The Microbiological Characteristics of *Acinetobacter Baumannii* Associated With Early Mortality in Patients With Bloodstream Infection

Chan Mi Lee,[1] Yunsang Choi,[2] Seong Jin Choi,[2] Song Mi Moon,[2] Eu Suk Kim,[2] Hong Bin Kim,[2] Sin Young Ham,[3] Jeong Su Park,[4] Jinki Yeom,[5,6,7,a] and Kyoung-Ho Song[2,a]

[1]Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Republic of Korea, [3]Department of Internal Medicine, Veterans Health Service Medical Center, Seoul, Republic of Korea, [4]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Republic of Korea, [5]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea, [6]Department of Microbiology and Immunology, Seoul National University College of Medicine, Seoul, Republic of Korea, and [7]Cancer Research Institute, Seoul National University, Seoul, Republic of Korea

Background. Despite rapid deaths resulting from *Acinetobacter baumannii* bacteremia, the clinical impact of the microbiological characteristics of *A baumannii* strains on early mortality (EM) is unclear. We aimed to identify the microbiological characteristics of *A baumannii* strains associated with EM.

Methods. Clinical information and isolates from patients with *A baumannii* bacteremia from January 2015 to December 2021 were collected. EM was defined as death within 3 days of the initial positive blood culture, whereas late mortality meant death within 5–30 days. The microbiological characteristics of *A baumannii* were analyzed using multilocus sequence typing, polymerase chain reactions, and a *Galleria mellonella* in vivo infection model.

Results. Among 130 patients, 69 (53.1%) died within 30 days and EM occurred in 38 (55.1% of 30-day deaths). Sequence type 191 (ST191) strain was more prevalent in patients with EM than in 30-day survivors (31.6% vs 6.6%). Regarding virulence genes, *bfmS* was more frequent (92.1% vs 47.5%), whereas *bauA* was less frequent (13.2% vs 52.5%) in patients with EM than in 30-day survivors. Higher clinical severity, pneumonia, and ST191 infection were identified as independent risk factors for EM. In the *G mellonella* infection model, ST191, *bfmS*+, and *bauA*– isolates showed higher virulence than non-ST191, *bfmS*–, and *bauA*+ isolates, respectively.

Conclusions. ST191 and *bfmS* were more frequently found in the EM group. ST191 infection was also an independent risk factor for EM and highly virulent in the in vivo model. Tailored infection control measures based on these characteristics are necessary for *A baumannii* bacteremia management.

Keywords. *Acinetobacter baumannii*; *bfmS*; early mortality; ST191; virulence.

Acinetobacter baumannii is a common cause of nosocomial infections, including catheter-related bloodstream infections (CRBSIs), urinary tract infections, and pneumonia [1–3]. The high prevalence of antibiotic resistance in *A baumannii*, coupled with limited treatment options and high mortality rates, imposes a substantial burden on healthcare systems [4, 5].

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We previously reported in a multicenter cohort study that a large proportion of *A baumannii* bacteremia with high severity progresses to a rapidly fatal course, resulting in early mortality (EM), regardless of the severity of underlying diseases or source of infection [6]. Therefore, additional research is needed to investigate the microbiological factors of the bacterium itself or the pathogen–host interactions in patients with EM. We aim to explore the clinical outcome-associated microbiological characteristics of *A baumannii* strains, focusing on those associated with EM in *A baumannii* bacteremia. Additionally, we demonstrate the virulence of specific *A baumannii* strains using a *Galleria mellonella* in vivo infection model.

METHODS

Study Patients and Clinical Data

The *A baumannii* bloodstream isolates were collected from patients with *A baumannii* bacteremia admitted to Seoul National University Bundang Hospital from January 2015 to December 2021. Vitek 2 system (BioMérieux, Marcy l'Etoile, France) was

used for species identification and antimicrobial susceptibility testing of bloodstream isolates, excluding colistin.

