Original article

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ON THE ROLE OF ENTEROCOCCI IN THE BLOODSTREAM: RESULTS OF A SINGLE-CENTER, RETROSPECTIVE, OBSERVATIONAL STUDY AT A GERMAN UNIVERSITY HOSPITAL

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This study assesses the clinical relevance of vancomycin-susceptible enterococci in bacteremic patients and compares it with bacteremia due to *Staphylococcus aureus* and *Escherichia coli*.

During a 5-year-study interval, clinical and diagnostic features of patients with enterococcal bacteremia were compared to those of patients with *E. coli* or *S. aureus* bacteremia. Each patient was only counted once per hospital stay.

During the 5-year study interval, data from 267 patients with enterococcal bacteremia and from 661 patients with bacteremia due to *E. coli* or *S. aureus* were evaluated. In spite of a comparable risk of death, patients with enterococci more frequently needed catecholamines and invasive ventilation. Furthermore, enterococci were more frequently associated with a mixed bacterial flora in bloodstream infections. While fatal sepsis due to *E. coli* and *S. aureus* was associated with typical shock symptoms, this association was not confirmed for enterococci.

Although enterococcal bacteremia is associated with a risk of dying comparable to that with bacteremia due to *E. coli* and *S. aureus,* a lower pathogenic potential of enterococci in bloodstream has to be acknowledged. Enterococci in the bloodstream are more likely to be an epiphenomenon of impending death than its major cause.

Keywords: enterococci, bloodstream infection, fatal outcome, *Enterococcus* spp., blood culture

Introduction

On human mucous membranes, particularly in the gut, enterococci usually persist as harmless to useful colonizers. A pathological role for enterococci is well established for urinary tract infections [1], in bacterial endocarditis [2], or in cases of translocation into primarily sterile compartments [3, 4]. Prophylaxis with combinations of cephalosporins and metronidazole works well during surgical operations on the open gut [5, 6], although enterococci are not susceptible to these antibiotics. This is regarded as an argument for the low etiological relevance of enterococci in abdominal infections [5]. In corpses, in contrast, enterococci spread readily throughout the body of the deceased after the breakdown of the gut–blood barrier [7] and drive their decomposition. In line with a presumably low pathogenicity, death after enterococcal infections is mainly observed in immunocompromised patients [8–11].

With respect to this overall low relevance of enterococci in deep-seated infections, this study was undertaken to determine their etiological relevance in bloodstream infections, which has not yet been satisfactorily assessed.

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Clinical and diagnostic features of *Enterococcus* spp.-associated bacteremia were assessed and compared with those of bacteremia due to *Staphylococcus aureus* (both methicillin-susceptible and -resistant) and *Escherichia coli*, both of which are well-established causative agents of sepsis [12, 13].

We have set up the working hypothesis that, unlike sepsis caused by established bacterial pathogens, enterococcal bacteremia is primarily encountered in two groups of patients: One is comparably healthy and suffers from transient bacteremia potentially associated with surgical procedures or inflamed mucous membranes. The other consists of pre-mortal patients with a disrupted gut–blood barrier allowing easy translocation of gut-inhabiting bacteria like enterococci from the gut into the bloodstream. To test this hypothesis, the following severity indicators were compared: automated ventilation, catecholamine requirement, and death of patients bacteremic for one of the three species. Identification of polymicrobial bacteremia was used as a criterion for translocation events in severely ill patients.

Methods

The study was designed as a retrospective observational study and carried out at the University Hospital of Rostock, Germany, covering a study interval from 2007 to 2011.

All patient samples were collected and processed according to the instructions of the accredited (DIN EN ISO 15189) Institute for Medical Microbiology, Virology and Hygiene of the Rostock University Hospital. The resulting data were analyzed with the SwissLab software (version 2.12.2.008848; SwissLab Ltd., Berlin, Germany) of the laboratory.

Assessment of the frequency of enterococci in blood cultures at the study site

The data from the 20 most frequent species isolated from blood culture bottles (BacT/ALERT SA/SN/FA/FN/PF, bioMérieux, Nürtingen, Germany) at the Institute for Medical Microbiology, Virology and Hygiene were used for comparison. Data sets were extracted from the SwissLab laboratory software and analyzed using the HyBase software version 6.1508.1 (Tieto Germany Ltd., Regensburg, Germany). Mean values were calculated from the data from the study interval.

Inclusion and exclusion criteria

For a more refined analysis, data from patients of the University Hospital Rostock suffering from *Enterococcus* spp. and/or *E. coli* and/or *S. aureus* (methicillin-susceptible or -resistant) bacteremia in the years 2007 to 2011 were included in the study. In the following sections, data sets from *S. aureus* and *E. coli* were occasionally combined for statistical reasons and are then referred to as "non-enterococci". Each bacterial species was counted only once per patient and hospital admission to avoid analyzing copy strains.

Assessment of clinical data

Available clinical data were retrospectively assessed from the medical records of the included patients. The assessment was performed anonymously to ensure the patients' rights to privacy. As well as age, gender, and death of patients during the hospital stay, a total of 20 clinical and laboratory features per patient were assessed. The assessment comprised differentiation of pure and mixed bacterial culture; surgical interventions in general and abdominal surgery in particular; presence of wounds, urinary catheters, and stomata; body temperature; blood pressure according to Riva-Rocci (RR) [14]; pulse rate; leukocyte count; C-reactive protein levels; procalcitonin levels; detection of leukocytes and bacteria in urine samples; need for treatment in intensive care units (ICUs); catecholamine administration or invasive ventilation at the ICU; the presence of bacterial isolates from bronchoalveolar lavage; sepsis as the main diagnosis; and administration of antibiotic therapy at the time of sample acquisition.

