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RESEARCH

Prognostic value of the identified genus of anaerobic bacteria in bloodstream infection beyond host-related risk factors: a five-year retrospective cohort study

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Abstract

Background Anaerobic bacteraemia is an uncommon but clinically significant bloodstream infection associated with substantial mortality. The respective roles of pathogen-specific and host-related factors in determining outcomes are not fully elucidated.

Aim To identify predictors of mortality in anaerobic bacteraemia and to evaluate whether genus-based microbiological classification offers prognostic value beyond established clinical risk factors.

Methods A retrospective cohort study was performed involving patients with anaerobic bacteraemia ($n=96$) treated at a tertiary-care centre in Hungary from January 2021 to December 2025. Survival was analysed using Kaplan–Meier estimates and Cox proportional hazards regression. The incremental prognostic value of anaerobic genus was assessed using nested multivariable models, with Firth-penalised logistic regression applied as a sensitivity analysis.

Results Among 96 patients (median age 68 years), the most common isolates were *Bacteroides* spp. (33%), *Clostridium* spp. (17%), *Actinomyces* spp. (16%), and *Fusobacterium* spp. (9%). Mortality rates were 43.8% at 30 days, and 54.2% at 180 days. Survival varied significantly by genus, with *Clostridium* spp. bacteraemia exhibiting the highest mortality (75.0%). Independent predictors of 180-day mortality were increasing age (HR 1.03, $p=0.037$), acute kidney injury (HR 2.27, $p=0.007$), liver cirrhosis (HR 2.83, $p=0.022$), hypertension (HR 1.95, $p=0.048$), and *Clostridium* spp. bacteraemia (HR 3.00, $p=0.006$). Microbiological classification significantly improved model performance (likelihood-ratio $p=0.016$). The final model had an apparent 180-day AUC of 0.81.

Conclusions Bacterial genus provides prognostic information beyond host-related risk factors in anaerobic bacteraemia. *Clostridium* spp. bacteraemia was associated with the highest mortality and independently predicted 180-day mortality.

Keywords Anaerobic bacteraemia · Bloodstream infection · *Clostridium* · Liver cirrhosis · Mortality · Prognostic factors

Introduction

Anaerobic bacteria play a pivotal role in the human microbiota and can cause bloodstream infections associated with substantial morbidity [1–3]. Although anaerobic bacteraemia accounts for a small proportion of all bloodstream infections, it is associated with high mortality rates ranging from 14% to 42% [4–7]. The epidemiology of anaerobic bloodstream infections has changed significantly in recent decades [2]. Factors such as an aging population, higher prevalence of malignancy and chronic comorbidities, increased use of immunosuppressive therapies, and

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improvements in microbiological diagnostics have contributed to the rising incidence of anaerobic bacteraemia [7–9]. Moreover, the implementation of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has substantially improved the identification of anaerobic pathogens [10].

Bacteroides species are the most frequently isolated anaerobic organisms in blood cultures, followed by *Clostridium* and *Fusobacterium* species [2]. Clinical presentations and outcomes vary among these genera. *Bacteroides* bacteraemia is primarily linked to intra-abdominal infections, while *Fusobacterium* bacteraemia usually arises from the oropharyngeal or gastrointestinal tract and is often associated with underlying malignancy [11, 12]. In contrast, *Clostridium* bacteraemia is more frequently linked to severe disease and poorer clinical outcomes [7].

It remains unclear whether these differences are attributable to the intrinsic pathogenicity of specific anaerobic genera or are primarily related to host factors. The majority of studies have focused on mortality, antimicrobial resistance, or individual genera, with limited data available from multivariable prognostic analyses. As a result, the relative contributions of microbiological characteristics, patient demographics, comorbidities, and infection-related factors to clinical outcomes are not well defined.

Accordingly, this retrospective study was conducted to characterise the clinical and microbiological features of anaerobic bacteraemia and to identify predictors of mortality. The study also assessed whether the anaerobic genus offers prognostic information beyond established host-related risk factors, utilizing multivariable Cox regression and complementary Firth-penalised logistic regression analyses.

Methods

Patients and definitions

This retrospective observational cohort study was performed at the Clinical Centre of the University of Debrecen (Great Forest Campus), a tertiary-care institution with over 1,700 beds in Hungary. The cohort comprised all patients diagnosed with anaerobic bloodstream infection between 1 January 2021 and 31 December 2025. Only the initial episode of anaerobic bacteraemia per patient was analysed. Anaerobic bacteraemia was defined as at least one positive anaerobic blood culture yielding an anaerobic pathogen. The date of the first positive blood culture was designated as the index blood culture date. Episodes involving *Cutibacterium acnes* or polymicrobial bloodstream infections were excluded. Polymicrobial anaerobic bloodstream infection was defined as the isolation of more than one clinically relevant microorganism during the same infectious episode, regardless of whether the additional organism was another anaerobic or a non-anaerobic pathogen. Therefore, episodes involving multiple anaerobic genera ($n = 5$) or an anaerobic organism together with one or more non-anaerobic pathogens ($n = 14$) were excluded to reduce ambiguity in genus-specific mortality analyses. Monomicrobial bacteraemia was defined as the isolation of a single clinically significant microorganism.

The cohort included patients with clinically significant bloodstream infections caused by bacterial species conventionally classified in clinical diagnostic microbiology as anaerobic microorganisms. Because physiological oxygen tolerance varies within certain genera, particularly *Actinomyces*, inclusion criteria were not based solely on a strict obligate anaerobe definition. *Actinomyces* isolates were retained in the primary cohort but analysed as a distinct microbiological group. To evaluate the robustness of the main findings, a sensitivity analysis excluding all *Actinomyces* cases was also performed.

The focus of bloodstream infection was determined through comprehensive review of available clinical, microbiological, and radiological data. Infections were classified as ear/nose/throat, abdominal, urinary/genital, pulmonary, or skin and soft tissue in origin based on overall clinical assessment rather than any single symptom or diagnostic investigation. Cases with insufficient evidence to confidently identify a focus were classified as primary bloodstream infections.

