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Antimicrobial resistance and therapy in critically ill patients

Edited by
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Antimicrobial resistance and therapy in critically ill patients

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Editorial: Antimicrobial resistance and therapy in critically ill patients

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KEYWORDS

antimicrobial resistance, critically ill, therapy, carbapenem-resistant organisms, carbapenem-resistant *Klebsiella pneumoniae*

Editorial on the Research Topic
Antimicrobial resistance and therapy in critically ill patients

Multidrug-resistant (MDR) microbes infection for critically ill patients is a big challenge in clinical practice and is associated with greatly increased mortality (1). This Research Topic includes three articles that explored the clinical and microbiological characteristics of MDR pathogen infection in patients admitted to intensive care units (ICU) in China (Wu H. et al., Wu H.-N. et al., Li et al.).

Wu H.-N. et al. retrospectively analyzed the distribution and antibiotic resistance of pathogens based on the clinical data of intensive care patients with bloodstream infections presented to a Chinese tertiary hospital and explored the value of procalcitonin (PCT) for the differentiated diagnosis of bloodstream infections caused by various pathogens. Gram-negative bacteria were the most frequently isolated microorganisms and were associated with a higher percentage of complications such as brain dysfunction, acute kidney injury, and thrombocytopenia. It was observed that PCT was a not good biomarker to distinguish bloodstream infections caused by various pathogens or fungi. Given that the mortality for patients with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) bloodstream infection is reported to be as high as 30%−70%, Wu H. et al. used a logistic analysis to assess the association between the neutrophil-to-lymphocyte ratio (NLR) on 4th-day and 28th-day mortality. After balancing the confounders, NLR on the 4th day was associated with the 28th-day mortality, whereas the appropriate initial therapy was an independent protective factor. Moreover, the authors suggested that the trend of the NLR during therapy may help to evaluate the efficacy of different anti-infection therapy strategies at an early stage. In another article, Li et al. described their experience in the management of post-neurosurgical central nervous system infection caused by MDR Gram-negative bacteria with combined intraventricular and intravenous polymyxin B administration. After a mean duration of 14 days of treatment, all six cases caused by CRKP or carbapenem-resistant *Acinetobacter baumannii* (CRAB) were cured and no obvious kidney injury occurred.

This Research Topic also includes three articles on the superinfection of patients with SARS-CoV-2 infection who were admitted to the ICU (Karlsson et al., Yoon et al., Casarotta et al.). Yoon et al. demonstrated that bacterial superinfections were common in a tertiary Korean academic hospital and that more than one-third of the bacterial superinfection cases were caused by multidrug-resistant pathogens. Moreover, bacterial superinfection was associated with significantly fewer ventilator-free days, longer ICU and hospital stays. As many studies reported that a CRAB-associated

bloodstream infection was the crucial risk factor for death in patients with COVID-19 (2), Casarotta et al. compared two different antibiotic strategies for CRAB infection in terms of microbiological negativization. The *Protocol* group, which was managed with combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam, was associated with a significantly higher microbiological clearance compared to the *Control* group, which consisted of patients treated with a combination of two antibiotics (100% vs. 36.4% respectively). In a prospective longitudinal study in Sweden conducted by Karlsson et al., the authors evaluated the complicated bacteriuria and antibiotic resistance for ICU-admitted COVID-19 patients. They found that the vast majority of patients received antibiotics on ICU admission. Longer stays in ICUs linearly correlated with bacteriuria, and the authors proposed that biofilms in urinary catheters act as a reservoir of pathogenic bacteria with the potential to develop and disseminate antibiotic resistance.

This Research Topic also comprises an uncommon but interesting study by Bushuven et al. to evaluate the feasibility of hand hygiene in a manikin cardiopulmonary resuscitation (CPR) study, given that CPR scenarios are at high risk for healthcare-associated infections. By studying Advanced Cardiovascular Life Support (ACLS) courses in a manikin simulation, they found more than half of hand-cleaning indications could have been accomplished without delaying patient resuscitation and they concluded that hand disinfection can be implemented without compromising quality in acute care. In patients with severe acute pancreatitis (SAP), secondary MDR pathogen infection plays a vital role in increased mortality and prolonged hospital and ICU stays (3). MDR pathogen infection in patients with SAP is lethal and generally associated with excessive antibiotic exposure. As shown in the study by Shajiei et al., which used a previously reported SAP trial data, PCT-guided antibiotics management significantly reduced antibiotic usage but it did not translate into a detectable change in antimicrobial resistance.

This Research Topic also includes an original study by Byrnes et al. that aimed to identify the optimal tissue source of both naïve and cytokine pre-activated mesenchymal stromal cells (MSCs) to enhance the resolution of late-phase organizing pneumonia caused by CRKP. Organizing pneumonia is a pattern of lung-tissue repair after injury and it can be cryptogenic or a response to a specific lung injury in many diverse clinical contexts. Given the therapy for organizing pneumonia is empirical and few therapies have been confirmed besides systemic glucocorticoid therapy (4), they demonstrated that delayed MSC therapy enhanced the resolution of lung injury induced by CRKP infection and favorably modulated immune cell profile, which indicates the potential role of MSC to facilitate the resolution of pulmonary organization after MDR pathogen infection.

Author contributions

QL: Writing—original draft. S-JZ: Writing—review and editing. J-CZ: Writing—original draft, Writing—review and editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Value of Neutrophil-To-Lymphocyte Ratio for Evaluating Blood Stream Infection Caused by Carbapenem-Resistant *Klebsiella pneumoniae*: A Retrospective Cohort Study

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Background: The neutrophil-to-lymphocyte ratio (NLR) is a useful marker of inflammation. However, the prognostic function of the NLR in patients with carbapenem-resistant *Klebsiella pneumoniae* (CRKP) blood stream infection (BSI) remains largely unknown. The aim of this study was to explore the potential relationship between the NLR and mortality in these patients.

Methods: We performed a retrospective cohort study based on data retrieved from the computerized patient record system in a tertiary hospital from 1 January 2017 to 31 October, 2020. A total of 134 inpatients with CRKP BSI were enrolled in this study, including 54 fatal cases and 80 survival cases, 28 days after the onset of CRKP BSI. A logistic analysis was performed to assess the association between the NLR on the 4th day and 28-day mortality. Multivariate analyses were used to control for the confounders.

Results: The overall 28-day mortality rate of patients with a CRKP BSI episode was 40.3% (54/134). We conducted a multivariate analysis of the data of 134 patients and found that the NLR on the 4th day [odds ratio (OR) 1.148, 95% confidence interval (CI) 1.076–1.225, $p < 0.001$] and antibiotic exposure before BSI onset (OR 3.847, 95% CI 1.322–11.196, $p = 0.013$) were independent risk factors for 28-day mortality of patients with CRKP BSI, while appropriate initial therapy (AIT, OR 0.073, 95% CI 0.017–0.307, $p < 0.001$) was an independent protective factor. Among patients treated with AITs, the Cox proportional hazards regression analysis revealed a significant difference in prognosis ($p = 0.006$) between the ceftazidime/avibactam contained (CAZ) group and non CAZ-AVI groups. After dividing the non CAZ-AVI group into the tigecycline (TGC), colistin (COL), and TGC + COL groups, there were no differences between the CAZ-AVI group and the TGC group ($p = 0.093$), but CAZ-AVI group showed lower 28-day mortality than

COL ($p = 0.002$) and TGC + COL ($p = 0.002$) groups. Meanwhile, there was no difference in NLR on the 1st day ($p = 0.958$) of patients in different groups but significant difference in NLR on the 4th day ($p = 0.047$).

Conclusions: The NLR on the 4th day is a readily available and independent prognostic biomarker for patients with CRKP BSI. This marker may have the potential for use in evaluating the efficacy of different anti-infection therapy strategies at an early stage.

Keywords: neutrophil-to-lymphocyte ratio, carbapenem-resistant *Klebsiella pneumoniae*, blood stream infection, prognosis, therapy strategies

INTRODUCTION

Klebsiella pneumoniae is one of the most common bacteria in the class Enterobacteriales; it is ubiquitous and can cause nosocomial infections, such as pneumonia, urinary tract infection, catheter-related infection, and blood stream infection (BSI) (1, 2). *K. pneumoniae* isolates can develop resistance by producing extended-spectrum β-lactamases (ESBLs) (3, 4). Carbapenems are the first-line therapy for severe infections caused by ESBL-producing KPs (5). However, with the increasing clinical use of carbapenems over the last few years, carbapenem-resistant *K. pneumoniae* (CRKP) has risen at an alarming rate, and is considered a serious threat to human health worldwide. It has been recorded in the China antimicrobial surveillance network that from 2005 to 2021, the proportion of *K. pneumoniae* isolates resistant to imipenem increased from 3.0 to 25.5% in China (http://www.chinets.com/).

Patients infected with CRKP have higher mortality rates than those infected with carbapenem-susceptible *Klebsiella pneumonia* (CSKP) (6). It was reported that the mortality of patients with CRKP infection, mainly BSI, was up to 70% (7), and a high readmission rate of survivors (∼72%) within 90 days of discharge was reported (8). The proposed hypotheses for this increased mortality include (1) severe comorbidities, (2) increased virulence of carbapenemase-producing strains, (3) low effectiveness and high toxicity of drugs used for treatment of these infections, and (4) a low probability of receiving appropriate initial antibiotic therapy (9).

Since CRKP BSIs would result in worse clinical outcomes, early and accurate evaluation is essential for the treatment and prognosis of these patients. The neutrophil-to-lymphocyte ratio (NLR) is a measure of systemic inflammation derived from the white blood cell (WBC) count, one of the most common infection markers. It has been used as a predictor of cardiovascular diseases (10) and cancer (11). Zahorec proposed the use of the NLR as an additional infection marker in clinical intensive care unit practice based on the phenomenon that the physiological immune response of circulating leukocytes to various stressful events is often characterized by an increase in neutrophil counts and a decline in lymphocyte counts (12). Additionally, according to Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sepsis-related Organ Failure Assessment (SOFA) scores, it was found that NLR correlated well with the severity of disease and outcome (13). de Jager et al. evaluated the performance of NLR and other markers of infection in predicting bacteraemia in adults and found it to be a better predictor than C-reaction protein (CRP) levels and WBC counts (14).

Nevertheless, the value of the NLR in predicting the prognosis of patients with CRKP BSI is rarely reported. Thus, we performed a cohort study to evaluate NLR as a predictor of the prognosis of these patients.

Abbreviations: NLR, neutrophil to lymphocyte count ratio; CRKP, carbapenem-resistant Klebsiella pneumoniae; CSKP, carbapenem- susceptible Klebsiella pneumoniae; BSI, blood stream infection; OR, odd ratio; WBC, white blood cell count; CRP, C-reaction protein; ICU, intensive care unit; IQR, inter quartile range; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sepsis-related Organ Failure Assessment scores; ROC, receiver operating characteristic; AUC, the area under curve; CAZ-AVI, ceftazidime/avibactam; TGC, tigecycline; COL, colistin.

METHODS AND MATERIAL

Ethics

All isolates present in this study were stored in the Department of Microbiology of a tertiary hospital, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine. The study was approved by the ethical research committee of Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University. The ethics committee approved the waiver of patients' informed consent, with the justification that this was a retrospective and analytical study whose information was obtained from medical records and that the data were stripped of identifying information and anonymously analyzed. The study was performed in accordance with the Declaration of Helsinki and its amendments.

Privacy statement: the authors guarantee confidentiality of the patient data (Ethics No. 20170301-3).

Study Design and Data Source

We performed a single-center, retrospective cohort study based on data retrieved from the Computerized Patient Record System of the Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China. Patients aged ≥18 years who were confirmed to present with CRKP BSI were included from January 1, 2017 to October 31, 2020. Episodes of CRKP BSI were identified based on blood culture results. All patients with CRKP BSI were followed up for 28 days and were divided into surviving and deceased groups. The database contains comprehensive clinical data, including patient

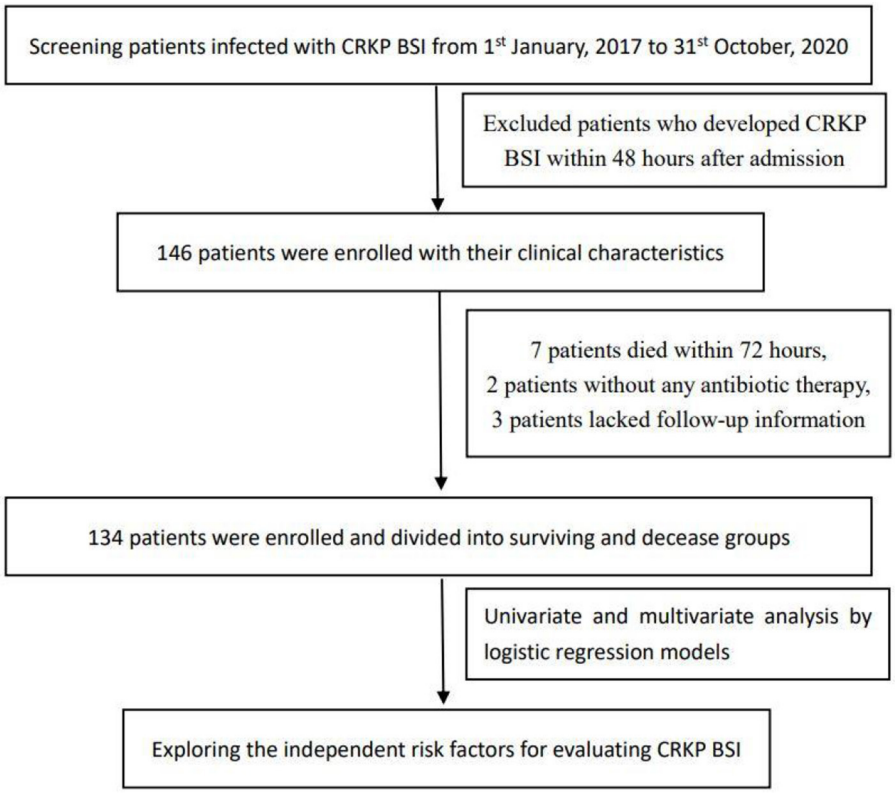

FIGURE 1 | Flowchart of the study design.

characteristics, laboratory outcomes, clinical diagnoses, and medical records (**Figure 1**).

Inclusion and Exclusion Criteria

We included patients with CRKP BSI from the database with their medical records. The exclusion criteria were as follows: (I) non-adult patients, (II) patients who developed CRKP BSI within 48 hours after admission, (III) patients who died within 72 h after the first positive blood culture or those with no antibiotic therapy records, and (IV) lack of records regarding laboratory examinations on the onset of BSI or the 4th (±1) day. A total of 134 CRKP BSI inpatients were included in this study, including 54 fatal cases and 80 survival cases on day 28 after the onset of CRKP BSI.

Definitions

CRKP BSI was defined as a positive blood culture for *K. pneumoniae* with resistance to any carbapenem combined with symptoms of infection and elevated CRP or procalcitonin (PCT), including those sampled from a peripherally inserted central catheter or central venous catheter. The date when the first positive blood culture was sampled was considered to be the onset of BSI and recorded as the first day. Renal damage was defined as a creatinine clearance of <30 mL/min, while liver damage was defined as an alanine transaminase or aspartate aminotransferase level >3-fold of the upper normal limit. Antibiotic exposure meant that antibiotics were administered intravenously or orally for more than 48 h within the past 14 days. Appropriate initial therapy (AIT) was defined as antibiotics, confirmed to be active against CRKP by a susceptibility test, administered within 72 hours after BSI onset. All-cause mortality was defined as death from any cause during hospitalization.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range), and categorical variables are presented as frequencies and percentages. Continuous variables with an abnormal distribution were compared using Student's *t*-test or Mann–Whitney *U*-test. Continuous variables in more than two groups were compared using Analysis of Variance (ANOVA). The chi-square or Fisher's exact tests were used to compare categorical variables. Risk factors for mortality from CRKP BSI were analyzed using binary logistic regression. Variables with $p < 0.10$ and clinical relevance in the univariate analysis were selected for logistic regression models for the multivariate analysis to evaluate risk factors for 28-day mortality of CRKP BSI.

TABLE 1 | Clinical and demographic characteristics of 134 patients with CRKP BSI and risk factors for 28-day mortality.

	Total ***N* = 134**	**28d-Surviving** ***N* = 80**	**28d-Deceased** ***N* = 54**	***p*-value**
Demographic variables				
Age	66 (55, 73)	66 (52, 73)	68 (59, 73)	0.577
Male	92 (68.6)	58 (72.5)	34 (63.0)	0.243
Body mass index (<18.5 or >24)	68 (50.7)	41 (51.2)	27 (50.0)	0.887
Underlying diseases				
Hypertension	56 (41.8)	36 (45.0)	20 (37.3)	0.359
Diabetes mellitus	27 (20.1)	18 (22.5)	9 (25.9)	0.208
Cardiovascular and cerebrovascular diseases	15 (11.2)	9 (11.2)	6 (18.5)	0.237
Chronic lung diseases	32 (23.9)	20 (25.0)	12 (35.2)	0.203
Liver failure	15 (11.2)	10 (12.5)	5 (14.8)	0.700
Renal failure	11 (8.2)	6 (7.5)	5 (14.8)	0.175
Solid tumor	34 (25.4)	25 (31.2)	9 (25.9)	0.506
Hematological malignancy	6 (4.5)	3 (3.8)	3 (9.3)	0.267
Immunosuppressive therapy	11 (8.2)	6 (7.5)	5 (14.8)	0.175
Age-adjusted Charlson comorbidity index	4.90 ± 2.45	4.55 ± 2.34	5.41 ± 2.54	**0.047**
Two weeks before the onset of BSI				
ICU stay	72 (53.0)	40 (50.0)	32 (59.2)	0.292
Antibiotic exposure	56 (41.8)	24 (30.0)	32 (59.2)	**0.001**
Tigecycline	3 (5.4)	1 (4.2)	2 (6.2)	0.999
Quinolones	1 (1.8)	1 (4.2)	0 (0)	0.429
β-Lactam/lactamase combinations	23 (41.1)	11 (45.8)	12 (37.5)	0.530
Carbapenems	29 (33.9)	11 (45.8)	18 (56.2)	0.440
The day of BSI onset				
APACHE II	20 (16, 26)	17 (12, 23)	22 (19, 29)	**<0.001**
SOFA	6 (4, 9)	5 (3, 8)	8 (5, 10)	**<0.001**
Hospitalized days before BSI onset	14 (2, 23)	12 (1, 20)	18 (11, 35)	**0.041**
Appropriate Initial Therapy	112 (83.6)	75 (93.8)	37 (68.5)	**0.001**
White blood cell (10^9/L)	13.36 ± 7.77	13.62 ± 7.90	12.97 ± 7.64	0.637
Neutrophil to lymphocyte count ratio	30.97 ± 38.16	28.69 ± 34.14	34.35 ± 43.56	0.402
C-reaction protein (mg/L)	133.64 ± 78.95	134.12 ± 82.30	132.93 ± 74.47	0.932
Procalcitonin (ng/mL)	19.24 ± 40.21	20.12 ± 42.72	17.94 ± 36.57	0.769
Hemoglobin (g/L)	85.04 ± 22.06	86.16 ± 22.18	83.39 ± 22.00	0.477
Platelet (10^9/L)	154.12 ± 117.31	163.29 ± 116.10	140.54 ± 118.87	0.272
Hematocrit	26.11 ± 7.58	26.07 ± 6.72	26.17 ± 8.76	0.939
Alanine transaminase (U/L)	58.63 ± 128.10	56.20 ± 124.28	62.24 ± 134.67	0.790
Aspartate aminotransferase (U/L)	81.49 ± 222.38	90.38 ± 272.81	68.48 ± 115.83	0.579
Total bilirubin (μmol/L)	61.45 ± 79.84	54.00 ± 75.97	72.49 ± 84.76	0.190
Direct bilirubin (μmol/L)	44.52 ± 61.16	38.46 ± 57.70	53.49 ± 65.48	0.175
Albumin (g/L)	29.10 ± 4.70	29.73 ± 4.79	28.17 ± 4.43	0.059
Creatinine (μmol/L)	135.40 ± 147.77	132.40 ± 150.45	139.83 ± 144.99	0.776
The 4th day after BSI onset				
APACHE II	18 (13, 24)	15 (9, 20)	23 (19, 29)	**<0.001**
SOFA	5 (4, 8)	4 (2, 7)	8 (5, 10)	**<0.001**
White blood cell count (10^9/L)	10.56 ± 6.29	9.36 ± 4.98	12.34 ± 7.54	**0.007**
Neutrophil-to-lymphocyte ratio	19.06 ± 24.72	9.42 ± 6.41	33.34 ± 33.53	**<0.001**
C-reaction protein (mg/L)	109.00 ± 69.16	87.08 ± 62.65	141.48 ± 65.97	**<0.001**
Procalcitonin (ng/mL)	9.82 ± 21.30	7.57 ± 19.32	13.15 ± 19.80	0.107
Hemoglobin (g/L)	79.94 ± 22.60	81.37 ± 25.25	77.83 ± 18.01	0.377
Platelet (10^9/L)	136.55 ± 130.37	171.91 ± 138.25	84.17 ± 97.35	**<0.001**
Hematocrit	25.43 ± 5.50	26.55 ± 5.57	23.78 ± 4.99	**0.004**

(Continued)

TABLE 1 | Continued

	Total $N = 134$	28d-Surviving $N = 80$	28d-Deceased $N = 54$	*p*-value
Alanine transaminase (U/L)	39.79 ± 61.41	35.09 ± 34.22	46.48 ± 86.57	0.298
Aspartate aminotransferase (U/L)	45.89 ± 56.10	40.09 ± 40.92	54.17 ± 72.12	0.158
Total bilirubin (μmol/L)	68.90 ± 88.42	51.36 ± 74.43	94.88 ± 101.04	**0.008**
Direct bilirubin (μmol/L)	48.68 ± 65.67	34.60 ± 53.73	69.53 ± 76.02	**0.004**
Albumin (g/L)	28.62 ± 5.11	29.69 ± 4.91	27.02 ± 5.03	**0.003**
Creatinine (μmol/L)	105.16 ± 87.79	93.40 ± 69.31	122.57 ± 107.99	0.083
Adverse events				
Renal damage	18 (13.4)	9 (11.2)	9 (16.7)	0.367
Liver damage	15 (11.2)	9 (11.2)	6 (11.1)	0.980

The bold values means p < 0.05, with a statistical significance.

TABLE 2 | Multivariate logistic regression analysis of risk factors for 28-day mortality of patients with CRKP BSI.

	Exp (B)	95%CI Exp (B)	*P*-value
Appropriate initial therapy	0.073	(0.017, 0.307)	**<0.001**
Antibiotic exposure	3.847	(1.322, 11.196)	**0.013**
NLR on 4th day	1.148	(1.076, 1.225)	**<0.001**
APACHE II score on 4th day	1.096	(0.987, 1.218)	0.086
SOFA on score 4th day	1.020	(0.814, 1.277)	0.863

The bold values means p < 0.05, with a statistical significance.

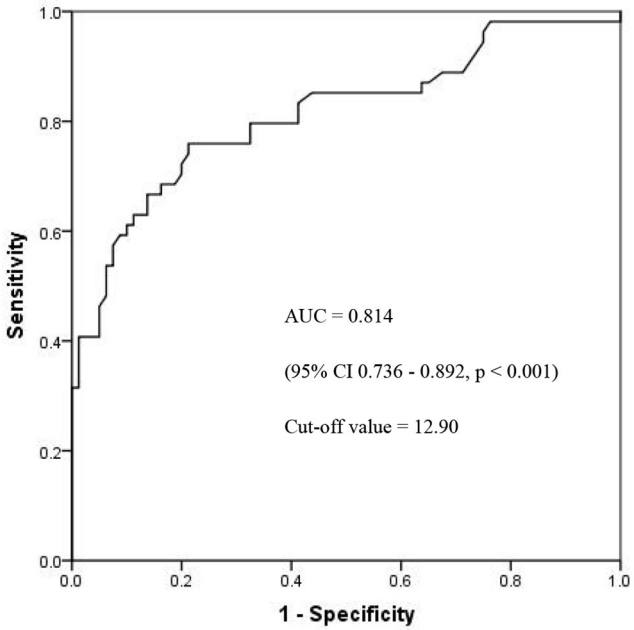

FIGURE 2 | The ROC curves of NLR and 28-day mortality. ROC, receiver operating characteristic; NLR, neutrophil- to-lymphocyte count ratio; AUC, area under curves.

RESULTS

Clinical and Demographic Characteristic of Patients With CRKP BSI and Risk Factors for 28-Day Mortality

The overall 28-day mortality rate of patients with a CRKP BSI episode was 40.3% (54/134). The clinical and demographic characteristics of cohort patients with CRKP BSI isolates are shown in **Table 1** according to the 28-day survival status.

To identify the potential risk factors for 28-day mortality of CRKP BSI, we conducted univariate analyses between the 28 day-surviving and 28 day-deceased groups. The potential risk factors included the age-adjusted Charlson comorbidity index ($p = 0.047$), antibiotic exposure in the past 2 weeks [odds ratio (OR) 3.394, $p = 0.001$], APACHE II ($p < 0.001$) and SOFA scores ($p < 0.001$) on the 1st day, hospitalized days before BSI onset ($p = 0.041$), AIT (OR 0.145; $p = 0.008$), and many factors on the 4th day after BSI onset, such as APACHE II scores ($p < 0.001$), SOFA scores ($p < 0.001$), WBC counts ($p = 0.007$), the NLR ($p < 0.001$), CRP levels ($p < 0.001$), platelet counts ($p < 0.001$), haematocrit values ($p = 0.004$), total bilirubin levels ($p = 0.008$), direct bilirubin levels ($p = 0.004$), and albumin levels ($p = 0.003$). After considering the univariate relationship with outcome and clinical relevance, we then conducted a multivariate analysis of these 134 patients and found that the NLR on the 4th day (OR 1.148, 95% CI 1.076–1.225, $p < 0.001$) and antibiotic exposure (OR 3.847, 95% CI 1.322–11.196, $p = 0.013$) were significant risk factors for 28-day mortality of patients with CRKP BSI, while AIT (OR 0.073, 95% CI 0.017–0.307, $p < 0.001$) was the only independent protective factor (**Table 2**).

According to the results of the multivariate analysis of surviving and deceased groups, the NLR on the 4th day after onset was one of the significant risk factors for 28-day mortality (OR 1.148, 95% CI 1.076–1.225, $p < 0.001$). The receiver operating characteristic (ROC) curves of the NLR and 28-day mortality are shown in **Figure 2**, and the area under the curve (AUC) was 0.814 (95% CI 0.736–0.892, $p < 0.001$). In this study cohort, we found that the NLR value with the highest

TABLE 3 | Baseline characteristics and NLR of 116 patients treated with AIT.

