* bacteremia at the time of hospital admission. *Am J Med Sci*. 2002;323(3):117–123. doi:10.1097/00000441-200203000-00001
35. Katip W, Oberdorfer P. A monocentric retrospective study of AUC/MIC ratio of vancomycin associated with clinical outcomes and nephrotoxicity in patients with enterococcal infections. *Pharmaceutics*. 2021;13(9):1378. doi:10.3390/pharmaceutics13091378

36. Issarangoon Na Ayuthaya S, Katip W, Oberdorfer P, Lucksiri A. Correlation of the vancomycin 24-h area under the concentration-time curve ( $AUC_{24}$ ) and trough serum concentration in children with severe infection: a clinical pharmacokinetic study. *Int J Infect Dis.* 2020;92:151–159. doi:10.1016/j.ijid.2019.12.036
37. Katip W, Jaruratanasirikul S, Pattharachayakul S, Wongpoowarak W, Jitsurong A, Lucksiri A. The pharmacokinetics of vancomycin during the initial loading dose in patients with septic shock. *Infect Drug Resist.* 2016;9:253–260. doi:10.2147/IDR.S121513
38. Ferrer R, Martin-Loeches I, Phillips G, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. *Crit Care Med.* 2014;42(8):1749–1755. doi:10.1097/CCM.0000000000000330
39. Falcone M, Bassetti M, Tiseo G, et al. Time to appropriate antibiotic therapy is a predictor of outcome in patients with bloodstream infection caused by KPC-producing *Klebsiella pneumoniae*. *Crit Care.* 2020;24(1):29. doi:10.1186/s13054-020-2742-9
40. Kim DH, Lee JH, Ha JS, Ryoo NH, Jeon DS, Kim JR. Evaluation of the usefulness of selective chromogenic agar medium (chromID VRE) and multiplex PCR method for the detection of vancomycin-resistant enterococci. *Korean J Lab Med.* 2010;30(6):631–636. doi:10.3343/kjlm.2010.30.6.631
41. Seo JY, Kim PW, Lee JH, et al. Evaluation of PCR-based screening for vancomycin-resistant enterococci compared with a chromogenic agar-based culture method. *J Med Microbiol.* 2011;60(Pt 7):945–949. doi:10.1099/jmm.0.029777-0
42. Kang CM, Chen XJ, Chih CC, et al. Rapid identification of bloodstream bacterial and fungal pathogens and their antibiotic resistance determinants from positively flagged blood cultures using the BioFire FilmArray blood culture identification panel. *J Microbiol Immunol Infect.* 2020;53(6):882–891. doi:10.1016/j.jmii.2020.03.018
43. Chan WS, Chan TM, Lai TW, et al. Complementary use of MALDI-TOF MS and real-time PCR-melt curve analysis for rapid identification of methicillin-resistant staphylococci and VRE. *J Antimicrob Chemother.* 2015;70(2):441–447. doi:10.1093/jac/dku411

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