Demographic characteristics, comorbidities, microbiological findings, laboratory parameters, and infection focus were retrospectively extracted from electronic medical records. Acute kidney injury at the time of bacteraemia was assessed according to the serum creatinine criteria of the Kidney Disease: Improving Global Outcomes (KDIGO) definition [13]. Acute kidney injury was defined as an increase in serum creatinine of ≥ 26.5 µmol/L within 48 h or to ≥ 1.5 times the baseline value within the preceding 7 days. Urine-output criteria were not assessed because reliable retrospective urine-output data were not consistently available. Moderate-to-severe chronic kidney disease was defined as established pre-existing chronic kidney disease stage G3b–G5 (eGFR < 45 mL/min/1.73 m^2), including kidney failure requiring chronic dialysis [14]. Chronic kidney disease classification was based on previous medical documentation and/or prior renal function data supporting chronicity for at least 3 months, in accordance with KDIGO criteria; a single reduced eGFR value during the acute bacteraemic episode was not considered sufficient to establish chronic kidney disease. Renal status could be assessed in 92 of 96 patients. Liver cirrhosis was defined as a documented

diagnosis based on the treating physician's assessment and supporting clinical, laboratory, radiological, and/or histological findings. Severe neutropenia was defined as an absolute neutrophil count less than 500 cells/μL at the time of bloodstream infection. Gastrointestinal surgery was defined as any abdominal surgical procedure involving opening of the gastrointestinal lumen within 30 days prior to bacteraemia onset.

Patients were followed from the date of the index blood culture until death or until 30 and 180 days after bloodstream infection onset. Survival status was determined by retrospective review of electronic medical records. Complete 180-day follow-up data were available for all included patients, and no patients were lost to follow-up.

Blood cultures were processed in accordance with standard institutional microbiological protocols. The Bact/Alert 3D system (bioMérieux SA, Marcy-l'Étoile, France) was utilized throughout the study period. The number of positive anaerobic blood culture bottles was retrospectively recorded for each patient. The number of anaerobic bottles collected was not standardised across patients. Samples from positive bottles were plated onto Schaedler agar (bioMérieux SA, Marcy-l'Étoile, France) containing 5% v/v horse blood, haemin, and Vitamin K1 to facilitate anaerobic bacterial isolation. Plates were incubated in an anaerobic environment with an atmosphere of 90% N_2, 5% H_2, and 5% CO_2 using an anaerobic chamber (Whitley A45 anaerobic incubator, Don Whitley Scientific, UK). Incubation was maintained for 2–5 days at 37 °C. Anaerobic isolates were identified by MALDI-TOF analysis. Identification was performed using a Bruker Biotyper Microflex LT instrument (Bruker Corporation, Billerica, MA, USA) following standard formic acid extraction and α-cyano-4-hydroxycinnamic acid treatment. Log(score) values of 2.0 or higher were considered acceptable for species-level identification.

Statistical analysis

Statistical analyses were conducted using R statistical software (R Foundation for Statistical Computing, Vienna, Austria; version 4.5.0). Baseline characteristics were summarised by anaerobic genus. Continuous variables are reported as medians with interquartile ranges (IQR) and compared using the Kruskal–Wallis test. Categorical variables are presented as frequencies and percentages and compared using Fisher's exact test. Overall survival was assessed at 30 and 180 days following the index blood culture. Survival probabilities were estimated using the Kaplan–Meier method and compared using the log-rank test. Risk factors for mortality were initially evaluated using univariable Cox proportional hazards regression analyses. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated. The variables included in the univariable analyses were age, sex, underlying comorbidities, infection focus, and microbiological genus. Variables demonstrating an association with mortality at $p<0.05$ in the univariable analyses were selected for further evaluation. Of these, those deemed clinically relevant and directly related to the study question were incorporated into the multivariable clinical model. The anaerobic genus was then added to the clinical model to evaluate its independent association with mortality and its additional prognostic value beyond the established clinical predictors. For genus-specific comparisons in the Cox regression analyses, *Bacteroides* spp., the largest microbiological group in the cohort, was used as the reference category. To determine whether microbiological classification improved prognostic performance beyond host-related clinical factors, nested Cox regression models were compared using likelihood-ratio testing. The proportional hazards assumption was assessed using Schoenfeld residuals. The discriminative performance of the final multivariable Cox model was evaluated using Harrell's concordance index (C-index). Time-dependent receiver operating characteristic (ROC) curve analysis was also performed, with discrimination quantified by the area under the curve (AUC) for both 30-day and 180-day mortality.

As a complementary analysis, 180-day all-cause mortality was evaluated using Firth-penalised logistic regression. Due to small subgroup sizes and sparse data, Firth penalisation was applied to reduce small-sample bias and improve estimate stability. Odds ratios (ORs) with 95% confidence intervals were reported. Model discrimination was assessed using ROC curve analysis and quantified by the AUC. The optimal probability threshold was identified using the Youden index, and the corresponding sensitivity and specificity were calculated.

Sensitivity analyses were conducted to assess the robustness of the findings. First, the multivariable Cox model was repeated after excluding patients with *Actinomyces* spp. bacteraemia, using 180-day all-cause mortality as the endpoint. As an additional sensitivity analysis, the model was repeated in the *Actinomyces*-excluded cohort using 30-day all-cause mortality as the endpoint. Internal bootstrap validation was performed using bootstrap resampling with 1000 iterations to assess model stability and potential overfitting. Calibration slope estimates were obtained for both the full genus model and a simplified binary microbiological model comparing *Clostridium* spp. with all other anaerobic genera. Bootstrap-derived hazard ratios for *Clostridium* spp. bacteraemia were additionally calculated to evaluate the robustness of the primary microbiological association.

Patients with unavailable renal classification ($n=4$) were excluded from models including acute kidney injury or chronic kidney disease using complete-case analysis.