	CAZ-AVI *N* = 14	TGC *N* = 59	COL *N* = 11	COL + TGC *N* = 32	*P*-value
Age	61 (47, 65)	63 (49, 69)	69 (61, 80)	68 (61, 70)	0.125
Male	9 (64.3)	44 (74.6)	8 (72.7)	19 (59.4)	0.483
Body mass index (<18.5 or >24)	6 (42.8)	34 (57.6)	5 (45.5)	13 (40.6)	0.434
Hypertension	7 (50.0)	24 (40.7)	5 (45.5)	12 (37.5)	0.870
Diabetes mellitus	2 (14.3)	17 (28.8)	2 (18.2)	7 (21.9)	0.726
CCD	4 (28.6)	19 (32.2)	3 (27.3)	6 (18.8)	0.632
Chronic lung diseases	0	11 (18.6)	2 (18.2)	4 (12.5)	0.314
Liver failure	3 (21.4)	6 (10.2)	3 (27.3)	2 (6.2)	0.130
Renal failure	2 (14.3)	6 (10.2)	2 (18.2)	1 (3.1)	0.281
Solid tumor	4 (28.6)	23 (39.0)	4 (36.4)	11 (34.4)	0.675
Hematological malignancy	0	4 (6.8)	0	4 (12.5)	0.542
Immunosuppressive therapy	4 (28.6)	5 (8.5)	0	3 (9.4)	0.123
aCCI	3.71 ± 1.98	4.63 ± 2.31	6.09 ± 2.35	4.78 ± 2.50	0.103
NLR on the 1st day	31.48 ± 30.45	33.10 ± 41.44	25.70 ± 19.50	31.84 ± 46.35	0.958
NLR on the 4th day	9.21 ± 4.72	16.50 ± 16.66	34.60 ± 40.30	23.17 ± 31.98	**0.047**

CAZ-AVI, ceftazidime-avibactam; TGC, tigecycline; COL, colistin; CCD, Cardiovascular and Cerebrovascular diseases; aCCI, Age-adjusted Charlson comorbidity index. The bold values means p < 0.05, with a statistical significance.

Youden index was 12.90, which was considered as the cut-off value of the NLR (**Figure 2**). All patients were divided into two groups based on their NLR on the 4th day using the 12.90 as cut-off value, and the Kaplan–Meier curves are shown in **Figure 3**.

The Exposure of Antibiotics in the Past 2 Weeks Could Increase 28-Day Mortality

Together with the NLR on the 4th day, antibiotic exposure was another risk factor (OR 3.847, 95% CI 1.322–11.196, $p = 0.013$). Patients in the 28 day-deceased group had a higher rate of antibiotic exposure than those in the 28 day-surviving group (59.2 vs. 30.0%). In the 28 day-deceased group, 32 patients had a history of antibiotic use, including carbapenems (18/32), β-lactam/lactamase combinations (12/32), and tigecycline (2/32), during the last 2 weeks. In the 28 day-surviving group, 24 patients were treated with antibiotics, including carbapenems (11/24), β-lactam/lactamase combinations (11/24), quinolones (1/24), and tigecycline (1/24), before BSI onset. There were no statistically significant differences in the usage ratios between the two groups.

Appropriate Initial Therapy Could Reduce 28-Day Mortality

According to the results of the multivariate logistic regression analysis, AIT was the only independent protective factor with an OR value of 0.073 (95% CI 0.017–0.307, $p < 0.001$). AIT included major antibiotics, such as ceftazidime/avibactam (CAZ-AVI), colistin, and tigecycline, which were active against the isolate in each case. In this study cohort, 116 of 134 (86.6%) patients received AIT, and this proportion in the 28 day-surviving group was higher than that in the 28 day-deceased group (93.8 vs. 63.5%, $p = 0.001$).

CAZ-AVI May Offer an Important Advancement in Treating CRKP BSI With a Low NLR on the 4th Day

Since it has been proven that AIT is an independent protective factor for 28-day mortality of patients with CRKP BSI, we performed further analyses by dividing the patients treated with AIT into four groups: the CAZ-AVI group (14 patients), TGC group (59 patients), COL group (11 patients), and TGC + COL group (32 patients). The baseline characteristics and clinical outcomes are shown in **Table 3**. There were no differences in age, sex, body mass index, and underlying disease between the four groups. There was no difference in the NLR on the first day of disease in the different groups; however, there was a significant difference in the NLR on the 4th day. The Cox proportional hazards regression analysis revealed a significant difference ($p = 0.006$) between the CAZ-AVI group and non CAZ-AVI groups (**Figure 4A**). After dividing the non CAZ-AVI groups into the TGC, COL, and TGC + COL groups, there were no differences between the CAZ-AVI group and the TGC group ($p = 0.093$). Meanwhile, patients in the CAZ-AVI group had a lower mortality than the patients in the COL ($p = 0.002$) and TGC + COL ($p = 0.012$) groups (**Figure 4B**).

DISCUSSION

The emergence of carbapenem resistance of *K. pneumoniae* is becoming challenging to treat and significantly impacts patient mortality, especially BSI (6, 7). It has been reported that drug resistance is associated with increased mortality, as patients tend to receive inappropriate empirical antibiotic therapy (9). Only few antibiotics, such as CAZ-AVI, colistin (COL), and tigecycline (TGC), have been confirmed to be effective in treating CRKP BSI *in vitro* and *in vivo*. Together with active antibiotics, early and accurate evaluation is an important method for improving outcomes. In clinical practice and previous studies, we observed that 2–3 days after initiation of the initial therapy is a recommended time point to evaluate the efficacy of the antibiotic therapy and do some adjustments if necessary in patients with BSI (15, 16). During the early period of infection, the inflammatory response can stimulate the production of neutrophils and speed up the apoptosis of lymphocytes, leading to multiorgan dysfunction (17, 18). Exploring the therapeutic effect and prognostic value of potential biomarkers in this period is important. Considering that the average time for patients to receive the first dose of appropriate initial therapy was 1.21 ± 1.32 day (116 samples) after the blood was sampled (containing the time for pathogen culture) in our center, the 3rd or 4th day after BSI onset seemed to be a target time point. A stable concentration is an important factor for antibiotics to have a therapeutic effect. Finally, we decided to use the 4th day after BSI onset as a target observation time point, as well as the 1st day of BSI onset, to ensure that we enrolled patients with stable concentrations. A suitable biomarker must provide additional information to what is presently available; it should be able to predict outcomes or evaluate the efficacy of treatment, and it should be immediately available and cost-effective (19). The present study aimed to evaluate factors, including clinical and demographic characteristics, blood biomarkers, and different therapy strategies. It was found that the NLR on the 4th day and antibiotic exposure within the past 2 weeks were independent risk factors for 28-day mortality of patients with CRKP BSI, while appropriate initial therapy was an independent protective factor.

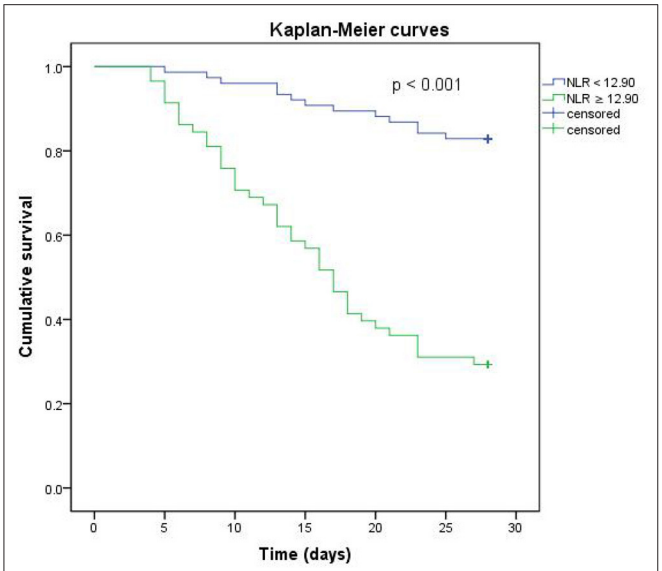

FIGURE 3 | Kaplan-Meier analysis of NLR on the 4th day. The blue line represents NLR < 12.90. The green line represents NLR ≥ 12.90.

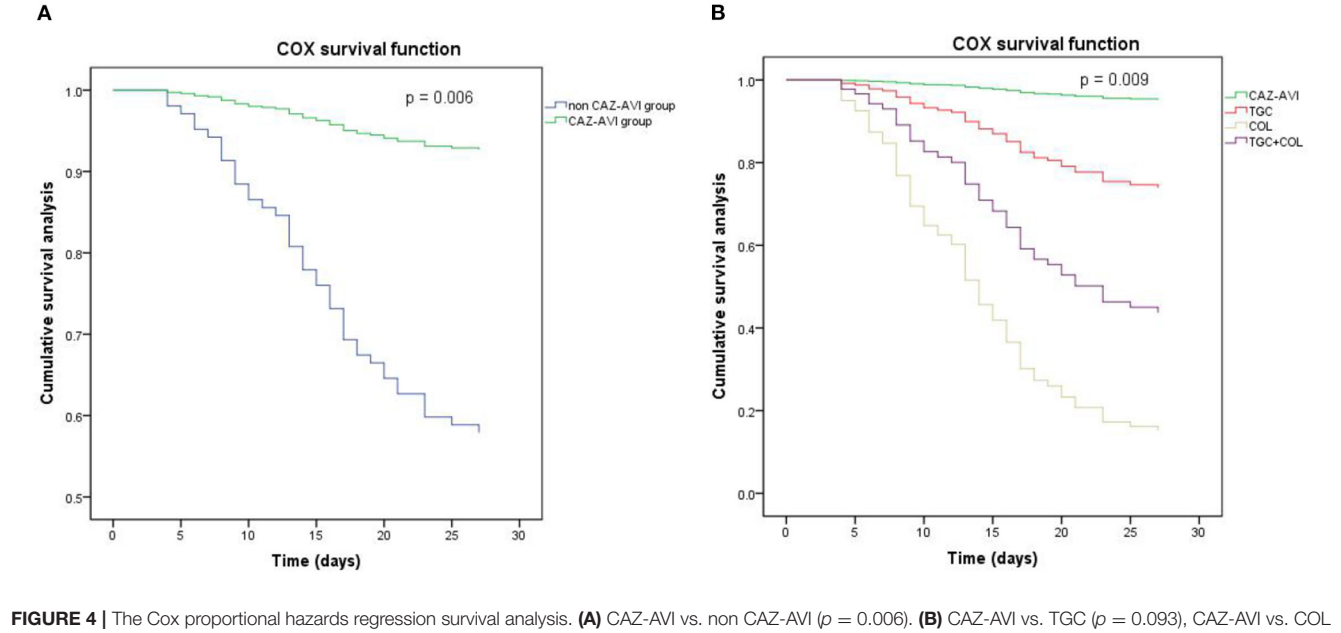

FIGURE 4 | The Cox proportional hazards regression survival analysis. **(A)** CAZ-AVI vs. non CAZ-AVI ($p = 0.006$). **(B)** CAZ-AVI vs. TGC ($p = 0.093$), CAZ-AVI vs. COL ($p = 0.003$), CAZ-AVI vs. TGC + COL ($p = 0.012$). CAZ-AVI, therapy contained ceftazidime/avibactam; TGC, therapy contained tigecycline; COL, therapy contained colistin, TGC + COL, tigecycline and colistin combined therapy.

The early phase of infection is considered a proinflammatory state mediated by neutrophils, macrophages, and monocytes with the release of inflammatory cytokines, such as tumor necrosis factor-α and interleukin 1 and 6. Neutrophils may function as killers as part of the innate response in this state with the suppression of apoptosis, causing injury (17). At the same time, lymphocyte apoptosis is increased in the thymus and spleen, leading to immune system suppression, multiorgan dysfunction, and death (18). The NLR has been used as a guide to the prognosis in various clinical conditions, such as cancer (11), ischaemic heart disease (10), and community-acquired pneumonia (20). The NLR has been observed in some studies to be more efficient than regular inflammation biomarkers in adults (14). It has also been reported that the NLR could function as a predictor of pediatric sepsis (21). Lowsby et al. evaluated the performance of the NLR as an early indicator of BSI and concluded that it may offer some diagnostic utility when taken into account as part of the overall assessment but fail to guide the clinical management of patients with suspected BSI itself (22). We conducted this study to evaluate the NLR as a predictor of the prognosis of patients infected with CRKP BSI, along with other biomarkers and therapy strategies. We found that the NLR on the 4th day after BSI onset could be an independent risk factor for 28-day mortality, and the AUC of the ROC was 0.814 with a cut-off value of 12.90. The survival rate of patients with an NLR value of <12.90 on the 4th day was significantly higher (**Figure 3**), which may offer a fresh insight into the evaluation of prognosis. To the best of our knowledge, this is the first study to explore the value of the NLR in predicting the 28-day mortality of patients with CRKP BSI.

According to the results of the multivariate analysis, antibiotic exposure within the past 2 weeks was an independent risk factor. The patients in the 28 day-deceased group had a higher rate of antibiotic use than those in the 28 day-surviving group. Carbapenems and β-lactam/lactamase combinations were the most commonly used. Previous studies have demonstrated that a history of antibiotic use increases the risk of CRKP infection (23), and a meta-analysis determined the OR results of previous antibiotic use (OR = 3.31), exposure to carbapenems (OR = 4.01), aminoglycosides (OR = 2.05), glycopeptides (OR = 2.40), quinolones (OR = 2.28), and anti-pseudomonal penicillins (OR = 2.67) (24). In our study, the OR of antibiotic exposure within the previous 2 weeks was 3.847 ($p = 0.013$) for 28-day mortality, while there was no difference in carbapenems, β-lactam/lactamase combinations, tigecycline, and quinolone usage between the two groups. The data showed that antibiotic exposure not only increased the risk of CRKP BSI but also increased mortality. Thus, a rational use of antibiotics can contribute to the decrease in morbidity and mortality associated with CRKP BSI.

Kohler et al. performed a meta-analysis (7 studies, 658 patients) of the relationship between AIT and mortality and concluded that AIT was a protective factor (unadjusted OR = 0.5) in both CSKP and CRKP bacteraemia (9). Another study that focused on CRKP BSI in high-risk hematological patients showed that AIT was the only independent factor able to protect against death ($p = 0.02$) (25). In the present study, we demonstrated that AIT is the only independent protective factor for 28-day mortality of patients with CRKP BSI, indicating that the use of at least one active antibiotic within 72 h can improve the prognosis. In addition to antimicrobial susceptibility tests, healthy conditions, underlying diseases, and economic burden should be considered when developing an appropriate therapy strategy, and an early evaluation of curative effectiveness is also essential. Thus, we performed further analyses of the data of patients who received AIT using a Cox proportional hazards regression model; we found that patients in the CAZ-AVI group had lower 28-day mortality rates than the non CAZ-AVI group. Furthermore, we divided the non CAZ-AVI group into the TGC, COL, and TGC + COL groups, based on the major antibiotics in the therapy strategies. Variance in therapy contributes to different outcomes. Patients who received CAZ-AVI as AITs had significantly improved 28-day mortality, compared to those with COL or TGC + COL, but no significant improvement when compared to the TGC group. We also found that there was no difference in NLR levels on the first day of CRKP BSI between the four groups; however, on the 4th day, the patients in the different therapy groups had significantly different NLRs. It was mentioned that the killer role of neutrophils and the apoptosis of lymphocytes at the early stage of infection can cause injury (17, 18), and these physiological processes can lead to changes in the NLR in the peripheral blood. Combined with clinical manifestations, the NLR may be an important factor for making decisions of appropriate therapy strategies, which can reduce the damage caused by the dysfunction of neutrophils and lymphocytes in each patient. This advantage can be observed on the 4th day of CRKP BSI. In addition to predicting the prognosis, the 4th day NLR may have the potential to evaluate the efficacy of the antibiotics at an early time. In clinical practice, physicians are required to evaluate and adjust the therapy strategy at the early stage of CRKP BSI due to poor outcomes; together with experience, a quantified marker is therefore desperately needed. From our perspective, the NLR, an available and economic marker, may have the function of indicating whether anti-infection therapy is suitable for patients, and is worthy of further study.

This study was limited by its retrospective, single-center design and small patient population. Due to the study size, the further analyses on therapy strategies might be limited. Among these 134 patients, only 3 patients were diagnosed as catheter-related blood stream infections, we failed to perform subgroup analyses for this special kind of infection. Limited by the retrospective design of our study and the missing data during the development of BSIs, we could not perform a dynamic profile analysis of NLR, which may offer more useful information.Culture-negative CRKP BSI was not considered in the present study because of prior antibiotic use, inadequate sampling techniques, or organisms that are difficult to identify. The primary endpoint we used was all-cause mortality, which may increase the influence on mortality caused by CRKP BSI.

To conclude, our study revealed that the NLR on the 4th day and antibiotic exposure within the previous 2 weeks were independent risk factors for the 28-day mortality of patients with CRKP BSI, while appropriate initial therapy was an independent protective factor. In this study cohort, we also found the NLR on the 4th day may have the potential to evaluate the efficacy of the

antibiotics at an early stage and allow screening for suitable target therapy for every patient.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Research Committee of Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HW and YM contributed to both in the conception and design of the study and performed the statistical analysis. HW and XD organized the data together. FZ performed the strain identification and the antimicrobial susceptibility tests. HW wrote the first draft of the manuscript. YM, XD, and FZ wrote sections of manuscript. YY and YJ helped perform the analysis with constructive discussions. All authors contributed to manuscript revision, read, and approved the submitted version.

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Microbiological and Clinical Characteristics of Bloodstream Infections in General Intensive Care Unit: A Retrospective Study

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Background: Bloodstream infections (BSI) are one of the common causes of morbidity and mortality in hospitals; however, the pathogenic spectrum and bacterial antibiotic resistance vary across the world. Therefore, identifying the pathogenic spectrum and changes in bacterial antibiotic resistance is critical in controlling BSI and preventing the irrational use of antibiotics. This study evaluated the microbiological and clinical data of BSI patients in the intensive care unit (ICU) of Tianjin Medical University General Hospital in Tianjin, China, to guide the selection of empirical antibiotic therapy.

Methods: This study retrospectively analyzed the distribution and antibiotic resistance of pathogens based on the clinical data of BSI patients presented in the ICU of a tertiary teaching hospital from 2018 to 2020. Test performance for the prediction of pathogen species was assessed by receiver operating characteristic (ROC) analysis.

Results: The analysis of the data of 382 BSI cases (10.40 cases per thousand patient day) revealed the most frequently isolated microorganisms to be *Klebsiella pneumonia* (11.52%), followed by *Escherichia coli* (9.95%), *Staphylococcus epidermidis* (9.95%), *Candida parapsilosis* (8.12%), and *Enterococcus faecium* (8.12%). Out of the isolated *E. coli* and *K. pneumonia* strains, 52.63, and 36.36%, respectively, were extended-spectrum β-lactamase (ESBL) positive. The antibiotic-resistance rate of the ESBL-positive strains was 30.56% for piperacillin/tazobactam, 5.56% for imipenem, and 11.11% for tigecycline. In addition, most *A. baumannii* belonged to the group of multidrug-resistant (MDR) strains, with an antibiotic-resistance rate of 90.48% for meropenem and 16.00% for amikacin. However, polymyxin-resistant *A. baumannii* strains were not detected. Four strains of methicillin-resistant *S. aureus* (MRSA) (4/21, 19.05%) and one strain of vancomycin-resistant enterococci (VRE) were detected, with a resistance rate of 4.76 and 2.32%, respectively. Among the isolated 55 fungal strains, *C. parapsilosis* was the most common one (30/55, 56.36%), with an antibiotic-resistance rate of 5.77% for voriconazole, fluconazole, and itraconazole. The presence

of amphotericin B-or flucytosine-resistant strains was not observed. Compared with the patients with Gram-positive and fungal pathogens, patients with Gram-negative bacteria exhibited the highest sequential organ failure assessment (SOFA) score ($P < 0.001$), lowest Glasgow Coma Scale (GCS) ($P = 0.010$), lowest platelet (PLT) value ($P < 0.001$), highest plasma creatinine (Cr) value ($P = 0.016$), and the highest procalcitonin (PCT) value ($P < 0.001$). The AUC in the ROC curve was 0.698 for the differentiation of Gram-negative BSI from Gram-positive BSI. A cutoff value of 8.47 ng/mL for PCT indicated a sensitivity of 56.9% and a specificity of 75.5%. The AUC in the ROC curve was 0.612 for the differentiation of bacteremia from fungemia. A cutoff value of 4.19 ng/mL for PCT indicated a sensitivity of 56.8% and a specificity of 62.7%.

Conclusion: Among the bloodstream infection strains in ICU, Gram-negative bacteria have the highest drug resistance rate, and will cause more serious brain damage, renal function damage and thrombocytopenia. So clinician should pay more attention to the treatment of Gram-negative bacteria in patients with bloodstream infection in ICU. The test index of PCT can be used to distinguish Gram-negative bacteremia from Gram-positive and bacteremia from fungemia but not as an effective indicator, thereby indicating the need for further large-scale research.

Keywords: bloodstream infection, antibiotic resistance, procalcitonin, multidrug-resistance, vancomycin-resistant enterococci (VRE)

INTRODUCTION

Bloodstream infections (BSI) are a life-threatening condition affecting patients in intensive care units (ICUs). The timely and effective application of antibiotics is crucial for managing the morbidity and mortality of the infection (1, 2). Antibiotics are useful for infection control, but their overuse or misuse could induce antibiotic resistance in various pathogens (3–5). For example, penicillin resistance was first reported 80 years ago (6). Currently, antibiotics and antibiotic-resistance genes have been reported in surface water, effluents from sewage treatment plants, soils, and animal wastes (7). Owing to this wide distribution, WHO declared it as a serious public health crisis of the 21st century (8). Generally, the results of etiological tests become available after 3–5 days only. Therefore, before obtaining the etiological results, most clinicians follow the empirical anti-infective therapy for the choice of the antibiotic regimen. The international Surviving Sepsis Campaign (SSC) recommended the empirical broad-spectrum therapy to be initiated immediately, with one or more intravenous antimicrobials to cover all likely pathogens (9). Thus, the use of broad-spectrum antibiotics is closely related to the emergence of bacterial resistance. All these factors lead to ICU being not only the ward with the highest use of broad-spectrum antibiotics but also the high-risk area of antibiotic-resistant bacterial infections.

Owing to increasing bacterial resistance, the choice of empirical antibiotics has become the focus of clinical treatments. In this research, we analyzed the clinical data and bacterial antibiotic resistance of BSI patients presented in ICU from January 2018 to December 2020. In addition, we explored the relationship between the changes in clinical data and bloodstream infection agents to guide the selection of empirical antibiotic therapy.

According to the National Antimicrobial Stewardship Campaign in China, to control the irrational consumption of antibiotics, especially the prediction usage in surgery, all antibiotic prescriptions should follow the drug use list (10).

MATERIALS AND METHODS

This retrospective study was conducted in Tianjin Medical University General Hospital in Tianjin, China, from January 2018 to December 2020 and was approved by the hospital's ethics committee (NO. IRB2021-YX-062–01). This tertiary teaching hospital is a 2,468-bed facility, and the General Intensive Care Unit (GICU) comprises 42 beds. All patients over 18 years of age with a confirmed BSI during ICU admission were included in this study. The ICU-acquired BSI was defined as bacteremia or fungemia diagnosed from day 3 onward of ICU stay, with the initial day of ICU admission being designated as day 0.

Blood culture is the golden criterion for the diagnosis of BSI. In our department, blood culture was performed for patients with infectious symptoms, such as fever, cold, shivering, and low blood pressure, before administering antimicrobial therapy. The specimens were sent to the microbiology laboratory, and the VITEK-2 compact automated system was used for bacterial identification and antibiotic susceptibility testing. The ATB-Fungus 3 system was used for antifungal susceptibility testing. The antimicrobial susceptibility tests were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). For results not included in CLSI, the guidelines prescribed by the European Committee on

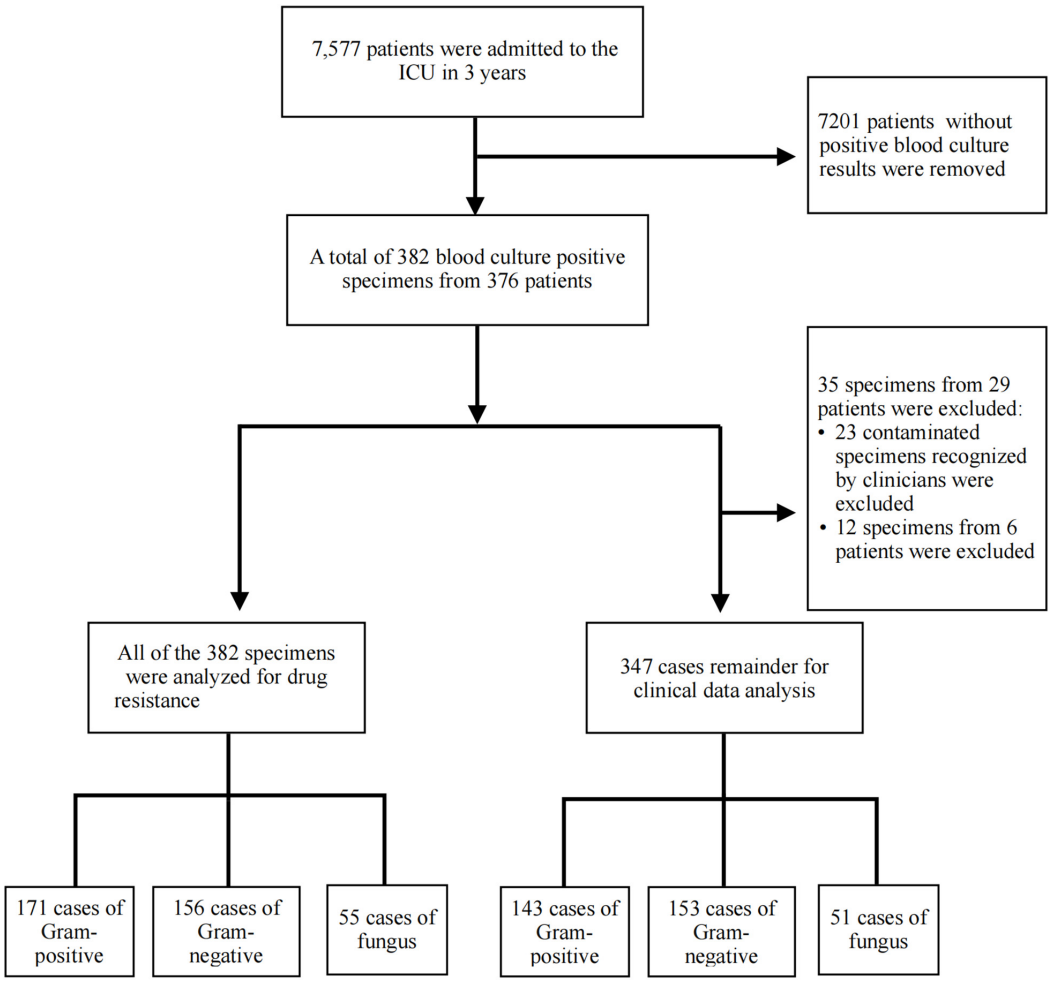

FIGURE 1 | Study flow diagram. A total of 382 blood culture positive specimens were analyzed for drug resistance, including 171 cases of Gram-positive bacteria, 156 cases of Gram-negative bacteria, and 55 cases of fungus. After excluding contaminated strains recognized by clinicians and one bottle with more than one organism, 347 cases of bloodstream infection (BSI) remainder were included in the clinical data analysis, including 143 cases of Gram-positive bacteria, 153 cases of Gram-negative bacteria, and 51 cases of fungus.