Clinical characteristics of patients, including demographic data, comorbidities, clinical severity of infection, bacteremia, antibiotic therapy, and mortality, were collected. Death within 3 days of the initial positive blood culture was classified as EM, whereas death occurring within 5–30 days was classified as late mortality (LM). Deaths 4 days after the initial positive blood culture were not classified as either EM or LM [6]. Age-adjusted Charlson's comorbidity index was used to assess comorbidities [7]. Clinical severity was assessed using the Sequential Organ Failure Assessment (SOFA) and Pitt bacteremia scores. Empirical antibiotic therapy was defined as the initial antibiotics administered more than 24 hours after the first positive blood culture and classified as appropriate if the blood culture isolate was susceptible to the antibiotics administered.

Multilocus Sequence Typing

Seven housekeeping genes, *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*, were sequenced, and allele numbers were assigned according to the multilocus sequence typing (MLST) database (http://pubmlst.org/databases/). MLST, following the Oxford scheme, was used to assign sequence types (STs) to isolates [8]. If allele numbers and STs were not designated in the MLST database, new allele sequences were submitted to the databases and new allele numbers and STs were assigned.

Polymerase Chain Reactions of Virulence Genes

The presence of 9 virulence genes (*ompA*, *adeB*, *smpA*, *bfmS*, *csuE*, *epsA*, *abaI*, *basD*, and *bauA*) was investigated by polymerase chain reaction (PCR) using specific primers (Supplementary Table 1) [9–13]. Each PCR reaction mixture contained 2 μL of 5× buffer, 0.2 μL of each forward and reverse primers, 0.1 μL of Taq polymerase (Bioline, London, UK), 1 μL of DNA, and distilled water in a final volume of 10 μL. The amplification was performed with following conditions: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 40 seconds, annealing at a specific temperature (Supplementary Table 1) for 40 seconds, extension at 72 °C for 40 seconds, and a final extension at 72 °C for 5 minutes. PCR products were separated on a 2% agarose gel and visualized using an ultraviolet transilluminator. *A baumannii* ATCC19606 was used as a positive control.

Colistin Susceptibility Testing

The minimum inhibitory concentration of colistin was measured using a broth microdilution test following Clinical and Laboratory Standards Institute guidelines [14]. Solutions of colistin (concentration range, 0.125–64 μg/mL) and *A baumannii* isolates of 5×10^5 colony-forming units/mL were dispensed into the 96-well plates. Plates were incubated for 24 hours at 35 ± 2 °C. Colistin minimum inhibitory concentration ≥ 4 μg/mL was classified as resistance [15].

G MELLONELLA INFECTION MODEL

Fifteen healthy *G mellonella* larvae (200–250 mg) were injected 10 μL of 1×10^8 colony-forming units/mL *A baumannii* suspension in the last left proleg. The health index score of larvae was calculated by summing the scores for activity, cocoon formation, melanization, and survival listed in Supplementary Table 2 [16]. All healthy larvae scored 10 before injection and dead larvae typically scored 0. Following injection, the larvae were kept in a 12-well Petri dish at 37 °C for 96 hours and checked every 24 hours. The health index scores of 15 larvae were summed every 24-hour time point.

Statistical Analysis

Categorical variables were compared using the chi-square test or Fisher exact test, whereas continuous variables were compared using the Student *t*-test or Mann–Whitney *U* test. Risk factors for early mortality were identified using backward stepwise logistic regression. Variables with a *P* value <.1 in univariate analysis were included in the multivariable analysis. To avoid multicollinearity, separate multivariable models were used for the SOFA and Pitt bacteremia scores as well as for carbapenem resistance and appropriate empirical antibiotic therapy. The Hosmer-Lemeshow test was used to evaluate the goodness of fit in stepwise logistic regression. The virulence of *A baumannii* isolates was compared using repeated-measures analysis of variance based on health index scores from the *G mellonella* infection model. *P* < .05 was considered significant. All statistical analyses were performed using SPSS Statistics software (version 26.0; IBM Corp., Armonk, NY, USA).