All statistical tests were two-sided, and a p-value less than 0.05 was considered statistically significant.

Results

During 2021–2025, a total of 84,602 blood culture bottles were processed, of which 18,788 (22.2%) were positive. The annual numbers of processed/positive blood culture bottles were 11,730/2,431 (20.7%) in 2021; 11,965/2,462 (20.6%) in 2022; 16,111/3,987 (24.7%) in 2023; 20,943/4,663 (22.3%) in 2024; and 23,853/5,245 (22.0%) in 2025. Anaerobic bacteria were recovered from 15, 23, 50, 38, and 53 bottles annually (179 overall), corresponding to 0.21% of all processed and 0.95% of all positive blood culture bottles.

A total of 96 patients with anaerobic bacteraemia were included in the study. Baseline characteristics of the study population stratified by anaerobic genus are shown in Table 1. Several baseline characteristics differed across anaerobic genera, including age, haematological malignancy, neutropenia, and the distribution of selected infection foci (Table 1). The median age was 68 years (IQR 58.8–77.0). *Bacteroides* spp. were the most frequently isolated organisms ($n=32$; 33%), followed by *Clostridium* spp. ($n=16$; 17%), *Actinomyces* spp. ($n=15$; 16%), and *Fusobacterium* spp. ($n=9$; 9%) (Supplementary Table S1). Regarding blood culture positivity, one anaerobic bottle was positive in 80 patients (83.3%), whereas ≥2 anaerobic bottles were positive in 16 patients (16.7%) (Table 1). Haematological malignancies were most frequent in patients with *Clostridium* spp. bacteraemia (37.5%, $p<0.001$) (Table 1). The distribution of bacteraemia foci varied significantly among anaerobic genera, particularly for abdominal infections and primary bloodstream infections ($p=0.021$ and $p=0.018$, respectively) (Table 1).

Species-level analysis demonstrated substantial heterogeneity within the major anaerobic genera (Supplementary Table S2). Among the 16 *Clostridium* bloodstream infections, *C. perfringens* ($n=5$) and *C. tertium* ($n=4$) were the most frequently isolated species, followed by *C. hathewayi* ($n=2$); *C. innocuum*, *C. paraputrificum*, *C. ramosum*, *C. septicum*, and *C. sordellii* were each identified in a single patient. Within the *Bacteroides* genus, *B. fragilis* predominated, accounting for 26 of 32 isolates (81%), whereas *B. thetaiotaomicron* was identified in four patients and *B. nordii* and *B. vulgatus* in one patient each. Crude 180-day mortality varied substantially across species. Among *Clostridium* isolates, mortality was highest for *C. perfringens* (80%), compared with 50% for both *C. tertium* and *C. hathewayi*. Within the *Bacteroides* genus, the predominant species, *B. fragilis*, was associated with a crude 180-day mortality of 65%. Because most individual species were represented by very small numbers of patients, these analyses were considered descriptive only, and all analyses were performed at the genus level.

Overall mortality reached 54.2% at 180 days, with most deaths occurring during the first 30 days after bacteraemia onset. Thirty-day mortality was 43.8% (42/96), increasing to 51.0% at 90 days and 54.2% (52/96) at 180 days. Notably, 80.8% of deaths during the 180-day follow-up occurred within the first 30 days after bacteraemia onset. Mortality varied significantly among microbiological groups. Kaplan–Meier analysis demonstrated significant differences in survival among anaerobic genera at 180 days (log-rank $p=0.033$) but not at 30 days (log-rank $p=0.062$) (Fig. 1). Patients with *Clostridium* spp. bacteraemia showed the poorest survival, with crude mortality rates of 62.5% at 30 days and 75.0% at 180 days. Similarly, reduced survival was observed among patients with *Fusobacterium* spp. (55.6% and 66.7%, respectively) and *Bacteroides* spp. bacteraemia (53.1% and 62.5%, respectively). In contrast, patients with *Actinomyces* spp. bacteraemia had the most favourable survival, with mortality rates of 13.3% at 30 days and 20.0% at 180 days.

In univariable Cox analyses, increasing age, hypertension, acute kidney injury, and liver cirrhosis were associated with mortality. A multivariable clinical Cox model identified increasing age (HR 1.03, 95% CI 1.00–1.05; $p=0.025$), acute kidney injury (HR 2.14, 95% CI 1.20–3.81; $p=0.010$), and liver cirrhosis (HR 2.54, 95% CI 1.06–6.11; $p=0.037$) as independent predictors of mortality (Fig. 2). To determine whether microbiological classification provided prognostic information beyond established host-related risk factors, anaerobic genus was subsequently incorporated into the multivariable Cox model. The addition of bacterial genus to the clinical model significantly improved model fit compared with the clinical model alone (likelihood-ratio test $\chi^2 = 13.95$, df = 5; $p=0.016$). In the extended model, increasing age (HR 1.03, 95% CI 1.00–1.05; $p=0.037$), acute kidney injury (HR 2.27, 95% CI 1.25–4.14; $p=0.007$), hypertension (HR 1.95, 95% CI 1.01–3.79; $p=0.048$), liver cirrhosis (HR 2.83, 95% CI 1.16–6.86; $p=0.022$), and *Clostridium* spp. bacteraemia (HR 3.00, 95% CI 1.37–6.54; $p=0.006$) were independently associated with 180-day mortality (Fig. 2). No violation of the proportional hazards assumption was detected (global Schoenfeld test $p=0.879$). Due to the limited number of events per variable in the full genus, a sensitivity analysis was conducted using a simplified binary microbiological classification of *Clostridium* spp. bacteraemia with all other anaerobic bloodstream infections (Supplementary Table S3). In this analysis, *Clostridium* spp. remained strongly and independently associated with 180-day mortality (HR 3.48, 95% CI 1.74–7.00; $p<0.001$) (Supplementary Table S3). Incorporating the binary *Clostridium*

Table 1 Baseline demographic, clinical and microbiological characteristics according to anaerobic genus

