Clinical Characteristics And Risk Factors In Mixed-Enterococcal Bloodstream Infections

Purpose: Although the enterococcal bloodstream infections (EBSI) are often observed in clinic, the mixed-EBSI are few reported. The aim of this study was to investigate the clinical characteristics and risk factors of mixed-EBSI in comparison with monomicrobial EBSI (mono-EBSI).

Methods: A single-center retrospective observational study was performed between Jan 1, 2013 and Dec 31, 2018 in a tertiary hospital. All patients with EBSI were enrolled, and their data were collected by reviewing electronic medical records.

Results: A total of 451 patients with EBSI were enrolled including 157 cases (34.8%) with mixed-EBSI. The most common co-pathogens were Coagulase-negative Staphylococcus (26.86%), followed by Acinetobacter baumannii (23.43%) and Klebsiella pneumoniae (8.57%). In multivariable analysis, burn injury (adjusted odds ratio [aOR], 7.39; 95% confidence interval [CI], 2.69–20.28), and length of prior hospital stay (aOR, 1.01; 95% CI, 1.00–1.02) were associated with mixed-EBSI. Patients with mixed-EBSI developed with more proportion of septic shock (19% vs. 31.8%, p=0.002), prolonged length of intensive care unit (ICU) stay [9(0.25) vs. 15(2.5,36), p<0.001] and hospital stay [29(16,49) vs. 33(18.5,63), p=0.031]. The mortality was not significantly different between mixed-EBSI and mono-EBSI (p=0.219).

Conclusion: A high rate of mixed-EBSI is among EBSI, and Acinetobacter baumannii is the second predominant co-existed species, except for Coagulase-negative Staphylococcus. Burn injury and length of prior hospital stay are independent risk factors for mixed-EBSI. Although the mortality is not different, patients with mixed-EBSI might have poor outcomes in comparison with mono-EBSI, which merits more attention by physicians in the future.

Keywords: bloodstream infections, mixed-enterococcal bloodstream infections, monomicrobial enterococcal bloodstream infections, clinical characteristics, risk factors

Introduction

Due to potentially serious consequences, bloodstream infections (BSI) are a growing worldwide concern. Enterococci is an important pathogen of BSI, which ranks the second leading cause of central line-associated bloodstream infection (16%) after Coagulase-negative Staphylococcus (CNS) (34.1%) according to the National Healthcare Safety Network’s report. The Enterococci becomes a significant pathogen, resulting from its ubiquitous distribution in the intestinal flora, the widespread uses of antibiotics and immunosuppressants, and the increase of invasive medical examinations and treatments in recent years. Enterococcal bloodstream infections (EBSI) are associated with significant morbidity (9%) and mortality (20–50%). In a recent Chinese report, Enterococcus accounted for 20% bloodstream infections with a mortality rate of 24%. Thus,
EBSI is becoming a serious threat to public health with its rising prevalence, high morbidity and mortality, and huge care cost.\(^{11}\)

Most of BSI are monomicrobial, but the trend of polymicrobial BSI is rising which accounted for 6–34% of BSI in previous studies.\(^{12-14}\) Polymicrobial BSI is generally associated with a higher acute physiology and chronic health evaluation II (APACHE II) scores, prolonged ICU and hospital stay, and a more severe prognosis than monomicrobial BSI in adults.\(^{12,14-17}\) In these previous studies,\(^{12,14-17}\) some limitations are existed as follows: (1) The clinical significance and outcomes of polymicrobial versus monomicrobial BSI were in indeed investigated, but few reports focused on a specific pathogen. Thus, the specific clinical features and outcomes between mixed-EBSI and mono-EBSI are still largely unknown. (2) The outcomes like 28-day mortality were poor in patients with polymicrobial BSI than monomicrobial BSI,\(^{14,16}\) while other studies showed that mixed-EBSI were not independently associated with mortality.\(^{18}\)

Thus, the clinical outcomes between polymicrobial BSI and monomicrobial BSI are still controversial. (3) Some risk factors like recent chemotherapy/radiation and recent antibiotic exposure were observed for mixed-EBSI,\(^{18}\) but the main subjects were African Americans and Caucasians. In addition, the sample size in the study was relatively small (284 episodes). Herein, we performed the study for better understanding of the clinical characteristics and risk factors of mixed-EBSI in Chinese population.

**Materials And Methods**

**Patients And Study Design**

This single-center retrospective cohort study was conducted from January 2013 to December 2018 in the Second Affiliated Hospital, Zhejiang University School of Medicine, a 3200-bed tertiary health-care facility in Hangzhou, China. The present study received human research ethics approval (No. 2019–194) from the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine, and made sure that the personal data should be kept confidential. Due to the retrospective nature of the study, the Ethics Committee determined that patient consent was not required. In addition, a statement of permission from patients for submission the present study was not required as the study did not include any personal information.