RESULTS

Clinical Characteristics of Patients According to Mortality

A total of 130 nonreplicate bloodstream isolates were obtained from patients with *A baumannii* bacteremia, of which 83 (63.8%) *A baumannii* isolates were carbapenem resistant. Of the 69 patients with 30-day mortality, 38 (55.1%) were classified as EM and 27 (39.1%) as LM.

The clinical characteristics of patients with *A baumannii* bacteremia from whom clinical isolates were collected are presented in Table 1. The SOFA and Pitt bacteremia scores were the highest in the EM group. CRBSI occurred more frequently in the 30-day survivor group than in the EM group and tended to occur more frequently in the LM group compared to the EM group. The proportion of hepatobiliary infection was also significantly higher in the 30-day survivor group compared to the EM group and tended to be higher in the LM group than in the EM group. Compared with the 30-day survivor group, both the EM and LM groups showed significantly higher percentages of pneumonia. The proportion of carbapenem-resistant isolates was higher in the EM and LM groups than in the 30-day survivor group. The proportion of appropriate

Table 1. Clinical Characteristics of Patients With *Acinetobacter baumannii* Bacteremia According to Mortality Outcomes

Variables	EM (n = 38)	LM (n = 27)	30-d Survivor (n = 61)	P^a	P^b	P^c
Age (y), mean ± SD	69.7 ± 15.1	70.6 ± 12.0	65.8 ± 13.4	.796	.185	.114
Male	27 (71.1)	20 (74.1)	38 (62.3)	>.999	.372	.282
CCWI	7.0 (5.0–9.0)	6.0 (5.0–9.0)	5.0 (4.0–8.0)	.881	.082	.171
Clinical severity	…	…	…	…	…	…
SOFA score, median (IQR)	15.5 (11.8–18.0)	11.0 (6.0–14.0)	4.0 (2.0–7.0)	.002	<.001	<.001
Pitt bacteremia score, median (IQR)	9.0 (7.0–10.0)	5.0 (2.0–8.0)	2.0 (0.5–3.5)	<.001	<.001	<.001
Source of infection	…	…	…	…	…	…
Primary bacteremia	7 (18.4)	3 (11.1)	15 (24.6)	.503	.473	.148
CRBSI	6 (15.8)	9 (33.3)	22 (36.1)	.098	.029	.805
Phlebitis	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
Vascular device	0 (0.0)	1 (3.7)	0 (0.0)	.415	—	.307
Surgical site infection	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
Skin and soft tissue infection	1 (2.6)	1 (3.7)	0 (0.0)	>.999	.384	.307
Pneumonia	21 (55.3)	10 (37.0)	4 (6.6)	.147	<.001	.001
Urinary tract infection	0 (0.0)	0 (0.0)	5 (8.2)	—	.153	.318
Intra-abdominal infection	3 (7.9)	1 (3.7)	2 (3.3)	.636	.369	>.999
Hepatobiliary infection	0 (0.0)	2 (7.4)	11 (18.0)	.169	.006	.329
Carbapenem resistance	35 (92.1)	22 (81.5)	23 (37.7)	.260	<.001	<.001
Appropriate empirical antibiotic therapy	3 (7.9)	10 (37.0)	28 (45.9)	.004	<.001	.439

Abbreviations: —, statistical analysis is not applicable; CCWI, Charlson's comorbidity-weighted index; CRBSI, catheter-related bloodstream infection; EM, early mortality; IQR, interquartile range; LM, late mortality; SD, standard deviation; SOFA, sequential organ failure assessment.

[a]*P* values between the EM and LM groups.

[b]*P* values between the EM and 30-d survivor groups.

[c]*P* values between the LM and 30-d survivor groups.

empirical antibiotic therapy was significantly lower in the EM group than in the 30-day survivor group and the LM group.

The clinical characteristics of subgroups, including patients with carbapenem-resistant *A baumannii* (CRAB) bacteremia, are shown in Supplementary Table 3. The EM group showed a significantly higher percentage of pneumonia than the 30-day survivor group (60.0% vs 13.0%, *P* < .001).