Variable	Overall, $n=96$[1]	*Actinomyces* $n=15$[1]	*Bacteroides* $n=32$[1]	*Clostridium* $n=16$[1]	*Fusobacterium* $n=9$[1]	Other $n=19$[1]	*Prevotella* $n=5$[1]	p-value[2]
Demographics								
Age, years	68.0 (58.8–77.0)	56.0 (1.0–73.0)	72.5 (66.5–81.0)	65.0 (58.5–72.5)	65.0 (53.0–71.0)	71.0 (54.0–81.0)	66.0 (65.0–78.0)	0.020
Sex								0.803
Female	41 (42.7%)	6 (40.0%)	16 (50.0%)	8 (50.0%)	3 (33.3%)	6 (31.6%)	2 (40.0%)	
Male	55 (57.3%)	9 (60.0%)	16 (50.0%)	8 (50.0%)	6 (66.7%)	13 (68.4%)	3 (60.0%)	
Comorbidities and predisposing conditions								
Hypertension	54 (56.3%)	5 (33.3%)	22 (68.8%)	5 (31.3%)	6 (66.7%)	13 (68.4%)	3 (60.0%)	0.052
Type 1 or 2 diabetes mellitus	26 (27.1%)	3 (20.0%)	6 (18.8%)	5 (31.3%)	4 (44.4%)	6 (31.6%)	2 (40.0%)	0.557
Acute kidney injury[3]	37/92 (40.2%)	2/13 (15.4%)	15/32 (46.9%)	7/15 (46.7%)	2/8 (25.0%)	9/19 (47.4%)	2/5 (40.0%)	0.374
Moderate-to-severe chronic kidney disease	24/92 (26.1%)	3/13 (23.1%)	9/32 (28.1%)	2/15 (13.3%)	2/8 (25.0%)	5/19 (26.3%)	3/5 (60.0%)	0.531
Liver cirrhosis	10 (10.4%)	0 (0.0%)	3 (9.4%)	2 (12.5%)	1 (11.1%)	4 (21.1%)	0 (0.0%)	0.501
Solid malignancy	27 (28.1%)	2 (13.3%)	13 (40.6%)	4 (25.0%)	2 (22.2%)	4 (21.1%)	2 (40.0%)	0.416
Haematological malignancy	13 (13.5%)	3 (20.0%)	1 (3.1%)	6 (37.5%)	3 (33.3%)	0 (0.0%)	0 (0.0%)	0.001
Neutropenia (<0.5 G/L)	5 (5.2%)	1 (6.7%)	0 (0.0%)	3 (18.8%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0.047
Corticosteroid treatment	19 (19.8%)	4 (26.7%)	8 (25.0%)	3 (18.8%)	1 (11.1%)	3 (15.8%)	0 (0.0%)	0.854
Abdominal surgery	18 (18.8%)	1 (6.7%)	9 (28.1%)	2 (12.5%)	2 (22.2%)	4 (21.1%)	0 (0.0%)	0.501
Prosthetic device	16 (16.7%)	2 (13.3%)	7 (21.9%)	3 (18.8%)	0 (0.0%)	3 (15.8%)	1 (20.0%)	0.786
Pancreatitis	4 (4.2%)	1 (6.7%)	2 (6.3%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0.540
Severity of illness								
Intensive care unit admission	22 (22.9%)	7 (46.7%)	5 (15.6%)	3 (18.8%)	0 (0.0%)	6 (31.6%)	1 (20.0%)	0.096
Invasive mechanical ventilation	13 (13.5%)	5 (33.3%)	2 (6.3%)	2 (12.5%)	1 (11.1%)	2 (10.5%)	1 (20.0%)	0.215
Central venous catheter	28 (29.2%)	7 (46.7%)	8 (25.0%)	4 (25.0%)	4 (44.4%)	3 (15.8%)	2 (40.0%)	0.325
Urinary catheter	52 (54.2%)	6 (40.0%)	18 (56.3%)	7 (43.8%)	4 (44.4%)	13 (68.4%)	4 (80.0%)	0.414
Number of positive anaerobic blood culture bottles								
1 bottle	80 (83.3%)	14 (93.3%)	25 (78.1%)	9 (56.3%)	9 (100.0%)	18 (94.7%)	5 (100.0%)	
≥2 bottles	16 (16.7%)	1 (6.7%)	7 (21.9%)	7 (43.8%)	0 (0.0%)	1 (5.3%)	0 (0.0%)	
Focus of infection								
Primary bloodstream infection	45 (46.9%)	12 (80.0%)	10 (31.3%)	6 (37.5%)	6 (66.7%)	10 (52.6%)	1 (20.0%)	0.018
Abdominal focus	27 (28.1%)	0 (0.0%)	14 (43.8%)	5 (31.3%)	1 (11.1%)	5 (26.3%)	2 (40.0%)	0.021
Ear/nose/throat focus	4 (4.2%)	3 (20.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (20.0%)	0.006
Urinary/genital focus	6 (6.3%)	0 (0.0%)	3 (9.4%)	1 (6.3%)	1 (11.1%)	1 (5.3%)	0 (0.0%)	0.896
Pulmonary focus	3 (3.1%)	0 (0.0%)	1 (3.1%)	1 (6.3%)	1 (11.1%)	0 (0.0%)	0 (0.0%)	0.529
Skin/soft tissue focus	9 (9.4%)	0 (0.0%)	4 (12.5%)	1 (6.3%)	0 (0.0%)	3 (15.8%)	1 (20.0%)	0.468
Vascular focus	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (6.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.469
Osteoarticular focus	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	>0.999

[1]Median (Q1–Q3); n (%)

[2]Kruskal-Wallis rank sum test; Fisher's exact test

[3]Acute kidney injury and moderate-to-severe chronic kidney disease could be assessed in 92 of 96 patients; percentages for these variables were calculated among patients with available data

variable significantly improved model fit (likelihood-ratio χ^2 = 10.22, df=1; $p=0.001$). Internal bootstrap validation with 1000 resamples yielded optimism-corrected calibration slopes of 0.66 for the full genus model and 0.86 for the binary *Clostridium* model. Bootstrap resampling further supported the stability of the *Clostridium* effect, with a median hazard ratio of 3.67 and a 95% bootstrap percentile interval of 1.94–8.54. These findings suggest that *Clostridium* spp. bacteraemia provides additional prognostic information beyond host-related clinical risk factors and is independently associated with mortality in patients with anaerobic bloodstream infection.