The results were adjusted to remarkable findings as depicted and defined in *Table 2* from the Results section. All data were included in a SAS database, version 9.4 (SAS Institute Inc., Cary, NC, USA), which was also used for all statistical assessments as described below.

Comparison of the enterococcus group and the non-enterococcus group regarding hard endpoints

Hard endpoints for patients with enterococcal or non-enterococcal bacteremia were defined as need for invasive ventilation, need for catecholamine administration, and death at any time point in the hospital stay after the diagnosis of bacteremia. The data from the enterococcus and the nonenterococcus groups were compared with the χ^2 test.

Assessment of the enterococcus group and the non-enterococcus group for single species or mixed bacterial species in blood cultures

In the first step, differences between the frequencies of mixed-species bacterial culture in the enterococcus group and the nonenterococcus group were assessed based on odds ratios. In the second step, the same calculation was repeated for deceased patients only.

Comparison of factors associated with survival and death for the enterococcus group and the nonenterococcus group

To identify factors associated with death specifically due to enterococcal bacteremia, all recorded clinical features were analyzed for associations with survival and death in both the enterococcus group and the nonenterococcus group. Taken together, this assessment should discriminate whether dying patients from the enterococcus group were more severely affected than patients from the nonenterococcus group. Furthermore, it should distinguish the extent of differences in clinical illness between dying and surviving patients in the two bacteremic groups.

A logistic regression (model: binary logit; optimization: Fischer's scoring) was performed to indicate the impact of score parameters on the fatal outcome for patients with enterococcal and nonenterococcal bacteremia. Then, an adapted logistic regression (model: binary logit; optimization: Fischer's scoring) with stepwise elimination was added to indicate only significant variables and two-way interactions regarding fatal outcome for patients with enterococcal and nonenterococcal bacteremia. Significance was assessed by χ^2 testing.

Comparison of factors associated with other hard endpoints in the enterococcus group and the nonenterococcus group

The initially chosen hard endpoint parameters of death, need for catecholamine administration, and need for invasive ventilation were analyzed for independence. After reciprocal dependence of the three parameters had been proven by crosstabs (data not shown), logistic regression (model: binary logit; optimization: Fischer's scoring) with stepwise elimination was performed to indicate only significant variables and two-way interactions regarding the composite outcome "need for catecholamines and need for invasive ventilation and fatal outcome" for patients with enterococcal and nonenterococcal bacteremia. Significance was assessed by χ^2 testing.

Ethics statement

Ethical clearance for the study, including the anonymized retrospective assessment of patients' data from their medical records without informed consent of the patients, was approved by the ethics committee of the University Medicine Rostock (study registration number: A 2015-0078) in accordance with relevant guidelines and regulations.

Results

Frequency of enterococci in blood cultures at the study site

In the list of the 20 most frequently isolated species from blood cultures at the University Hospital Rostock during the study period *(Supplementary Material 1), Enterococcus faecium* was at position no. 6 and *Enterococcus faecalis* at position no. 8. Next to typical skin contaminants such as coagulase-negative staphylococci and *Propionibacterium* spp., *E. coli* and *S. aureus* were the two most frequently isolated species with probable or at least presumptive etiological relevance for severe deep-seated infections or sepsis *(Supplementary Material 1)*.

Included and excluded patients and samples

During the study interval, 32,394 blood culture bottles were assessed at the University Medicine Rostock. Adjusted for samples from the same patient during the same hospital stay, there were 3992 patients with one or more positive blood cultures and 12,266 patients with negative blood cultures. The distribution of analyzed blood culture samples as assessed by the laboratory statistics is depicted in *Table 1*.

Table 1. Statistics of assessed blood culture samples during the study interval from 2007 to 2011 at the University Medicine Rostock

Groups	2007–2011	2007	2008	2009	2010	2011
Assessed blood culture samples (total)	32394	6324	6165	6092	6924	6889
Assessed blood culture samples (corrected by copy strains)	16258	3020	3078	3209	3433	3518
Positive blood culture samples (total)	5640	1053	1134	1036	1177	1240
Positive blood culture samples (corrected by copy strains)	3992	758	777	750	810	897
Negative blood culture samples (total)	26754	5271	5031	5056	5747	5649
Negative blood culture samples (corrected by copy strains)	12266	2262	2301	2459	2623	2621
Detected enterococci (total)	567	133	98	92	140	104
Detected enterococci (corrected by copy strains)	369	88	66	63	86	66
Included patients with enterococci into the assessment	267*	48	40	30	49	41
Detected *Enterococcus faecalis* (total)	221	48	34	46	55	38

Table 1. *(cont'd)*

Groups	2007–2011	2007	2008	2009	2010	2011
Detected *Enterococcus faecalis* (corrected by copy strains)	147	30	26	31	30	30
Included patients with *Enterococcus faecalis* into the assessment	112**	17	18	16	13	22
Detected *Enterococcus faecium* (total)	312	75	62	37	77	61
Detected *Enterococcus faecium* (corrected by copy strains)	196	51	39	25	50	31
Included patients with *Enterococcus faecium* into the assessment	141†	29	22	11	35	16
Detected *S. aureus* (including MRSA, total)	650	84	135	126	165	140
Detected *S. aureus* (including MRSA, corrected by copy strains)	363	51	77	70	84	81
Included patients with *S. aureus* (including MRSA) into the assessment	286‡	30	52	49	49	61
Detected MSSA (total)	434	49	84	98	107	96
Detected MSSA (corrected by copy strains)	275	37	54	57	67	60
Included patients with MSSA into the assessment	219§	21	35	38	35	44
Detected MRSA (total)	216	35	51	28	58	44
Detected MRSA (corrected by copy strains)	88	14	23	13	17	21
Included patients with MRSA into the assessment	67¶	9	17	11	4	17
Detected *E. coli* (total)	659	105	132	119	135	168
Detected *E. coli* (corrected by copy strains)	463	75	91	87	87	123
Included patients with *E. coli* into the assessment	375#	42	57	54	46	94