Microbiological Characterization

The distribution of STs according to mortality outcomes using MLST is presented in Table 2. Clinical isolates with new STs assigned based on submitted data were classified as "others." Compared with that in the 30-day survivor group, ST191 was more frequent in the EM and LM groups. The proportion of ST195 was higher in the LM group than in the 30-day survivor group. The proportion of newly assigned STs was higher in the 30-day survivor group than in the EM and LM groups.

The distribution of STs in the 130 *A baumannii* isolates throughout the study period is shown in Supplementary Figure 1. ST191 was the most prevalent ST, consistently identified throughout the study period. ST451 was mainly identified between 2015 and 2016, whereas ST784 was detected between 2016 and 2020. A comparison of the STs between CRAB and carbapenem-susceptible *A baumannii* (CSAB) isolates is shown in Supplementary Table 4. The proportions of ST191, ST451, and ST784 were higher among CRAB than among CSAB. The proportion of newly assigned STs was lower among CRAB than among CSAB.

The frequencies of 9 virulence factors according to mortality outcomes are shown in Table 3. The *ompA* and *adeB* genes were detected in all *A baumannii* isolates. The *bfmS* gene was detected more frequently in the EM and LM groups than in the 30-day survivor group. Compared with the 30-day survivor group, in the EM and LM groups, *bauA* was detected less frequently. The *csuE* gene was detected more frequently in the LM group than in the 30-day survivor group.

The frequency of virulence genes according to ST is presented in Supplementary Table 5. The *bmfS* gene was identified more frequently in ST191 than in non-ST191 (100.0% vs 62.5%, *P* < .001), in ST451 than in non-ST451 (93.3% vs 67.0%, *P* = .038), and in ST784 than in non-ST784 (100.0% vs 66.9%, *P* = .018). The *bauA* gene was detected less frequently in ST191 than in non-ST191 (0.0% vs 41.3%, *P* < .001), in ST451 than in non-ST451 (0.0% vs 37.4%, *P* = .002), and in ST784 than in non-ST784 (0.0% vs 36.4%, *P* = .008).

RISK FACTORS FOR EARLY MORTALITY IN *A BAUMANNII* BACTEREMIA

The results of the univariate and multivariable analyses identifying risk factors for EM are shown in Table 4. In multivariable analyses, SOFA and Pitt bacteremia scores were independently associated with EM. Additionally, pneumonia and ST191 were independent risk factors for EM. Appropriate empirical antibiotic therapy was the only protective factor against EM.

Subgroup analyses to identify the risk factors for EM among CRAB bacteremia are shown in Supplementary Table 6. SOFA score (adjusted odds ratio [aOR], 1.44; 95% confidence interval [CI], 1.18–1.75; $P < .001$), Pitt bacteremia score (aOR, 1.96; 95% CI, 1.33–2.88; $P = .001$), and pneumonia (aOR, 10.33; 95% CI, 1.35–79.19; $P = .025$) were independent risk factors for EM. Only CRBSI was associated with a decreased risk of EM (aOR, 0.03; 95% CI, 0.00–0.71; $P = .029$). Definitive antibiotic therapies for patients with LM and 30-day survivors are shown in Supplementary Table 7.

VIRULENCE IN *G MELLONELLA* INFECTION MODEL

Using repeated-measures analysis of variance, the health index scores over time from the *G mellonella* infection model were compared between the groups. There was a significant difference in the health index scores between the EM and 30-day survivor groups ($P = .022$; Figure 1A). Additionally, changes in scores over time were significantly different between ST191 and non-ST191 ($P < .001$; Figure 1B). The scores of isolates with and without *bfmS* were significantly different ($P = .001$; Figure 1C), as were the scores of isolates with and without *bauA* ($P = .002$; Figure 1D). The health index scores by *G mellonella* infection model over time are presented as mean ± standard deviation at every 24-hour time point in Supplementary Table 8.

