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ORIGINAL RESEARCH

Clinical Characteristics And Risk Factors In Mixed-Enterococcal Bloodstream Infections

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Cheng Zheng,[1,2,*] Jiachang Cai,[3,*] Haizhou Liu,[1,4,*] Shufang Zhang,[5] Li Zhong,[1,6] Nanxia Xuan,[1] Hongwei Zhou,[3] Kai Zhang,[1] Yesong Wang,[1] Xijiang Zhang,[2] Baoping Tian,[1] Zhaocai Zhang,[1] Changming Wang,[2] Wei Cui,[1] Gensheng Zhang[1]

[1]Department of Critical Care Medicine, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, People's Republic of China; [2]Department of Critical Care Medicine, Taizhou Municipal Hospital, Taizhou, Zhejiang, People's Republic of China; [3]Clinical Microbiology Laboratory, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, People's Republic of China; [4]Department of Critical Care Medicine, Zhejiang Rehabilitation Hospital, Hangzhou, Zhejiang, People's Republic of China; [5]Department of Cardiology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, People's Republic of China; [6]Department of Critical Care Medicine, Huzhou First People's Hospital, Huzhou, Zhejiang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Gensheng Zhang
Department of Critical Care Medicine, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, People's Republic of China
Tel +86-571-8778-3636
Fax +86-571- 87022776
Email genshengzhang@zju.edu.cn

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.[1] *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.[2,3] 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.[4,5] Enterococcal bloodstream infections (EBSI) are associated with significant morbidity (9%) and mortality (20–50%).[6–9] In a recent Chinese report, *Enterococcus* accounted for 20% bloodstream infections with a mortality rate of 24%.[10] 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., *Streptococci, 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*,

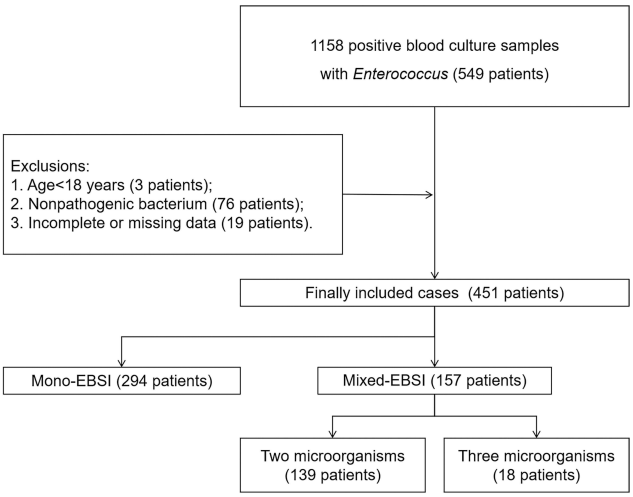

Figure 1 Flowchart of study participant enrollment.
Abbreviation: EBSI, enterococcal bloodstream infection.

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 χ^2 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 (56.5% 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.

Table 1 Demographic And Clinical Characteristics Of The Patients With Mono-EBSI Or Mixed-EBSI

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(4.0.19.0)	8.5(3.0.15.3)	12.0(5.0.21.0)	**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.

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

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 (×10^9/L) (IQR)	10.0(6.8,13.9)	10.0(7.1,14.0)	9.3(6.3,13.9)	0.215
Hematocrit (%) (IQR)	26.5(22.3,31.9)	27.1(22.3,32.4)	25.0(22.3,30.8)	0.068
Platelet (×10^9/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(5.51,12.33)	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(10.5,30.5)	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.

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 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
Pitt 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(0.55,2.342)	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.

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%).[9,11,21,24] Previous studies[14,25] 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.[25] 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%).[5,9,21,26] A constant increase in the rate of *E. faecium* BSI was observed.[4] In fact, the incidence of *E. faecium* BSI exceeding *E. faecalis* BSI was observed in a Swiss study and two Chinese studies.[10,27,28] 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*[29] and enhanced virulence by acquiring new virulence factors.[30]

Like in previous studies,[16,25,31–33] 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.[34] Although recent chemotherapy/radiation and recent antibiotic exposure were positively associated with mixed-EBSI in a previous study,[18] 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.[35] 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%).