*Missing documentation of isolation year for 59 data sets
**Missing documentation of isolation year for 26 data sets
†Missing documentation of isolation year for 28 data sets
‡Missing documentation of isolation year for 55 data sets
§Missing documentation of isolation year for 46 data sets
¶Missing documentation of isolation year for 9 data sets
#Missing documentation of isolation year for 82 data sets

Over the 5-year-interval of the study, 267 patients with enterococci in blood cultures, comprising 121 *E. faecalis,* 141 *E. faecium,* 4 *E. avium,* 1 *E. casseliflavus,* 1 *E. cecorum*, and 6 not further characterized *Enterococcus* spp., and 661 patients with nonenterococci, comprising 375 *E. coli,* 219 methicillin-susceptible *S. aureus,* and 67 methicillin-resistant *S. aureus* (MRSA), respectively, were identified and included in the study *(Table 1)*. Each patient was counted only once per hospital stay, irrespective of the number of positive blood cultures or the time between them. As also detailed in *Table 2,* the coverage of data assessment of the affected patients ranged between one-half and three-quarters of the total number of patients with bacteremia due to enterococci, *E. coli,* or *S. aureus*. The incompleteness is due to the fact that not all patient files were assessable for logistic reasons.

The mean age of patients with enterococcal bacteremia was 63.8 years; that of patients with nonenterococcal bacteremia was 65.5 years. Among the individuals with enterococcal bacteremia, 67% were male; among the ones with nonenterococcal bacteremia, 56% were male.

Assessment of clinical data

Clinical characteristics were compared for enterococcal and nonenterococcal bacteremia *(Table 2),* ensuring sufficiently high patient numbers for statistical assessments as described in the following.

Comparison of the enterococcus group and the non-enterococcus group regarding hard endpoints

Considering the two endpoints, i.e., need for mechanical ventilation and need for catecholamines, the risk was considerably increased in patients with enterococcal bacteremia in comparison with patients with *E. coli* or *S. aureus* bacteremia. The risk of dying, however, was identical in both groups *(Table 3)*. There were no relevant differences between *E. faecium*- and *E. faecalis*-associated bacteremia.

Assessment of the enterococcus group and the non-enterococcus group for one or more bacterial species in blood cultures

The presence of more than one bacterial species in the blood culture medium was significantly more frequent in patients with enterococcal bacteremia than in patients with bacteremia due to *E. coli* or *S. aureus*. If only deceased patients were analyzed, the significance of this difference disappeared *(Table 4)*. Again, there were no detectable differences between *E. faecalis* and *E. faecium*.

Table 2. Part 1. Characteristics of surviving and deceased patients with enterococcal or nonenterococcal bacteremia

	Enterococcal bacteremia					Nonenterococcal bacteremia				
	Survived		Deceased		Incomplete data sets	Survived		Deceased		Incomplete data sets
	(*n*)	(%)	(*n*)	(%)	(*n*)	(*n*)	(%)	(*n*)	(%)	(*n*)
Differentiation of pure and mixed bacterial culture										
Pure	110	69	39	20	59	386	91	91	90	138
Mixed	49	31	10	80		36	9	10	10	
Surgical interventions occurred										
Yes	100	64	25	56	62	125	31	26	26	152
No	57	36	21	44		284	69	74	74	
Abdominal surgery occurred										
Yes	46	29	12	25	62	42	12	12	12	142
No	111	71	36	75		388	88	89	88	
Presence of wounds										
Yes	35	23	14	29	63	78	19	26	26	145
No	120	77	35	71		337	81	75	74	
Presence of stomata										
Yes	61	39	20	41	61	54	13	21	21	143
No	96	61	29	59		363	87	80	79	
Body temperature (°C)										
≥38.5	70	48	12	28	75	229	56	46	46	156
<38.0	29	20	23	50		70	17	32	32	
38.0–38.4	47	32	10	22		107	26	21	21	
Registered blood pressure according to Riva-Rocci (RR, mmHg)										
<100/60	32	22	13	28	77	80	20	41	41	171
100/60–120/80	104	72	32	70		248	63	54	55	
>120/80	8	6	1	2		63	16	4	4	
Pulse rate										
≥100/min	47	33	18	39	188	127	32	47	47	170
<100/min	95	67	28	61		265	68	52	53	
Leukocyte count										
≥11.5 × 10^9/l	74	51	28	64	79	219	55	57	59	164
<11.5 × 10^9/l	70	49	16	36		181	45	40	41	
C-reactive protein (CRP)										
CRP ≥0.05 g/l	142	99	44	98	79	391	98	100	100	162
CRP <0.05 g/l	1	1	1	2		8	2	0	0	

Table 2. Part 2. Characteristics of surviving and deceased patients with enterococcal or nonenterococcal bacteremia

	Enterococcal bacteremia					Nonenterococcal bacteremia				
	Survived		Deceased		Incomplete data sets	Survived		Deceased		Incomplete data sets
	(*n*)	(%)	(*n*)	(%)	(*n*)	(*n*)	(%)	(*n*)	(%)	(*n*)
Procalcitonin										
≥0.5 × 10^{-5} g/l	26	90	18	95	219	70	89	33	97	548
<0.5 × 10^{-5} g/l	3	10	1	5		9	11	1	3	
Presence of urinary catheters										
Yes	152	98	48	98	63	359	85	96	97	142
No	3	2	1	2		61	15	3	3	
Detection of leukocytes in urine										
Yes	45	48	6	29	152	172	58	25	53	315
No	49	52	15	71		127	42	22	47	
Detection of bacteria in urine samples										
Yes	33	42	7	30	165	146	57	30	64	357
No	46	58	16	70		111	43	17	36	
Treatment on intensive care unit required										
Yes	103	66	36	77	63	125	30	64	63	148
No	54	34	11	23		287	70	37	37	
Need for catecholamines										
Yes	65	42	31	66	64	67	16	52	51	147
No	91	58	16	34		346	84	49	49	
Need for invasive ventilation										
Yes	60	38	30	64	63	46	11	36	36	145
No	97	62	17	36		369	89	65	64	
Antibiotic therapy at time point of sample acquisition										
Yes	100	65	41	85	64	173	42	71	71	146
No	55	35	7	5		242	58	29	29	
Main diagnosis sepsis										
Yes	18	11	10	20	61	159	38	30	30	141
No	139	89	39	80		260	62	71	70	
Isolates from bronchoalveolar lavage										
Yes	1	3	2	25	229	3	12	3	27	624
No	29	97	6	75		23	88	8	73	