DISCUSSION

To the best of our knowledge, this is the first study to investigate the microbiological factors associated with clinical outcomes, particularly EM, and find that ST191 is an independent risk factor for EM in *A baumannii* bacteremia. Additionally, ST191 was confirmed to be highly virulent in a *G mellonella* infection model.

In a retrospective study conducted in Korea, pneumonia as the source of bacteremia was more frequent in 30-day deaths than in 30-day survivors [17]. Consistent with these findings, our study showed a higher proportion of pneumonia and lower proportion of CRBSI as the focus of infection in the EM group than in the 30-d survivor group. Moreover, pneumonia was identified as an independent risk factor for EM in *A baumannii* bacteremia, whereas CRBSI was associated with a reduced risk of EM in CRAB. These findings suggest that EM may be

Table 2. Sequence Types of *Acinetobacter baumannii* Isolates According to Mortality Outcomes

ST	EM (n = 38)	LM (n = 27)	30-d Survivor (n = 61)	P^a	P^b	P^c
191	12 (31.6)	7 (25.9)	4 (6.6)	.621	.001	.030
195	1 (2.6)	5 (18.5)	2 (3.3)	.074	>.999	.026
231	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
357	1 (2.6)	0 (0.0)	0 (0.0)	>.999	.384	—
368	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
369	5 (13.2)	2 (7.4)	1 (1.6)	.689	.690	.222
436	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
447	3 (7.9)	0 (0.0)	0 (0.0)	.260	.054	—
451	8 (21.1)	3 (11.1)	4 (6.6)	.338	.338	.671
469	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
491	1 (2.6)	1 (3.7)	1 (1.6)	>.999	>.999	.522
585	0 (0.0)	1 (3.7)	0 (0.0)	.415	—	.307
784	4 (10.5)	4 (14.8)	4 (6.6)	.709	.479	.243
1386	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
1482	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
1911	0 (0.0)	1 (3.7)	0 (0.0)	.415	—	.307
2098	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
2787	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999
Others[d]	3 (7.9)	3 (11.1)	36 (59.0)	.686	<.001	<.001
Unclassified	0 (0.0)	0 (0.0)	1 (1.6)	—	>.999	>.999

Abbreviations: —, statistical analysis is not applicable; EM, early mortality; LM, late mortality; ST, sequence type.

[a]*P* values between the EM and LM groups.

[b]*P* values between the EM and 30-d survivor groups.

[c]*P* values between the LM and 30-d survivor groups.

[d]Newly assigned STs by submitting data of isolates to multilocus sequence typing database (http://pubmlst.org/databases/).

Table 3. Frequency of Virulence Factors in *Acinetobacter baumannii* Isolates According to Mortality Outcomes

Virulence Factors	Function	EM (n = 38)	LM (n = 27)	30-d Survivor (n = 61)	P^a	P^b	P^c
ompA	Biofilm formation	38 (100.0)	27 (100.0)	61 (100.0)	—	—	—
adeB	Drug resistance	38 (100.0)	27 (100.0)	61 (100.0)	—	—	—
smpA	Membrane integrity	38 (100.0)	27 (100.0)	60 (98.4)	—	>.999	>.999
bfmS	Membrane integrity	35 (92.1)	23 (85.2)	29 (47.5)	.437	<.001	.001
csuE	Biofilm formation	27 (71.1)	24 (88.9)	41 (67.2)	.085	.689	.033
epsA	Capsule	1 (2.6)	6 (22.2)	8 (13.1)	.017	.147	.346
abaI	Quorum sensing	38 (100.0)	27 (100.0)	59 (96.7)	—	.522	>.999
basD	Siderophore	38 (100.0)	27 (100.0)	57 (93.4)	—	.295	.308
bauA	Siderophore	5 (13.2)	5 (18.5)	32 (52.5)	.729	<.001	.003

Abbreviations: —, statistical analysis is not applicable; EM, early mortality; LM, late mortality.

[a]*P* values between the EM and LM groups.

[b]*P* values between the EM and 30-d survivor groups.

[c]*P* values between the LM and 30-d survivor groups.