Patients with *Actinomyces* bacteraemia exhibited a distinct clinical profile compared with those infected by other anaerobic genera. Most episodes (12/15, 80%) were classified as primary bloodstream infections, and no abdominal

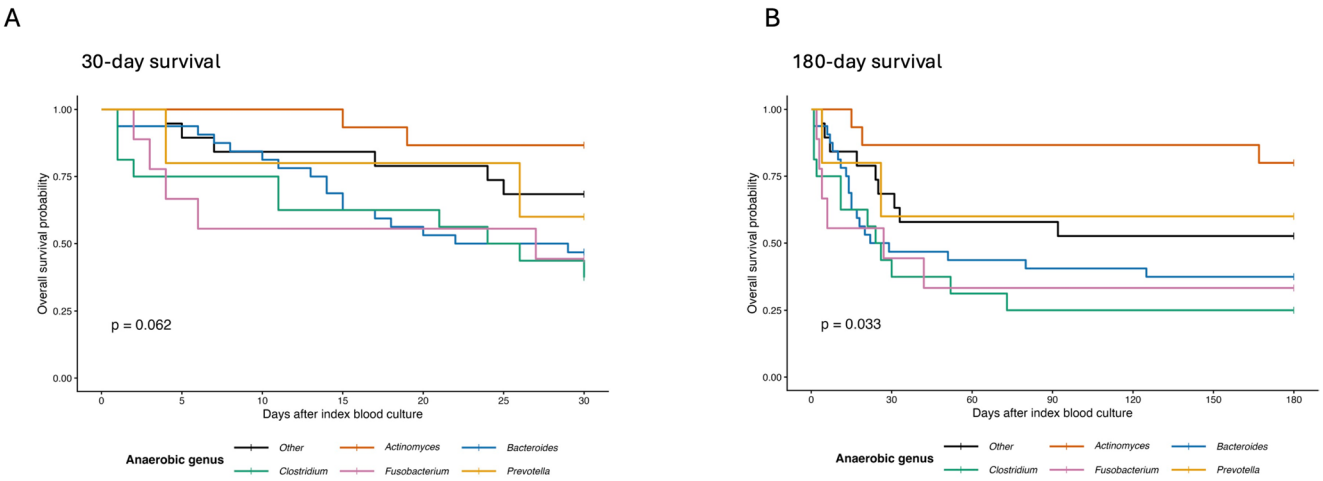

Fig. 1 Kaplan–Meier survival curves according to anaerobic genus. (**A**) Thirty-day survival and (**B**) 180-day survival among patients with anaerobic bacteraemia stratified by anaerobic genus. Survival differed significantly among microbiological groups at 180 days (log-rank $p=0.033$), whereas the difference at 30 days did not reach statistical significance (log-rank $p=0.062$). Patients with *Clostridium* spp. bacteraemia had the poorest survival, whereas *Actinomyces* spp. bacteraemia was associated with the most favourable outcomes. Tick marks indicate censored observations

source was identified. Liver cirrhosis was absent, and acute kidney injury was less frequently observed than in patients with *Bacteroides* bacteraemia. Only three patients (20%) died within 180 days, representing the lowest crude mortality among the major anaerobic genera. Given the markedly lower mortality observed among patients with *Actinomyces* spp. bacteraemia, a sensitivity analysis excluding these cases was performed. The principal findings remained unchanged. *Clostridium* spp. bacteraemia (HR 2.84, 95% CI 1.30–6.22; $p=0.009$), liver cirrhosis (HR 2.79, 95% CI 1.14–6.80; $p=0.024$), and acute kidney injury (HR 2.02, 95% CI 1.09–3.73; $p=0.025$) remained independently associated with 180-day mortality. An additional sensitivity analysis was performed in the *Actinomyces*-excluded cohort using 30-day all-cause mortality as the endpoint. Similar results were observed for 30-day mortality, with *Clostridium* spp. bacteraemia (HR 2.33, 95% CI 1.01–5.39; $p=0.047$), liver cirrhosis (HR 3.18, 95% CI 1.31–7.75; $p=0.011$), and acute kidney injury (HR 2.21, 95% CI 1.12–4.33; $p=0.021$) remaining independently associated with mortality. The global Schoenfeld test did not indicate violation of the proportional hazards assumption ($p=0.411$).

The final multivariable Cox model showed moderate-to-good apparent discrimination. Harrell's concordance index was 0.74 (SE 0.037). Time-dependent receiver operating characteristic analysis yielded an AUC of 0.81 for both 30-day and 180-day mortality prediction (Fig. 3). These apparent performance measures suggest moderate-to-good discrimination; however, the optimism-corrected calibration slope indicated overfitting of the full genus model.

As a complementary analysis of the survival analysis findings, a Firth-penalised logistic regression model was fitted using 180-day all-cause mortality as the endpoint (Supplementary Table S4). Increasing age showed a borderline association with mortality (OR 1.03, 95% CI 1.00–1.07; $p=0.060$), whereas acute kidney injury (OR 3.02, 95% CI 1.12–8.89; $p=0.029$) and *Clostridium* spp. bacteraemia (OR 5.04, 95% CI 1.08–30.49; $p=0.038$) were independently associated with mortality. The model demonstrated good discriminative performance, with an AUC of 0.82 (95% CI 0.732–0.905). At the optimal probability threshold of 0.60 determined by the Youden index, sensitivity was 0.73 and specificity was 0.83 (Supplementary Table S4).