If any microorganisms other than *Enterococcus* were found in the same blood culture, the cases were retained. If only *Enterococcus* was found in multiple blood cultures of the same patient, the patients were only included once at the time of the first BSI with *Enterococci*. Exclusion criteria were as follows: a) Age<18 years old; b) Cases data were incomplete or missed; c) *Enterococcus* was considered as nonpathogenic bacterium. Common skin contaminant organisms (e.g., *Bacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., *Streptococcus* spp., *Lactobacillus* spp. and CNS) were considered as pathogens only when they were present in two or more consecutive blood cultures from separate blood draws. Thus, a total of 1158 blood culture specimens containing enterococcus were initially included, and final 451 cases were recruited with 157 cases for mixed-EBSI and 294 cases for mono-EBSI (Figure 1).

**Data Collection**

The patients’ data were collected by reviewing electronic medical records. The demographic data like age and gender, the clinical data including underlying diseases, sequential organ failure assessment (SOFA) score, Pitt bacteremia score, the Charlson Comorbidity Index (CCI) score, the APACHE II score in the first 24 h following the onset of BSI, the hospitalization wards, nosocomial infection or not, previous exposures (length of prior hospital stay, previous treatment such as surgical procedures, immunosuppressive agents, chemotherapeutic agents, radiation therapy, hyperalimentation, mechanical ventilation, renal replacement therapy, blood transfusion), and outcomes (length of hospital stay, length of ICU stay, cause septic shock and 28-day mortality) were collected. The microbiological data like species of *Enterococcus*,

![Figure 1: Flowchart of study participant enrollment.](image-url)

**Abbreviation:** EBSI, enterococcal bloodstream infection.
likely source of BSI, and sensitivity to antibiotics were also recorded. If the source of a BSI could not be attributed to any known source, it was classified as a primary BSI.19

Species Identification And Antibiotic Sensitivity Test
Blood was cultured using a BacT/ALERT 3D system (Becton-Dickinson, Sparks, MD, USA) in the microbiology laboratory. Species identification was performed using Bruker Daltonics DataAnalysis. Antibiotic susceptibility testing was performed using the VITEK 2 (Card number: AST-GN16; AST-GP67) system or the Kirby–Bauer Disk Diffusion method (Oxoid, UK) according to the recommendations proposed by the Clinical and Laboratory Standards Institute (CLSI).

Definitions
Diagnosis of EBSI was based on CDC definition for Bloodstream Infection Event.19 Onset of BSI was defined as the date when the blood culture was collected. Mixed-EBSI were defined as at least one nonenterococcal bacterial species isolated from one single blood culture sample.18 Nosocomial BSI was defined as the first positive blood culture obtained ≥48 h after hospital admission and with no evidence of infection at admission.9,20 Nonpathogenic bacterium was considered as contaminants, defined as one single positive blood culture in the absence of clinical manifestations.21 Appropriate antibiotic therapy was defined as an antibiotic regimen to which the index enterococcal isolate and co-pathogen (when applicable) were susceptible in vitro based on Clinical and Laboratory Standards Institute guidelines. Delayed antibiotic therapy was defined as therapy given more than 48 hrs after release of antibiotic susceptibility results.22 Sepsis and Septic shock were defined according to the new definition of Sepsis-3.23

Statistical Analysis
Statistical analysis was performed with SPSS 20.0 (IBM Corp, Armonk, NY, USA) software. Continuous variables were presented as mean ± standard deviation if normally distributed, and as median and interquartile range (IQRs) if nonnormally distributed. Continuous variables were compared by Student t test or Mann–Whitney U-test and enumeration variables were compared by Pearson χ² or Fisher exact test, where appropriate. Variables that had significance at a p<0.05 level in the univariate analysis were considered candidates for the building of stepwise logistic regression multivariable models. A two-tailed p<0.05 was considered statistically significant.