Table 4. Associated Factors for Early Mortality in Patients With *Acinetobacter baumannii* Bacteremia

Variables	EM (n = 38)	30-d Survivor (n = 61)	Univariate OR (95% CI)	P	Multivariable aOR (95% CI)	P
Age	69.7 ± 15.1	65.8 ± 13.4	1.02 (0.99–1.05)	.186	—	—
Male	27 (71.1)	38 (62.3)	1.49 (0.62–3.55)	.373	—	—
CCWI	7.0 (5.0–9.0)	5.0 (4.0–8.0)	1.11 (0.97–1.26)	.123	—	—
Clinical severity	…	…	…		…	…
SOFA score	15.5 (11.8–18.0)	4.0 (2.0–7.0)	1.54 (1.31–1.80)	<.001	1.61 (1.28–2.02)	<.001
Pitt bacteremia score	9.0 (7.0–10.0)	2.0 (0.5–3.5)	1.98 (1.55–2.53)	<.001	1.89 (1.43–2.50)	<.001
Infection focus	…	…	…		…	…
Primary bacteremia	7 (18.4)	15 (24.6)	0.69 (0.25–1.89)	.474	—	—
CRBSI	6 (15.8)	22 (36.1)	0.33 (0.12–0.92)	.034	—	—
Phlebitis	0 (0.0)	1 (1.6)	—	>.999	—	—
Surgical site infection	0 (0.0)	1 (1.6)	—	>.999	—	—
Skin and soft tissue infection	1 (2.6)	0 (0.0)	—	>.999	—	—
Pneumonia	21 (55.3)	4 (6.6)	17.60 (5.31–58.36)	<0.001	16.00 (2.06–124.32)	.008
Urinary tract infection	0 (0.0)	5 (8.2)	—	.999	—	—
Intra-abdominal infection	3 (7.9)	2 (3.3)	2.53 (0.40–15.88)	.322	—	—
Hepatobiliary infection	0 (0.0)	11 (18.0)	—	.999	—	—
Carbapenem resistance	35 (92.1)	23 (37.7)	19.38 (5.32–69.87)	<.001	6.32 (0.88–45.38)	.067
Appropriate empirical antibiotic therapy	3 (7.9)	28 (45.9)	0.10 (0.03–0.36)	<.001	.03 (0.01–0.44)	.009
ST	…	…	…		…	…
Non-ST191	26 (68.4)	57 (93.4)	1.00		1.00	…
ST191	12 (31.6)	4 (6.6)	6.58 (1.94–22.34)	.003	11.69 (1.39–98.71)	.024
Virulence factors	…	…	…		…	…
ompA	38 (100.0)	61 (100.0)	—	—	—	—
adeB	38 (100.0)	61 (100.0)	—	—	—	—
smpA	38 (100.0)	60 (98.4)	—	>.999	—	—
bfmS	35 (92.1)	29 (47.5)	12.87 (3.58–46.38)	<.001	—	—
csuE	27 (71.1)	41 (67.2)	1.20 (0.50–2.89)	.689	—	—
epsA	1 (2.6)	8 (13.1)	0.18 (0.02–1.49)	.112	—	—
abaI	38 (100.0)	59 (96.7)	—	.999	—	—
basD	38 (100.0)	57 (93.4)	—	.999	—	—
bauA	5 (13.2)	32 (52.5)	0.14 (0.05–0.40)	<.001	—	—

Abbreviations: —, statistical analysis is not applicable; aOR, adjusted odds ratio; CCWI, Charlson's comorbidity-weighted index; CI, confidence interval; CRBSI, catheter-related bloodstream infection; EM, early mortality; OR, odds ratio; SOFA, sequential organ failure assessment; ST, sequence type.

associated with appropriate source control of infection. An ineradicable focus was identified as an independent risk factor for 30-day mortality in CRAB bacteremia [18].