Discussion

This retrospective study of anaerobic bacteraemia demonstrated substantial mortality, with 30-day mortality of 43.8%, increasing to 54.2% at 180 days. These findings indicate that anaerobic bloodstream infections remain associated with poor outcomes despite advances in microbiological diagnostics and antimicrobial therapy. Increasing age and host-related clinical factors, particularly acute kidney injury and liver cirrhosis, were associated with mortality, while *Clostridium* spp. bacteraemia remained independently associated with reduced survival after multivariable adjustment. Notably, incorporation of anaerobic genus into the multivariable model significantly improved model performance, demonstrating that anaerobic genus contributes independent prognostic information beyond established host-related risk factors and comorbidities. Previous studies have reported mortality rates ranging from 15% to 40%, although direct comparisons are limited by differences in study populations,

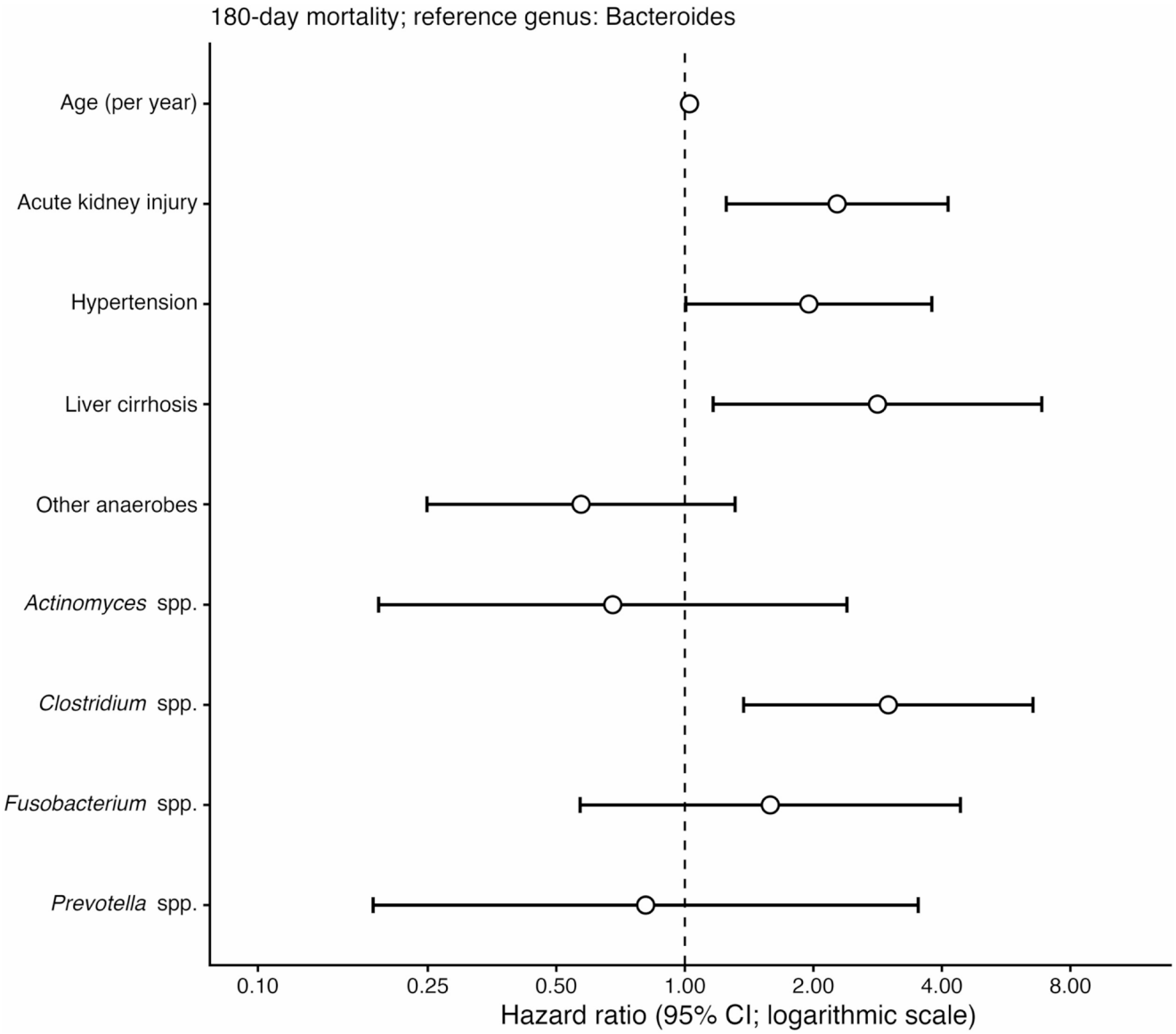

Fig. 2 Forest plot of the multivariable Cox proportional hazards model for 180-day mortality. Adjusted hazard ratios (HRs) and 95% confidence intervals are shown for clinical and microbiological predictors of all-cause mortality within 180 days after anaerobic bacteraemia. The vertical dashed line indicates an HR of 1.0 (no effect). Increasing age, acute kidney injury, hypertension, liver cirrhosis, and *Clostridium* spp. bacteraemia were independently associated with 180-day mortality in the clinical-plus-genus model

study design, follow-up duration, and outcome definitions [5, 6, 10, 15]. The higher mortality observed in this cohort may reflect the tertiary-care setting and the advanced age and comorbidity burden of the study population. Additionally, the extended follow-up period likely captured deaths related not only to the acute infectious episode but also to severe underlying conditions frequently associated with anaerobic bacteraemia [2, 7]. Another potential explanation for the adverse outcome of anaerobic bloodstream infections is the time required for definitive microbiological identification. In our laboratory, species identification was performed from colony growth after anaerobic subculture of positive blood culture bottles, which prolongs the time to definitive identification compared with many aerobic pathogens. Although this may delay optimization of antimicrobial therapy in some patients, the timing and appropriateness of antimicrobial treatment were not evaluated in the present study; therefore, no causal relationship can be inferred.