Antimicrobial Susceptibility Testing (EUCAST) were referred. Our hospital has developed a critical value reporting system, according to which, if the blood culture results are positive, the microbiology laboratory must report Gram-positive, Gram-negative, or candida as soon as possible; the rapid reporting could direct the clinician's decision and reduce the delay in initiating the antibiotic treatment. However, generally, it takes 3–5 days to get the final results.

The clinician explained the blood culture results according to the clinical symptoms, treatment effects, bacterial species (e.g., coagulase-negative Staphylococci, Corynebacterium species, *Propionibacterium acnes*, and other skin colonizers), etc., to find contaminants. The bacterial contaminants were recorded in the clinical course record. The clinical course records were reviewed in this study, and the contaminant cases were excluded.

The patients' data, including gender, age, diagnosis, Acute Physiology, and Chronic Health Evaluation II score (APACHE II), Sequential Organ Failure Assessment (SOFA) score, Glasgow Coma Scale (GCS), mechanical ventilation, catheter insertion, hemofiltration, prescription, vital signs, vasopressors, length of stay in ICU, antibiotics consumption, and prognosis were extracted from the health information system (HIS). The laboratory information system (LIS) provided the patients' examination results, such as microorganisms isolated from samples, resistance to antimicrobials, blood routine, blood creatine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), PCT, and arterial blood gas analysis. All examination results were submitted within 24 h after blood culture. APACHE II score, SOFA score and GCS score were calculated within 24 h after blood culture.

According to the blood culture results, we divided the patients into the following three groups: Gram-positive bacteremia, Gram-negative bacteremia, and fungemia. The collected etiological results were analyzed for drug resistance. To exclude the influence of simultaneous infection of two or more pathogens on patients' clinical symptoms and laboratory indexes, the data

TABLE 1 | The characteristics of the included patients, except for one case with more than one strain.

Characteristics	G^+ (143)	G^- (153)	Fungi (51)	F/χ^2 Value	*P*-value
Male (%)	93 (65.035)	86 (56.209)	28 (54.902)	2.953[b]	0.228
Age mean ± SD	61.22 ± 16.835	62.13 ± 15.396	66.24 ± 14.685	1.894[a]	0.152
Community acquired infection (*n*, %)	46 (32.168)	57 (37.255)	9 (17.647)	6.729[b]	**0.035**
Hospital acquired infection (except ICU) (*n*, %)	23 (16.084)	27 (16.647)	8 (15.686)	0.175[b]	0.916
ICU acquired infection (*n*, %)	74 (51.748)	69 (45.098)	34 (66.667)	7.174[b]	**0.028**
History of hormone or immunosuppressant use (*n*, %)	21 (14.69)	31 (20.26)	5 (9.80)	3.584[b]	0.167
Elective surgery (*n*, %)	43 (30.70)	52 (33.99)	14 (27.45)	0.889[b]	0.641
Emergency surgery (*n*, %)	24 (16.78)	28 (18.30)	16 (10.46)	5.251[b]	0.072**
Mechanical ventilation (hour)	333.69 (0–4729)	312.70 (0–2885)	521.882 (0–6254)	1.722[a]	0.180
Catheter insertion					
Internal jugular vein catheterization (*n*, %)	87 (60.84)	91 (59.48)	31 (60.78)	0.065[b]	0.968
Subclavian vein catheterization (*n*, %)	18 (12.59)	17 (11.11)	9 (17.65)	1.478[b]	0.478
Femoral vein catheterization (*n*, %)	57 (30.89)	60 (39.22)	21 (41.18)	0.013[b]	0.993
ICU length of stay (days) (median, IQR)	30.50 (1–261)	25.14 (1–249)	44.25 (1–865)	2.205[a]	0.112
Apache II score (median, IQR)	21.58 (7–41)	23.14 (8–45)	21.69 (10–41)	1.662[a]	0.191
SOFA score (median, IQR)	7.54 (1–20)	9.36 (0–20)	6.43 (0–18)	9.712[a]	**< 0.001**
GCS score (median, IQR)	6.61 (3–15)	5.93 (3–15)	8.92 (3–15)	4.719[a]	**0.010**
Clinical data					
WBC (*10^9/L) mean ± SD	14.396 ± 9.735	15.174 ± 10.965	13.554 ± 7.438	0.559[a]	0.573
PLT (*10^9/L) (median, IQR)	140.06 (6–521)	97.52 (1–469)	137.04 (7–339)	8.565[a]	**< 0.001**
PCT (ng/mL) (median, IQR)	14.52 (0.021–243.33)	42.618 (0.05–298.01)	12.14 (0.13–110.93)	13.522[a]	**< 0.001**
Cr (μmol/L) (median, IQR)	168.82 (15–768)	222.45 (17–1430)	153.80 (17–523)	4.208[a]	**0.016**
ALT (U/L) (median, IQR)	114.03 (4–2928)	214.10 (6–3480)	148.49 (6–1981)	2.461[a]	0.087*
AST (U/L) (median, IQR)	168.24 (4–3004)	316.59 (12–7992)	230.35 (9–4311)	1.666[a]	0.191*
TBIL (μmol/L) (median, IQR)	48.30 (4.9–399.0)	67.86 (3.5–563.5)	53.99 (5.0–366.4)	2.109[a]	0.123*
Vasopressors (*n*, %)	49 (34.27)	70 (45.75)	14 (27.45)	7.118[b]	**0.028**
Mean arterial pressure (mmHg) (median, IQR)	80.9 (42–161)	75.59 (31–131)	81.78 (44–128)	2.910[a]	0.056*
Lactate (mmol/L) mean ± SD	3.363 ± 2.665	3.821 ± 3.624	2.600 ± 2.666	3.015[a]	**0.050****
A-aDO_2 (mmHg) (x ± 95% CI)	238.415 (215.414, 261.416)	258.866 (234.729, 283.002)	234.586 (198.411, 270.762)	1.561[b]	0.458

G^+: gram positive; G^-: gram negative; F: ANOVA test; χ^2: chi-square test; p-value: <0.05 was considered as statistically significant. [a]for F, [b]for χ^2; for $p < 0.05$, comparison between Gram-negative BSI and Gram-positive BSI; ** for $p < 0.05$, comparison between bacteremia and fungemia.*

of cases with two or more pathogens in one culture medium were excluded from this study (**Figure 1**).

DATA ANALYSIS

The WHONET 5.6 software was used to evaluate the blood culture results and the antimicrobial-resistance trends.

Statistical analyses were performed using the SPSS 24.0 software. Categorical data were described as percentages. Continuous variables were given as mean [standard deviation (SD)] for normal data and median [interquartile range (IQR)] for non-normal data. The differences between the observed and expected frequencies were calculated using the Chi-square test for the categorical variables, whereas the *T*-test and ANOVA were used to compare the continuous variables. The Wilcoxon rank sum test is applied to the count data of non-normal distribution. The value of $P < 0.05$ was considered as statistically significant.

Receiver operating characteristic (ROC) curves were used to determine the predictive performance of procalcitonin

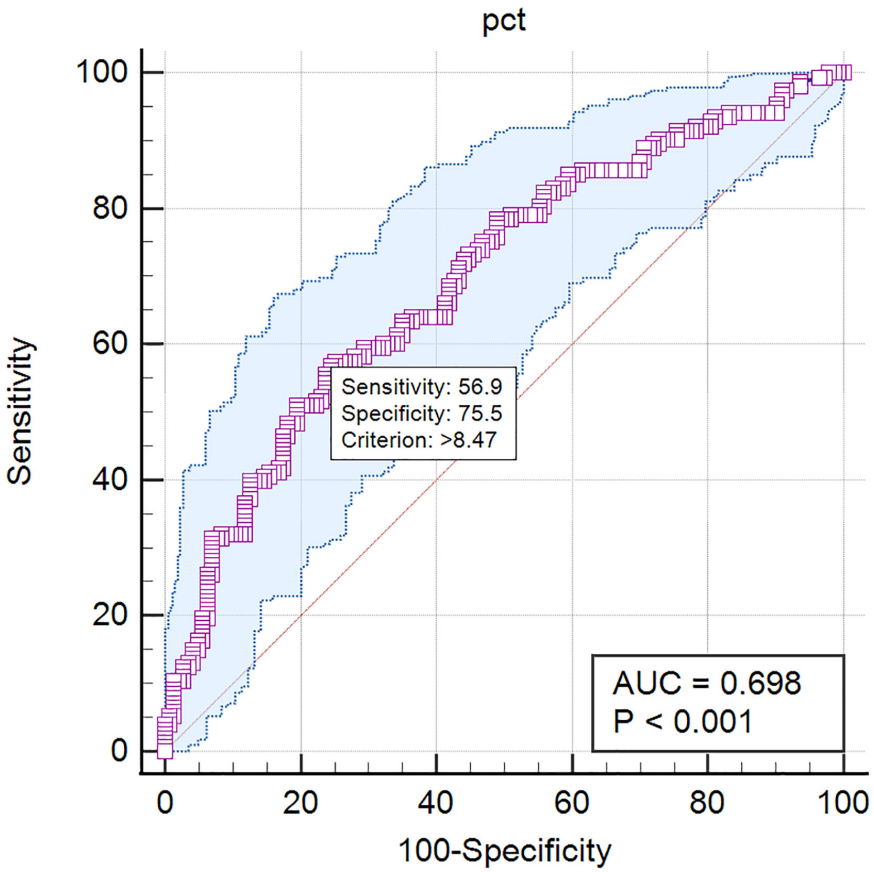

FIGURE 2 | The ROC curves of PCT predicting Gram-negative bacteremia from Gram-positive bacteremia. PCT: AUC = 0.698, cutoff point 8.47 ng/mL, sensitivity 56.9%, specificity 75.5%.

(PCT) for pathogen species. MedCalc software was used for ROC analysis.

RESULTS

General Situation

From January 2018 to December 2020, the total number of patients admitted to the ICU was 7,577 (36,716 patient day), including 2,398 (12,119 patient day) in 2018, 2,455 (12,158 patient day) in 2019, and 2,724 (12,439 patient day) in 2020. Furthermore, the mortality rate was 4.14% (99/2398) in 2018, 3.75% in 2019 (92/2455), and 2.06% in 2020 (56/2724). In this study, a total of 382 BSI cases (10.40/1,000 patient day) were included, with 171 cases of Gram-positive bacterial infections (44.76%), 156 cases of Gram-negative bacterial infections (40.84%), and 55 cases of fungal infections (14.40%). Out of the 382 BSI cases, cases with contaminated bacteria and more than one organism were excluded, leading to 347 BSI cases, as depicted in **Figure 1**. The analysis of the 347 cases revealed 143 Gram-positive bacterial infections, with a mortality rate of 30.77% (44/143); 153 Gram-negative bacterial infections, with a mortality rate of 33.99% (52/153); and 51 fungal infections, with a mortality rate of 39.22% (20/51). The year-wise analysis indicated 93 BSI cases in 2018, with a mortality rate of 40.86% (38/93); 120 BSI cases in 2019, with a mortality rate of 35.00% (42/120); and 134 BSI cases in 2020, with a mortality rate of 27.61% (37/134).

Clinical Data Analysis

The remaining patients ' data were analyzed except for the cases with two or more strains in the same culture medium (**Table 1**).

Results indicated that Gram-negative bacteria were the most common strains in the community-acquired BSI cases (57/112, 50.89%), Gram-positive bacteria were the most common strains in the ICU-acquired BSI cases (74/177, 41.81%), and fungemia mainly occurred in the ICU-acquired BSI patients (34/51, 66.67%). Gram-negative bacteria were the most pathogenic bacteria in patients treated with hormones or immunosuppressants (31/57, 54.39%, $P = 0.167$); however, the difference was not statistically significant. The duration of ICU hospitalization (44.25 days, $P = 0.112$) and mechanical ventilation (521.882 h, $P = 0.180$) of patients with fungemia were longer than the other two groups, without any statistically significant difference.

Acute Physiology, and Chronic Health Evaluation II score and SOFA score are widely used in ICUs to evaluate the severity of the

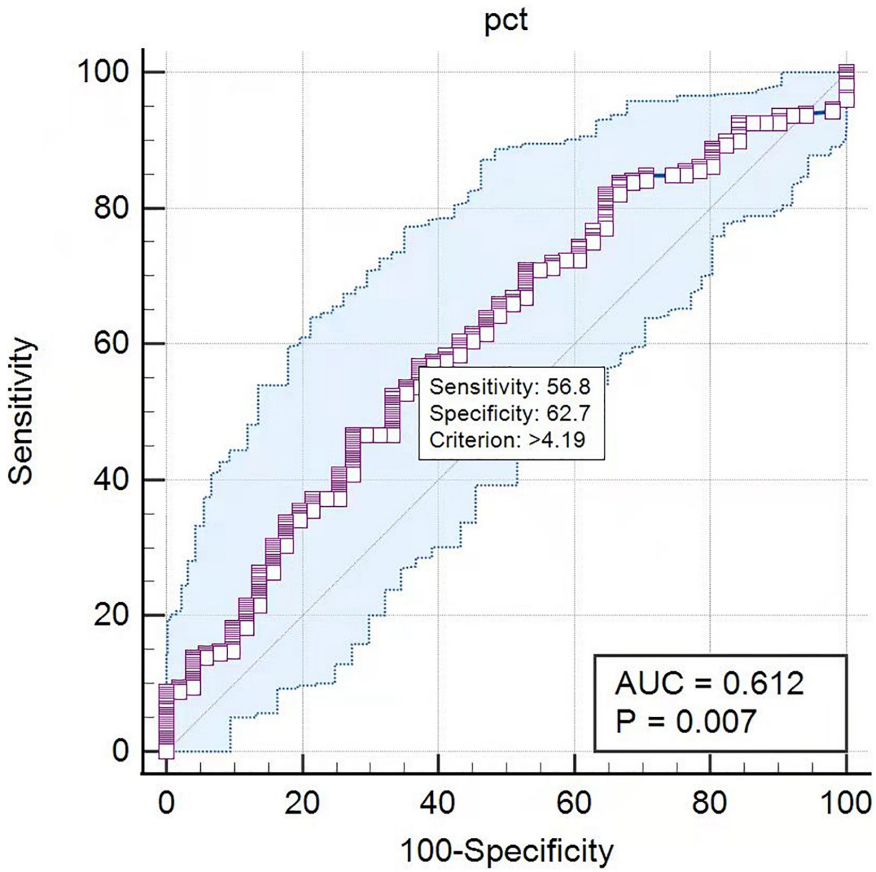

FIGURE 3 | The ROC curves of PCT predicting bacteremia from fungemia. PCT: AUC = 0.612, cutoff point 4.19 ng/mL, sensitivity 56.8%, specificity 62.7%.

disease, the higher the score, the higher the severity of the disease. The average score of APACHE II was greater than 20 in the three groups, higher than the critical condition standard of 15. The highest SOFA score was obtained for the Gram-negative group ($P < 0.001$). In the Gram-negative group, an increased number of patients used vasopressor ($P = 0.028$) and exhibited the lowest mean blood pressure ($P = 0.056$) and highest arterial blood lactate levels ($P = 0.050$). However, the differences for mean blood pressure and arterial blood lactate levels were not statistically significant. The GCS score can objectively reflect patient's coma severity. The Gram-negative group exhibited the lowest GCS score ($P = 0.010$), which suggested the most serious degree of brain injury in this group. In addition, the Gram-negative group exhibited lowest PLT value ($P < 0.001$), highest plasma CR value ($P = 0.016$), and highest PCT value ($P < 0.001$). However, no differences in white blood cell (WBC) count ($P = 0.573$) were observed among the three groups. Subgroup comparison showed Gram-negative group patients with lower mean blood pressure ($P = 0.035$) and higher ALT ($P = 0.010$), AST ($P = 0.039$), and TBIL ($P = 0.025$) values compared with Gram-positive group patients. Compared with fungemia patients, bacteremia patients showed higher arterial lactic acid level ($P = 0.001$).

Procalcitonin is a marker of infection correlated with the severity of microbial invasion. In this study, PCT levels were calculated to differentiate pathogen species. The AUC in the ROC curve was 0.698 for the differentiation of Gram-negative BSI from Gram-positive BSI. A cutoff value of 8.47 ng/mL for PCT indicated a sensitivity of 56.9% and a specificity of 75.5% (**Figure 2**). The AUC in the ROC curve was 0.612 for the differentiation of bacterial BSI from fungal BSI. A cutoff value of 4.19 ng/mL for PCT indicated a sensitivity of 56.8% and a specificity of 62.7% (**Figure 3**). In the subgroup analysis, no differences in PCT concentrations were observed among the patients infected with *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *A. Baumannii* ($P = 0.200$). In addition, in blood culture, *E. coli* and *P. aeruginosa* were associated with two or three times higher PCT values than *K. pneumonia* and *A. baumannii* (**Table 2**).

TABLE 2 | PCT values associated with different pathogens.

Pathogen species detected from blood cultures	Number	PCT (IQR)	*P*-value
E. coli	38	66.272 (37.325, 95.218)	0.200
K. pneumonia	44	29.170 (16.180, 42.160)	
P. aeruginosa	16	69.706 (17.537, 121.874)	
A. baumannii	25	19.460 (6.251, 32.668)	

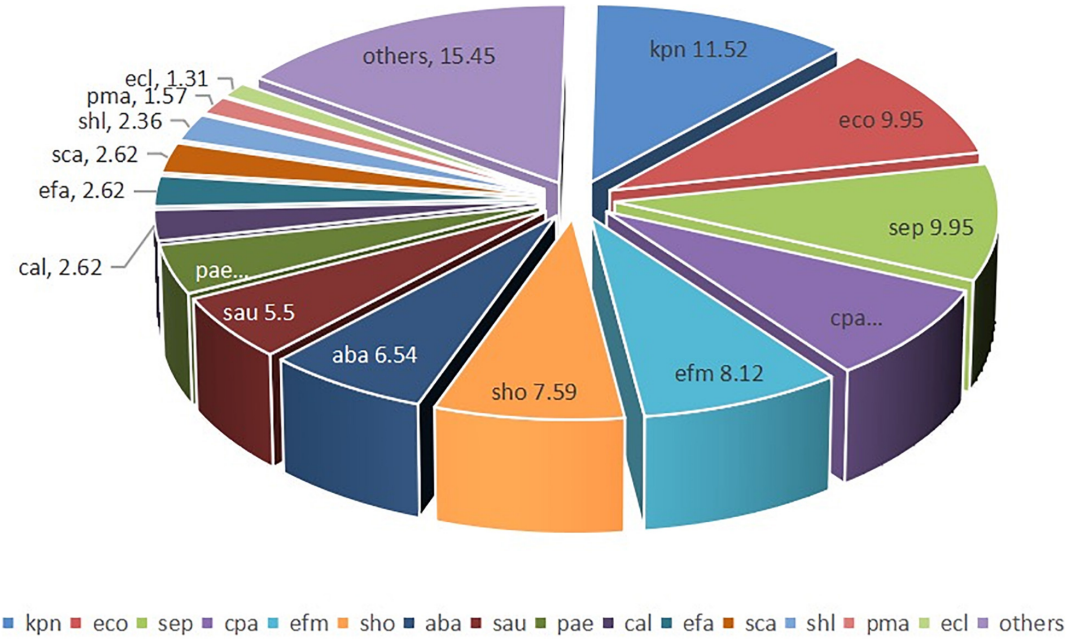

FIGURE 4 | Distribution of the isolated pathogens in blood culture samples (%). kpn, *K. pneumonia*; eco, *E. coli*; sep, *S. epidermidis*; cpa, *C. parapsilosis*; efm, *E. faecium*; sho, *S. hominis*; aba, *A. baumannii*; sau, *S. aureus*; pae, *P. aeruginosa*; cal, *C. albicans*; efa, *E. faecalis*; sca, *S. capitis*; shl, *S. haemolyticus*; pma, *Stenotrophomonas maltophilia*; and ecl, *E. cloacae.*

Bacteriological Results

The most frequently isolated microorganisms were *K. pneumonia* (44/382, 11.52%), *E. coli* (38/382, 9.95%), *S. epidermidis* (38/382, 9.95%), *C. parapsilosis* (31/382, 8.12%), *E. faecium* (31/382, 8.12%), *S. hominis* (29/382, 7.59%), *A. baumannii* (25/382, 6.54%), *S. aureus* (21/382, 5.50%), *P. aeruginosa* (16/382, 4.19%), and *C. albicans* (10/382, 2.62%) (**Figure 4**).

Gram-Negative Bacteria

The most commonly isolated Gram-negative bacteria were *K. pneumonia.* Out of the 44 isolated strains, 16 were extended-spectrum β-lactamase (ESBL) positive (16/44, 36.37%). This group of bacteria exhibited an antibiotic-resistance rate of approximately 50% for cefuroxime, ceftazidime, ceftriaxone, and cefepime and 22.73% (10/44) for carbapenems. Carbapenemase assay was not performed during the period of this study in our hospital (**Figure 5A**). The resistance rate for β-lactase inhibitor combinations was in the range of 36.37–40.91%.

The second commonly isolated Gram-negative bacteria was *E. coli*, with 38 strains detected; out of these, 20 cases were ESBL positive (20/38, 52.63%). This group exhibited antibiotic-resistance rates, with 58.82% for cefuroxime, 52.26% for ceftriaxone, 55.26% for quinolones, and 66.67% for ciprofloxacin. The antibiotic-resistance rates for piperacillin/tazobactam and cefoperazone sulbactam were 13.16%, whereas for amoxicillin/clavulanic acid 18.42%. Only one carbapenem-and tigecycline-resistant *E. coli* strain was detected (**Figure 5B**).

A total of 36 ESBL-positive strains were detected, including 20 (20/36, 55.56%) in the *E. coli* group and 16 (16/36, 44.44%) in the *K. pneumonia* group. In addition, the number of ESBL-positive strains increased from 2018 (27.78%; 5/18) to 2021 (50.00%; 16/32). The antibiotic resistance of ESBL-positive strains was 33.33% for amoxicillin/clavulanic acid, 36.11% for cefoperazone sulbactam, 30.56% for piperacillin/tazobactam, 5.56% for imipenem, and 11.11% for tigecycline (**Table 3**).

A total of 25 strains of *A. baumannii* were detected, with most of them being multidrug-resistance (MDR) strains. The antibiotic-resistance rate of these strains was 90.48% for meropenem, 80.00% for ciprofloxacin, 85.71% for ticarcillin clavulanic acid, 84.00% for piperacillin/tazobactam, 56% for cefoperazone sulbactam, 86.36% for ceftazidime, 22.73% for minocycline, and 16.00% for amikacin. However, polymyxin-resistant strains were not detected (**Figure 5C**).

A total of 16 strains of *P. aeruginosa* were isolated, including three carbapenem-resistant strains. These strains exhibited an antibiotic-resistance rate of 100% for amoxicillin, 21.42% for clavulanic acid, 6.67% for cefepime, 7.14% for meropenem, 25.00% for piperacillin, tazobactam, and levofloxacin, and 6.25% for tobramycin. However, amikacin-, gentamicin-, and colistin-resistant strains were not observed (**Figure 5D**).

One strain (1/38, 2.63%) of carbapenem-resistant *E. coli* (CRE) and 10 strains (10/44, 22.73%) of carbapenem-resistant *K. pneumonia* (CRKP) were detected. Three strains (3/16, 18.75%) of *P. aeruginosa* were resistant to carbapenems (CRPA). One strain (1/38, 2.63%) of *E. coli*, seven strains (7/44, 15.91%) of *K. pneumonia,* and four strains (4/25, 16.00%) of *A. baumannii* were resistant to tigecycline.