Table 3. Differences regarding catecholamine administration, requirement for automated ventilation, and fatal outcome for patients with enterococcal bacteremia vs. patients with *S. aureus* or *E. coli*. The results were confirmed by a forward modeled logistic regression model

	No catecholamines*	Catecholamines*	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia	107	96	3.0	2.1–4.2	<0.0001
Nonenterococcal bacteremia	397	119	Ref.		
	No catecholamines*	Catecholamines*	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	7	2	0.9	0.2–4.6	0.95
Bacteremia due to *E. faecalis*	54	31	1.9	1.2–3.1	0.01
Bacteremia due to *E. faecium*	46	63	4.6	3.0–7.0	<0.0001
Nonenterococcal bacteremia	397	119	Ref.		
	No automated ventilation**	Automated ventilation**	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia	114	90	4.2	2. 9–6.0	<0.0001
Nonenterococcal bacteremia	434	82			
	No automated ventilation**	Automated ventilation** ‘	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	8	1	0.7	0.1–5.4	0.7
Bacteremia due to *E. faecalis*	58	27	2.5	1.5–4.1	0.0006
Bacteremia due to *E. faecium*	48	62	6.8	4.4–10.7	<0.0001
Nonenterococcal bacteremia	434	82	Ref.		
	Survival†	Fatal outcome†	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	159	49	1.3	0.9–1.9	0.2
Nonenterococcal bacteremia	422	101			
	Survival†	Fatal outcome†	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	7	2	1.2	0.2–5.8	0.8
Bacteremia due to *E. faecalis*	67	19	1.1	0.7–2.1	0.55
Bacteremia due to *E. faecium*	85	28	1.4	0.8–2.2	0.2
Nonenterococcal bacteremia	422	101	Ref.		

*, **, † Incomplete information for 21%, 23%, and 22% of data sets, respectively

Table 4. Frequency of single and mixed bacterial species from patients with enterococcal bacteremia or patients with *S. aureus*/*E. coli* bacteremia with respect to the full sample size (upper table) and the subgroup of deceased patients (lower table)

All patients					
	2 or More species*	Single species*	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia	73	194	3.8	2.6–5.6	<0.0001
Nonenterococcal bacteremia	59	600	Ref.		
All patients					
	2 or More species*	Single species*	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	6	8	7.63	2.6–22.7	0.0003
Bacteremia due to *E. faecalis*	29	83	3.6	2.2–5.9	<0.0001
Bacteremia due to *E. faecium*	38	103	3.7	2.4–5.9	<0.0001
Nonenterococcal bacteremia	59	600	Ref.		
Deceased patients					
	2 or More species	Single species	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia	10	39	2.3	0.9–6.0	0.08
Nonenterococcal bacteremia	10	91	Ref.		
Deceased patients					
	2 or More species*	Single species*	Odds ratio	95% Confidence interval	*P*
Enterococcal bacteremia (other than *E. faecalis* or *E. faecium*)	1	1	9.1	0.5–156.9	0.13
Bacteremia due to *E. faecalis*	4	15	2.4	0.7–8.7	0.17
Bacteremia due to *E. faecium*	5	23	2.0	0.6–6.3	0.25
Nonenterococcal bacteremia	10	91	Ref.		

*Incomplete data for 2 data sets each

Table 5. Logistic regression (model: binary logit; optimization: Fischer's scoring) indicating the impact of score parameters on the fatal outcome for patients with enterococcal or nonenterococcal bacteremia. Significance was assessed by χ^2 testing

Odds ratio estimates and profile-likelihood confidence intervals*						
	Enterococcal bacteremia			Nonenterococcal bacteremia		
Effect	Estimate	95% Confidence limits	*P*	Estimate	95% Confidence limits	*P*
Pure culture vs. mixed culture	1.30	0.49–3.44	0.60	0.96	0.40–2.30	0.92
Surgery vs. no surgery	0.52	0.16–1.66	0.27	0.34	0.16–0.74	0.0063
Any wounds vs. no wounds	0.91	0.36–2.32	0.85	0.92	0.49–1.76	0.81
Any stomata vs. no stomata	0.85	0.29–2.46	0.76	0.97	0.46–2.04	0.94
Abdominal surgery yes vs. no	1.19	0.43–3.26	0.74	1.58	0.67–3.73	0.30
Temp. 38 °C–38.4 °C versus temp. <38 °C	0.21	0.07–0.64	0.0059	0.51	0.24–1.12	0.09
Temp. ≥38.5 °C vs. temp. <38 °C	0.29	0.11–0.82	0.019	0.66	0.33–1.28	0.22
Pulse rate ≥100/min vs. <100/min	1.79	0.66–4.82	0.25	2.04	1.17–3.55	0.012
Leukocytes ≥11.5 × 10^9/l vs. <11.5 × 10^9/l	1.07	0.46–2.53	0.87	1.10	0.63–1.92	0.73
CRP ≥0.05 g/l vs. < 0.05 g/l	0.15	0.01–3.06	0.22	>999.99	<0.01 to >999.99	0.99
Urinary catheter yes vs. no	0.79	0.06–10.90	0.86	2.31	0.65–8.16	0.20
Need for intensive care treatment vs. no need	0.24	0.04–1.40	0.11	1.69	0.79–3.61	0.18
Need for catecholamines vs. no need	1.09	0.32–3.78	0.89	2.38	1.04–5.44	0.039
Need for invasive ventilation yes vs. no	9.35	1.99–44.98	0.0047	2.05	0.81–5.18	0.13
Antibiotic therapy at time point of sample acquisition vs. no such therapy at this time point	3.06	0.95–9.91	0.06	2.57	1.46–4.53	0.0011
RR <100/60 vs. RR 100/60–120/80 (mmHg)	1.16	0.43–3.17	0.77	1.94	1.09–3.44	0.024
RR >120/80 vs. RR 100/60–120/80 (mmHg)	1.41	0.13–15.38	0.78	0.47	0.15–1.47	0.19
Main diagnosis sepsis vs. other than sepsis	1.11	0.35–3.54	0.86	0.46	0.25–0.85	0.014