A primary objective of this study was to determine whether differences in outcome are primarily attributable to genus-specific characteristics or host-related factors. Although substantial differences in crude mortality were observed between anaerobic genera, several associations were attenuated after adjustment for demographic and

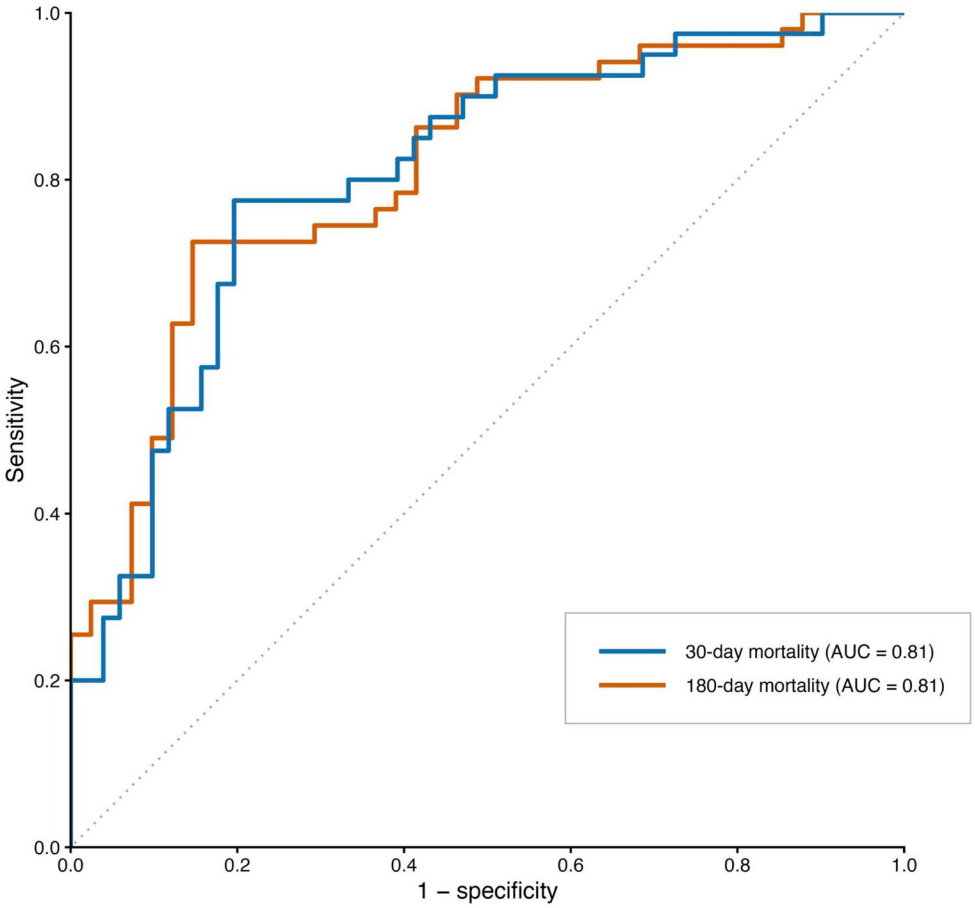

Fig. 3 Time-dependent receiver operating characteristic (ROC) analysis of the final multivariable Cox model. ROC curves demonstrating the discriminative performance of the final prognostic model for prediction of 30-day and 180-day all-cause mortality following anaerobic bacteraemia. The model showed good discrimination for both outcomes, with an area under the curve (AUC) of 0.81 for both 30-day and 180-day mortality

clinical variables. This finding suggests that much of the observed variation in mortality is attributable to patient characteristics rather than genus-related microbiological factors alone. Previous studies have similarly identified host-related factors as major determinants of outcome. Vena et al. reported that the Charlson comorbidity index and septic shock independently predicted mortality, while Zouggari et al. identified sepsis and chronic kidney disease as important risk factors for adverse outcomes [10, 15]. Older age was independently associated with 180-day mortality in the clinical, full genus, and simplified binary *Clostridium* Cox models, although the association was borderline in the complementary Firth-penalised logistic regression, while liver cirrhosis and acute kidney injury also emerged as significant prognostic factors in the multivariable survival analysis, whereas moderate-to-severe chronic kidney disease was not significantly associated with mortality in univariable analysis.

Liver cirrhosis remained independently associated with 180-day mortality in the primary analysis and with 30-day mortality in the Actinomyces-excluded sensitivity analysis [16]. This association is biologically plausible, as cirrhosis is characterised by profound immune dysfunction, impaired bacterial clearance, and increased bacterial translocation from the gastrointestinal tract. Previous studies have demonstrated that bacterial infections substantially worsen prognosis in cirrhotic patients and frequently precipitate sepsis, organ dysfunction, and clinical decompensation [17, 18].

Despite the predominant influence of host-related factors, *Clostridium* spp. bacteraemia remained independently associated with mortality after multivariable adjustment. This observation aligns with previous reports demonstrating *Clostridium* spp. as particularly virulent anaerobic pathogens associated with poor clinical outcomes [7, 19]. Watanabe et al. also identified *Clostridium* spp. as an independent predictor of in-hospital mortality, while Zouggari et al. observed a significantly higher prevalence of *Clostridium* bacteraemia among non-survivors, supporting the hypothesis that *Clostridium* infections represent a particularly high-risk subgroup of anaerobic bloodstream infections [10, 19]. The adverse outcomes associated with *Clostridium* bacteraemia may partly reflect the ability of several *Clostridium* species to produce potent exotoxins and tissue-destructive enzymes, which can contribute to rapid clinical deterioration and severe systemic inflammatory responses [20]. Furthermore, *Clostridium* bacteraemia frequently occurs in patients with serious underlying illnesses, including haematological malignancies, solid cancers and immunosuppressive

conditions [21, 22]. In this cohort, *Clostridium* infections were also disproportionately associated with haematological malignancy, supporting this interpretation.