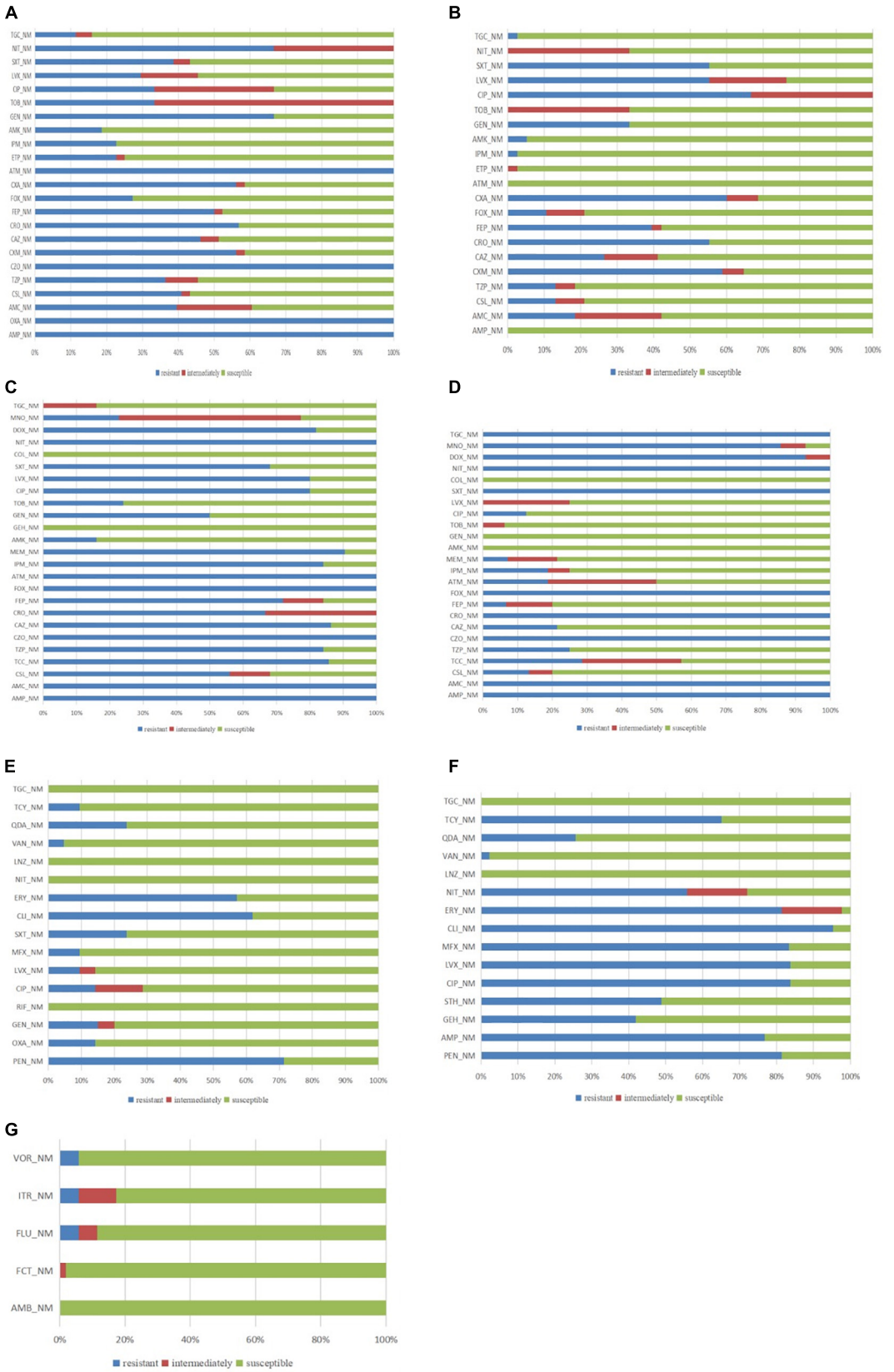

FIGURE 5 | (Continued)

FIGURE 5 | Antibiotic sensitivity patterns of panel **(A)** *K. pneumonia*; **(B)** *E. coli*; **(C)** *A. baumannii*; **(D)** *P. aeruginosa*; **(E)** *S. aureus*; **(F)** Enterococcus; and **(G)** fungal strains; AMB: Amphotericin B; AMC: Amoxicillin + Clavulanate; AMK: Amikacin; AMP: Ampicillin; ATM: Aztreonam; CAZ: Ceftazidime; CIP: Ciprofloxacin; CLI: Clindamycin; COL: Colistin; CRO: Ceftriaxone; CSL: Cefoperazone + Sulbactam; CXM: Cefuroxime; CZO: Cefazolin; DOX: Doxycycline; ERY: Erythromycin; FEP: Cefepime; FCT: Fluorocytosine; FLU: Fluconazole; FOX: Cefoxitin; GEN: Gentamicin; GEH: High concentration gentamicin; IPM: Imipenem; ITR: Itraconazole; LNZ: Linezolid; LVX: Levofloxacin; MEM: Meropenem; MFX: Moxifloxacin; MNO: Minocycline; NIT: Nitrofurantoin; OXA: Oxacillin; PEN: Penicillin; QDA: Quinuptin/dafoptin; RIF: Rifampicin; STH: High concentration streptomycin; SXT: Trimethoprim + Sulfamethoxazole; TCC: Ticarcillin + Clavulanate; TCY: Tetracycline; TGC: Tigecycline; TOB: Tobramycin; TZP: Piperacillin + Tazobactam; VAN: Vancomycin; and VOR: Voriconazole.

TABLE 3 | The antibiotic resistance of ESBL-positive strains.

Antibiotic name	Number	R + I%	95% CI	MIC50
Amoxicillin/Clavulanic acid	36	33.33	19.1–51.1	16
Cefoperazone/Sulbactam	36	36.11	21.3–53.8	16
Piperacillin/Tazobactam	36	30.56	16.9–48.3	16
Cefuroxime	35	97.14	83.4–99.9	64
Ceftazidime	32	59.38	40.8–75.8	32
Ceftriaxone	36	100	88.0–100	64
Cefepime	36	77.78	60.4–89.3	32
Cefoxitin	36	11.11	3.6–27.0	4
Ertapenem	36	2.78	0.1–16.2	0.125
Imipenem	36	5.56	1.0–20.0	0.25
Amikacin	35	8.57	2.2–24.2	2
Levofloxacin	36	58.33	40.9–74.0	8
Trimethoprim/Sulfamethoxazole	36	75	57.5–87.3	384
Tigecycline	36	11.11	3.6–27.0	0.5

Gram-Positive Bacteria

Among the Gram-positive bacteria, 21 strains of *S. aureus* were detected, including four methicillin-resistant *S. aureus* (MRSA) (4/21, 19.05%). Among the penicillin-resistant strains, the production rate of β-lactamase was 80.95%, the resistance rates for penicillin, vancomycin, and levofloxacin were 71.43, 4.76, and 9.52%, respectively (**Figure 5E**).

A total of 43 cases of enterococci were detected, including one vancomycin-resistant enterococci (VRE) strain. The overall antibiotic-resistant rates of the enterococcal strains were 81.40, 83.72, 95.23, and 2.32% for penicillin, levofloxacin, clindamycin, and vancomycin, respectively. However, tigecycline-and linezolid-resistant strains were not detected (**Figure 5F**).

Fungus

A total of 55 fungal strains were detected, including 31 *C. parapsilosis,* 10 *C. albicans,* 3 *Cryptococcus neoformans,* 3 *C. tropicalis,* and 8 other strains. Compared with the high incidence of *C. parapsilosis,* the occurrence of *C. albicans* was lower (**Figure 6**). The antibiotic-resistance rate of these strains for voriconazole, fluconazole, and itraconazole was 5.77%. However, amphotericin B-and flucytosine-resistant strains were not observed (**Figure 5G**).

DISCUSSION

According to the Hour-1 Bundle of SCC, antibiotic treatment should be initiated within 1 h, and etiological examination should be performed to treat critical patients with severe infection and septic shock (9). A delay in prescribing an adequate empiric antibiotic therapy may result in increased mortality, whereas the early prescription of effective antimicrobial treatment is linked to improved clinical outcomes (11–15). In the absence of blood culture reports, clinicians in ICU prescribe empiric broad-spectrum therapy with one or more intravenous antimicrobials to cover all likely pathogens (9). The use of broad-spectrum antibiotics is closely related to the emergence of bacterial resistance, with Gram-negative bacilli exhibiting the highest resistance. Intrinsic, adaptive, and acquired antimicrobial resistance led to the emergence of MDR, XDR, and PDR strains (16, 17). This study found that among the indexes related to the severity of the disease, patients with Gram-negative BSI used more vasopressor, exhibited the highest SOFA score, highest CR level, lowest GCS score, and lowest PLT count. Subgroup comparison showed Gram-negative group patients with lower mean blood pressure and higher ALT, AST, and TBIL values compared with Gram-positive group patients. Therefore, clinicians should pay more attention to infections caused by Gram-negative strains.

The β-lactamase producing Gram-negative bacteria threaten critical care of patients, as they often cause MDR infections. Major β-lactamase families include plasmid-mediated ESBLs, AmpC cephalosporinases, and carbapenemases (18). In this research period, ESBLs were the most common β-lactamases, while carbapenemases were not checked in our hospital.

Extended-spectrum β-lactamases are one of the most popular reasons for intrinsic resistance in bacteria. According to our report, 52.63% of *E. coli* were ESBL positive, which is similar to the rate in China (19) but higher than that in the United States (20). Apart from *E. coli,* 36.37% of *K. pneumonia* were ESBL positive (16/44, 36.36%). Increasing rates of ESBL-positive pathogens have been reported in many countries, leading to an increased focus on this group (21, 22). In our study, the ESBL positivity rate increased from 22.22% in the first half of 2018 to 52.38% in the second half of 2020 (**Appendix 1**); however, the difference was not statistically significant, which may be related to the limited data. Moreover, the carbapenems-resistance rate in *K. pneumonia* was 22.73%. According to the China Antimicrobial Surveillance Network (CHINET), the prevalence of meropenem-resistant *K. pneumonia* increased in China from 9.0% in 2011 to 26.3% in 2018 and fluctuated between 24.2–27.1% from 2019 to 2021. Carbapenem is the preferred treatment for infections outside of the urinary tract caused by ESBL-E (23). The link between carbapenem

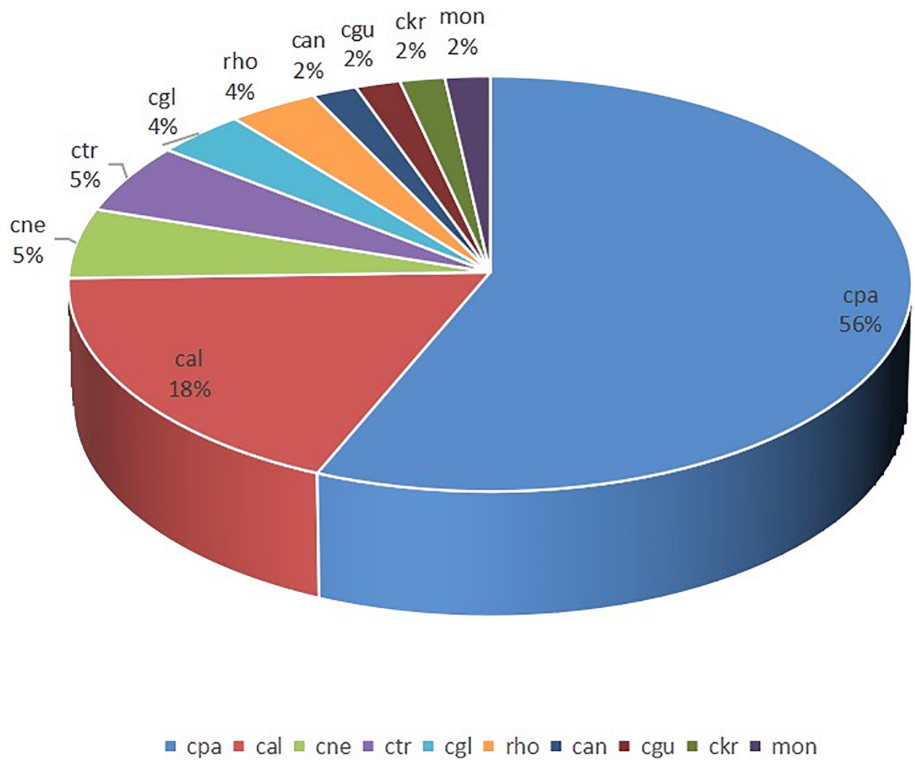

FIGURE 6 | Distribution of fungal strains (%). cpa, *C. parapsilosis;* cal, *C. albicans;* cne, *Cryptococcus neoformans;* ctr, *C. tropicalis;* cgl, *C. glabrata;* rho, *Rhodotorula;* can, *Candida spp;* cgu, *Candida guilliermondii;* ckr, *C. krusei;* and mon, *Monilia.*

consumption and the emergence of carbapenem resistance has been indicated in various studies (24). Therefore, reducing the irrational use of carbapenem antibiotics is critical for healthcare systems. Mark D. Lesher et al. reported that removing the ESBL designation from microbiology reports could decrease the prescription of carbapenems from 48.4 to 16.1% and increase the use of β-lactam–β-lactamase inhibitor combinations from 19.4 to 61.3% (25). In fact, piperacillin/tazobactam is our center's most widely used antibiotic (**Appendix 2**) for suspected Gram-negative bacterial infections. But the susceptibility of *E. coli*, *K. pneumonia,* and *P. aeruginosa* to piperacillin is lower (66/98, 67.35%) than that to carbapenems (82/98, 83.67%). The data of ESBL positive strains is few, but the increasing trend of ESBL-positive rate from 2018 (27.78%; 5/18) to 2021 (50.00%; 16/32) is observed. Whether the increasing trend is related to the high usage of piperacillin tazobactam remains to be further studied. According to a study by Patrick N A Harris et al. among patients with *E coli.* or *K. pneumoniae* BSI and ceftriaxone resistance, compared with meropenem treatment, definitive treatment with piperacillin-tazobactam did not result in non-inferior 30-day mortality (26). Stewart AG et al. reported that piperacillin-tazobactam might lead to more microbiological failures among patients with bloodstream infection due to AmpC producers (27). Piperacillin tazobactam is not suitable for empirical treatment of ESBL positive bloodstream infection. According to our research results, the drug resistance rate of tigecycline, polymyxin, and amikacin against Gram-negative bacteria is lower than that of piperacillin/tazobactam and carbapenem, which can be a choice for Gram-negative bacteria infection. However, the plasma concentration of tigecycline is low and is not recommended for patients with bloodstream infection. Aminoglycoside antibiotics and colistin have adverse reactions of nephrotoxicity. Patients with bloodstream infection are usually complicated with organ function injury. AKI is a common complication. So the application of aminoglycoside drugs and colistin in ICU critical patients is limited. Aminoglycoside antibiotics and colistin are more suitable for combination therapy (23).

In this report, the detection rate of Gram-positive strains, especially *S. epidermidis,* in blood culture was high, which is close to the detection rate reported by CHINET but lower than that of Diekema et al. (28). Coagulase-negative staphylococcus was a common colonization bacterium on the skin. However, partial coagulase-negative Staphylococcus strains were identified in the BSI patients and included in the statistical analysis during the retrospective analysis. *S. epidermidis* was the most detected, with 38 strains in 3 years and 13 discharged cases with contaminated strains. Twenty-five patients with positive *S. epidermidis* in blood culture were treated with vancomycin or linezolid. Most were critical patients with damaged skin barriers

caused by skin damage or dermatic cellulitis, low immune function, and disposed to Gram-positive cocci infections. During the 3 years of this study, the use of vancomycin showed a gradual upward trend, and the possibility of antibiotic abuse could not be excluded. In our study, the Gram-positive BSI maintained a low resistance rate to vancomycin, indicating that vancomycin could be used as an empirical drug. However, the use of vancomycin against coagulase-negative staphylococcus should be restrained to reduce medical consumption and the emergence of antibiotic-resistant bacteria.

Risk factors for invasive candidiasis include the extensive use of invasive procedures and devices, broad-spectrum antimicrobial agents, advanced life support, and aggressive chemotherapy (29). In this study, the incidence of fungemia in ICU was 0.73% (55 patients/7,577 ICU admissions), which was higher than that of 0.32% reported by Guo F et al. (306 patients/96,060 ICU admissions) (30). Nearly two-thirds of fungemia cases were ICU-acquired infections. Moreover, the occurrence of *C. parapsilosis* was higher than that of *C. albicans*, which was following the results of Pfaller MA et al. who reported a decreased detection of *C. albicans* and increased isolation of *C. glabrata* and *C. parapsilosis* (31). Previous studies have indicated that patients requiring prolonged use of a central venous catheter or indwelling device are at an increased risk of *C. parapsilosis* infection (32). Therefore, it is suggested that antifungal treatment should be added to the treatment regime of high-risk patients in ICU. In this study, the overall resistance rate of fluconazole was 10.91%, indicating its use as an empirical antifungal drug. Because of the high incidence of *C. parapsilosis* and to reduce the cases of BSI caused by *C. parapsilosis*, catheter maintenance in clinical operations should be given importance. The high positivity rates of methicillin-resistant *S. epidermidis* and *C. parapsilosis* suggest that our hospital needs to strengthen further the prevention and control of ICU-acquired infections or catheter-related infections (33).

Many studies have employed next-generation sequencing (NGS) to reduce the waiting time for blood culture reports (34, 35). Moreover, rare microorganisms could be detected by NGS. However, because of the high expense, this technology could not be used in our hospital. In this study, the level of peripheral blood leukocytes, an indicator of inflammatory reaction, did not vary among the Gram-positive, Gram-negative, and fungal groups. The level of PCT in the Gram-negative group was significantly higher than that of the other two groups. Studies have confirmed that PCT, a common clinical monitoring index, can be used to distinguish infectious fever from non-infectious fever (36). Dynamically monitoring the changes in PCT levels could determine the time to initiate antibiotic treatment and discontinue the same, thereby reducing the duration of antibiotic use without affecting the prognosis (37–39). The plasma PCT levels of patients with Gram-negative bacterial infections were higher than that of the patients with Gram-positive and the plasma PCT levels were higher in bacterial BSI group than that in fungal BSI group, which was consistent with the results of earlier studies (40, 41). However, it was not an effective indicator to distinguish, thereby indicating the need for further large-scale research. Although no differences in PCT concentrations were observed among the cases with *E. coli, K. pneumonia, P. aeruginosa,* and *A. baumannii* infections, blood culture reports revealed two or three times higher PCT values in *E. coli* and *P. aeruginosa* infections compared with that of *K. pneumonia and A. baumannii* infections. Daniel O. Thomas-Rüddel et al. reported that Streptococci, *E. coli*, and other Enterobacteriaceae detected in blood culture were associated with three times higher PCT values in a linear regression model (40). Although many studies have demonstrated the advantages of PCT in the diagnosis and treatment of BSI, especially Gram-negative BSI, more experimental data and/or more inflammatory indicators are needed for the differential diagnosis of infectious pathogens.

CONCLUSION

Among the bloodstream infection strains in ICU, Gram-negative bacteria have the highest drug resistance rate, and will cause more serious brain damage, renal function damage and thrombocytopenia. So clinician should pay more attention to the treatment of Gram-negative bacteria in patients with bloodstream infection in ICU. The test index of PCT can be used to distinguish Gram-negative bacteremia from Gram-positive and bacteremia from fungemia but not as an effective indicator, thereby indicating the need for further large-scale research. This study has the following limitations: (1) since the included cases were single-center samples, the findings of this study cannot be generalized to other regions; (2) this study is prone to bias because of its retrospective characteristic; (3) because of the small sample size, the research results and conclusions are only for reference; and (4) due to the limited conditions, the carbapenem-resistance gene was not detected.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tianjin Medical University General Hospital, China. The ethics committee waived the requirement of written informed consent for participation. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

H-NW, E-YY, and MP contributed to the conception and design of the study. H-NW organized the database and wrote the first draft of the manuscript. W-BL and E-YY performed the statistical analysis. Q-YZ, K-lX, H-NW, and MP wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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APPENDIX

Appendix 1 | ESBL-positivity rate in the first half of the years 2018–2020.

	2018 First half	2018 second half	2019 First half	2019 second half	2020 First half	2020 second half	*P*-value
ESBL positivity rate (%)	22.22	33.33	38.46	50.00	45.45	52.38	0.143

Appendix 2 | Antibiotic consumption in the first half of the years 2018–2020.

Antibiotic consumption	2018 First half	2018 second half	2019 First half	2019 second half	2020 First half	2020 second half	*P*-value
Piperacillin/tazobactam	1300.82	1425.86	1721 .89	1491.75	1381.18	1536.43	0.566
Cefuroxime	218.50	369.25	622.75	675.00	703	611.5	**0.041**
Cefotaxime sodium	82.125	34.625	114.25	62.375	48.625	157.25	0.397
Cefepime	769	739.5	1224.5	1425.5	1376	1023.5	0.201
Cefoperazone Sulbactam Sodium	546	622.125	820.875	937.125	657	696.375	0.477
Cefatriaxone	136	185	194	286.5	147.5	282.5	0.223
Cefazolin	62.67	65.00	96	167	101.33	178.33	**0.049**
Cefoxitin	271.83	227.67	391.67	371.83	196	350.33	0.729
Levofloxacin	58	71	102	145	102	144	**0.037**
Imipenem cilastatin	159	232.5	573.5	435	244	295.5	0.701
Meropenem	103.5	117.75	284.5	123.75	64.5	57.75	0.489
Tigecycline	193.5	177.5	564	352.5	304.5	205.5	0.875
Linezolid	151.83	226.33	509.83	436.67	287.33	207.17	0.781
Vancomycin	60.25	77.75	85.25	116.25	105	129.5	**0.004**
Fluconazole	192	154	293	161	202	240	0.622
Caspofungin	3	13	16	14	7	84	0.139
Voriconazole	31.5	83	170.5	151.5	30	205.5	0.317
Ceftazidime	67.75	55.5	78	120.25	177.25	221.5	**0.005**
Total	4407.275	4877.36	7862.515	7473	6134.215	6626.635	0.247

Antibiotic consumption was expressed in defined daily dose(DDD) in every six months. The rising consumption was detected in Cefuroxime, Cefazolin, Levofloxacin, Vancomycin and Ceftazidime in three years.

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BRIEF RESEARCH REPORT
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Antibiotic Treatment of *Acinetobacter baumannii* Superinfection in Patients With SARS-CoV-2 Infection Admitted to Intensive Care Unit: An Observational Retrospective Study

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Introduction: In COVID-19 patients on mechanical ventilation, VAP from *Acinetobacter baumannii* remains a crucial risk factor for death. Antibiotic resistance represents an important problem in treating this infection. This study aims to describe the evolution of the superinfection from *PDR Acinetobacter baumannii* in patients with acute respiratory failure from SARS-CoV-2 infection admitted to ICU and compare the impact of two different antibiotic strategies on microbiological negativization.

Methods: *S*ingle-center observational retrospective study, including patients admitted to our ICU from March 2020 to May 2021 for acute respiratory failure from SARS-CoV-2 infection who developed *PDR Acinetobacter baumannii* superinfection. Clinical data at ICU admission were collected, as well as the timing of isolation of *Acinetobacter baumannii*, its resistance profile, the site of infection, and the antibiotic therapy.

Results: Of the 32 patients enrolled, 10 patients (31.2%) were treated with the combination of high-dose ampicillin/sulbactam, high-dose tigecycline, intravenous and inhaled colistin *(Protocol)*, the other 22 (68.8%) were treated with the combination of two antibiotics *(Control)*. Of the 10 patients in the *Protocol* group, 8 patients (80%) received also fosfomycin. All patients (100%) in the *Protocol* group had microbiological negativization, while in the *Control* group microbiological negativization was observed in 8 (36.4%) patients, $p < 0.01$.

Conclusion: Our report shows microbiological negativization in all patients treated with the combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam. This combination of antibiotics seems to be a useful alternative when other treatments are not available or fail.

Keywords: ***Acinetobacter baumannii*, superinfection, SARS-CoV-2, acute respiratory failure, antibiotics**

INTRODUCTION

In December 2019 new cases of pneumonia of unknown origin came to light in China (1). The new virus was recognized as a coronavirus able to cause the severe acute respiratory syndrome (2). Therefore, it was named SARS-CoV-2, and the pathology derived from it was called Coronavirus Disease 2019 (COVID-19) (2). It is widely known that the clinical presentation of the illness may vary considerably. In some cases, the disease may be asymptomatic, while 5–15% of patients may experience dyspnea and respiratory effort and require endotracheal intubation and mechanical ventilation (3, 4).

In the case of intubated patients in intensive care units (ICUs), Ventilator-Associated Pneumonia (VAP) remains a crucial risk factor for death (5). A VAP is diagnosed when new pneumonia is detected after 2 days from the patient being intubated and mechanically ventilated. As for causative agents, the most common pathogens include *Staphylococcus spp., Enterococcus spp., Klebsiella pneumonia, Enterobacter spp., Escherichia coli, Acinetobacter spp.,* and *Pseudomonas spp.* (6).

Furthermore, a significant percentage of patients, admitted to ICU, is treated with broad-spectrum antibiotics, which increase the risk of developing hospital-acquired infections, particularly from multi-drug resistant (MDR) pathogens. Among them, *Multi-Drug Resistant Acinetobacter baumannii* (MDR-AB) represents a causative agent for almost half of Ventilator-Associated Pneumonia (VAP) (7) and is a severe problem in patients with COVID-19 in ICU (8, 9).

The most important risk factors for VAP from *Acinetobacter baumannii* are high blood pressure, chronic obstructive pulmonary disease (COPD), length of stay in ICU, at least one organ failure, chronic renal impairment, and reduced blood oxygenation level. Interestingly, these features are usually common to COVID-19 patients in ICU, who, therefore, become highly susceptible to the infection (10–12). *Acinetobacter baumannii* is a Gram-negative bacterium, opportunistic, pleomorphic, and non-motile. It can colonize dry surfaces and devices surviving up to 33 days (13–15). Moreover, the pathogen can develop resistance to numerous classes of antibiotics more rapidly than other bacteria. Therefore, it has been considered a major health problem in the international medical community (16). In regards to antimicrobial therapy, in the case of a *Multi Drug-Resistant Acinetobacter baumannii*, carbapenems still represent the treatment of choice. Unfortunately, the resistance to carbapenems has increased making the pathogen *eXtensively Drug-Resistant* (XDR), while other strains have been named *Pan Drug-Resistant* (PDR) when they showed resistance to polymyxins, especially colistin, and tigecycline (17). As for XDR AB, one of the last options is colistin which is highly nephropathic and neurotoxic (18). However, by changing the way of administration, the risk of nervous and renal damage can be decreased. When colistin is given by inhalation, the systemic distribution of the drug is reduced (19). Therefore, nebulized colistin seems to be a reasonable choice in the case of *Carbapenem-Resistant Acinetobacter baumannii* in patients with COVID-19 in ICU (5).

The higher incidence of *Pan Drug-Resistant Acinetobacter baumannii* causing VAP is observed particularly in Greece, Spain, and Italy, implying the need for new therapeutic strategies (20).

Thus, some authors proposed to use of a combination of antibiotics to exploit the synergistic effect of different classes (21).

In 2019, *Assimakopoulos et al.* reported positive results in treating 10 ICU patients with VAP from *Pan Drug-Resistant Acinetobacter baumannii* with a combination of antibiotics, which consisted of a high dose of tigecycline and ampicillin/sulbactam, and colistin, given both by inhalation and intravenously (22). As for sulbactam, its use is justified by its intrinsic activity against several strains of AB (23, 24).

The present brief report aimed to describe retrospectively the evolution of the superinfection from *PDR Acinetobacter baumannii* in patients with SARS-CoV-2 infection admitted to ICU. In addition, it assessed the incidence of negativization between patients treated with the combination of at least three antibiotics, according to a treatment protocol applied in our ICU, and those who received a combination of two antibiotics.

MATERIALS AND METHODS

We retrospectively collected the data from adult patients admitted to a single COVID-ICU (Anesthesia and Intensive Care Unit, University Hospital "Ospedali Riuniti" of Ancona, Italy) for acute respiratory failure from SARS-CoV-2 infection and *Acinetobacter baumannii* superinfection. We collected demographic data, including age, sex, body mass index (BMI) and comorbidities, and clinical data at ICU admission among which respiratory parameters and blood tests including lymphocytes, leukocytes, and procalcitonin. We calculated the SOFA *(Sequential Organ Failure Assessment)* score and the *Charlson Comorbidity Index* at ICU admission. The immunomodulatory and immunosuppressive therapies, if administered before admission, were noted. Any microbiological tests performed at the beginning and during the stay in ICU were reviewed. We noted the date of positivity to SARS-CoV-2, detected with the reverse transcriptase-polymerase chain reaction (RT-PCR) on the nasopharyngeal swab, performed before ICU admission. We also noted the precise timing of isolation of *Acinetobacter baumannii*, its resistance profile (MDR, XDR, PDR), and the site of infection. *Acinetobacter baumannii* strains from all kinds of cultures were identified in our microbiology laboratory with the new-generation mass spectrometry microbial identification system, VITEK® MS PRIME *(bioMérieux, Marcy-l'Étoile, France)*. To test the antimicrobial susceptibility was used the VITEK®2 System *(bioMérieux, Marcy-l'Étoile, France)* for all antibiotics. The resistance to colistin detected with the VITEK®2 System was confirmed with the broth microdilution method. The results were interpreted following the latest EUCAST breakpoints for *Acinetobacter baumannii* spp. available.