*Detection of bacteria in bronchoalveolar lavage or urine samples as well as the parameters leukocytes in urine and elevated procalcitonin levels were excluded because of incomplete data sets

The species distribution of the bacteria accompanying enterococci, *E. coli,* and *S. aureus* in mixed species blood cultures showed an inconclusive pattern *(Supplementary Material 2)*. The low numbers of observed isolates did not allow for statistical analysis. However, enterococci were more frequently associated with coagulase-negative staphylococci than *S. aureus* or *E. coli*.

Factors associated with survival or death of patients with enterococcal or nonenterococcal bacteremia

Logistic regression *(Table 5)* and logistic regression with stepwise elimination of factors *(Supplementary Material 3)* indicated that patients with bacteremia due to *E. coli* and *S. aureus* were more likely to die in case of tachycardia with a pulse rate ≥100/min, low blood pressure (RR) of <100/60, and need for catecholamines. For enterococcal bacteremia, this association was not observed.

Comparison of factors associated with a combined endpoint comprising "need for invasive ventilation, need for catecholamines, and death" in patients with enterococcal or nonenterococcal bacteremia

When analyzed in cross tables, the three endpoints "need for invasive ventilation, need for catecholamines, and death" showed reciprocal dependence (data not shown). Therefore, an additional analysis that regarded the three parameters as a single composite endpoint was performed. Specifically for patients with *E. coli* and *S. aureus* bacteremia, the presence of stomata and intravenous catheters was associated with an increased risk. Specifically for patients with enterococcal bacteremia, fever combined with the presence of stomata was the only identified risk factor. Associations of shock parameters were no longer observed *(Supplementary Material 4)*.

Discussion

The study was performed to address the predisposing factors for and etiological relevance of enterococci in blood culture. Bacteremia due to *E. coli* and *S. aureus,* a likely cause of sepsis [12, 13], was chosen as a reference for the comparison. As described for other European hospitals [15, 16], *E. faecium* quantitatively dominated in comparison to the therapeutically less problematic *E. faecalis*. This is in contrast to other regions such as Australia, where *E. faecalis* dominates [17]. Based upon data from the German National Surveillance System, an annual incidence of about 30,000 nosocomial *E. faecium* infections has been calculated, comprising 13.2% vancomycin-resistant isolates [18], demonstrating the importance of vancomycin-susceptible strains.

The study produced several main results. First of all, patients with enterococcal bacteremia are typically more severely ill than patients with typical causative agents of sepsis like *E. coli* and *S. aureus* in their bloodstream. In spite of a comparable risk of death, patients with enterococci more frequently need catecholamines and invasive ventilation. In addition, enterococcal bacteremia was more frequently associated with at least one additional bacterial species in the bloodstream. Finally, fatal sepsis due to *E. coli* and *S. aureus* is usually associated with shock symptoms such as tachycardia, hypotonia, and need for catecholamines, whereas this association was not identified for the enterococci. The synopsis of these results suggests that severely ill patients more frequently suffer from enterococcal bacteremia, while patients who are still able to show a systemic inflammatory reaction resulting in shock are more likely to die from sepsis due to *E. coli* and *S. aureus*.

Data on the risk for bacteremia with vancomycin-susceptible enterococci are still rare [9]. In particular, comparisons with the features of infections due to nonenterococcal causes of sepsis are usually absent. Bacteremia due to enterococci is usually hospital acquired, with urinary tract infections, intra-abdominal infections, and infective endocarditis as the major sources [10]. In India, the rate of enterococcal bacteremia has been estimated at 25.4 episodes per 1000 admissions in trauma patients [19]. Preliminary recent data suggest increased mortality in cases of repeated detection of positive blood cultures with enterococci, in particular in case of prolonged bloodstream infections lasting more than 6 days [20].

This study implies that enterococcal bacteremia and *E. coli* and *S. aureus* bacteremia display identical risks of dying, being 18.4% and 19.3% of the respective patients. This mortality matches that in a Polish study on enterococcal bacteremia, which indicated a 14-day mortality of 18.1% [21]. In pediatric patients with enterococcal bacteremia after hematopoietic stem cell transplantation, a 30-day mortality of 20% has been reported irrespective of vancomycin susceptibility or resistance traits of the strains [9]. In a Danish assessment, 30-day mortality of 21.4% due to *E. faecalis* and even of 34.6% due to *E. faecium* has been reported, with age, comorbidity, and hospital-acquired infections as predictors of deadly courses [10]. Another analysis described a 7-day mortality of 13% and a 30-day mortality of 25% in patients with bacteremia due to vancomycin-susceptible enterococci [8]. Prolonged healthcare exposure and increased comorbidity are associated with enterococcal bacteremia [8, 22]. The mortality risk in vancomycin-resistant enterococci (VRE) patients is generally higher [23, 24].