We previously reported that the SOFA and Pitt bacteremia scores were independent risk factors for EM [6]. Another study reported that a higher Acute Physiology and Chronic Health Evaluation II score was a risk factor for 14-day mortality in *A baumannii* bacteremia [19]. Here, the SOFA and Pitt bacteremia scores were consistently identified as independent risk factors for EM. Appropriate empirical antibiotic therapy has been identified as a protective factor against EM. This aligns with previous findings that appropriate antibiotic therapy is associated with a reduced risk of mortality in *A baumannii* bacteremia [19, 20].

ST191 was the predominant ST, followed by ST784 and ST451. A previous multicenter study in Korea reported that most *A baumannii* blood isolates were ST191, ST784, and ST451 [21]. In a study conducted in China, 3 main STs were

ST191, ST195, and ST208, indicating regional variations in STs [22]. Furthermore, this previous study showed that the proportion of patients with septic shock and 3-day mortality (early mortality) were higher in patients with ST191/195/208 than in those with other STs. Thus, clinical severity and outcomes may differ depending on the ST. We also identified ST191 as an independent risk factor for EM in *A baumannii* bacteremia and showed that ST191, ST451, and ST369 were prevalent in EM. In studies conducted in Korea, ST191 was associated with 30-day mortality in *A baumannii* bacteremia [21], and ST191 and ST369 were independent risk factors for 7-day mortality in CRAB bacteremia [23]. In the 30-day survivor group, initially unclassified STs were frequent and no specific ST was dominant, suggesting that the unclassified STs, which were classified as others in this study, were not highly virulent.

The *bfmS* gene is associated with biofilm formation, adherence to cells, and antibiotic susceptibility [24], and *bauA* plays
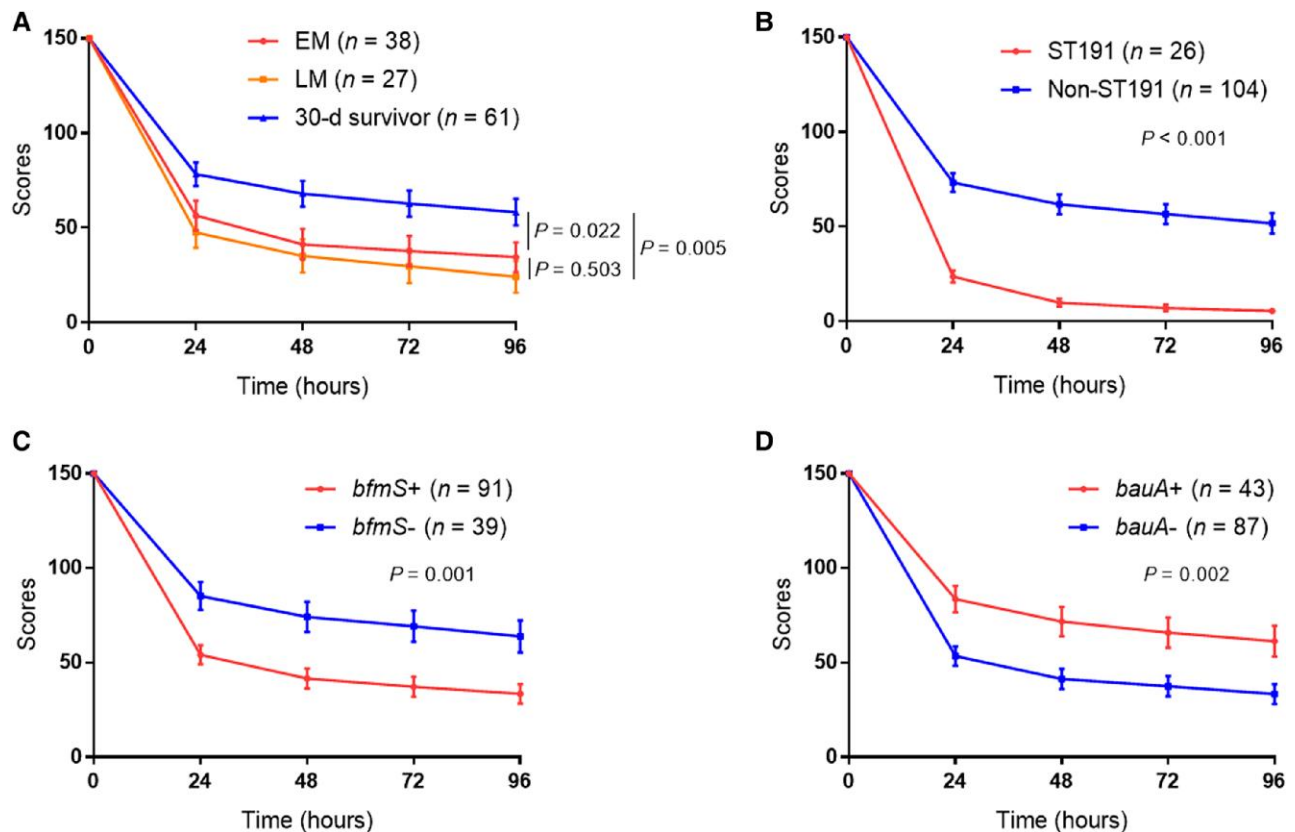
Figure 1. The virulence of *Acinetobacter baumannii* isolates by *Galleria mellonella* infection model. *A*, Health index scores of larvae injected with isolates from the early mortality (EM), late mortality (LM), and 30-day survivor groups. *B*, Health index scores of larvae injected with ST191 and non-ST191. *C*, Health index scores of larvae injected with isolates carrying *bfmS* and those without *bfmS*. *D*, Health index scores of larvae injected with isolates carrying *bauA* and those without *bauA*. The circles, squares, and triangles represent the mean, and the lines indicates standard error of the mean. The health index score of larvae was calculated by summing the scores for activity, cocoon formation, melanization, and survival. Health index scores of 15 larvae were summed at every 24-hour time point.