Following the definition of the resistance profile (17), we considered as *Multi Drug-Resistant* (MDR) *Acinetobacter baumannii* resistant to at least three classes of antimicrobial

Abbreviations: COVID-19, COronaVIrusDisease-19; ICU, Intensive Care Unit; SARS-CoV-2, Severe Acute Respiratory Syndrome CoronaVirus-2; VAP, Ventilator Associated Pneumonia; MDR, Multi Drug-Resistant; XDR, eXtensively Drug-Resistant; PDR, Pan Drug-Resistant; SOFA, Sequential Organ Failure Assessment; AKI, Acute Kidney Injury; MIC, Minimum Inhibitory Concentration.

agents (all penicillins and cephalosporins, including inhibitor combinations, fluoroquinolones, and aminoglycosides), *eXtensively Drug-Resistant (XDR)* the *MDR Acinetobacter baumannii* resistant also to carbapenems and *Pan Drug-Resistant (PDR)* the *XDR Acinetobacter baumannii* resistant to polymyxins and tigecycline. In addition, data regarding antimicrobial treatment were collected. Starting from the second wave of the pandemic, when the problem of *PDR Acinetobacter baumannii* superinfection became very consistent in COVID-19 patients admitted to our ICU, impacting the length of stay and the outcome, we started to apply a protocol of antibiotic therapy based on the case series study of *Assimakopoulos et al.* (22). Patients with *PDR Acinetobacter baumannii* superinfection received combination therapy with intravenous colistin at the loading dose of 9 million IU followed by a maintenance dose of 4.5 million IU every 12 h, intravenous tigecycline at the dose of 100 mg every 12 h, intravenous ampicillin/sulbactam, administered in a continuous infusion, at the dose of 12 gr per day and inhaled colistin at the dose of 3 million IU every 6 h, added in case of respiratory tract infection. Sometimes, in patients with particularly severe clinical conditions, we added also fosfomycin at the dose of 12 gr per day. The maintenance dose of intravenous colistin in patients with the impaired renal function was adjusted with the use of the colistin calculator, based on the pharmacokinetic modeling data published by *Garonzik et al.* (25). We also reduced the dose of ampicillin/sulbactam in patients with a creatinine clearance less than 30 ml/min, according to the Cockcroft-Gault equation. Considering that rapid molecular systems to detect the pathogen were not routinely used in the period of study, we used to start this combination of antibiotics about 48–72 h after the cultural tests, as soon as the microbiological examinations reports were made available. Before the application of this protocol of the three antibiotics in combination, patients with *PDR Acinetobacter baumannii* were treated with nebulized and intravenous colistin alone or combined with an antibiotic of another class. To define the resolution of the infection, we considered both the clinical improvement in the signs of infection and the laboratory or instrumental parameters and the negativization from *Acinetobacter baumannii* in control culture tests. We also reported the complications of antibiotic therapy. According to the KDIGO guidelines (26), we defined *Acute Kidney Injury (AKI)* as the presence of any of the following criteria: an increase in serum creatinine by $\geq$ 0.3 mg/dl ($\geq$ 26.5 μmol/l) within 48 h or an increase in serum creatinine to $\geq$ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or urine volume $<$ 0.5 ml/kg/h for 6 h.

Statistical Analysis

The statistical analysis was performed using STATA 17.0 BE – Basic Edition *(StataCorp, Texas, United States)*. Categorical data were expressed as absolute and relative frequencies, numerical data as mean ± standard deviation, if normally distributed, or median [interquartile range], if not normally distributed. Normality of distribution was assessed using the Shapiro-Wilk test. Dichotomous data were compared using the chi-square test or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's *t*-test for unpaired data or the Wilcoxon rank-sum test, as appropriate. A $p < 0.05$ was used to indicate the statistical significance.

Given the descriptive nature of the primary objective, a sample size calculation was not necessary.

Ethical Aspects

The study protocol was approved by the local Ethics Committee (Comitato Etico Regionale delle Marche). All the data were anonymously analyzed. Written informed consent was not applicable due to the retrospective nature of the study.

RESULTS

We considered 32 patients, admitted to our ICU from March 2020 to May 2021 for acute respiratory failure consequent to SARS-CoV-2 infection who developed *PDR Acinetobacter baumannii* superinfection. The MIC (Minimum Inhibitory Concentration) values of the *PDR Acinetobacter baumannii* in the study population are presented in **Table 1**. In 30 patients (93.7%) the site of *PDR Acinetobacter baumannii* superinfection was the respiratory tract, in 2 patients (6.3%) the microorganism was isolated firstly in the rectal swab and then also in the respiratory tract cultures. The median age of patients was 59.5 [54–66] years and 28 (87.5%) were males. Of the 32 patients, 10 patients (31.2%) were treated with the combination of high-dose ampicillin/sulbactam, high-dose tigecycline, intravenous and inhaled colistin *(Protocol)*, the other 22 (68.8%) were treated with the combination of two antibiotics *(Control)*. Of the 10 patients in the *Protocol* group, 8 patients (80%) received also fosfomycin. In all the 10 patients of the *Protocol* group, the *PDR Acinetobacter baumannii* was isolated only in the respiratory swab. The demographic and clinical characteristics of the two groups of patients are presented in **Table 2**.

Between the two groups of patients, no significant differences were observed in demographic and clinical characteristics at admission to the ICU. Considering the therapy received before the ICU admission, 12 patients (54.5%) in the *Control* group and 7 (70%) in the *Protocol* group had already been treated with antibiotics, $p = 0.28$. There was no significant difference in the duration of the steroid therapy received before the ICU admission

TABLE 1 | Minimum Inhibitory Concentration (MIC) values of the *PDR Acinetobacter baumannii* in the study population.

Antimicrobial agent	MIC values (mg/L)	MIC breakpoints (mg/L)* R >
Amikacin	≥ 64	8
Ciprofloxacin	≥ 4	1
Colistin	> 2	2
Gentamicin	≥ 16	4
Meropenem	≥ 16	8
Trimethoprim/Sulfamethoxazole	≥ 320	4
Tigecycline	2-4	–

**EUCAST Clinical breakpoints tables, v. 10.0 and 11.0.*
R = resistant; – = no EUCAST breakpoint available.

TABLE 2 | Demographic and clinical characteristics at Intensive Care Unit (ICU) admission of the two groups of patients.

Characteristics	Protocol (*n* = 10)	Control (*n* = 22)	p-value*
Male sex, n (%)	8 (80)	20 (90.9)	0.57
Age, years	58.5 [55–66]	60 [53–66]	0.97
BMI, kg/m^2	33.5 [26–35.9]	27.8 [25.5–31.2]	0.12
Charlson Comorbidity Index, points	2.2 ± 2.2	2.1 ± 1.5	0.84
PaO_2/FiO_2	74.5 [63–82]	78.5 [61–87]	0.67
WBC, ×10^3/mm	12.1 [7.8–14]	11.8 [7.8–19.2]	0.81
Lymphocytes, ×10^3/mm	0.98 [0.63–1.24]	0.56 [0.4–0.69]	0.07
Procalcitonin, ng/ml	0.33 [0.17–0.93]	0.39 [0.11–1.03]	0.96
SOFA score	6.5 ± 1.6	7.7 ± 2.3	0.13

Data are presented as absolute and relative frequencies, mean ± standard deviation, median [interquartile range].
**Chi-squared test or Fisher's exact test for categorical variables and Student's t-test or Wilcoxon rank-sum test for numerical variables, as appropriate.*
BMI = body mass index; PaO_2/FiO_2 = ratio of partial pressure of arterial oxygen to fraction of inspired oxygen; WBC = white blood cells; SOFA = Sequential Organ Failure Assessment.

[8 (2–11) days in the *Control* group vs. 4 (1–10) days in the *Protocol* group, $p = 0.52$]. The mean length of stay in the ICU of patients in the *Control* group was 25.2 ± 17.3 days, instead, for patients in the *Protocol* group was 36.1 ± 32.6 days, $p = 0.36$. All patients, 100% (95% CI: [69–100]%), in the *Protocol* group had microbiological negativization, while in the *Control* group microbiological negativization was observed in 36,4% (95% CI: [17–59]%) of patients, $p < 0.01$. Considering the side effects of the antibiotic therapy, 40% (95% CI: [12–73]%) of patients in the *Protocol* group developed AKI, while in the *Control* group only 4,5% (95% CI: [0,1–22]%) of patients, $p = 0.01$. All patients with AKI, in both groups, received renal replacement therapy and, in all patients, the renal function recovered before ICU discharge. No other relevant side effects related to antibiotic therapy were observed in both groups. All patients, 100% (95% CI: [69–100]%) in the *Protocol* group were discharged alive from ICU, while, in the *Control* group, 36.4% (95% CI: [17–59]%) of patients survived, $p < 0.01$, **Table 3**.

TABLE 3 | Outcomes in the study population.

Outcomes	Protocol* (*n* = 10)	Control° (*n* = 22)	p-value**
Negativization, n (%)	10 (100)	8 (36.4)	<0.01
Complication – AKI, n (%)	4 (40)	1 (4,5)	0.01
ICU Survivors, n (%)	10 (100)	8 (36.4)	<0.01

**Protocol = colistin 9 million IU + 4.5 million IU every 12 h, intravenous tigecycline 100 mg every 12 h, intravenous ampicillin/sulbactam 12 gr per day and inhaled colistin 3 million IU every 6 h.*
°Control = nebulized and intravenous colistin alone or combined with an antibiotic of another class.
***Chi-squared test or Fisher's exact test, as appropriate. AKI = acute kidney injury.*

DISCUSSION

The present brief report aimed to retrospectively describe the evolution of the superinfection from *PDR Acinetobacter baumannii* in patients with SARS-CoV-2 infection admitted to ICU and assess the incidence of negativization between patients treated with the combination of at least three antibiotics, according to the protocol applied in our ICU, and those who received a combination of two antibiotics. Our study shows that all patients in the *Protocol* group had microbiological negativization together with the clinical resolution of the infection and all of them were discharged alive from ICU. Considering the side effects of the antibiotic therapy, the patients in the *Protocol* group had a significantly higher incidence of AKI, which was managed in all cases with renal replacement therapy. However, the renal function recovered without sequelae in all patients before ICU discharge. Regarding the outcome, it is important to mention that the causes of death of patients in the *Control* group were not exclusively related to the complications of the *PDR Acinetobacter baumannii* superinfection. In fact, this study was focused on this single specific infection and the impact of this treatment protocol on microbiological negativization. No other co-infections, as well as other possible complications, were considered and the study itself was not designed to assess a cause-effect relationship with the outcome. However, considering the impact of this superinfection, the fact that all patients in the *Protocol* group survived was important to point out.

Carbapenem-Resistant Acinetobacter baumannii, as well as *Enterobacterales* and *Pseudomonas aeruginosa* resistant to carbapenems, were first on the WHO's list of resistant bacteria for 2016-2017 as they threaten public health globally (27). In particular, among the 12000 annual infections in the United States, more than 60% of them were caused by *MDR Acinetobacter baumannii*, as remarked by the American CDC report in 2013 (28). The management of *MDR Acinetobacter baumannii* is currently based on carbapenems if the isolated microorganisms show susceptibility to this antibiotics class (17). With regards to *XDR Acinetobacter baumannii*, it is associated with a mortality rate higher than 50% (29, 30). Its recommended treatment consists of polymyxins and tigecycline. Whether colistin alone or in a combined therapy gives advantages or not, is still debatable (17, 30). As regards tigecycline, although standard doses did not seem to have an effect, high-dose tigecycline, defined as a loading dose of 200 mg followed by 100 mg every 12 h, lead to better results in terms of outcome (31).

A recent metanalysis by *Jung et al.*, regarding *MDR/XDR Acinetobacter baumannii*, showed that sulbactam, both at a normal and at a high dose, had the best survival benefit. Fosfomycin and colistin came second, followed by a combination of colistin given both by inhalation and intravenously, while monotherapy with high-dose of tigecycline and colistin came last (32). Only sulbactam showed activity against *Acinetobacter baumannii* but in most European countries, such as Greece and Italy, the only available combination is ampicillin/sulbactam (33). As *Acinetobacter baumannii* becomes *Pan-Drug Resistant*, treatment options significantly decrease in number. The problem of *Pan-Drug Resistant* Gram-negative bacteria is increasing worldwide, but the management of the *PDR Acinetobacter baumannii* infections is particularly hard (21). *Karakonstantis et al.,* in their cohort study, showed that in-hospital mortality

is significantly higher in patients with *PDR Acinetobacter baumannii* infections compared to those with *PDR Acinetobacter baumannii* colonization (34). Moreover, typically affecting patients with critical illness, multimorbidity, and exposure to invasive procedures, the *PDR Acinetobacter baumannii* infections considerably prolong the length of hospitalization (34). To cope with the lack of effective treatments available, several studies have been performed to establish the effectiveness of current options available such as ceftazidime/avibactam and ceftolozane/tazobactam, but they did not show any significant advantages (35). Following this, if some data regarding the effectiveness of antibiotics against *XDR Acinetobacter baumannii* exist, no clinical data are available for *PDR Acinetobacter baumannii* (36–38). For this reason and given the difficulty in treating *PDR Acinetobacter baumannii*, *Assimakopoulos et al.* used a combination therapy, which seemed to have promising results *in vitro*. By administering colistin both nebulized and intravenous with high-dose tigecycline and high-dose ampicillin/sulbactam, they demonstrated a high rate of clinical response and the hitherto highest percentage of survival at 28 days (90%) (22).

Nonetheless, we must mention one of the newest cephalosporins, cefiderocol, which was inserted into the list of antimicrobials suitable for *MDR* Gram-negative infections, mainly for *MDR Acinetobacter baumannii* (39). As *Bassetti et al.* remarked in their review, in Europe, it has been used since 2020, while in the United States it was already approved in 2019 (39). Given its high costs and its initial no-refunds policy in Italy, it was not used routinely. The Italian Drug Agency (AIFA) did not approve its refundability until June 2021 and only for patients with limited or no further options of treatment. Nevertheless, cefiderocol showed advantageous clinical cure rates compared to the best available therapy in Gram-negative pneumonia caused by carbapenem-resistant Enterobacteriaceae, complicated urinary tract infections, bloodstream infections, and sepsis. Despite this, the all-cause mortality was found higher in patients treated with cefiderocol (39). Therefore, its use is restricted to those aged equal to or more than 18 years old with no other options (39).

Our study has some limitations. First of all, the retrospective design of the study does not allow to control for all confounding factors. Moreover, our attention, as mentioned above, was focused on a single infection and the impact of the treatment protocol, applied in our ICU, on microbiological negativization: we did not collect data about other possible co-infections. Regarding the side effects of antibiotic therapy, it was difficult to assess and report the exact incidence of the neurotoxicity of colistin. It is established that colistin, interacting with neurons, can cause a wide spectrum of neurological manifestations, such as peripheral and orofacial paresthesias, visual disturbances, vertigo, mental confusion, ataxia, and seizures (40). All these manifestations are difficult to assess in patients sedated and intubated in ICU. Furthermore, it is now known that the SARS-CoV-2 infection itself can lead to neurological effects (41) as well as hospitalization in ICU, which is related to the "critical illness polyneuropathy" (40). Furthermore, to date, we have not yet collected the data on COVID-19 patients, admitted to our ICU in a period following the study population, who developed *PDR Acinetobacter baumannii* superinfection, treated with cefiderocol. It may be useful to compare the two treatment strategies in terms of effectiveness and side effects.

CONCLUSION

Our brief report shows microbiological negativization as well as the clinical resolution of the *PDR Acinetobacter baumannii* superinfection in all patients treated with the combination therapy of nebulized and intravenous colistin, high-dose tigecycline, and high-dose ampicillin/sulbactam. This combination of antibiotics seems to be a useful alternative to eradicate *PDR Acinetobacter baumannii* when cefiderocol is not easily accessible or may fail therapeutically.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Regionale delle Marche – Azienda Ospedaliero Universitaria "Ospedali Riuniti," Ancona. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

EC collected the data, performed the statistical analysis, interpreted the data, and drafted the manuscript. EB contributed to drafting the manuscript and reviewing the literature. SV and RG collected the data. RD contributed to drafting and revising the manuscript. AC, ED, and CS participated in the interpretation of the results. VG, SP, and BM revised the manuscript. AD and EA designed the study, participated in the statistical analysis and interpretation of the data, and revised the manuscript. All authors approved the submitted version of the manuscript and agreed both to be personally accountable for the author's contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature, and read and approved the final manuscript.

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Outcome of Using Intraventricular Plus Intravenous Polymyxin B in Post-neurosurgical Patients With Multi/Extensively Drug-Resistant Gram-Negative Bacteria-Induced Intracranial Infection

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Introduction: Post-neurosurgical central nervous system (CNS) infection caused by multidrug-resistant (MDR)/extensively drug-resistant (XDR) Gram-negative bacteria remains a major clinical challenge. This study describes our experience of treating such patients with combined intraventricular (IVT) and intravenous (IV) polymyxin B administration.

Methods: This retrospective study included six patients with post-neurosurgical CNS infections of carbapenem-resistant *Acinetobacter baumannii* (CRAB) or carbapenem-resistant *Klebsiella pneumoniae* (CRKP). All patients were treated in the intensive care unit (ICU) of First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China) between November 2020 and November 2021, and all received IVT plus IV polymyxin B. Data including patients' characteristics, therapeutic process, symptoms, cerebrospinal fluid (CSF) examination, laboratory tests, and complications were collected.

Results: Six patients with post-neurosurgical CNS infection were enrolled in the study. The patients comprised five males and one female, and the average age was 58 years (range, 38–73 years). Four out of the six cases were CRAB-positive in CSF culture, while two cases were CRKP-positive. The mean duration of polymyxin B administration was 14 ± 5.69 days (range, 6–20 days). The average period of patients reaching CSF sterilization was 10.33 ± 3.67 days (range, 5–14 days). All six cases were cured without acute kidney injury or epilepsy.

Conclusion: IVT plus IV polymyxin B is a safe and effective treatment for post-neurosurgical patients with intracranial infection caused by MDR/XDR Gram-negative bacteria.

Keywords: intraventricular polymyxin B, colistin, neurosurgery, central nervous system (CNS) infection, multidrug-resistant (MDR)/extensively drug-resistant (XDR) bacteria

INTRODUCTION

Central nervous system (CNS) infection is one of the most common complications following neurosurgery and can be difficult to treat due to the blood–brain barrier (BBB). The BBB is crucial for the protection of the CNS against microbial entry from the circulation, but can also prevent various drugs from entering the brain.

The microbes responsible for CNS infection are predominantly Gram-negative and Gram-positive bacteria. According to the 2021 China Antimicrobial Surveillance Network (CHINET) report,[1] the detection rate of Gram-negative bacteria *Acinetobacter baumannii* (AB) and *Klebsiella pneumoniae* (KP) in the CNS from patients with intracranial infection was 11.3 and 9.1%, respectively. Moreover, multidrug-resistant (MDR)/extensively drug-resistant (XDR) bacterial strains in critical-care patients have become much more common in recent years, making the treatment of CNS infections increasingly difficult. Furthermore, meningitis-related mortality is significantly associated with carbapenem resistance (1). Fortunately, the MDR/XDR pathogens remain susceptible to the polymyxins, although only a small proportion of the intravenous (IV) polymyxin dosage reaches the CNS infection site due to limited penetration of the BBB by this class of antibiotics (2, 3). Consequently, the cerebrospinal fluid (CSF) distribution of polymyxin is very low.

According to the 2017 guidelines of the American Society for Infectious Diseases (IDSA) (4), intraventricular (IVT) injection combined with IV polymyxin B or colistin is recommended for the treatment of intracranial MDR Gram-negative bacterial infections. Multiple studies have suggested IVT+IV colistin or polymyxin B is the optimal treatment option for CNS infections with MDR/XDR Gram-negative bacteria (5–7). Current IDSA guidelines for IVT polymyxin B recommend a dose of 5 mg once daily for 10–21 days (4). However, there are limited data on the safety, efficacy, and optimal dose/duration of polymyxin B for patients with intracranial infection. The present study describes our experience of six patients with post-neurosurgical CNS infections of carbapenem-resistant *Acinetobacter baumannii* (CRAB) or carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and their treatment with IVT+IV polymyxin B.

METHODS

In this retrospective case series, the medical records of six patients with post-neurosurgical CNS infections of CRAB/CRKP, who were hospitalized in the intensive care unit (ICU) of First Affiliated Hospital, Zhejiang University School of Medicine (Hangzhou, China) between November 2020 and November 2021, were reviewed.

Inclusion and Exclusion Criteria

Inclusion criteria for enrollment in the study included: (1) patients ≥18 years old; (2) cases with both clinical and imaging evidence of post-neurosurgical CNS infection; (3) laboratory tests (routine and biochemical tests) of CSF suggestive of CNS infection; (4) detection of CRAB/CRKP in CSF culture; (5) concomitant use of IVT and IV polymyxin B.

Exclusion criteria for enrollment in the study were: (1) history of intracranial infection before craniotomy; (2) previous treatment with IV polymyxin B/colistin for intracranial infection or infection at other positions in the past 3 months; (3) administration of either IV polymyxin B or IVT polymyxin B alone.

Microbiological Methods

The broth microdilution method was used for bacterial strain identification and antimicrobial susceptibility tests. One exception was the method for ceftazidime-avibactam susceptibility, which was tested by the Kirby-Bauer method. Antimicrobial susceptibility results are presented in **Table 1**.

Treatments

After diagnosis of a CNS infection, the external ventricular drain (EVD) was removed and replaced. Before the detection of CRAB/CRKP was confirmed, systemic control of infection with intravenous antibiotics—including meropenem, vancomycin, cefoperazone-sulbactam, and piperacillin-tazobactam—was performed based on clinical experience. Polymyxin B administration was initiated once positive culture of CRAB/CRKP was confirmed in the CSF. IVT polymyxin B at a dose of 5 mg (50,000 units) per 24 h, plus IV polymyxin B at a dose of 1.5 mg/kg every 12 h was given (4, 8). IVT polymyxin B was diluted in 5 mL of 0.9% saline solution and injected into the ventricle through the EVD. Thereafter, the EVD was flushed with 2 mL of 0.9% saline solution and clamped for 2 h. After 3 days of treatment, CSF samples were collected from the EVD every 2 days.

Clinical Outcomes

Clinical cure was defined as: (1) remission of the clinical symptoms and signs of CNS infection; (2) routine and biochemical tests of CSF back to normal; (3) three consecutive negative CSF cultures.

Data Collection

For each case, collected data included clinical characteristics (age, gender, primary disease, surgeries, foreign body), therapeutic process (antibiotic usage prior to positive CSF culture, concomitant infection, time from symptoms onset to IVT polymyxin B administration, IVT+IV polymyxin B duration, CSF sterilization time), symptoms and signs [temperature, Glasgow Coma Scale (GCS)], CSF examination (culture, susceptibility, polymorphonuclear leukocytes, glucose, protein), laboratory tests (white blood cell (WBC), C-reactive protein (CRP), procalcitonin (PCT), creatinine, glucose), and complications [hydrocephalus, additional surgery of ventriculoperitoneal shunt (VPS) for hydrocephalus, epilepsy, acute kidney injury (AKI), and skin hyperpigmentation]. Adverse events related to polymyxin B were graded using the Common Toxicity Criteria for Adverse Events (CTCAE), version 5.0.

[1]http://www.chinets.com

TABLE 1 | Antibiotic minimum inhibitory concentration (MIC, μg/mL)/susceptibility of CSF isolates from patients enrolled in the study.

Antibiotic	Case (CSF isolate)					
	1 (AB)	2 (AB)	3 (AB)	4 (AB)	5 (KP)	6 (KP)
Polymyxin	≤0.5/S	≤0.5/S	≤0.5/S	≤0.5/S	1/S	1/S
Tigecycline	4/I	4/I	4/I	2/S	1/S	0.5/S
*Ceftazidime-Avibactam	NT	NT	NT	NT	27 mm/S	25 mm/S
Meropenem	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R	NT
Imipenem	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R	≥16/R
Piperacillin-tazobactam	≥128/R	≥128/R	≥128/R	≥128/R	≥128/R	≥128/R
Cefoperazone-sulbactam	≥64/R	≥64/R	32/R	≥64/R	≥64/R	≥64/R
Ticarcillin-clavulanic acid	≥128/R	≥128/R	≥128/R	≥128/R	≥128/R	NT
Ceftazidime	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R	≥64/R
Cefepime	≥32/R	≥32/R	≥32/R	≥32/R	≥32/R	≥32/R
Amikacin	4/R	8/R	≤2/S	NT	≥64/R	≥64/R
Tobramycin	≥16/R	≥16/R	≤1/S	≤1/S	≥16/R	NT
Ciprofloxacin	≥4/R	≥4/R	≥4/R	≥4/R	≥4/R	NT
Levofloxacin	≥8/R	≥8/R	≥8/R	≥8/R	≥8/R	≥8/R
Doxycycline	≥16/R	≥16/R	1/S	≥16/R	≥16/R	NT
Minocycline	8/I	8/I	≤1/S	≥16/R	≥16/R	NT
Sulfamethoxazole-trimethoprim	≤20/S	≥320/R	≥320/R	≥320/R	≥320/R	≥320/R

*The broth microdilution method was used for all antibiotics except Ceftazidime-Avibactam, which was tested by the Kirby-Bauer method.
CSF, cerebrospinal fluid; AB, Acinetobacter baumannii; KP, Klebsiella pneumoniae; S, susceptible; I, intermediate; R, resistant; NT, not tested.

Data Analysis

Statistical analysis was performed using RStudio (version 2021.09.1+372). Numeric data with a normal distribution were shown as mean ± standard deviation, and a paired *t*-test was conducted for the comparison between pre- and post-treatment variables. Non-normally distributed data were presented as median with interquartile range, and statistical analysis was performed by the Wilcoxon test. $P < 0.05$ was considered statistically significant.

RESULTS

Between November 2020 and November 2021, six patients with post-neurosurgical CNS infection treated with IVT+ IV polymyxin B were enrolled in the study, including five males and one female. The age of the patients ranged from 38 to 73 years with an average of 58 years. Primary neurological diseases and surgical methods are shown in **Table 2**. An EVD catheter was placed for all patients during the operation.