The patient numbers with 112 *E. faecalis* and 141 *E. faecium* bacteremic isolates did not allow for a statistically meaningful analysis. From the literature, it is known that invasive procedures, surgery, chronic skin ulcers, and indwelling devices are risk factors in particular for *E. faecalis* infections [25]. Community-acquired bacteremia due to *E. faecalis* was shown to be associated with infective endocarditis in 25% of instances [10]. For *E. faecium,* in contrast, it could be demonstrated that certain clones of

increased pathogenic potential may cause invasive disease with bacteremia [26]. In cancer patients, *E. faecium* bacteremia is independently associated with more severe underlying illness [27, 28].

Enterococci can lead to systemic infections if the gut–blood barrier fails and if the intestinal flora is disrupted by antibiotic treatment, resulting in domination of the gut flora by enterococcal species [29]. The higher rate of mixed bacterial flora in patients with enterococcal bacteremia in this study could be due to a disturbed gut–blood barrier in severely ill patients. While 27.4% of mixed bacterial infections were observed in this study, a previous assessment suggested up to 39% bacteremia with enterococci plus other species [10]. Interestingly, in this study, typically skin-inhabiting coagulase-negative staphylococci (CNS) predominated as partners in enterococcal bacteremia, while typical gut colonizers were less frequently observed. There is a formal possibility that CNS in blood cultures result from improper skin disinfection during specimen sampling [30, 31]. However, the legally enforced annual surveillance on infectious disease data of the Rostock University Hospital demonstrates CNS rates in blood cultures at or below the average of the hospitals in this region. In addition, CNS were more frequently associated with enterococcal but not with nonenterococcal bacteremias. Therefore, the occurrence of enterococci could be interpreted as a warning sign for critical disease courses with failing gut–blood barrier. However, risk of dying due to polymicrobial bacteremia is 21% [32] and thus comparable with the general risk of dying as observed in this study.

The study has several limitations. First of all, the number of assessed samples does not allow for detailed sub-group analyses. Future studies with higher numbers of included patients should address the impact of immunosuppression of affected patients and the question whether comparable results can be achieved for patients with repeated proof of enterococci in blood culture. In addition, it would be interesting to compare healthcare-associated from community-acquired infections with enterococci, which was not analyzed by this approach.

Summary

In summary, it was shown that patients with enterococcal bacteremia exhibit a risk of dying comparable to that for *E. coli* and *S. aureus* bacteremia and an even higher morbidity as expressed by need for catecholamines and invasive ventilation. Thus, patients dying with enterococcal bacteremia are usually more severely ill than patients with nonenterococcal bloodstream infections. Together with the lack of association between typical signs of shock and death due to enterococcal bacteremia, the low pathogenic potential of enterococci in the bloodstream has to be acknowledged when compared with bacteremia due to *E. coli* and *S. aureus*. Thus, in many cases, enterococcal bacteremia could rather be an epiphenomenon of impending death than one of its true causes. The higher frequency of mixed bacterial blood cultures indicating failure of the gut–blood barrier further supports this interpretation for patients with enterococcal bacteremia.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Ronald A: The etiology of urinary tract infection: traditional and emerging pathogens. Dis Mon 49, 71–82 (2003)
2. Megran DW: Enterococcal endocarditis. Clin Infect Dis 15, 63–71 (1992)
3. Bayer AS, Seidel JS, Yoshikawa TT, Anthony BF, Guze LB: Group D enterococcal meningitis. Clinical and therapeutic considerations with report of three cases and review of the literature. Arch Intern Med 136, 883–886 (1976)
4. Tornero E, Senneville E, Euba G, Petersdorf S, Rodriguez-Pardo D, Lakatos B, Ferrari MC, Pilares M, Bahamonde A, Trebse R, Benito N, Sorli L, del Toro MD, Baraiaetxaburu JM, Ramos A, Riera M, Jover-Sáenz A, Palomino J, Ariza J, Soriano A; European Society Group of Infections on Artificial Implants (ESGIAI): Characteristics of prosthetic joint infections due to *Enterococcus* sp. and predictors of failure: a multi-national study. Clin Microbiol Infect 20, 1219–1224 (2014)
5. Mittelkötter U: Antimicrobial prophylaxis for abdominal surgery: is there a need for metronidazole? J Chemother 1, 27–34 (2001)
6. Solomkin JS, Mazuski JE, Bradley JS, Rodvold KA, Goldstein EJ, Baron EJ, O'Neill PJ, Chow AW, Dellinger EP, Eachempati SR, Gorbach S, Hilfiker M, May AK, Nathens AB, Sawyer RG, Bartlett JG: Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. Clin Infect Dis 50, 133–164 (2010)
7. Epstein EZ, Kugel MA: The significance of postmortem bacteriological examination: With special reference to streptococci and enterococci. J Infect Dis 44, 327–334 (1929)
8. McBride SJ, Upton A, Roberts SA: Clinical characteristics and outcomes of patients with vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* bacteraemia – a five-year retrospective review. Eur J Clin Microbiol Infect Dis 29, 107–114 (2010)
9. Vydra J, Shanley RM, George I, Ustun C, Smith AR, Weisdorf DJ, Young JA: Enterococcal bacteraemia is associated with increased risk of mortality in recipients of allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 55, 764–770 (2012)
10. Pinholt M, Ostergaard C, Arpi M, Bruun NE, Schønheyder HC, Gradel KO, Søgaard M, Knudsen JD; Danish Collaborative Bacteraemia Network (DACOBAN): Incidence,