a crucial role in the pathogenicity of *A baumannii* by associating with siderophore acinetobactin receptor [25]. Several virulence factors and their associations with various functions in *A baumannii* isolates have been investigated. Additionally, a previous study found differences in virulence factors based on the STs of *A baumannii* isolates [26]. Here, the EM group showed different frequencies of *bfmS* and *bauA* compared with the 30-day survivor group, and ST-specific differences in the frequency of virulence factors were observed. This suggests that virulence factors may be tailored to their surroundings, inducing different frequencies of virulence factors in *A baumannii* isolates.

The *G mellonella* larvae infection model serves as a major invertebrate model for investigating host–microbe interactions. This model has been used in a range of studies on fungi, such as *Aspergillus fumigatus*, and various bacteria, such as *Staphylococcus aureus* and *A baumannii* [27–29]. We confirmed that ST191 and isolates with *bfmS*, frequently found in EM, showed low health index scores in the *G mellonella* infection model. This finding supports the hypothesis that *A baumannii*

isolates that cause EM exhibit high virulence in the *G mellonella* infection model.

This study had several limitations. First, *A baumannii* isolates were collected from a single tertiary care hospital, which may limit the generalizability of our findings. Second, the number of CRAB cases was relatively small, which limited the identification of microbiological risk factors for EM in the subgroup analysis of CRAB. Nonetheless, we demonstrated that ST191 is an independent risk factor for EM in *A baumannii* bacteremia, even after adjusting for carbapenem resistance. Finally, the clinical variables of patients with *A baumannii* bacteremia were retrospectively collected.

In conclusion, our findings showed that the independent risk factors for EM were ST191, pneumonia as the infection source, and the severity of illness. Comparing *A baumannii* isolates based on clinical outcomes, the proportions of ST191 and *bfmS* were significantly higher in strains causing EM than in survivors. Furthermore, ST191 and isolates with *bfmS* exhibited high virulence in the *G mellonella* infection model. Therefore, differences in microbiological characteristics, including STs

and virulence factors, contribute to the different outcomes. Elucidating the microbiological characteristics of *A baumannii* strains that cause EM may aid tailoring infection control measures focusing on these strains to improve clinical outcomes through prevention.

Supplementary Data

Notes

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