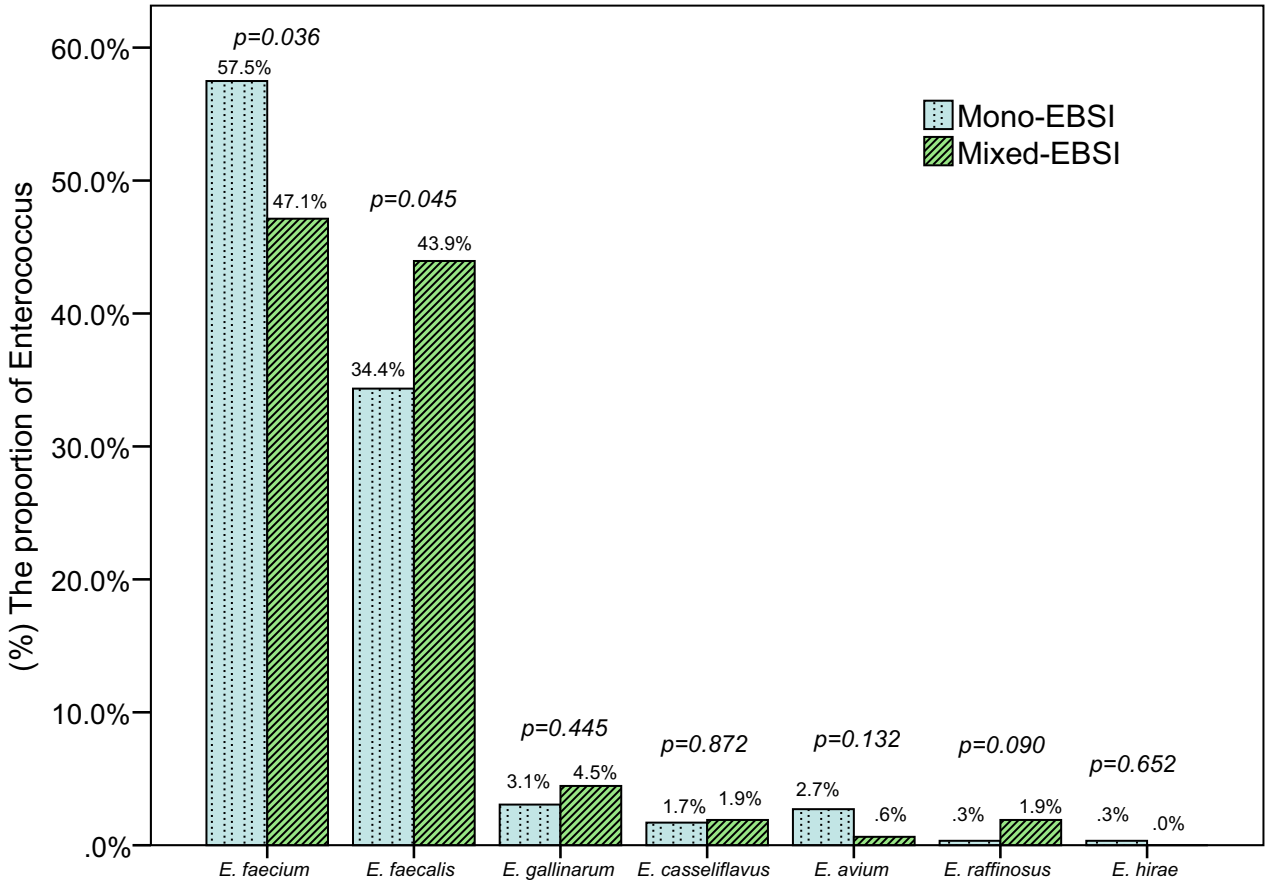

Figure 2 The distribution comparison of enterococcus species isolated from mixed-EBSI and mono-EBSI.
Abbreviation: EBSI, enterococcal bloodstream infection.

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[18] 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*.[36–38] 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.[39] 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.[40–42] 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

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

	Total (n=451)	Mono-EBSI (n =294)	Mixed-EBSI (n =157)	P-value
Source of BSIs				
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%)	**0.016**
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 *Enterococcus*[b]				
Ampicillin (285 vs. 154)[c]	229(52.2%)	163(57.3%)	66(42.9%)	**0.004**
Ciprofloxacin (294 vs. 157)[c]	255(56.5%)	172(58.5%)	83(52.9%)	0.250
Tetracycline (208 vs. 112)[c]	156(48.8%)	93(44.7%)	63(56.2%)	**0.049**
Erythromycin (236 vs. 113)[c]	249(71.3.0%)	172(72.9%)	77(68.1%)	0.359
Levofloxacin (235 vs. 121)[c]	210(59.0%)	148(63.0%)	62(51.2%)	**0.033**
Nitrofurantoin (239 vs. 132)[c]	115(31.8%)	84(35.1%)	31(25.2%)	0.054
Teicoplanin (57 vs. 43)[c]	1(1.0%)	1(1.8%)	0(0.0%)	1
Linezolid (288 vs. 152)[c]	71(16.1%)	41(14.2%)	30(19.7%)	0.136
Vancomycin (294 vs. 157)[c]	10(2.2%)	8(2.7%)	2(1.3%)	0.505
Treatment after the onset of BSIs				
Delayed antibiotic therapy	74(16.4%)	45(15.3%)	29(18.5%)	0.387

Notes: Bold indicates P<0.05; [a]Submandibular gland, joint; [b]Not all agents listed tested in all isolates; [c]the numbers in parentheses represent the total numbers of *Enterococcus* performed susceptibility test.

Abbreviation: EBSI, enterococcal bloodstream infections.

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 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.

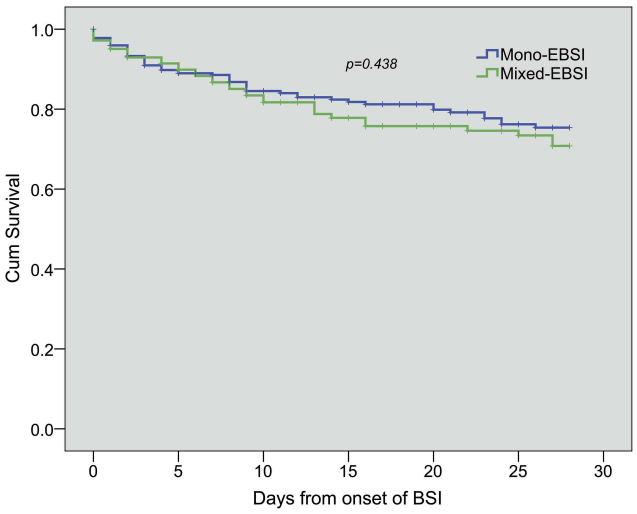

Figure 3 Kaplan-Meier estimates of survival in patients with mixed-enterococcal bloodstream infections and monomicrobial enterococcal bloodstream infections.
Abbreviation: EBSI, enterococcal bloodstream infection.

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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Disclosure

The authors report no conflicts of interest in this work.

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