Of the six cases, four patients were confirmed with CRAB infection in CSF culture, three of whom also showed CRAB in sputum samples. The other two cases were CRKP-positive in both CSF and sputum culture. Before CSF culture results were available, all patients were treated with meropenem and vancomycin (**Table 3**). Additionally, three patients received cefoperazone-sulbactam and one received piperacillin-tazobactam. The time between onset of symptoms and known bacterial susceptibility was 3 days, then the treatment of IVT+IV polymyxin B was administered based on drug susceptibility tests. The mean duration of IVT+IV polymyxin TB administration was 14 ± 5.69 days (range, 6–20 days), while the mean duration to achieve sterile CSF was 10.33 ± 3.67 days (range, 5–14 days) (**Table 3**).

TABLE 2 | Clinical characteristics of patients enrolled in the study.

Case	Age (years)	Gender	Primary disease	Surgeries	Foreign body
1	38	Male	Aneurysm	Aneurysm embolization + Hematoma evacuation + EVD	EVD
2	64	Male	Moyamoya disease	Hematoma evacuation + EVD	EVD
3	61	Male	TBI	Hematoma evacuation + EVD	EVD
4	73	Female	TBI	Hematoma evacuation + EVD	EVD
5	57	Male	ICH	Hematoma evacuation + EVD	EVD
6	55	Male	Cerebellar infarction	Decompressive craniectomy + EVD	EVD

TBI, traumatic brain injury; ICH, intracerebral hemorrhage; EVD, external ventricular drain.

Clinical features and laboratory data before and after treatment are presented in **Table 4**. The median CSF/blood glucose ratio of the six patients increased significantly following IVT plus IV polymyxin B administration (0.013 pre-treatment vs. 0.684 post-treatment, $P < 0.05$). Glucose in CSF (0.1 vs. 4.95 mmol/L, $P < 0.05$) and GCS score (5.00 ± 2.28 vs. 7.17 ± 3.43, $P < 0.05$) also improved markedly after the polymyxin B treatment. Meanwhile, the number of polymorphonuclear leukocytes in CSF

TABLE 3 | Cerebrospinal fluid (CSF) culture and treatment strategies for each patient.

Case	CSF isolate	Susceptibility	Antibiotics usage prior to positive CSF culture	Concomitant infection	Time from onset of symptoms to IVT+IV polymyxin B administration (days)	Dose of IVT polymyxin B (mg/day)	Dose of IV polymyxin B (mg/kg/12 h)	Duration of IVT+IV polymyxin B (days)	CSF sterilization time (days)
1	AB	Carbapenem-resistant; Susceptible to polymyxin	Cefoperazone-sulbactam; Meropenem; Vancomycin	Pneumonia (AB+PA)	5	5	1.5	15	13
2	AB	Carbapenem-resistant; Susceptible to polymyxin	Meropenem; Vancomycin	No	3	5	1.5	9	7
3	AB	Carbapenem-resistant; Susceptible to polymyxin	Cefoperazone-sulbactam; Meropenem; Vancomycin	Pneumonia (AB)	5	5	1.5	6	5
4	AB	Carbapenem-resistant; Susceptible to polymyxin	Piperacillin-tazobactam; Meropenem; Vancomycin	Pneumonia (AB)	7	5	1.5	20	13
5	KP	Carbapenem-resistant; Susceptible to polymyxin	Cefoperazone-sulbactam; Meropenem; Vancomycin	Pneumonia (KP)	6	5	1.5	14	10
6	KP	Carbapenem-resistant; Susceptible to polymyxin	Meropenem; Vancomycin	Pneumonia (KP)	4	5	1.5	20	14

CSF, cerebrospinal fluid; AB, Acinetobacter baumannii; KP, Klebsiella pneumoniae; PA, Pseudomonas aeruginosa; IVT, intraventricular; IV, intravenous.

TABLE 4 | Clinical symptoms and laboratory data in patients.

Laboratory measurement	Beginning of CNS infection	Resolution of CNS infection	*P*-value
Temperature (°C)	39.3 ± 0.45	37.13 ± 0.21	<0.001
GCS	5.00 ± 2.28	7.17 ± 3.43	0.027
Serum			
WBC count ($\times 10^9$/L)	18.85 ± 6.41	7.99 ± 2.21	0.004
CRP (mg/L)	152.03 ± 48.96	19.23 ± 11.92	0.002
PCT (ng/mL)	0.86 ± 0.55	0.21 ± 0.20	0.023
Creatinine (μmol/L)	63.00 ± 38.13	58.50 ± 28.02	0.45
CSF			
Leukocytes (cells/μL)	11,525 (5,275, 21,412.5)	218 (31, 391.5)	0.028
Glucose (mmol/L)	0.1 (0.1, 0.7)	4.95 (4.0, 5.9)	0.028
CSF/blood glucose	0.013 (0.0, 0.1)	0.684 (0.7, 0.7)	0.028
Protein (g/L)	5.96 (3.4, 8.0)	2.41 (1.5, 4.2)	0.116

CNS, central nervous system; GCS, Glasgow Coma Scale; WBC, white blood cell; CRP, C-reactive protein; PCT, procalcitonin; CSF, cerebrospinal fluid. Data are presented as the mean ± standard deviation or median (interquartile range).

showed a significant decrease compared with pre-treatment tests (11,525 vs. 218 cells/μL, $P < 0.05$). Serum creatinine was not significantly different from the baseline level (63.00 ± 38.13 vs. 58.50 ± 28.02 μmol/L, $P = 0.45$).

TABLE 5 | Clinical outcomes and complications.

Outcome/complication	No. cases (%)	No. adverse events (%)	
		Grade 1–2	Grade 3 or higher
Cured case	6 (100)	–	–
28-day mortality	0 (0)	–	–
Adverse events			
Epilepsy	0 (0)	0 (0)	0 (0)
Acute kidney injury (AKI)	0 (0)	0 (0)	0 (0)
Skin hyperpigmentation	4 (66.6)	4 (66.6)	0 (0)

Clinical cure was achieved in all patients (100%). During treatment and follow-up, three patients (50%) presented with hydrocephalus because of severe infection and two of them subsequently underwent VPS surgery for this complication. Four patients (66.6%) had skin hyperpigmentation after polymyxin usage. No drug-related epilepsy or AKI were found (**Table 5**). Furthermore, no adverse events of grade 3 or higher were observed.

DISCUSSION

CHINET (see footnote 1) online data for 2021 show that the isolation rates of AB and KP in CSF were 11.3 and 9.1%,

TABLE 6 | Studies of CNS infections treated with intrathecal (IT) or intraventricular (IVT) polymyxin B*.

Author/Year	No. cases	Pathogens (No.)	IT/IVT antibiotic and dosage	Duration of IT/IVT antibiotics (days)	Outcome
Segal-Maurer et al. (13)	1 (Adult)	Ceftazidime-Resistant *K. pneumoniae*	Polymyxin B 5 mg/day	7	Cured
Piparsania et al. (14)	1 (32-week-old infant)	Carbapenem-resistant *A. baumannii*	Polymyxin B 4 mg/2 days	28	Cured
Guo et al. (15)	1 (Adult)	Carbapenem-resistant *A. baumannii*	Polymyxin B 5 mg/day for 10 days, then 2.5 mg/12 h	N/A	Cured
Pan et al. (7)	23 (Adults)	Carbapenem-resistant *A. baumannii*	Polymyxin B 5 mg/day	N/A	21/23 cured
Chen et al. (16)	28 (Adults)	Carbapenem-resistant *A. baumannii* (14); Carbapenem-resistant *K. pneumoniae* (9); Carbapenem-resistant *Pseudomonas aeruginosa* (3); Carbapenem-resistant *Enterobacter cloacae* (2)	Polymyxin B 5 mg/day	14.96 ± 4.28	23/28 cured
Zhong et al. (18)	1 (Adult)	Carbapenem-resistant *A. baumannii*	Polymyxin B 10 mg/day for 4 days, then 10 mg/2 days	19	Cured
Xing et al. (19)	1 (14-year-old adolescent)	Carbapenem-resistant *A. baumannii*	Polymyxin B 5 mg/day for 4 days, then 5 mg/2 days	9	Cured
Li et al. (20)	1 (Adult)	Carbapenem-resistant *A. baumannii*	Polymyxin B 5 mg/day + tigecycline 5 mg/day	16	Cured
Chen et al. (17)	21 (Adults)	Carbapenem-resistant *A. baumannii*	Polymyxin B 5 mg/day	18.19 ± 12.36	17/21 cured
Present study	6 (Adults)	Carbapenem-resistant *A. baumannii* (4); Carbapenem-resistant *K. pneumoniae* (2)	Polymyxin B 5 mg/day	14 ± 5.69	All cured

CNS, central nervous system; IT, intrathecal; IVT, intraventricular; K. pneumoniae, Klebsiella pneumoniae; A. baumannii, Acinetobacter baumannii; N/A, Not applicable. Data of duration are presented as the mean ± standard deviation.

**Database: PubMed; Keywords: [intraventricular polymyxin B(Title/Abstract)] OR [intrathecal polymyxin B(Title/Abstract)].*

respectively, ranking second and third. Furthermore, a marked increase in drug resistance of AB to imipenem was seen from 31% in 2005 to 71.5% in 2021, and a similar increase was seen in the resistance rate of AB to meropenem—from 39% in 2005 to 72.3% in 2021. Increased resistance of KP to these two antibiotics was also observed (from 3.0% in 2005 to 23.1% in 2021 for imipenem, and from 2.9% in 2005 to 24.4% in 2021 for meropenem). Clinical data on CRAB/CRKP in our hospital exhibited similar trends.

CNS infection is one of the most common complications after neurosurgery (9), and such CNS infections caused by MDR/XDR Gram-negative bacteria after neurosurgery have a higher mortality rate. Treatment of these infections is difficult due to the limited choice of antibiotics and the presence of the BBB (10). Polymyxins are one of the most effective classes of antibiotics against MDR/XDR Gram-negative bacteria (11). However, polymyxins have difficulty penetrating through the BBB when administered by the IV route due to the polycationic structure and higher molecular weight of these antibiotics (12). Consequently, the CSF distribution of polymyxin after IV injection is relatively low, with a reported CSF-to-serum concentration ratio of 0.07 (3). To increase the concentration of polymyxin B in the CSF, the IVT route was conducted.

The IDSA guidelines state that if carbapenem resistance is suspected then a combination of IV and IVT polymyxin B is recommended, especially when systemic IV medication is not effective (4). Pan et al. (7) collected 61 cases with CSF culture of CRAB and found that the intrathecal/intracerebral polymyxin B group had significantly lower 28-day mortality and higher rates of microbiological clearance compared with the IV group. Therefore, the IVT+IV polymyxin B approach is suggested as the optimal treatment option for CNS infections with MDR/XDR Gram-negative bacteria. In the current study, IVT+IV polymyxin B was used to treat patients with CRAB or CRKP in CSF cultures, and all patients were successfully cured without severe adverse events or 28-day mortality.

Despite the success of IVT+IV polymyxin B treatment, the optimal dose/duration of IVT polymyxin B remains unclear. Current IDSA guidelines for IVT polymyxin B recommend a dose of 5 mg/day for 10–21 days (4). In the International Consensus Guidelines for the Optimal Use of the Polymyxins, the dose is 5 mg/day for IVT polymyxin B with a mean duration

of 18 days (8). However, the quality of evidence is low as the guidelines have stated. Literature in the PubMed database with the keywords of intraventricular/intrathecal polymyxin B was reviewed (**Table 6**). Most of the identified papers/studies were case reports, including infants and pediatric patients. Chen et al. (16) reported a total of 28 patients with MDR/XDR Gram-negative bacilli intracranial infection after neurosurgery. IVT polymyxin B at a dose of 5 mg/day was used for 14.96 ± 4.28 days (range, 9–23 days) and the clinical cure rate was 82.1% (23/28). Chen et al. (17) recruited a total of 21 patients with MDR/XDR-AB-induced intracranial infection after neurosurgeries, all of which received IVT polymyxin B at a dose of 5 mg/day for 18.19 ± 12.36 days, and the clinical cure rate was 81.0% (17/21). In the present study, a daily IVT dose of 5 mg polymyxin B was used. The mean duration of administration was 14 ± 5.69 days (range, 6–20 days), the mean duration to achieve sterile CSF was 10.33 ± 3.67 days (range, 5–14 days), and the clinical cure rate was 100% (6/6). Based on existing evidence and data from the current study, a daily IVT dose of 5 mg polymyxin B is considered the optimal adult dose, and the treatment duration of IVT polymyxin B should be at least 10 days.

Regretfully, the concentration of polymyxin B in CSF, which might be able to guide the optimal dosage of polymyxin B, was not monitored in most studies. In a recent study, Ni et al. (21) administered colistin (also known as polymyxin E) at 100,000 units/24 h *via* IVT to 10 patients with CNS infections. Three days later, the concentration of colistin in the CSF was determined by selective ultra-performance liquid chromatography (UPLC) at 2, 4, 6, 8, 12, and 24 h after IVT colistin administration. The measured trough concentration was 1.12–8.33 μg/mL, which was above the minimum inhibitory concentration (MIC) of 0.5 μg/mL. Microbial cure was observed in all patients. However, information on the CSF pharmacokinetics of polymyxin B is lacking.

Polymyxin B has been reported to have side effects such as nephrotoxicity and skin hyperpigmentation. Using the definition from Kidney Disease Improving Global Outcomes (KDIGO) guidelines (22), no cases of AKI were observed in the current study. Furthermore, the incidence and severity of nephrotoxicity was reported to be rare and mild in other studies (7, 16). This suggests the dosage of combined administration of polymyxin B in the present study was comparatively safe for renal function. The kidneys are the primary sites of polymyxin elimination, thus the dosage must be carefully monitored (23), and it is therefore recommended to monitor the blood/CSF concentration of polymyxin B. In the current study, all patients were successfully cured without AKI, epilepsy or other adverse events of grade 3 or higher. Recent case reports have mentioned the adverse effect of skin hyperpigmentation following IV polymyxin B treatment (24–26), but this is usually mild and can gradually disappear after discontinuation of medication (27). In the present study, skin hyperpigmentation was observed in four patients (66.6%).

There are some limitations to the current study, including the small sample size, the retrospective nature of the study, the lack of control groups, and the absence of monitoring the concentration of polymyxin in the CSF or blood. These limitations must be considered when drawing conclusions from the study.

CONCLUSION

In conclusion, IVT combined with IV polymyxin B is a safe and effective treatment for post-neurosurgical patients with intracranial infection caused by MDR/XDR Gram-negative bacteria. Furthermore, a daily IVT dose of 5 mg polymyxin B is considered the optimal adult dose. However, the small sample size and lack of a control group limit the reliability of such conclusions. Further large-scale randomized controlled trials are warranted to confirm the findings from this study.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Clinical Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HL, WY, and GW contributed to data collection. HL and WY performed the statistical analysis. HL wrote the first draft of the manuscript. WY, GW, and HC wrote sections of the manuscript. WY and HC reviewed the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Indications for hand and glove disinfection in Advanced Cardiovascular Life Support: A manikin simulation study

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Background and aim: There are no investigations on hand hygiene during cardiopulmonary resuscitation (CPR), even though these patients are at high risk for healthcare-associated infections. We aimed to evaluate the number of indicated hand hygiene per CPR case in general and the fraction that could be accomplished without delay for other life-saving techniques through standardized observations.

Materials and methods: In 2022, we conducted Advanced Cardiovascular Life Support (ACLS) courses over 4 days, practicing 33 ACLS case vignettes with standard measurements of chest compression fractions and hand hygiene indications. A total of nine healthcare workers (six nurses and three physicians) participated.

Results: A total of 33 training scenarios resulted in 613 indications for hand disinfection. Of these, 150 (24%) occurred before patient contact and 310 (51%) before aseptic activities. In 282 out of 310 (91%) indications, which have the highest impact on patient safety, the medication administrator was responsible; in 28 out of 310 (9%) indications, the airway manager was responsible. Depending on the scenario and assuming 15 s to be sufficient for alcoholic disinfection, 56–100% (mean 84.1%, SD ± 13.1%) of all indications could have been accomplished without delaying patient resuscitation. Percentages were lower for 30-s of exposure time.

Conclusion: To the best of our knowledge, this is the first study investigating the feasibility of hand hygiene in a manikin CPR study. Even if the feasibility is overestimated due to the study setup, the fundamental conclusion is that a

relevant part of the WHO indications for hand disinfection can be implemented without compromising quality in acute care, thus increasing the overall quality of patient care.

KEYWORDS

BLS (Basic Life Support), hand disinfection, infection prevention, CPR - cardiopulmonary resuscitation, life support, ACLS (Advanced Cardiovascular Life Support), glove disinfection, hospital acquire infection

Introduction

Background/rationale

In Germany, ∼84 of every 100,000 persons annually suffer an acute cardiac arrest requiring early cardiopulmonary resuscitation (CPR), activation of the emergency chain, Advanced Cardiovascular Life Support, transportation, and integrated critical care (1). Hospital-acquired infections (HAI), mainly device-associated bloodstream infections, urinary tract infections, and pneumonia, significantly impact the mortality and morbidity of these patients, especially in those with hypoxemic brain injury (2, 3). Proper hand hygiene, especially before aseptic procedures, can significantly reduce these infections (4), especially in critical care settings. Under the recognition of national recommendations, the overall objective is to accomplish 80% of all indicated hand disinfection (5, 6). These comprise five main indications according to the five moments of hand hygiene: before touching the patient (WHO-1), before clean/aseptic procedures (WHO-2), after body fluid exposure/risk (WHO-3), after touching a patient (WHO-4), and after touching the patient's surroundings (WHO-5) (4, 6).

Currently, there are no investigations on the significance of infection prevention and control (IPC) in out-of-hospital cardiac arrest (OHCA) or in-hospital cardiac arrest (IHCA). Learning material for rescue service staff considers hand hygiene to be "good medical practice" and partially shows hand disinfection in educational videos provided by the American Heart Association (AHA) (7, 8). However, the need for IPC and especially hand hygiene is poorly emphasized in educational material, despite the effect of hand hygiene on nosocomial infection in general and in the ICU is high and considered a cornerstone of patient safety (9–13).

In general, hand hygiene is not conducted consistently in emergency situations such as trauma resuscitation (14) or Advanced Cardiovascular Life Support (ACLS). These situations involve potentially hazardous invasive procedures under time pressure, such as intravenous or intraosseous catheter placement, medication preparation and administration, endotracheal intubation, endotracheal suctioning, thoracocentesis, and in some cases, mini-thoracotomy, pericardiocentesis, or even clamshell-thoracotomy (15). All of these interventions are aseptic clean procedures according to the WHO's moments of hand disinfection (4, 6), and it may appear that they can be sacrificed to save time because the immediate demand for life-saving procedures precludes the time-consuming hand or glove disinfection.

Survivors of sudden cardiac arrest who require critical care are susceptible to infections with devastating effects like sepsis. In addition, post-hypoxic brain tissue and its penumbra are most vulnerable to inflammation, and outcomes may be even worse with fever (16). Therefore, rational infection prevention should be an integral part of life support from the first patient contact.

Healthcare providers are trained in CPR proficiency according to international or national recommendations for resuscitation provided by the International Liaison Committee on Resuscitation (ILCOR). The most widely used training concepts include scenarios provided by the Advanced Cardiovascular Life Support (ACLS) and Pediatric Advanced Life Support (PALS) programs that are available all around the world (8). ACLS and PALS primarily consist of standardized simulation-based learning for groups of six (about 4–7 persons) on manikins. These individuals share roles and responsibilities for different CPR actions (see Figure 1).

1. TL – Team leader
   Guides and supervises the team
2. A – Airway manager
   Conducts bag mask ventilation, oxygen supplementation, airway or tube suctioning, and intubation
3. C – Compressor
   Completes the BLS-Check and provides chest compressions
4. MD – Monitor/Defibrillation
   Attaches electrodes and the monitor, delivers electrotherapy, and supervises the quality of chest compressions and ventilations
5. T – Timekeeper
   Records the amount of time taken and documents the CPR
6. IV – Medication administrator
   Establishes venous or intraosseous access and prepares and administers IV/IO medication

FIGURE 1
A prototypical ACLS training scenario with six members: the team leader (TL) and timekeeper (T) normally do not interact with the patient and do not perform invasive procedures. The compressor (C) and monitor/defibrillator (MD) may change roles and provide chest compressions to maintain cerebral and coronary perfusion. They also both typically do not perform invasive procedures. The monitor/defibrillation manager (MD) attaches electrodes to the patient's chest, analyses the ECG, and delivers shocks as indicated. The airway manager (A) ventilates with a bag valve mask, clears the airway if it is obstructed, and administers oxygen. If indicated, the airway manager places a supraglottic or endotracheal airway device. Hence, invasive procedures are sometimes performed by the airway manager or an assisting person (e.g., M or C), depending on the situation and crew resources. The medication administrator (IV) establishes intravenous or intraosseous access and prepares and administers medications according to the CPR or ROSC algorithm as identified and communicated by the team leader. The medication administrator is the person with the most expected invasive procedures and therefore the most hand hygiene indications. After each scenario, the roles were changed. It is noted that individuals are not wearing hospital clothing or personal protective equipment due to the training settings. N95 respirators were worn due to the COVID-19 pandemic. All depicted persons gave written informed consent for photography.

During these courses, team performance and communication are evaluated and reflected on while debriefing. The training is conducted by certified course instructors who guide the trainees through different prototypical case vignettes of pre-arrest, arrest, and combined scenarios (17), as shown in Figure 2.

Objective

This observational study aimed to evaluate how many indications are followed for hand hygiene per CPR and according to the five moments of hand hygiene and per case occurrence, how many of these indicated hand disinfections could be accomplished without delaying patient resuscitation.

We hypothesized that more than 80% of all WHO moments indicating the need for hand hygiene could be performed without losing time for other life-saving actions in the ACLS algorithms.

Methods

Study design and setting

In 2022, we held ACLS courses over 4 days with 4–5 providers practicing 33 ACLS case vignettes in an ACLS course (2 days), an ACLS refresher course (1 day), and an ACLS course for experienced providers (1 day). The case vignettes (see Tables 1, 2 and Figure 2) consisted of either vignette Type A1, A2, B, or C. In the ACLS courses, provided by NOTIS e.V (Notfallmedizinisches Trainingszentrum in Singen, a registered association), we used an AmbuMan Airway Manikin (Ambu GmbH, Bad Nauheim, Germany) and an ALSi Monitor (iSimulate, 3b Scientific GmbH, Hamburg, Germany). In addition, we used real-life equipment typically available in German hospitals and emergency medical services. This included a bag valve mask with oxygen supply, a backpack with ampules, sterile syringes, suction, an IO access device (Arrow EZIO, Teleflex, Morrisville, USA), IV catheters (Vasofix Safety, B. Braun, Melsungen, Germany), infusion bags, and documentation cards.

Participants

Recruited participants ($n = 9$) were ICU nurses and anesthesiologists from different institutions in southern Germany. All participants were informed about the observation and agreed to participate. Further instructions were not needed as participants were not expected to simulate or conduct hand hygiene or other IPC measures that would have deviated from the AHA course protocol. All participants rotated through the roles with different scenarios and were evaluated in the role of the team leader.

According to the Ethical Committee of the Physician Board Association of Baden-Württemberg, no ethical approval was needed. The data was obtained anonymously. The study protocol aligns with the Declaration of Helsinki and the German Physician Professional Code: there was no intervention in the personal, psychological, or somatic integrity of the participants, no data that could be retraced to a single person, and there was no data retrieved from patients.

Variables and data sources

The primary variables consisted of the following:

a) The number of observed hand disinfection indications according to the WHO

b) The type of moment indicating hand disinfection (WHO 1–5)
c) The time from the indication of medical action to the *de facto* conduction of the action ("action time")
d) The person responsible for hand disinfection according to the ACLS – roles
e) Type of CPR scenario and first identified heart rhythm

The secondary variables included arrest time and chest-compression-fraction (CCF– a surrogate parameter for CPR quality). CCF, arrest time (AT), and compression time (CT) were simultaneously measured using a stopwatch. CCF was calculated as the index of CT/AT.

Hand disinfection was "feasible" if the response time between the indication and the conduction thereof was at least 60 s, which includes 15 s of exposure time to the disinfectant (18). Furthermore, an additional 45 s for an aseptic procedure were granted according to the consensus of six specialists for CPR training.

The whole data was collected by the principal investigator as a certified ACLS instructor, specialist for infection control, and medical educator (single observer approach).

Bias

We addressed the observer bias in this single-researcher approach by using prototypical case vignettes with easily reproducible choreography to maintain validity and reliability. One can question not using video recording but rather "observed" results. However, the prototypical cases are standardized internationally with a clearly defined structure, so we decided not to use videos because they could distract trainees and are not an integral part of certified AHA (American Heart Association) courses. Therefore, we combined the measurements of hand disinfection [which are used the same way in classical audits of hand disinfection (19)] with instructor-based observations (including measurements of CCF and team performance).

The performance bias could have occurred if there would have been any feedback on hand hygiene to the trainees and therefore improved performance in hand hygiene. However, at this point in the project, we did not provide any feedback on hand hygiene to limit this bias and maintain the ACLS training structure.

Study size

We aimed for at least 30 ACLS training scenarios for better reproducibility and to rule out outlier scenarios.