Beyond confirming the adverse prognostic impact of *Clostridium* spp., these findings demonstrate that inclusion of bacterial genus significantly improved model fit compared with a model based solely on clinical variables, supporting the independent prognostic contribution of anaerobic genus classification. This observation complements previous studies that primarily focused on mortality associated with individual anaerobic genera and suggests that genus classification – primarily *Clostridium* spp. bacteraemia – may contribute meaningful prognostic information in the management of anaerobic bloodstream infections [19, 23].

Although the primary analyses were performed at the genus level, species-level identification remains clinically relevant. In our cohort, *C. perfringens* was associated with the highest crude mortality among the more frequently isolated *Clostridium* spp., consistent with previous reports describing its rapidly progressive clinical course and high mortality. Likewise, *C. septicum*, although represented by a single case in our cohort, has been consistently associated with colorectal and haematological malignancies, whereas *C. sordellii* is recognised as a cause of fulminant toxic shock syndrome. Within the *Bacteroides* genus, *B. fragilis* accounted for the vast majority of isolates, in line with previous epidemiological studies identifying it as the predominant cause of *Bacteroides* bloodstream infection [1–5]. Nevertheless, because most species were represented by only a few patients, our data do not permit reliable species-specific comparisons. Larger multicentre studies will be required to determine whether prognosis differs between individual anaerobic species beyond the genus level.

These findings have several potential clinical implications. The high mortality associated with anaerobic bacteraemia underscores the importance of early recognition and prompt initiation of antimicrobial therapy [24]. Previous studies have demonstrated that inadequate empirical treatment and delayed source control are associated with worse outcomes in anaerobic bloodstream infections [10, 15]. Identification of *Clostridium* spp. in blood cultures may provide clinically useful prognostic information beyond standard patient characteristics. Recognition of this high-risk subgroup could support more intensive monitoring, earlier source control interventions, and closer clinical follow-up. In addition to prognostic implications, species-level identification may provide broader clinical information. Recent population-based studies have demonstrated strong associations between bloodstream infections caused by *Fusobacterium* spp., selected *Bacteroides* species, Gram-positive anaerobic cocci, and certain *Clostridium* species and subsequent diagnosis of colorectal cancer [25]. These observations suggest that anaerobic bacteraemia may occasionally serve as a marker of occult gastrointestinal pathology and warrant further diagnostic evaluation in selected patients [25, 26].

An unexpected finding of our study was the remarkably favourable prognosis observed among patients with *Actinomyces* bacteraemia. Although *Actinomyces* spp. are traditionally considered within the spectrum of clinically relevant anaerobic bacteria, the genus is physiologically heterogeneous with regard to oxygen tolerance. Despite this physiological heterogeneity, the inclusion of *Actinomyces* in studies of anaerobic bloodstream infections is not unprecedented. Several previous studies investigating anaerobic bacteraemia or anaerobic organisms recovered from blood cultures have included *Actinomyces* spp. within their study populations. These include the Italian nationwide ITA-NAEROBY survey by Di Bella et al., the study of rapid identification of anaerobic bacteria from blood cultures by Rassolie and Özenci, and the recent European survey of anaerobes recovered from paediatric and adult blood cultures by Boattini et al. [1, 2, 27]. In addition, the clinical relevance of *Actinomyces* bloodstream isolates has recently been specifically examined by Ayantunde et al. [28]. It is noteworthy that most patients in our cohort presented with primary bloodstream infection and lacked an identifiable abdominal focus. Furthermore, differences in host-related characteristics and infection focus may have contributed to the favourable prognosis observed in the *Actinomyces* group. These findings suggest that the favourable prognosis observed in our cohort is more likely related to differences in patient characteristics and infection focus than to intrinsic differences in bacterial virulence. Because of the retrospective design and limited number of cases, we could not determine whether some episodes reflected transient mucosal translocation or catheter-associated bloodstream infection, and these possibilities warrant further investigation.

Nevertheless, several limitations should be highlighted regarding the current study. The relatively small size of the *Clostridium* subgroup should be considered when interpreting the effect estimates. Although *Clostridium* spp. bacteraemia remained independently associated with mortality in the full genus model and in the binary sensitivity analysis, the subgroup included only 16 patients. Therefore, the magnitude of the hazard ratio should be interpreted cautiously and requires confirmation in larger multicentre cohorts. Another limitation concerns the distinction between true bloodstream infection and blood culture contamination. This is particularly relevant for organisms such as *C. perfringens*, which may occasionally represent contamination [29]. Although all *C. perfringens* cases were individually examined and considered clinically significant, misclassification cannot be completely excluded because of the

retrospective study design. In addition, disease severity scores such as SOFA or APACHE II score were not consistently available. Furthermore, the timing and appropriateness of antimicrobial therapy were not evaluated. Because of the retrospective design, reliable classification of empirical therapy as appropriate or inappropriate was not feasible. Consequently, differences in antimicrobial management may have contributed to the observed differences in mortality between microbiological groups.

In conclusion, anaerobic bacteraemia remains associated with substantial short- and long-term mortality. Although patient-related factors were the principal determinants of outcome, *Clostridium* spp. bacteraemia provided independent prognostic information beyond clinical risk factors. These findings support further evaluation of anaerobic genus classification as a component of clinical risk stratification in patients with anaerobic bloodstream infection. Further multicentre studies are required to validate genus-specific associations and to determine whether species-level classification can further improve risk prediction.

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Data availability The data that support the findings of this study are available from the corresponding authors upon reasonable request.

Declarations

Ethical approval The authors confirm that this study was conducted in accordance with the ethical standards outlined in the journal's guidelines for authors and that all applicable ethical requirements were fulfilled. Ethical approval was obtained from the institutional ethics committee of the University of Debrecen, Regional and Institutional Research Ethics Committee (DE RKEB/IKEB) (approval number: 6508 − 2023).

Competing interests The authors declare no competing interests.

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