Results
Demographic Characteristics
The demographic characteristics of these patients are summarized in Table 1. The median age was 63 years (IQR, 50,72), and 71% (320/451) of them were male. Solid tumor was the most common comorbidity (23.3%), followed by trauma (19.5%) and diabetes mellitus (16.2%). The most ward of EBSI occurrence was ICU (61.6%), followed by surgical ward (29%) and medical ward (9.1%). There was no significant difference in age or gender between groups of mixed-EBSI and mono-EBSI. In terms of co-morbidities, a significant high percentage of trauma or burn injuries was observed in mixed-EBSI compared with mono-EBSI (both p<0.05). In comparison with mono-EBSI, patients with mixed-EBSI presented a more severe condition, evidenced by a higher APACHE II score (median, 18 vs. 15, p=0.001), a higher SOFA score (median, 6 vs. 5, p=0.005) and a higher Pitt Bacteremia Score (median, 4 vs. 3, p<0.001), and displayed more need of ICU admission (56.3% vs. 71%, p=0.002) or invasive mechanical ventilation (63.3% vs. 78.3%, p=0.001). Although patient with mixed-EBSI was negatively correlated with admission to surgical wards (21% vs. 33.7%, p=0.005), which was not related to surgery (52.9% vs. 47.3%, p=0.258) and the use of parenteral nutrition (55.3% vs. 45.9%, p=0.125). Blood transfusion was significantly often in patients with mixed-EBSI than those with mono-EBSI (15.9% vs. 8.2%, p=0.012). A significant increase in central line indwelling was observed in mixed-EBSI compared with mono-EBSI (50.3% vs. 39.8%, p=0.032), but not for indwelling of urinary catheter or intraperitoneal drainage tube (both p>0.05). In addition, a longer hospital stay before onset of BSI was often seen in patients with mixed-EBSI than mono-EBSI (median, 12 vs. 8.5, p=0.001).

Biological Indicators
A comparison of biological indicators between mixed-EBSI and mono-EBSI is shown in Table 2. Procalcitonin (PCT) was higher in patients with mixed-EBSI than that with mono-EBSI (median, 0.405 vs. 0.76, p=0.003), whereas there were no significant differences in blood routine test, liver & kidney function.
Independent Risk Factors For Mixed-EBSI

As shown in Table 3, multivariate logistic regression model analysis showed that the independent risk factors of mixed-EBSI were burn injury (adjusted odds ratio [aOR], 7.39; 95% confidence interval [CI], 2.69–20.28), and the days of prior hospital stay before onset of BSI (aOR, 1.01; 95% CI, 1.00–1.02).

Species Distribution Of Enterococcal Bloodstream Infections

The most common Enterococcus species was Enterococcus faecium (E. faecium), which comprised 53.88% (243/451) of all episodes, followed by Enterococcus faecalis (E. faecalis) (37.69%, 170/451) and Enterococcus gallinarum (3.55%, 16/451) (Supplemental Figure 1). Of the 451 episodes of

| Characteristics          | Total (n=451) | Mono-EBSI (n =294) | Mixed-EBSI (n =157) | P-value |
|--------------------------|--------------|---------------------|---------------------|---------|
| Age, median years (IQR)  | 63.0(50.0,72.0) | 63.0(51.0,73.0) | 61.0(47.0,71.0) | 0.317   |
| Male sex                 | 320(71.0%)   | 212(72.1%)         | 108(68.8%)         | 0.460   |
| Co-morbidities           |              |                     |                    |         |
| Diabetes mellitus        | 73(16.2%)    | 44(15.0%)          | 29(18.0%)          | 0.336   |
| Chronic kidney disease   | 29(6.4%)     | 20(6.8%)           | 9(5.7%)            | 0.659   |
| Chronic liver disease    | 17(3.8%)     | 11(3.7%)           | 6(3.8%)            | 0.966   |
| COPD or Severe asthma    | 27(6.0%)     | 19(6.5%)           | 8(5.1%)            | 0.560   |
| Chronic cardiac insufficiency | 38(8.4%) | 26(8.8%)       | 12(7.6%)          | 0.662   |
| Solid tumour             | 105(23.3%)   | 75(25.0%)          | 30(19.1%)          | 0.125   |
| Trauma                   | 88(19.5%)    | 45(15.3%)          | 43(27.4%)          | 0.002   |
| Burn injury              | 29(6.4%)     | 7(2.4%)            | 22(14.0%)          | <0.001  |
| Cerebrovascular accident | 69(15.3%)    | 45(15.3%)          | 24(15.3%)          | 0.996   |
| CCI, median (IQR)        | 3(2.5)       | 4(2.5)             | 3(1.5)             | 0.089   |
| APACHE II score, median (IQR) | 16(11.21) | 15(10.20)       | 18(13.22)          | 0.001   |
| SOFA score, median (IQR) | 5(4.9)       | 5(3.8)             | 6(4.9)             | 0.005   |
| Pitt Bacteremia Score, median (IQR) | 4(2.6) | 3(1.5)       | 4(3.6)             | <0.001  |
| Hospitalization ward     |              |                     |                    |         |
| Medical                  | 41(9.1%)     | 29(9.9%)           | 12(7.6%)           | 0.435   |
| Surgical                 | 132(29.0%)   | 99(33.7%)          | 33(21.0%)          | 0.005   |
| ICU                      | 278(61.6%)   | 166(56.5%)         | 112(71.0%)         | 0.002   |
| Previous treatment       |              |                     |                    |         |
| Hyperalimentation         | 219(48.6%)   | 135(45.9%)         | 84(55.3%)          | 0.125   |
| Mechanical ventilation    | 309(68.5%)   | 186(63.3%)         | 123(78.3%)         | 0.001   |
| Antibiotic exposure       | 426(94.5%)   | 279(94.9%)         | 147(93.6%)         | 0.575   |
| Surgery                  | 222(49.2%)   | 139(47.3%)         | 83(52.9%)          | 0.258   |
| Chemotherapy/radiation    | 11(2.4%)     | 6(2.0%)            | 5(3.2%)            | 0.453   |
| Renal replacement therapy | 38(8.4%)     | 25(8.5%)           | 13(8.3%)           | 0.935   |
| Blood transfusion         | 49(10.9%)    | 24(8.2%)           | 25(15.9%)          | 0.012   |
| Invasive devices          |              |                     |                    |         |
| Central line             | 196(43.5%)   | 117(39.8%)         | 79(50.3%)          | 0.032   |
| Indwelling urinary catheter | 321(71.2%) | 203(69.0%)      | 118(75.2%)         | 0.172   |
| Intraperitoneal drainage  | 87(19.3%)    | 58(19.7%)          | 29(18.5%)          | 0.747   |
| Prior hospital stay, median days (IQR) | 9,0(4.0190) | 8,5(3.0153) | 12,0(5,021,0)     | 0.001   |
| Nosocomial infection      | 396(87.8%)   | 253(86.1%)         | 143(91.1%)         | 0.120   |