Statistical methods

We used descriptive statistics of the scenarios with standard measurements of CCF and hand hygiene indications. Statistics,

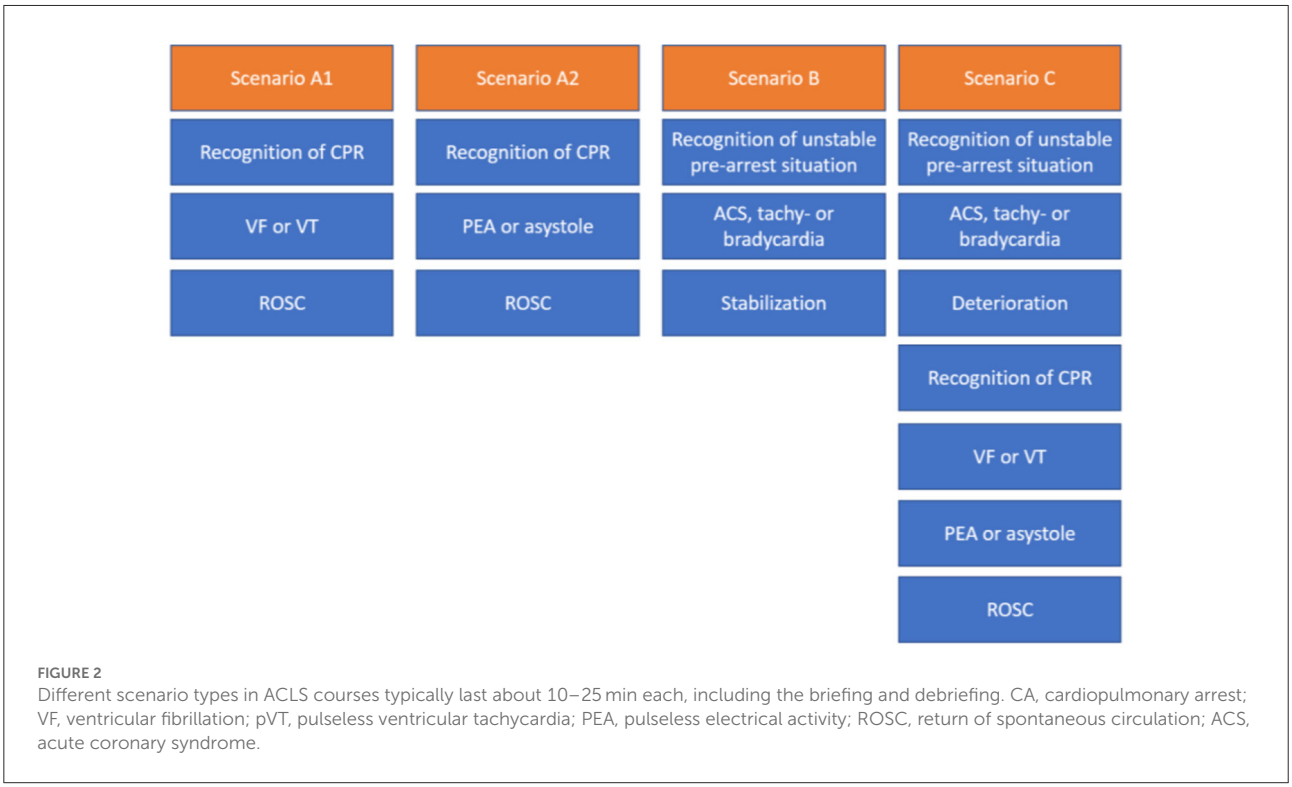

FIGURE 2
Different scenario types in ACLS courses typically last about 10–25 min each, including the briefing and debriefing. CA, cardiopulmonary arrest; VF, ventricular fibrillation; pVT, pulseless ventricular tachycardia; PEA, pulseless electrical activity; ROSC, return of spontaneous circulation; ACS, acute coronary syndrome.

TABLE 1 Scenarios in ACLS (Advanced Cardiovascular Life Support Course), in ACLS-R (ACLS-refresher course for providers with preceding certification in ACLS, and ACLS-EP (ACLS for experienced providers with more complex or rare cases of cardiac arrest and peri-arrest).

No	Scenario description	Group	Team	Type	WHO2 count	WHO-2 realisable	Initial rhythm	Initial rhythm detection time	Seconds to first i.v-drug indicated
1	Lifeless person at the train station, asystole	ACLS	5	A	10	50.00%	ASY	30	30
2	ED Patient with acute deterioration and ventricular fibrillation	ACLS	5	A	7	100.00%	VT/VF	62	180
3	Unresponsive person at the lakes, hypoglycaemia, ventricular fibrillation	ACLS	5	A	14	78.57%	VT/VF	50	180
4	Obstetric Ward, collapsed visitor, ventricular fibrillation	ACLS	5	A	6	66.67%	VT/VF	148	180
5	Geriatric patient after fracture of the femoral neck, deterioration after aspiration, pulseless ventricular tachycardia	ACLS	5	A	10	80.00%	VT/VF	55	180
6	Oncological patient, asystole	ACLS	5	A	15	26.67%	ASY	60	60
7	Post ACS patient at the rehabilitation hospital, initially stable bradycardia, i.v already placed, later deterioration	ACLS	5	B	6	33.33%	BRADY	30	30
8	ED patient with ACS, instable bradycardia, i.v already placed	ACLS	5	B	4	100.00%	BRADY	105	105
9	Patient in the recovery room after cholecystectomy, initially stable supraventricular tachycardia; i.v already placed	ACLS	5	B	6	100.00%	SVT	18	
10	Elderly patient at the traumatological ward, fracture of the femoral neck, irregular instable supraventricular tachycardia, i.v. in place	ACLS	5	B	7	57.14%	SVT	27	
11	Unconscious patient in the park with acute coronary syndrome, instable bradycardia	ACLS	5	B	9	100.00%	BRADY	61	61
12	Patient with STEMI in the emergency department, instable broad complex tachycardia, then ventricular fibrillation and later asystole i.v. in place	ACLS	5	C	7	57.14%	BRADY	40	40
13	Old lady with abdominal pain due to NSTEMI, bradycardia, later ventricular fibrillation	ACLS	5	C	9	44.44%	BRADY	36	36
14	Patient with alcohol intoxication at a parking garage, instable superventricular tachycardia later ventricular fibrillation and asystole	ACLS	5	C	5	100.00%	SVT	67	

(Continued)

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TABLE 1 (Continued)

No	Scenario description	Group	Team	Type	WHO2 count	WHO-2 realisable	Initial rhythm	Initial rhythm detection time	Seconds to first i.v-drug indicated
15	Mass casualty incident / overcrowding in the emergency department, patient with STEMI and instable bradycardia, later pulseless ventricular tachycardia and pulseless electrical activity, i.v. in place	ACLS	5	C	9	55.56%	BRADY	40	40
16	Dialysis patient with acute coronary syndrome and hyperkalaemia, instable bradycardia, ventricular fibrillation, later asystole	ACLS	5	C	11	54,55%	BRADY	40	40
17	Emergency department patient with STEMI, instable bradycardia, later ventricular fibrillation and asystole, i.v. in place	ACLS	5	C	11	63,64%	BRADY	28	28
18	Patient at the cardiological ward after percutaneous coronary intervention, STEMI, instable supraventricular tachycardia, then ventricular fibrillation and later pulseless electrical activity	ACLS	5	C	5	100.00%	SVT	27	
19	Emergency department patient with acute hemiparesis, suspected aortic dissection, bradycardia, ventricular fibrillation and later asystole, i.v.in place	ACLS	5	C	10	30.00%	BRADY	34	34
20	Patient at the urological ward, bradycardia, later ventricular fibrillation and asystole	ACLS	5	C	10	80.00%	BRADY	63	63
21	Patient at the orthopaedic ward after surgery, bradycardia, ventricular fibrillation and later pulseless electrical activity	ACLS	5	C	8	37.50%	BRADY	30	30
22	Lay rescuer CPR at the airport after two shocks by AED, persistent ventricular fibrillation	ACLS-R	4	C	8	37.50%	VT/VF	20	
23	Patient with instable bradycardia with AV-Block III° with conversion to ventricular fibrillation and later pulseless electrical activity	ACLS-R	4	C	13	76.92%	BRADY	64	64
24	Young athlete with initially stable supraventricular tachycardia and ventricular fibrillation after adenosine cardioversion (Wolff-Parkinson-White-Syndrome)	ACLS-R	4	C	10	100.00%	SVT	212	212
25	Elderly patient in an ice cream café, bradycardia due to NSTEMI, later ventricular fibrillation and pulseless electrical activity	ACLS-R	4	C	19	73.68%	BRADY	58	58

(Continued)

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TABLE 1 (Continued)

No	Scenario description	Group	Team	Type	WHO2 count	WHO-2 realisable	Initial rhythm	Initial rhythm detection time	Seconds to first i.v-drug indicated
26	Retirement Home, collapsed nurse with instable bradycardia and later ventricular fibrillation	ACLS-R	4	C	12	41.67%	BRADY	30	30
27	ICU-patient after robotic prostatectomy, acute coronary syndrome with supraventricular tachycardia, later pulseless ventricular tachycardia, and asystole	ACLS-R	4	C	8	100.00%	SVT	40	
28	Elderly women in long term caring home, AV-Block III degree, later ventricular fibrillation, and pulseless electrical activity	ACLS-R	4	C	14	64,29%	BRADY	45	45
29	Syncopal women at the supermarket, initially stable supraventricular tachycardia and later ventricular fibrillation	ACLS-R	4	C	8	100,00%	SVT	32	
30	Pregnant in 22nd gestational week, thrombosis with obstructive shock due to pulmonary embolus, supraventricular tachycardia and pulseless electrical activity, emergency c-section	ACLS-EP	4	C	14	85,71%	SVT	45	
31	Young women with "herbal" intoxication and initially stable supraventricular tachycardia and later ventricular fibrillation	ACLS-EP	4	C	8	100,00%	SVT	50	
32	Electricity worker working at the ceiling installations of a private swimming pool, high level fall after accidental electric shock from a ladder into the pool, traumatic brain injury, drowning, pulseless electrical activity	ACLS-EP	4	C	9	22.22%	ASY	25	25
33	Lightning strike at a music festival, mass casualty incident with 20 persons and two persons in cardiac arrest, pulseless electrical activity	ACLS-EP	4	C	8	62,50%	ASY	40	40

"Initial rhythm detection time" was the time from arrival of the crew to recognition of the underlying rhythm. "Seconds to first i.e., drug indicated" was the time after assessing the patient until the first i.e., medication was necessary according to the algorithms. Missed values in some scenarios were those with a variable time of first indication, e.g., in initial stable conditions leading to CPR later, ongoing therapy by "others" (team arrives during ongoing therapy), pure pre-arrest scenarios (without CPR), or difficult conditions with the need for evacuation/transportation of the victim.

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TABLE 2 Example of a prototypical case with possible indications for hand hygiene.

Timeline	Situation /Algorithm	TL	TK	C	A	M	IV
−3 min	Victim lies collapsed on the floor of the hospital hallway. Alarm on collapse, bystander BLS						
	ACLS Team informed	**WHO-1** Assignes roles and responsibilities	**WHO-1***	**WHO-1***	**WHO-1***	**WHO-1***	**WHO-1***
+ 0 m 0 s		ACLS Team approaches & Safety check					
+0 m 15 s	BLS Algorithm		Starts recording	BLS check, Tap & Shout and check for pulse and breathing			
+0 m 25 s	BLS Algorithm			Recognition of Arrest Start thorax compression			
+0 m 30 s	Arrest Algorithm				Start ventilation with mask - bag device and oxygene supply	Start ECG electrode placement	**WHO-2**** Prepare IV-Access
+0 m 40 s	VF/VT Algorithm	Communicates Algorithm				Recognition of VF Loading Defibrillator	
+0 m 50 s	VF/VT Algorithm			Re-Start CPR after shock	Re-Start ventilation after shock	Clear Team, Defibrillation	
+1 m 30 s	VF/VT Algorithm						**WHO-2*** Placement of i.v. Access, exposure to blood possible
+2 m 0 s	VF/VT Algorithm						**WHO-2/3*** Preparation of Infusion bag
+2 m 50 s	VF/VT Algorithm					2nd ECG Check recognition of VF loading defibrillator	
	VF/VT Algorithm			Re-Start CPR after shock	Re-Start ventilation after shock	Clear Team, SHOCK	
+3m 0 s	VF/VT Algorithm						**WHO-2*** Connection of crystalloid with venous access, possible contamination with blood
	VF/VT Algorithm						**WHO-2/3**** [Preparation of 1mg epinephrine]

(Continued)

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TABLE 2 (Continued)

Timeline	Situation /Algorithm	TL	TK	C	A	M	IV
+4m 0 s	VF/VT Algorithm						**WHO-2**** Administration of epinephrine
+4m 50 s	VF/VT Algorithm					2nd ECG Check Recognition of VF Loading defibrillator	**WHO-2/3**** [Prepare 2nd dose epinephrine]
	VF/VT Algorithm			Re-Start CPR after shock	Re-Start ventilation after shock	Clear Team, shock	
+5m 20 s	VF/VT Algorithm						**WHO-2**** [Prepare 1st dose Lidocaine or Amiodarone]
+6m 50 s	VF/VT Algorithm					3nd ECG Check Recognition of ROSC	
+7m 10 s	ROSC Algorithm	Communicates Algorithm		BLS Check Pulse, but unresponsive	Ventilation check	Apply 12-Lead ECG	
+7m 40 s	ROSC Algorithm STEMI Algorithm	Communicates Algorithm				ECG: Recognition of STEMI	**WHO-2*** Start preparation of ASS i.v.
+8m 10 s	ROSC Algorithm STEMI Algorithm			Preparation of Intubation e.g. by "C" for "A"	Suctioning Airway in case of regurgitation **WHO-3***		**WHO-2** Administration preparation of ASS i.v.
+9m 0 s	ROSC Algorithm STEMI Algorithm				Restarts Ventilation	**WHO-2*** Fingertip puncture	**WHO-2*** [Preparation of Heparine]
+10m 0 s	ROSC Algorithm STEMI Algorithm					Glucose measurement	**WHO-2*** Administration of Heparine
+10m 30 s	ROSC Algorithm STEMI Algorithm			Intubation ready (ET-Tube with guidewire)			**WHO-2**** [Preparation of hyponotic]
+11 m 0 s	ROSC Algorithm STEMI Algorithm				Preparation for Intubation		**WHO-2*** Administration of hypnotic
+11 m 30 s	ROSC Algorithm STEMI Algorithm			Assist intubation	**WHO 2*** Intubation attempt, suctioning airway	Recognize Monitor changes	
+11 m 45 s	ROSC Algorithm STEMI Algorithm			Fixation of the ET-Tube	**WHO 2/3**** Apply mechanical ventilation and capnography		

(Continued)

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TABLE 2 (Continued)

Timeline	Situation /Algorithm	TL	TK	C	A	M	IV
	ROSC Algorithm STEMI Algorithm			Prepare Transport of patient	Prepare Transport of patient	Prepare Transport of patient	Prepare Transport of patient
+X	Handover to second Team (cathlab)	Communicates with cath lab crew					
	Team dismisses	WHO-5*	WHO-5*	WHO-3/4*	WHO-3/4*	WHO-3/4*	WHO-3/4*
	Structured Debriefing	Communicates with ACLS crew					

In this algorithm-based case and a team with six members, there are altogether 30 indications for hand hygiene that can be counted. In this theoretical "algorithm-based" scenario, we assumed that each i.v. medication preparation needs a separate hand disinfection before (which is realistic whenever the airway manager is involved in other interrupting actions). Errors and deviations from the algorithm are not considered in this table, and thus reality can further deviate from these estimations. Preparations with the possibility of prefilled syringes are placed in brackets. Time estimation was considered 15 s for hand disinfection. *Feasible. **Feasible for 15 s but not for 30-s hand disinfection.

including explorative data analyses and Mann-Whitney U-test, were conducted using Microsoft Excel (Microsoft Corporation, Redmond, USA), Addinsoft XL STAT (Addinsoft Inc., New York, USA), and IBM SPSS 27.0 (IBM Corporation, Armonk, USA).

Results

Participants and descriptive data

Overall, we examined 33 scenarios conducted in two ACLS courses with five and four participants, respectively, and guided by three certified instructors each. The participants were six nurses and three anesthesiologists eligible for ACLS courses according to the AHA. The instructors were physicians with an AHA instructor certification in ACLS (Advanced Cardiovascular Life Support) and ACLS-EP (ACLS for Experienced Providers, a course giving deeper insight into life support and special conditions, such as drowning, pregnancy, and intoxications). The cases presented during the course are shown in Table 1. Chest compression fractions ranged from 55.8 to 97.0 % (mean 81.2%), mainly depending on the scenario type (see Table 1) and learning progress.

Main results

Overall, 613 indications for hand disinfection could be observed in the 33 scenarios. Of these indications, 150 occurred before touching a patient (WHO-1), 310 occurred before clean/aseptic procedures (WHO-2), three occurred after body fluid exposure risk (WHO-3), and 150 occurred after touching a patient (WHO-4) or after contact with the patient's surroundings (WHO-5) indications. WHO-1, WHO-4, and WHO-5 hand disinfection indications were considered appropriate to carry out before attending to the patient and after handover to other teams of the emergency chain. Due to the training setting on manikins, the WHO-3 indications varied depending on the case choreography (e.g., description of vomiting or dislocation of peripheral lines). The WHO-2 moments occurred most frequently and at different stages during the scenarios (e.g., IV medication administration or airway manipulation) and are therefore the most significant to analyze in detail.

Per one scenario, we detected between 14 and −27 WHO 1–5 indications (mean 18.6, SD 3.2) and between 4 and 19 WHO-2 indications (mean 9.0, SD 3.0). Depending on the scenario, 56–100% (mean 84.1%, SD = 13.1%) of all indications could have been accomplished without delaying patient resuscitation.

Of the 310 WHO-2 indications (before an aseptic procedure), 282 suggested the responsibility of the medication administrator (91.0%) and 28 of the airway manager (9.1%). For

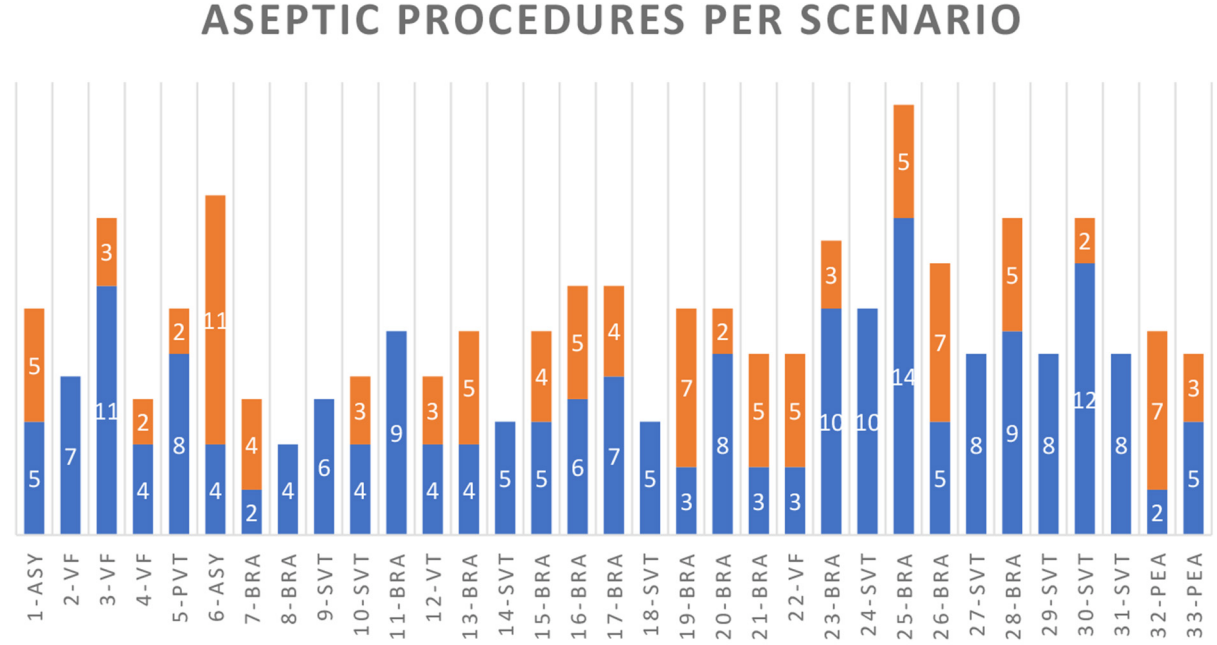

FIGURE 3
WHO-2 indications for cases 1–33 with the scenarios: asystole (ASY), ventricular fibrillation (VF), pulseless ventricular tachycardia (PVT), bradycardia (BRA), unstable VT with a pulse (VT), supraventricular tachycardia (SVT), and pulseless electrical activity (PEA). Blue columns indicate the hand or glove disinfection indications that were feasible and orange for those that were not.

each CPR scenario, the medication administrator had to expect 4–17 WHO-2 indications and airway managers 0–2 WHO-2 indications. There were no WHO-2 indications detected for other team members.

For the medication administrator, 186 of 282 hand disinfections (66.0%) and for the airway manager, 22 of 28 (78.6%) hand disinfections would have been feasible without delay for patient care according to ACLS algorithms (see Figure 3). In scenarios with an immediate need for IV access (unstable bradycardia, asystole, and PEA), the count of unfeasible hand disinfection was significantly higher than in scenarios with subsequent indications for IV drug administration, such as supraventricular tachycardia (SVT) and ventricular tachycardia/ventricular fibrillation (pVT/VF) ($p = 0.002$). In contrast, the count of feasible hand disinfection did not significantly differ between these scenario types ($p > 0.05$). Scenario 22 (VF) was an exception, as lay rescuer CPR with two given shocks occurred before the high-performance team arrived, and therefore an early administration of epinephrine was required.

The feasible hand hygiene indications for the airway manager (or any person assisting) include the preparation of the endotracheal tube with a guidewire, laryngoscopy, endotracheal intubation, and the ventilator setup for a patient not awakening after ROSC with sufficient time for hand or glove disinfection while the compressor repeats the BLS check according to the ROSC algorithm. On the contrary, a difficult airway during bag-mask ventilation was simulated in some cases ("cannot ventilate" – situation), which, to our interpretation, shows no opportunity for hand hygiene without delay for the patient's airway safety.

Regarding the medication administrator, participants placed an IV or IO access early with preparing and connecting a crystalloid infusion. However, as the identification of the heart rhythm determines the algorithm, IV access and administration of medications are necessary as soon as the algorithm is clear. In most ACLS cases, the approach to the patient, BLS check, attachment of the monitor, and correct identification of the heart rhythm ranged between 18 and 212 s (mean 51.85 s, SD 37.7). In CPR scenarios with the VT/VF algorithm, the first IV medication should be considered after the second shock (after 240 s of CPR/2 CPR cycles), providing enough time for IV drug preparation, IV access, and hand disinfection. The asystole or pulseless electrical activity algorithm recommends giving 1 mg of epinephrine as fast as possible. It should, therefore, ideally be administered directly after recognition of the rhythm. All four cases with initial asystole or PEA (scenarios 1, 6, 32, and 33) showed low feasibility of hand disinfection in WHO-2 indications in the first minutes (hand hygiene possible in 70.0%, 26.7%, 22.2%, and 62.5%). In contrast, the megacode scenarios with sequential asystole or PEA after VT/VF showed higher feasibility as the first i.v. medication is conducted after the second shock: after the first minutes of the peri-arrest

or arrest algorithms, nearly all indications for the medication administrator showed to be foreseeable; thus, hand hygiene was feasible. These "late" indications included the repetitive administration of epinephrine (every 3–5 min), amiodarone, or lidocaine (after the third shock); the use of ACS medication after ROSC in ACS and peri-arrest scenarios (acetylsalicylic acid, heparin, rt-PA, morphine, hypnotics, and muscle relaxants); and measurements of blood sugar.

Discussion

Key results

To the best of our knowledge, this is the first study investigating the feasibility of hand hygiene in a manikin CPR study. In this study, we demonstrated that hand or glove disinfection is indicated repeatedly in prototypical arrest and peri-arrest scenarios. Approximately, 90% of all hand hygiene indications and, in many cases, more than 80% of WHO-2 indications are achievable during CPR without delay for resuscitation. That would align with the WHO recommendation of at least an 80% compliance rate (4), even in acute cardiac arrest scenarios. A total of 90% of the highly significant WHO-2 indications had to be accomplished by the role of the medication administrator and about 10% by the airway manager. Lower rates of realizable hand hygiene were detected in primary cardiac arrest scenarios with asystole or PEA and unstable bradycardia needing early drug administration.

Limitations

This study has several limitations. First, this is a manikin study under ideal resuscitation conditions, with the likelihood of rapid IV access and a steep learning curve for the participants. The latter is shown by improving CCF rates and reducing the time to identify the first rhythm. These ideal conditions are not transferable to reality, where limited resources, different competencies, an additional need for team setup, and patient and environmental obstacles (e.g., difficult clothing, delayed rhythm identification, or difficult IV access) are common challenges. However, ACLS courses are widely acknowledged to effectively prepare staff for emergency situations (20–22), are satisfactory to participants (23), and can be considered a worldwide standard. Even if the feasibility is overestimated, the basic observation and statement remain true: a relevant part of the WHO indications could be implemented without delaying acute care, and thus, the overall quality of patient care could be increased.

Second, the selection bias must be mentioned as we examined 33 scenarios with 9 participants. From our viewpoint, the number of participants using highly standardized scenarios and algorithms does not play a significant role. If we had conducted the study with a new crew for each scenario and assumed adherence to AHA algorithms and choreographed scenarios, we would not expect significant changes to the number of hand hygiene indications, as these are mainly dependent on the scenarios. However, this hypothesis could be examined further, especially for errors in algorithms that may "produce" more or less indications. On the contrary, with succeeding simulation studies focusing on real-life hand hygiene protocol adherence, the number of participants would play a significant role as adherence is individually different and vulnerable to psychological effects (24, 25). In addition, other life support courses should be taken into account, such as PALS, ATLS, PHTLS, or ACiLS (26, 27) scenarios, to determine whether the number and opportunities for hand disinfection differ from ACLS scenarios. Consequently, real-time observations in real cases should clarify further differences between simulated and realistic cases (14).

Third, it is possible that the WHO-3 moments were under measured. These would have depended on the choreography of each case, which was not considered in this setting. Empirically, these indications may be relevant for all team members after contact with the patient's blood, esophageal regurgitation, vomiting, and respiratory secretions. Third, our study did not use video recording or other technically supported identification of WHO indications, possibly leading to observer bias. However, this presents an opportunity for further research with video-recorded observations, which add further data and enhance the validity of our findings.

A further limitation is that hand hygiene and the use of PSA were only indicated but not simulated. However, as a first approach, we decided not to alter the AHA course protocols for implementing PSA and hand hygiene. Our results show that hand hygiene could be implemented in several cases so that we are focusing on the realistic feasibility of the use of PSA and glove disinfection in a BLS follow-up study of our working group. Regarding observer bias, the use of videotaping or multiple observers in further examinations or real cases may strengthen test reliability.

Interpretation and generalizability

Our study shows that, concerning hand hygiene, two roles of the CPR team must be focused on: the medication administrator and the airway manager. The other roles had just two hand hygiene indications: when arriving at the scene and after handover (WHO-1 and -4 or -5). These can be considered feasible in all cases.

The airway manager (or an assisting person) must carry out bag-mask ventilation or intubation after ROSC in unconscious patients. After ROSC, the need for a 12-lead-ECG and additional medication takes some time. Therefore, the airway manager

and assisting crewmembers to have a foreseeable time window to prepare and conduct endotracheal intubation after hand hygiene (which is relevant long-term regarding the risks for nosocomial pneumonia).

For medication administrators, this is more complicated, as they have many tasks when arriving at the scene: place and open their medication backpack or trolley on the ground or a table, apply a tourniquet to the patient's arm, identify a puncturable vein, apply skin disinfection, unpack the IV set, perform hand disinfection, puncture the vein, secure the IV cannula, prepare a crystalloid infusion or saline syringe, connect the infusion bag, and prepare and administer IV medications. Especially in cases where there is a need for early drug administration (unstable bradycardia, asystole, PEA), this could be difficult. More time is available for the medication administrator to safely prepare and administer medications later in all algorithms or with a previously placed IV cannula.

It must be mentioned that most resuscitation teams might indicate the need for IV access according to the situation (a person in distress) and not the diagnosis made by examination and monitoring ("every emergency patient needs IV access as soon as possible"). This point is debatable, especially in peri-arrest emergencies when a greater focus should be placed on history-taking. This controversy should be considered in further investigations. For this study, we considered IV access indicated by the diagnosis, not by the situation.