clinical characteristics and 30-day mortality of enterococcal bacteraemia in Denmark 2006–2009: a population-based cohort study. Clin Microbiol Infect 20, 145–151 (2014)
11. Kajihara T, Nakamura S, Iwanaga N, Oshima K, Takazono T, Miyazaki T, Izumikawa K, Yanagihara K, Kohno N, Kohno S: Clinical characteristics and risk factors of enterococcal infections in Nagasaki, Japan: a retrospective study. BMC Infect Dis 15, 426 (2015)
12. Glauser MP, Zanetti G, Baumgartner JD, Cohen J: Septic shock: pathogenesis. Lancet 338, 732–736 (1991)
13. Laupland KB, Church DL: Population-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev 27, 647–664 (2014)
14. Lewis WH: The evolution of clinical sphygmomanometry. Bull N Y Acad Med 17, 871–881 (1941)
15. Top J, Willems R, Blok H, de Regt M, Jalink K, Troelstra A, Goorhuis B, Bonten M: Ecological replacement of *Enterococcus faecalis* by multiresistant clonal complex 17 *Enterococcus faecium*. Clin Microbiol Infect 13, 316–319 (2007)
16. Weisser M, Capaul S, Dangel M, Elzi L, Kuenzli E, Frei R, Widmer A: Additive effect of *Enterococcus faecium* on enterococcal bloodstream infections: a 14-year study in a Swiss tertiary hospital. Infect Control Hosp Epidemiol 34, 1109–1112 (2013)
17. Coombs GW, Pearson JC, Daley DA, Le T, Robinson OJ, Gottlieb T, Howden BP, Johnson PD, Bennett CM, Stinear TP, Turnidge JD; Australian Group on Antimicrobial Resistance: Molecular epidemiology of enterococcal bacteremia in Australia. J Clin Microbiol 52, 897–905 (2014)
18. Gastmeier P, Geffers C, Herrmann M, Lemmen S, Salzberger B, Seifert H, Kern W, Fätkenheuer G: Nosocomial infections and infections with multidrug-resistant pathogens – frequency and mortality. Dtsch Med Wochenschr 141, 421–426 (2016)
19. Rajkumari N, Mathur P, Thanbuana B, Sajan S, Misra MC: Magnitude of enterococcal bacteraemia in trauma patients admitted for intensive trauma care: a tertiary care experience from South Asian country. J Lab Physicians 7, 38–42 (2015)
20. Claeys KC, Zasowski EJ, Lagnf AM, Rybak MJ: Comparison of outcomes between patients with single versus multiple positive blood cultures for *Enterococcus:* infection versus illusion? Am J Infect Control 44, 47–49 (2016)
21. Gawryszewska I, Żabicka D, Bojarska K, Malinowska K, Hryniewicz W, Sadowy E: Invasive enterococcal infections in Poland: the current epidemiological situation. Eur J Clin Microbiol Infect Dis 35, 847–856 (2016)
22. Moses V, Jerobin J, Nair A, Sathyendara S, Balaji V, George IA, Peter JV: Enterococcal bacteraemia is associated with prolonged stay in the medical intensive care unit. J Glob Infect Dis 4, 26–30 (2012)
23. 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 41, 327–333 (2005)
24. Prematunge C, MacDougall C, Johnstone J, Adomako K, Lam F, Robertson J, Garber G. VRE and VSE bacteraemia outcomes in the era of effective VRE therapy: a systematic review and meta-analysis. Infect Control Hosp Epidemiol 37, 26–35 (2016)
25. Hayakawa K, Marchaim D, Palla M, Gudur UM, Pulluru H, Bathina P, Alshabani K, Govindavarjhulla A, Mallad A, Abbadi DR, Chowdary D, Kakarlapudi H, Guddati H, Das M, Kannekanti N, Vemuri P, Doddamani R, Mundra VR, Guddeti RR, Policherla R, Bai S, Lohithaswa S, Shashidharan SP, Chidurala S, Diviti S, Sukayogula K, Joseph M, Pogue JM, Lephart PR, Martin ET, Rybak MJ, Kaye KS: Epidemiology of vancomycin-resistant *Enterococcus faecalis:* a case-case-control study. Antimicrob Agents Chemother 57, 49–55 (2013)
26. Lu CL, Chuang YC, Chang HC, Chen YC, Wang JT, Chang SC: Microbiological and clinical characteristics of vancomycin-resistant *Enterococcus faecium* bacteraemia in Taiwan: implication of sequence type for prognosis. J Antimicrob Chemother 67, 2243–2249 (2012)
27. Conde-Estévez D, Grau S, Albanell J, Terradas R, Salvadó M, Knobel H: Clinical characteristics and outcomes of patients with vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* bacteraemia in cancer patients. Eur J Clin Microbiol Infect Dis 30, 103–108 (2011)
28. Gudiol C, Ayats J, Camoez M, Domínguez MÁ, García-Vidal C, Bodro M, Ardanuy C, Obed M, Arnan M, Antonio M, Carratalà J: Increase in bloodstream infection due to vancomycin-susceptible *Enterococcus faecium* in cancer patients: risk factors, molecular epidemiology and outcomes. PLoS One 2013;8:e74734 (2013)
29. Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG: Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis 55, 905–914 (2012)
30. Freeman JT, Chen LF, Sexton DJ, Anderson DJ: Blood culture contamination with Enterococci and skin organisms: implications for surveillance definitions of primary bloodstream infections. Am J Infect Control 39, 436–438 (2011)
31. Jindai K, Strerath MS, Hess T, Safdar N: Is a single positive blood culture for *Enterococcus* species representative of infection or contamination? Eur J Clin Microbiol Infect Dis 33, 1995–2003 (2014)
32. Reuben AG, Musher DM, Hamill RJ, Broucke I: Polymicrobial bacteraemia: clinical and microbiologic patterns. Rev Infect Dis 11, 161–183 (1989)