Notes: Bold indicates P<0.05.
Abbreviations: COPD, chronic obstructive pulmonary disorder; CCI, Charlson Comorbidity Index; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; IQR, interquartile range; EBSI, enterococcal bloodstream infections.
bacteremia, 294 (65.2%) were mono-EBSI and 157 (34.8%) were mixed-EBSI. The distribution comparison of Enterococcus species isolated from mixed-EBSI and mono-EBSI is shown in Figure 2, which showed the proportion of E. faecium or E. faecalis was significantly lower or higher in mixed-EBSI than that in mono-EBSI (47.1% vs. 57.5%, p=0.036; or 43.9% vs. 34.4%, p=0.045, respectively). A total of 175 other microorganisms in mixed-EBSI cases were isolated in 157 mixed-EBSI cases, with two microorganisms accounting for 88.5% (139/157) and three microorganisms for 11.5% (18/157). The most common co-pathogen was Gram-negative bacteria (57.1%), followed by Gram-positive bacteria (38.3%) and fungi (4.6%). In terms of the exacted microorganism, the most frequent pathogen was CNS (26.86%), followed by Acinetobacter baumannii (A. baumannii) (23.43%), Klebsiella pneumoniae (8.57%) and Staphylococcus aureus (S. aureus) (8%). The detailed distribution of additional organisms in mixed-EBSI is shown in Supplemental Figure 2.

The source of EBSI was mainly from intra-abdominal (34.4%, 155/451), followed by primary BSI (28.8%, 130/451) and pneumonia (13.7%, 62/451). Compared with mono-EBSI, the sources of mixed-EBSI were more often from central venous catheter (12.7% vs. 6.1%, p=0.016) and the skin/soft tissue (16.6% vs. 5.8%, p<0.001), but less from abdominal cavity (26.1% vs. 38.8%, p=0.007) (Table 4).

### Antibiotic Resistance And Appropriate Therapy

The resistance of Enterococcus to vancomycin and teicoplanin in both groups of mixed-EBSI and mono-EBSI was very low (less than 3%) (Table 4). In comparison with mono-EBSI, the ratio of resistance of Enterococcus to tetracycline was significantly higher in mixed-EBSI groups (44.7% vs. 56.2%, p<0.05), but it was lower to ampicillin (42.9% vs. 57.3%) or levofloxacin (51.2% vs. 63.0%) (both, p<0.05). A total of 16.4% (74/451) patients did not receive appropriate therapy within 48 hrs after the release of antibiotic susceptibility results, but there was no difference between the two groups (15.3% vs. 18.5%, p=0.387) (Table 4).

### Outcomes

The comparison of prognosis between mixed-EBSI and mono-EBSI is shown in Table 5. The median length of hospital stay was 31 days (IQR, 16,53), and the median length of ICU stay was 11 days (IQR, 0,28). In comparison with mono-EBSI, patients with mixed-EBSI developed with more proportion of septic shock (19% vs. 31.8%, p=0.002), prolonged length of ICU stay [9(0,25) vs. 15 (2.5,36), p<0.001] and hospital stay [29(16,49) vs. 33 (18.5,63), p=0.031]. The 7-day, 14-day or 28-day mortality.

### Table 2 Comparison Of Biological Indicators Between Groups Of Mixed-EBSI And Mono-EBSI

| Biological Indicators          | Total (n=451) | Mono-EBSI (n =294) | Mixed-EBSI (n =157) | P-value |
|-------------------------------|--------------|--------------------|---------------------|---------|
| Temperature (°C) (IQR)        | 39.0(38.4,39.3) | 39(38.3,39.3)       | 39(38.5,39.5)       | 0.057   |
| Blood routine test            |              |                    |                     |         |
| WBC (×10⁹/L) (IQR)            | 10.0(6.8,13.9) | 10.0(7.1,14.0)      | 9.3(6.3,13.9)       | 0.215   |
| Hematocrit (%)                | 26.5(22.3,31.9) | 27.1(22.3,32.4)     | 25.0(22.3,30.8)     | 0.068   |
| Platelet (×10⁹/L) (IQR)       | 156.0(101.1,246.0) | 158.0(103.0,237.3)  | 155.0(98.0,254.5)   | 0.870   |
| ANC (IQR)                     | 8.46(5.1,12.33) | 8.72(5.86,12.35)    | 7.59(5.15,12.25)    | 0.169   |
| Liver and kidney function     |              |                    |                     |         |
| Albumin (g/L) (mean±S.D.)     | 31.07±5.99    | 31.09±5.92          | 31.03±5.15          | 0.930   |
| GPT (U/L) (IQR)               | 37.0(20.0,60.0) | 34.5(19.0,63.3)     | 41.0(21.0,71.5)     | 0.227   |
| GOT (U/L) (IQR)               | 37.0(26.0,75.0) | 36.0(24.8,72.0)     | 39.0(18.8,81.0)     | 0.150   |
| ALP (U/L) (IQR)               | 105.0(72.0,150.0) | 108.0(71.8,153.5)   | 99.0(72.0,142.0)    | 0.394   |
| γ-GT (U/L) (IQR)              | 47.0(25.0,105.0) | 49.5(24.0,101.0)    | 47.0(27.0,113.0)    | 0.526   |
| LDH (U/L) (IQR)               | 290.0(211.0,401.0) | 283.0(210.8,385.3)  | 301.0(215.0,460.5)  | 0.267   |
| TBil (µmol/L) (IQR)           | 16.5(10.5,35.0) | 15.7(10.0,27.6)     | 17.8(11.9,33.3)     | 0.162   |
| SCr (µmol/L) (IQR)            | 62.0(44.0,89.0) | 62.5(44.0,88.0)     | 60.0(43.5,92.0)     | 0.725   |
| PCT (ng/mL) (IQR)             | 0.53(0.20,2.00)  | 0.405(0.17,1.52)    | 0.76(0.26,3.52)     | 0.003   |

Notes: Bold indicates P<0.05.

Abbreviations: WBC, white blood count; ANC, absolute neutrophil count; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; ALP, alkaline phosphatase; γ-GT, gamma glutamyl transpeptidase; LDH, lactic dehydrogenase; TBil, total bilirubin; SCr, serum creatinine; PCT, procalcitonin; IQR, interquartile range; EBSI, enterococcal bloodstream infections.
rates or in-hospital mortality in patients with mixed-EBSI were not different with those with mono-EBSI (Table 5, Figure 3).

Discussion

In the current study, several important results were found. First, mixed-EBSI was no longer a rare event, and *E. faecium* (53.88%) was the most common pathogen. Second, some risk factors were found to be associated with mixed-EBSI, including ICU admission, a higher APACHE II score, a higher SOFA score, trauma, blood transfusion, mechanical ventilation and central venous catheter indwelling (Table 1). Moreover, burn injury and length of prior hospital stay were independent risk factors for mixed-EBSI. Third, although CNS had the highest proportion as co-pathogens in mixed-EBSI, Gram-negative bacteria remained the main co-pathogens in mixed-EBSI in comparison with Gram-positive bacteria. Last, patients with mixed-EBSI might have poor outcomes including higher occurrence of septic shock, prolonged lengths of ICU stay and hospital stay in comparison with mono-EBSI.

A high proportion (34.8%) of mixed-EBSI among EBSI was observed in the current study, which was consistent with other studies (28%–44%). Previous studies showed that the rate of polymicrobial bacteremia was increasing over years, which might be explained by an increasing number of patients with central venous catheters and immunocompromised patients. In terms of the exact *Enterococcus* in the study, *E. faecium* (53.88%) was the most common pathogen, which was high than that in previous EBSI studies (less than 50%). A constant increase in the rate of *E. faecium* BSI was observed.