Supplementary Material 1. Most frequently isolated microbial species from blood cultures (total numbers of isolates without modifying algorithms of data assessment)

Isolated microorganisms	Mean number of isolates per year (averaged over 4 years)	Standard deviation (SD)
Staphylococcus epidermidis	230	20
Escherichia coli	98	18
Staphylococcus hominis	65	16
Staphylococcus aureus (methicillin susceptible)	62	5
Propionibacterium spp.	40	8
Enterococcus faecium	38	13
Staphylococcus haemolyticus	34	8
Enterococcus faecalis	30	3
Staphylococcus aureus (methicillin resistant)	20	5
Staphylococcus capitis	19	6
Candida albicans	15	5
Micrococcus spp.	15	4
Klebsiella pneumoniae	14	4
Pseudomonas aeruginosa	12	6
Streptococcus pneumoniae	11	5
Proteus mirabilis	10	1
Enterobacter cloacae	9	2
Streptococcus agalactiae	9	4
Candida glabrata	9	6
Klebsiella oxytoca	8	3

Supplementary Material 2. Distribution of the mixed detected flora by species or taxonomic group on enterococci, *E. coli*, and *S. aureus*

		Enterococci		*Staphylococcus aureus*		*Escherichia coli*	
		All patients	Dead patients	All patients	Dead patients	All patients	Dead patients
Anaerobic bacteria	N	0	0	0	0	1	1
	%	0%	0%	0%	0%	0%	1%
Enterobacteriaceae	N	14	4	2	1	8	2
	%	2%	3%	0%	0%	0%	1%
Non-fermentative Gram-negative rod-shaped bacteria	N	5	2	1	0	4	0
	%	0%	1%	0%	0%	0%	0%
Other Gram-negative rod-shaped bacteria	N	1	0	0	0	1	0
	%	0%	0%	0%	0%	0%	0%
Enterococci as part of the mixed flora	N	2	0	0	0	0	0

	%	0%	0%	0%	0%	0%	0%
Staphylococcus aureus as part of the mixed flora	N	0	0	0	0	1	1
	%	0%	0%	0%	0%	0%	1%
Coagulase-negative staphylococci	N	40	2	11	0	13	1
	%	4%	1%	1%	0%	1%	0%
Streptococcus spp.	N	3	0	2	1	5	1
	%	0%	0%	0%	0%	1%	1%
Other Gram-positive coccoid bacteria	N	2	0	1	0	0	0
	%	0%	0%	0%	0%	0%	0%
Gram-positive rod-shaped bacteria	N	2	0	2	0	2	1
	%	0%	0%	0%	0%	0%	1%
Fungi	N	5	2	1	0	1	1
	%	1%	1%	0%	0%	0%	1%

Supplementary Material 3. Logistic regression (model: binary logit; optimization: Fischer's scoring) with stepwise elimination to indicate only significant variables and two-way-interactions regarding fatal outcome for patients with enterococcal or nonenterococcal bacteremia. Significance was assessed by chi-square testing

Odds ratio estimates and profile-likelihood confidence intervals[a]						
	Enterococcal bacteremia			Nonenterococcal bacteremia		
Effect	Estimate	95% Confidence limits	P	Estimate	95% Confidence Limits	P
Any surgery vs. no surgery	0.33	0.12-0.83	0.02	0.36	0.20-0.63	<0.01
Temp. ≥38.5°C vs. temp. <38°C	0.29	0.12-0.71	<0.01	0.48	0.29-0.81	<0.01
Need for invasive ventilation yes vs. no	4.98	2.03-13.51	<0.01	2.53	1.30-5.04	<0.01
Temp. 38°C-38.5°C vs. temp. <38°C	0.29	0.08-0.63	<0.01	0.37	0.21-0.67	<0.01
Pulse rate ≥100 / min vs. <100 / min				1.82	1.16-2.86	<0.01
RR <100/60 vs. RR 100/60-120/80 (mm Hg)				1.82	1.14-2.89	0.01
Need for catecholamines vs. no need				2.06	1.16-3.64	0.01
Antibiotic therapy at time point of sample acquisition vs. no such therapy at this time point				2.48	1.56-4.01	<0.01

[a]Detection of bacteria in bronchoalveolar lavage or urine samples as well as the parameters leukocytes in urine and elevated procalcitonin levels were excluded because of incomplete data sets.

Supplementary Material 4. Logistic regression (model: binary logit; optimization: Fischer's scoring) with stepwise elimination to indicate only significant variables and two-way-interactions regarding the composite outcome need for catecholamines AND need for invasive ventilation AND fatal outcome for patients with enterococcal or nonenterococcal bacteremia. Reciprocal dependence of the three parameters of the composite outcome had been proven by crosstabs (data not shown). Significance was assessed by chi-square testing

Odds ratio estimates and Wald confidence intervals[a]						
	Enterococcal bacteremia			Nonenterococcal bacteremia		
Effect	Estimate	95% Confidence limits	P	Estimate	95% Confidence Limits	P
Intensive care unit vs. no-intensive care unit	13.05	5.23-36.20	<0.01	18.15	10.81-31.41	<0.01
Antibiotic therapy at time point of sample acquisition vs. no such therapy at this time point	3.08	1.27-7.71	<0.01	2.80	1.65-4.85	<0.01
Temperature >38.5°C with vs. without stomata	6.75	2.02-22.58	<0.01	-	-	-
Stomata vs. no stomata	-	-	-	3.04	1.46-6.49	<0.01
Catheter vs. no catheter	-	-	-	3.54	1.29-11.73	0.02

[a]Detection of bacteria in bronchoalveolar lavage or urine samples as well as the parameters leukocytes in urine and elevated procalcitonin levels were excluded because of incomplete data sets.