In fact, the incidence of *E. faecium* BSI exceeding *E. faecalis* BSI was observed in a Swiss study and two Chinese studies. The exact reasons underlying the increased incidence of *E. faecium* infections are not yet well known, but might be related to increased resistance of *E. faecium* and enhanced virulence by acquiring new virulence factors.

Like in previous studies, similar risk factors for mixed-EBSI in our study were found including ICU admission, a higher APACHE II score, a higher SOFA score, and a longer prior hospital stay before onset of BSI, burn injury or trauma, blood transfusion, mechanical ventilation and central venous catheter indwelling (Table 1). However, the CCI, reflecting the severity of underlying disease, did not show any difference in both groups (Table 1), which might be explained by the fact that CCI is inferior to APACHE II score to predict hospital mortality for ICU patients. Although recent chemotherapy/radiation and recent antibiotic exposure were positively associated with mixed-EBSI in a previous study, they were not independently associated with mixed-EBSI in our study. This might be due to a low proportion of patients (2.4%) receiving chemotherapy/radiation therapy in our study. Importantly, burn injury and length of prior hospital stay were independent factors for mixed-EBSI in the current study, which was consistent with a previous study showing that more than 12% of burn patients suffered from polymicrobial BSI. These results together suggest that burn patients are not only susceptible to BSI, but also to polymicrobial BSI including mixed-EBSI.

In our current study, the most common co-pathogen was CNS (26.86%), followed by *A. baumannii* (23.43%).

### Table 3 Multivariable Logistic Regression Of Factors Associated With Mixed-EBSI

| Variable                          | Unadjusted OR (95% CI) | p-Value | Adjusted OR (95% CI) | p-Value |
|----------------------------------|------------------------|---------|----------------------|---------|
| Trauma                           | 2.09(1.30,3.35)        | 0.002   | 1.10(0.62,1.96)      | 0.742   |
| Burn injury                      | 6.68(2.79,16.02)       | <0.001  | 7.39(2.69,20.28)     | <0.001  |
| APACHE II score                  | 1.04(1.02,1.07)        | 0.003   | 1.03(0.98,1.09)      | 0.289   |
| SOFA score                       | 1.05(1.01,1.11)        | 0.032   | 0.97(0.89,1.06)      | 0.556   |
| Pct Bacteremia Score             | 1.15(1.07,1.24)        | <0.001  | 1.09(0.95,1.26)      | 0.231   |
| ICU stay                         | 1.95(1.29,2.97)        | 0.002   | 0.98(0.42,2.30)      | 0.960   |
| Surgical                         | 0.52(0.33,0.83)        | 0.005   | 0.76(0.34,1.70)      | 0.499   |
| Prior Blood transfusion          | 2.13(1.17,3.87)        | 0.013   | 1.26(0.63,2.53)      | 0.523   |
| Central line                     | 1.53(1.04,2.26)        | 0.032   | 0.89(0.55,1.34)      | 0.610   |
| Mechanical ventilation           | 2.10(1.34,3.29)        | 0.001   | 1.13(0.54,2.34)      | 0.751   |
| Prior hospital stay              | 1.01(1.00,1.02)        | 0.006   | 1.01(1.00,1.02)      | 0.026   |

Notes: Bold indicates *p*<0.05. Abbreviations: ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; EBSI, enterococcal bloodstream infections.
It is worth noting that Gram-negative bacteria were still the main co-pathogen (57.1%) in comparison with Gram-positive bacteria (Supplemental Figure 2). Although CNS was the same most common co-pathogen, the second co-pathogen was A. baumannii in our study, whereas it was S. aureus in Lafnf’s study. A high percentage of central venous catheter source of mixed-EBSI (38.7%) was observed in Lagnf’s study, while it only accounted for 12.7% in the current study. It is well known that the common pathogen of catheter-related bloodstream infections is Gram-positive bacteria especially S. aureus. Thus, this might partially explain a high proportion of S. aureus as a co-pathogen among polymicrobial EBSI in Lagnf’s study. In addition, we also found A. baumannii accounted for 38.8%, while S. aureus accounted for only 3.74% in post-neurosurgical intracranial infections in our previous study. This means gram-negative bacteria, especially A. baumannii, is the main pathogen in our hospital-acquired infection, as also observed in the distribution of co-pathogens in mixed-EBSI (57.1% for Gram-negative bacteria, while 38.3% for Gram-positive bacteria) in the current study. Taken together, A. baumannii was the second co-pathogen in mixed-EBSI, except for CNS.

Although a higher PCT value was observed in mixed-EBSI than that in mono-EBSI [0.76(0.26,3.52) vs 0.405 (0.17,1.52), p=0.003] (Table 2), it may have no clinical meaning. It is worth noting that serum PCT level was often high in Gram-negative bacterium-induced BSI, whereas it was slightly increased or no effect after Gram-positive bacterium-mediated BSI. To this end, we stratified mixed-EBSI group into two sub-groups of mixed-EBSI with Gram-negative bacteria and mixed-EBSI with non-Gram-negative bacteria. Compared with mono-EBSI, PCT in mixed-EBSI with Gram-negative bacteria was significantly higher than that in mono-EBSI (median, 1.06 vs. 0.405, p<0.001), whereas it was similar to that in

Figure 2 The distribution comparison of enterococcus species isolated from mixed-EBSI and mono-EBSI. Abbreviation: EBSI, enterococcal bloodstream infection.
mixed-EBSI with non-Gram-negative bacteria (median, 0.380 vs. 0.405, p=0.582) (Supplemental Figure 3). These results suggest that we should keep in mind that mixed-EBSI including a Gram-negative bacterium might be present once EBSI is accompanied with a high serum PCT value.

Although patients with mixed-EBSI might have poor outcomes than those with mono-EBSI, the 28-day mortality was similar between the two groups (Table 5). This result was consistent with other studies showing that no correlation between polymicrobial EBSI and mortality was observed.10,11,21 Low percentage (less than 20%) of delayed antibiotic therapy, a high proportion (more than one third) of primary BSI as primary BSI has a lower mortality rate than secondary BSI,43 and a quite low proportion of vancomycin-resistant Enterococci (VRE) (Table 4), might be ascribed to the similar mortality observed in our study.

### Table 4 Comparison Of Microbiological Characteristics In Patients With Mono-EBSI Or Mixed-EBSI

| Source of BSIs                          | Total (n=451) | Mono-EBSI (n =294) | Mixed-EBSI (n =157) | P-value  |
|-----------------------------------------|---------------|--------------------|---------------------|----------|
| Intra-abdominal                         | 155(34.4%)    | 114(38.8%)         | 41(26.1%)           | 0.007    |
| Primary BSI                             | 130(28.8%)    | 84(28.6%)          | 46(29.3%)           | 0.871    |
| Pneumonia                               | 62(13.7%)     | 41(13.9%)          | 21(13.4%)           | 0.867    |
| Skin and Soft tissue infection          | 43(9.5%)      | 17(5.8%)           | 26(16.6%)           | <0.001   |
| Central venous catheter                 | 38(8.4%)      | 18(6.1%)           | 20(12.7%)           | 0.16     |
| Urinary tract infection                 | 12(2.7%)      | 9(3.1%)            | 3(1.9%)             | 0.470    |
| Intracranial                            | 5(1.1%)       | 5(1.7%)            | 0(0.0%)             | 0.168    |
| Endocarditis                            | 4(0.9%)       | 4(1.4%)            | 0(0.0%)             | 0.303    |
| Others a                                | 2(0.4%)       | 2(0.7%)            | 0(0.0%)             | 0.545    |

**Antibiotic resistance of Enterococci**b

| Antibiotic                          | Total (n=295) | Mono-EBSI (n =157) | Mixed-EBSI (n =138) | P-value  |
|-------------------------------------|---------------|--------------------|---------------------|----------|
| Ampicillin                          | 229(52.2%)    | 163(57.3%)         | 66(42.9%)           | 0.004    |
| Ciprofloxacin                       | 255(56.5%)    | 172(58.5%)         | 83(52.9%)           | 0.250    |
| Tetracycline                        | 156(48.8%)    | 93(44.7%)          | 63(56.2%)           | 0.049    |
| Erythromycin                        | 249(71.3%)    | 172(72.9%)         | 77(68.1%)           | 0.359    |
| Levofloxacin                        | 210(59.0%)    | 148(63.0%)         | 62(51.2%)           | 0.033    |
| Nafcillin                           | 115(31.8%)    | 84(35.1%)          | 31(25.2%)           | 0.054    |
| Teicoplanin                         | 1(1.0%)       | 1(1.8%)            | 0(0.0%)             | 1        |
| Linezolid                           | 71(16.1%)     | 41(14.2%)          | 30(19.7%)           | 0.136    |
| Vancomycin                          | 10(2.2%)      | 8(2.7%)            | 2(1.3%)             | 0.505    |

**Notes:** Bold indicates P<0.05; aSubmandibular gland, joint; bNot all agents listed tested in all isolates; cthe numbers in parentheses represent the total numbers of Enterococcus performed susceptibility test.

**Abbreviation:** EBSI, enterococcal bloodstream infections.

### Table 5 Comparison Of Outcomes In Patients With Mono-EBSI Or Mixed-EBSI

| Outcomes                             | Total (n=451) | Mono-EBSI (n =294) | Mixed-EBSI (n =157) | P-value  |
|--------------------------------------|---------------|--------------------|---------------------|----------|
| Total Hospitalization days (m, IQR)  | 31.0(16.0,53.0)| 29.0(16.0,49.0)    | 33.0(18.5,63.0)     | 0.031    |
| Total ICU residence days (m, IQR)    | 11.0(0.0,28.0)| 9.0(0.0,25.0)      | 15.0(2.5,36.0)      | <0.001   |
| Septic shock (n, %)                  | 106(23.5%)    | 56(19.0%)          | 50(31.8%)           | 0.002    |
| 7-day mortality (n, %)               | 72(16.0%)     | 44(15.0%)          | 28(17.8)            | 0.428    |
| 14-day mortality (n, %)              | 95(21.1%)     | 57(19.4%)          | 38(24.2)            | 0.232    |
| 28-day mortality (n, %)              | 111(24.6%)    | 67(22.8%)          | 44(28.0%)           | 0.219    |
| In-hospital mortality (n, %)         | 135(29.9%)    | 80(27.2%)          | 55(35.0%)           | 0.084    |

**Notes:** Bold indicates P<0.05.

**Abbreviations:** M, median; IQR, interquartile range; ICU, intensive care unit; EBSI, enterococcal bloodstream infections.
There were some limitations in this study. First, it was a retrospective study, and as a result, some important information or variable such as Glasgow coma scale score could not be obtained; In addition, it is hard to say cause and effect about the relationship of polymicrobial bacteremia and more serious condition, though patients with more severe illness and/or serious condition tend to get polymicrobial bacteremia. Second, although the data of this study were collected over a 6 years period in a tertiary hospital, it only represented a single center. In addition, the “primary BSI” described in the current study might have a bias, as the exact source of BSI was really hard to confirm by retrospective analysis. Thus, future multicenter prospective studies are needed to investigate the risk factors of mixed-EBSI.

**Conclusion**

Mixed-EBSI is not a rare event among total EBSI, and *A. baumannii* is the second predominant co-existed species, except for *Coagulase-negative Staphylococcus*. Many factors including trauma, burn injury, placement of central intravenous catheter, use of mechanical ventilation, need of blood transfusion, length of prior hospital stay, ICU admission, a higher APACHE II score, a higher SOFA score, and a higher Pitt Bacteremia score are associated with mixed-EBSI, whereas burn injury and length of prior hospital stay are independent risk factors. Although the mortality is not different, patients with mixed-EBSI might have poor outcomes, which merits more attention by physicians in the future.

**Abbreviations**

EBSI, enterococcal bloodstream infections; COPD, chronic obstructive pulmonary disorder; CCI, Charlson Comorbidity Index; WBC, white blood count; ANC, absolute neutrophil count; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxaloacetic transaminase; ALP, alkaline phosphatase; γ-GT, gamma glutamyl transpeptidase; LDH, lactic dehydrogenase; TBil, total bilirubin; SCr, serum creatinine; PCT, procalcitonin; mono-EBSI, monomicrobial enterococcal bloodstream infections; mixed-EBSI, mixed enterococcal bloodstream infections; OR, odds ratio; CI, confidence interval; ICU, intensive care unit; BSI, bloodstream infections; CNS, *Coagulase-negative Staphylococcus*; APACHE, acute physiology and chronic health evaluation; SOFA, sequential organ failure assessment; CDC, Centers for Disease Control and Prevention; IQR, interquartile range; CLSI, Clinical and Laboratory Standards Institute; *E. faecium*, Enterococcus faecium; *E. faecalis*, Enterococcus faecalis; *A. baumannii*, Acinetobacter baumannii; *S. aureus*, Staphylococcus aureus; VRE, vancomycin-resistant Enterococci; *K. pneumoniae*, Klebsiella pneumoniae; *E. coli*, Escherichia coli; *P. aeruginosa*, Pseudomonas aeruginosa; *S. maltophilia*, Stenotrophomonas maltophilia; *S. viridans*, Streptococcus viridans.

**Ethical Approval**

The present study received human research ethics approval from the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. We make sure to keep patient data confidential and compliance with the Declaration of Helsinki.

**Informed Consent**

Due to the retrospective nature of the study, the Ethics Committee determined that no patient consent was required.

**Author Contributions**

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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