Aside from using time-saving glove disinfection (28) without the problem of "wet hands in new gloves," it might be possible to optimize hand hygiene for the medication administrator by providing prefilled syringes. This might apply to sodium chloride (for an IV push dose), epinephrine, lidocaine, amiodarone, and atropine. In a classical VF/VT scenario of 20 min CPR time, this could reduce the number of indications from 12 (Five dosages of epinephrine, two dosages of amiodarone) to 5. This reduction in medication preparation time can free up the medication administrator to fulfill other tasks, especially in a CPR setting with combined roles due to a staff shortage. In addition to the benefits of aseptic preparation, prefilled syringes might be preferable for correct dosing (29), fewer errors in selecting the correct drug (30), finding the correct doses (especially in pediatrics) (31), and reducing the risk of needle stick injuries when using needles for preparation.

Concerning the time for hand disinfection, we allowed 15 s for hand disinfection as this is suitable for the reduction of bacterial contamination of hands or gloves, which is more relevant for patients' nosocomial infections than viruses transmitted by contact. (18). Considering 30 s for hand hygiene would lower the feasibility rates of hand hygiene in some cases, especially in asystole/PEA: in scenario 5, the rate of the feasible hand disinfections would be the same as with 15 s as in VT-algorithms there is enough time before the first administration of epinephrine and enough time to prepare further medication as the case progresses. In contrast, in case 7, the rate of feasible hand disinfection would drop from 100 to 33%. Regarding the prototypical scenario (see Table 2), it is likely that, especially when prefilled syringes are not available, there would be a significant drop in justifiable hand disinfections due to the time-consuming preparation times for i.v. medications. Consequently, aside from the need for further research on this topic, there is a need for consensus and recommendations by infection prevention and resuscitation experts as to whether 15 s would be sufficient in the special situation of resuscitation or not.

Next, we did not consider the whole spectrum of hand disinfection agents like ethanol, 1-propanol, or 3-propanol, or even the durability of gloves after disinfection (28). Furthermore, we did not consider the problem that most disinfection agents are not fully virucidal and need 60 s for efficacy (32). Under CPR conditions and as mentioned above, the virucidal spectra might be considered less significant for patients as they are mainly susceptible to bacterial contamination of devices leading to bloodstream infection and pneumonia. However, it is relevant to healthcare providers caring for a patient with a viral disease (e.g., COVID-19), creating the need for the CPR team to wear protective equipment (33).

Furthermore, hand disinfection under CPR conditions may be subjectively seen as dispensable due to the emergency setting ("necessity knows no rules" – in German: "*Not kennt kein Gebot*"). In general, the discussion about the study after the courses resulted in irritation and amusement among single participants. We all strongly agree that hand disinfection must not delay life-saving care. However, we could demonstrate that hand hygiene is feasible in most cases and should not be abandoned categorically or carelessly, especially as it might ruin the success of resuscitation after some days due to nosocomial infection, sepsis, and multiorgan dysfunction. To raise awareness among healthcare workers, the prevalence of device-associated "post-ROSC pneumonia" (that may be interpreted as aspiration pneumonia) and "post-ROSC blood stream infection" should be investigated further. In addition, the learning material and videos presented to course participants should outline the role of IPC and post-ROSC removal of not aseptically placed IV lines.

It has to be emphasized that these findings clearly indicate the need for more research on the feasibility of hand hygiene under CPR conditions and post-ROSC, we need practical strategies to lower barriers to accomplish it. As we mentioned above, prefilled syringes may reduce the number of indications and may lower the risks of contamination. The use of double gloves and glove disinfection may also reduce contaminations. Furthermore, BLS- and ALCS-crews need a safe opportunity for hand disinfection, e.g., by small and easily accessible disinfection bottles for belts or smock pockets and perhaps a single dispenser for the IV-Manager. In wards and medical facilities, the visuality of dispensers is a factor for its use (34). In addition, algorithm-based pauses in algorithms, like team-time-outs ("10 s for the

next 10 min") (35) may be evaluated to grant generic glove disinfection for the whole team. Large teams (that are seldom in OHCA, but may be more often in IHCA) could even further split the role of the "IV" into an "IV" and "IV-assistant" – with regard to overcrowding phenomena.

Finally, our data were obtained in scenarios created to test the ACLS candidates using different algorithms. These scenarios seldom occur in reality, which reduces generalizability: according to the German databank for resuscitation (36), in 2021, about 21% of 16,265 detected out-of-hospital-cardiac arrests (OHCA) showed an initial shockable rhythm, whereas the remaining OHCA are due to asystole and PEA. Hypothetically, with about 10 WHO-2 indications in every asystole and PEA case and only 7% adherence to IPC protocols (14), this would result in approximately 151,125 omitted hand disinfections. According to our four cases with low justifiability for hand hygiene in 22–70% of the cases, approximately between 33,550 and 105,878 indicated and feasible hand disinfections would have been omitted. However, lower rates in the majority of OHCA do not justify general abandonment of hand hygiene at all in any resuscitation attempt: bio-ethically (37) such a generalized abandonment would be questionable in terms of benevolence (provision of best survival conditions to the patient), non-maleficence (infection prevention and secondary brain damage), and justice [providing best chances for survival due to reduced mortality, appreciating the work of healthcare providers in the chain of survival, and limiting the economic burden of hospital-associated infections (38, 39)].

Overall, these findings indicate the need for rational use of IPC in CPR conditions whenever feasible. Therefore, further training, raising awareness among CPR providers, and improving the education material are necessary.

Conclusion

In our manikin study, we demonstrated that most hygienic hand or glove disinfection indications were feasible using the 15-s hand disinfection approach. Furthermore, we were able to show that the medication administrator faced most of the indications, of which more than 80% could be conducted. The situations in which hand hygiene was not performed were mainly in unstable peri-arrest rhythms, asystole, and pulseless electrical activity. Further work should concentrate on real-life scenarios, the role of prefilled syringes, investigating the role of device-associated post-ROSC nosocomial diseases, and education to reduce possible narratives that hand disinfection is dispensable in emergency situations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Commitee Physicians Association Baden Wurttemberg, Germany. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SBu: conceptualization, recruitment, primary manuscript draft, funding, and ethics. JB: medical validation (ERC recommendations). MB and SBe: medical validation (AHA recommendations). BG: validation, primary draft, and secondary draft of the manuscript (native speaker). NR-S: psychological expertise. SiSc: design, method, and manuscript. All authors contributed to the article and approved the submitted‘version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Impact of reduced antibiotic treatment duration on antimicrobial resistance in critically ill patients in the randomized controlled SAPS-trial

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Background: In the previously reported SAPS trial (https://clinicaltrials.gov/ct2/show/NCT01139489), procalcitonin-guidance safely reduced the duration of antibiotic treatment in critically ill patients. We assessed the impact of shorter antibiotic treatment on antimicrobial resistance development in SAPS patients.

Materials and methods: Cultures were assessed for the presence of multi-drug resistant (MDR) or highly resistant organisms (HRMO) and compared between PCT-guided and control patients. Baseline isolates from 30 days before to 5 days after randomization were compared with those from 5 to 30 days post-randomization. The primary endpoint was the incidence of new MDR/HRMO positive patients.

Results: In total, 8,113 cultures with 96,515 antibiotic test results were evaluated for 439 and 482 patients randomized to the PCT and control groups, respectively. Disease severity at admission was similar for both groups. Median (IQR) durations of the first course of antibiotics were 6 days (4–10) and 7 days (5–11), respectively ($p = 0.0001$). Antibiotic-free days were 7 days (IQR 0–14) and 6 days (0–13; $p = 0.05$). Of all isolates assessed, 13% were MDR/HRMO positive and at baseline 186 (20%) patients were MDR/HMRO-positive. The incidence of new MDR/HRMO was 39 (8.9%) and 45 (9.3%) in PCT and control patients, respectively ($p = 0.82$). The time courses for MDR/HRMO development were also similar for both groups ($p = 0.33$).

Conclusions: In the 921 randomized patients studied, the small but statistically significant reduction in antibiotic treatment in the PCT-group did not translate into a detectable change in antimicrobial resistance. Studies with larger differences in antibiotic treatment duration, larger study populations or populations with higher MDR/HRMO incidences might detect such differences.

KEYWORDS

antibiotics, antimicrobial resistance, procalcitonin, treatment duration, culture, randomized trial

Introduction

Antibiotic treatment should be optimized in terms of its spectrum and duration to maximize patient outcome whilst minimizing the development potential antimicrobial resistance (AMR) and other side effects (1–3). Efforts to limit AMR in the intensive care unit (ICU) are of particular importance (4–7). In the stop antibiotics on guidance of PCT study (SAPS) (8), that randomized 1,546 ICU patients to PCT-guidance or standard-of-care, we observed a safe reduction of antibiotic treatment duration (ABTD) to a median of 5 days compared to 7 days with standard-of-care.

Interventions that lead to a reduced overall antibiotic consumption would be expected to lead to a reduction in AMR. Some randomized studies outside the ICU indeed observed reduced AMR (9), but for ICU studies that randomized up to 604 patients no significant impact on AMR was seen (10–13). Thus, the effect of reduced ABTD on AMR might be detectable in the larger cohort of ICU patients that was used in the SAPS trial.

The aim of the current study was to assess if the reduced ABTD achieved in the PCT arm of the SAPS study had an impact on the development of AMR.

Methods

The SAPS trial design (14) and its findings (8) have been published previously. This study was approved for all centers by the Ethics Committee of the Amsterdam University Medical Center and is in compliance with the Helsinki Declaration. SAPS was performed in 15 hospitals in the Netherlands between 2009 and 2013 (https://clinicaltrials.gov/ct2/show/NCT01139489). Adult patients admitted to the ICU and treated for presumed bacterial infection, were randomized after informed consent. PCT was measured daily in the intervention arm. When PCT showed an absolute level of $\leq$0.5 μg/L or a relative decrease to $\leq$20% of the baseline level, a non-binding advice was given to consider to discontinue antibiotic treatment.

For the current substudy, seven of the participating institutions were able to provide the required complete culture and resistance data from their hospital information systems. Microbiological data (i.e., type of culture and microorganisms cultured) from specimens from all sources were obtained for −30 to +30 days relative to randomization and prospectively recorded in the case record form during the trial. All reported isolates were then combined into a single database with source of the material, cultured microorganism and resistances recorded in a standardized manner. Resistances were obtained after conclusion of the SAPS trial and were classified as sensitive, intermediate and resistant, following automated standardized antimicrobial susceptibility testing. To compare the impact of reduced ABTD, we compared baseline resistance data with data obtained after randomization. Since most cultures were obtained directly after ICU-admission and randomization, and because resistance typically does not become manifest within a few days (15), we chose as baseline period the interval from −30 to +5 days relative to randomization. This baseline period was compared with the subsequent period, i.e., +5 to +30 days. To determine the incidence of new MDR/HRMO positive patients, microorganisms were classified as MDR based on an international definition from 2012 (16). The HRMO-classification was based on Dutch guidelines[1,2] as also further detailed in the Supplementary material. Both classifications were dichotomized to negative and positive, where positive denotes any form of multidrug resistance. Since our key data concerned AMR, which is generally considered unsuitable for imputation, no techniques were used make data more complete. All available cultures were examined for multi-drug resistant (MDR) or highly drug resistant (HRMO) organisms and the change in MDR/HRMO status was the primary endpoint.

The chi-square, Mann–Whitney *U* and Student's *t*-tests were used for group comparisons with two-sided *p*-values. The actuarial cumulative percentages for the first occurrence of an MDR/HRMO isolate were compared with the Kaplan–Meier method for the PCT and control groups with the log-rank test.

Results

We evaluated 921 (60%) of the original 1,546 patients that were included in the SAPS trial. The numbers of patients randomized to PCT-guidance and standard of care were 439 and 482, respectively. The baseline characteristics of these groups are shown in Table 1. Severity of illness and other baseline indicators were similar. The most observed presumed infection was community acquired pneumonia. The median (IQR) durations of the first course of antibiotics were 6 days (4–10) and 7 days (5–11), respectively ($p = 0.0001$) with a difference of 1.03 days between the means (Table 2). ICU and hospital length of stay and 28 day mortality were similar.

In total 8,113 cultures with 96,515 antibiotic test results were obtained. Most of the cultures were obtained around the day of randomization (Supplementary Figure 1). In total 546 isolates (7%) were non-bacterial, mainly *Candida* species. The 10 most identified bacterial isolates are shown in the Supplementary Table 5, with *Escherichia coli* (18%) being the most prominent. In only two patients *Clostridium difficile* was cultured. The five most frequently used antibiotics were ceftriaxone, ciprofloxacin, amoxicillin-clavulanate, metronidazole and cefuroxime (Supplementary Figure 2, Supplementary Table 6). Overall 1,001 (12%) of the isolates were MDR and 562 (7%) were HRMO. On a patient basis (Table 2), 22 and 18% were MDR/HRMO positive at baseline in the PCT and control groups, respectively. There were no patients with more than one unique MDR/HRMO during the study period. Subsequently, 39 (8.9%) and 45 (9.3%) of the patients became MDR/HRMO positive while they were MDR/HRMO negative at baseline ($p = 0.82$). The time course of the cumulative MDR/HRMO incidence (Figure 1) did not show a difference between the two groups ($p = 0.33$).

Discussion

In this substudy of the SAPS trial, the baseline characteristics of the PCT and control groups were well balanced and a statistically significant difference of 1 day in ABTD was achieved. However the

Abbreviations: AMR, antimicrobial resistance; ABTD, antibiotic treatment duration; CRP, C-reactive protein; ICU, intensive care unit; MDR multidrug resistant; HRMO highly resistant microorganisms; PCT, procalcitonin; SAPS, stop antibiotics on guidance of procalcitonin trial.

1 https://www.rivm.nl/documenten/wip-richtlijn-brmo (accessed October 17 2022).

2 https://lci.rivm.nl/richtlijnen/brmo (accessed October 17, 2022).

TABLE 1 Baseline characteristics.

	PCT group ($n = 439$)	Standard-of-care group ($n = 482$)	p-Value
Age	64 (54–73)	64 (56–74)	0.41
Men	268 (61%)	281 (58%)	0.42
Severity of illness			
APACHE IV score	72 (51–90)	70 (54–89)	0.81
Sepsis/severe sepsis	358 (82%)	394 (82%)	1.00
Septic shock	81 (18%)	88 (18%)	
SOFA score	6 (3–8)	6 (3–8)	0.59
Acquisition of infection[a]			
Community-acquired	216 (49%)	229 (48%)	0.73
Hospital-acquired	111 (25%)	119 (25%)	
ICU-acquired	112 (26%)	134 (28%)	
Presumed infection site[a]			
Pulmonary	276 (63%)	309 (64%)	0.15
CNS	18 (4%)	22 (5%)	
Skin and soft tissue	9 (2%)	14 (3%)	
Catheter-related	7 (2%)	8 (2%)	
Intraabdominal	63 (14%)	86 (18%)	
Urinary tract	17 (4%)	16 (3%)	
ENT	4 (1%)	1 (0.2%)	
Bloodstream	4 (1%)	2(0.4%)	
Unknown	41 (9%)	24 (5%)	
Inflammatory parameters			
Procalcitonin (μg/L)	2.1 (0.4–14.9)	NA	
C-reactive protein (mg/L)	230 (119–2–324)	213(121–308)	0.58
Leukocytes (10^9/L)	14.5 (10.5–20.9)	14.8 (10.2–21.2)	0.88
Temperature (°C)	38.2 (37.5–38.9)	38.1 (37.5–38.8)	0.15

Comparison of patient characteristics at the time of randomization to procalcitonin-guide group (PCT) or control group that received standard-of-care.
Medians with (IQR) or numbers with (percentages) APACHE-IV, acute and chronic health evaluation IV score; SOFA, sequential organ failure score; CNS, central nervous system; ENT, ear nose and throat; ICU, intensive care unit.
[a]Due to rounding, percentages may not always add up to 100%.

lower ABTD in the PCT-arm was not associated with a detectable difference in changes in MDR/HRMO incidence.

Generally, ICUs are among the heaviest consumers of antibiotics, with an estimated 70% of patients receiving antibiotics during an ICU stay (7). Various observational and before-after studies show that duration of antibiotic therapy is linked to antibiotic resistance development, both in ICU (17, 18) and non-ICU (19–21) settings. A number of randomized trials have shown that targeted interventions can safely reduce the ABTD in ICU (11, 22, 23) or non-ICU (9, 10, 12, 13) patients. Measuring readily available markers of inflammation such as C-reactive protein (CRP) or procalcitonin (PCT) can help reduce unnecessarily prolonged antibiotic prescriptions as was shown in the PRORATA (24), SAPS (8), PIRATE (13), and PROGRESS (25) trials. Although shorter antibiotic treatment is generally considered desirable, it is not beneficial under all circumstances. For example, a trial that randomized children aged 6–24 months with otitis to either 5 or 10 days of amoxicillin observed a worse outcome in the 5 days group (26). Recently the multicenter iDIAPASON trial randomized 186 patients with *Pseudomonas aeruginosa* ventilator-associated pneumonia to an ABDT of 8 or 15 days (27). Although formally non-inferiority was found, there was a trend (27) toward a better outcome in the 15 days group.

Clearly in SAPS, there was no indication that antibiotic treatment in the PCT-arm was too short, as mortality in this arm was significantly lower in the SAPS study (8), with also a trend toward lower mortality in the current substudy (Table 2). We observed a slightly smaller difference in ABTD, with a between-group absolute mean difference in ABTD of 1.03 days (Table 2) compared to 1.22 days for the original SAPS-group (8).

As expected, a clear time-dependent rise of AMR in terms of MDR/HRMO was observed after ICU admission and initiation of antibiotic treatment, as depicted in Figure 1. But the time courses were similar for groups. Although 8,113 isolates were analyzed for the 921 patients, an even larger number of cultures might have allowed the detection of more subtle differences between the two trial arms. But obtaining more isolates is not trivial, not in the least because considerable costs are associated with culturing and AMR-testing. Of note, the PIRATE trial (13) that examined CRP-guidance in limiting antibiotic treatment only reports 13 cultures for 514 randomized patients.

Studies from Belgium (15), Canada or the USA (10, 19, 22, 26, 28), China (21), France (11, 18, 23), Italy and Israel (12), Korea (29), Singapore (30), and Switzerland (13) examined the relation of ABTD with AMR. Large observational or before/after studies do indicate that prolonged ABTD increases AMR, both in (17, 18) and outside the ICU (19–21). Several meta-analyses also suggest that reduced ABTD may lead to reduced AMR (31–33). But ICU studies that randomized respectively 249 (11), 504 (13), 517 (10), and 604 (12) patients, report no significant impact on AMR. In contrast, the recent PROGRESS trial from Greece (25) does report an effect on resistance. In 261 patients randomized to PCT-guidance or standard-of-care, median ABTDs of 5 and 10 days ($p < 0.001$), respectively were achieved. Acquired resistance defined as new *C. difficile* infection of MDRO infection occurred in 7.2% and 15.3% of the patients, respectively ($p = 0.045$) (25). Possibly, the ongoing Canadian-international BALANCE trial (34) that will randomize more than 3,000 critically ill patients with a bloodstream infection to an ABTD of 7 or 14 days should also be able to detect clear differences in AMR.

Although mortality reduction was not a primary goal of the SAPS trial, in the main study with 1,546 patients we did observe better survival in the PCT arm (8), although this difference was not significant for the 921 patients from the seven centers in the current substudy ($p = 0.11$). Better adequacy of the antibiotics, more appropriate consideration of other diagnoses, decrease organ-toxicity toxicity of antibiotics as well as a type I error may all account for the observed lower mortalities.

A number of limitations of our study deserve mentioning. First, due to practical issues such as accessibility of electronic lab systems and the original design of the SAPS-trial, we were only able to obtain AMR data from seven of the original 15 participating SAPS centers, although still representing 921 patients. The resultant separation of ABTD was somewhat lower, although as indicated in Table 1, the two

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TABLE 2 Outcomes.

	PCT group ($n = 439$)	Standard-of-care group ($n = 482$)	*p*-Value	Between-group absolute difference (95% CI)
Antibiotic use				
Daily defined doses in first 28 d	7.9 (4.0–13.0)	9.0(5.0–17.2)	0.002	2.25 (0.30 to 4.20)
Duration of first antibiotic course	6 (4–10)	7 (5–11)	0.0001	1.03 (0.20 to 1.85)
Antibiotic-free days in first 28 d	7 (0–14)	6 (0–13)	0.05	−1.26 (−2.28 to −0.24)
Selective decontamination of the digestive tract	188 (43%)	208 (43%)	0.95	0.3% (−6.2 to 6.8)
28-day mortality	84 (19%)	114 (24%)	0.11	4.5% (−9.8 to 0.8)
ICU length of stay (days)	9 (5–18)	9 (5–18)	0.80	0.04 (−2.54–2.46)
Hospital length of stay (days)	24 (14–41)	24 (14–42)	0.76	−0.88 (−4.87 to 3.11)
Antimicrobial resistance				
MDR present at baseline	89 (20%)	76 (16%)	0.085	−4.5% (−9.4 to 0.4)
HRMO present at baseline	57 (13%)	50 (10%)	0.22	−2.6% (−6.7 to 1.5)
MDR or HRMO present at baseline	98 (22%)	88 (18%)	0.14	−4.1% (−9.2 to 1.0)
New MDR compared to baseline	29 (6.6%)	40 (8.3%)	0.38	1.7% (−1.6 to 5.0)
New HRMO compared to baseline	21 (4.8%)	23 (4.8%)	1.0	0.0% (−2.7 to 2.7)
New MDR or HRMO compared to baseline	39 (8.9%)	45 (9.3%)	0.82	0.5% (−3.2 to 4.2)

The PCT group had a significantly lower duration of antibiotic treatment ($p = 0.0001$), although this difference only amounted to 1 day. The rates of MDR and HRMO at baseline and after randomization did not differ between the two trial arms.

MDR, multidrug resistant organism; HRMO, highly resistant micro-organism according to the definition for the Netherlands (https://www.rivm.nl/documenten/wip-richtlijn-brmo; https://lci.rivm.nl/richtlijnen/brmo) as explained in the Supplementary material.

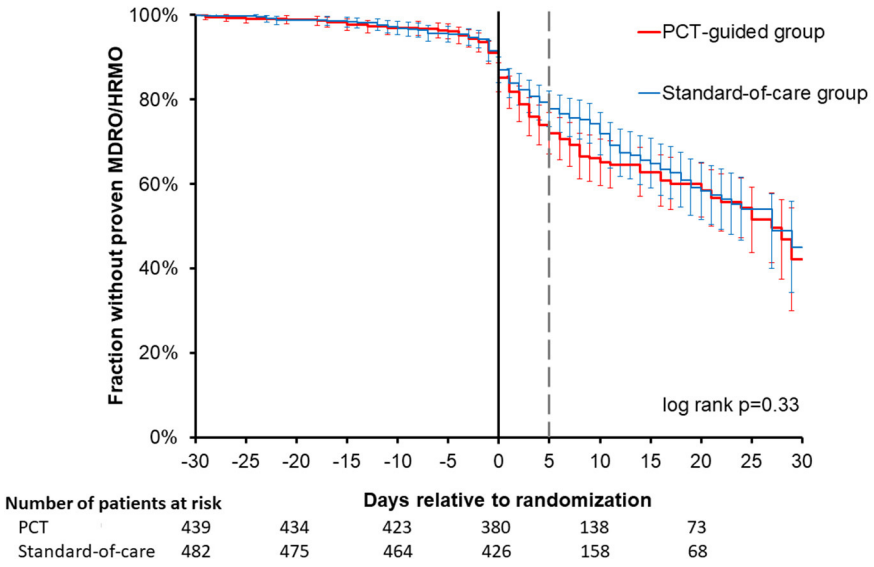

FIGURE 1
Time course of resistance development in the two trial arms. For all patients studied, the first occurrence of a multi-drug resistant (MDR) or highly resistant microorganism (HRMO) was compared between the procalcitonin (PCT) and standard-of-care groups from −30 to +30 days relative to randomization. No significant difference was observed between these two time courses. The actuarial cumulative percentages with 95% confidence intervals were plotted for the first occurrence of MDR/HRMO with the Kaplan-Meier method and compared for the PCT and control groups. The thinner, dashed vertical gray line represents the demarcation between baseline period (−30 days through +5 days) and the subsequent period (+5 days through +30 days) that we compared.

groups were still well matched. Second, since this sub-study of the SAPS trial combined data from different institutions with different classification systems and hospital information systems, data were not completely homogeneous. On the other hand, we believe these multicenter data well reflect routine health care in the Netherlands. Third, in the Netherlands overall antibiotic consumption is lower than many other countries (35), making it more difficult to achieve reductions larger than the 1 day reduction we achieved. Accordingly background AMR is comparatively low in the Netherlands (36). In our study, both the baseline AMR and the subsequent AMR were low when compared to many other studies that also achieved larger differences in (long) treatment durations, such as 7 vs. 14 days or

8 vs. 15 days (11, 12, 25, 26). Although randomization occurred on day 0, we somewhat arbitrarily selected day 5 as the cut-off between the baseline and subsequent periods. We chose this cut-off because of the limited number of cultures before day 0 and because divergence in ABDT as well as AMR would be expected after day 5. We did not perform subgroup analyses, since the number of patients, cultures and incidence of AMR also did not allow subgroup analyses. According to the Clinical Laboratory Standards Institute analyses should not be performed in subgroups with <30 first isolates.

With larger patient sets or larger differences in AB treatment duration or in settings with a higher background AMR, significant differences might be observed, such as in the aforementioned ongoing BALANCE trial (34). Lastly, the AMR data were obtained a decade ago—between 2009 and 2013. However we cannot conceive scientific arguments to assume that increased or decreased of AMR under PCT use would be fundamentally different in the present time, since we evaluated the relative AMR difference between PCT use and no PCT use.

In conclusion, although various types of evidence indicate that a lowered duration of antibiotic therapy protect leads to reduced subsequent AMR, our study could not demonstrate this. This may result from the small separation in antibiotic treatment duration between the two trial arms as well as the relatively low prevalence of drug-resistant organisms in the Netherlands. Future trials in large patient groups, with more marked differences in antibiotic treatment duration and in a context of higher background AMR, might be able to detect differences in subsequent AMR.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved for all centers by the Ethics Committee of the Amsterdam University Medical Center and is in compliance with the Helsinki Declaration (https://clinicaltrials.gov/ct2/show/NCT01139489). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study concept and design: AS, MB, CL, JO, AB, DL, EJ, and MN. Acquisition of data: AS, MN, PV, RK, BL, AR, LB-R, KL, TD, MO, and AvdB. Statistical analysis: AS, MB, CL, and MN. Analysis and interpretation of data: AS, MB, and CL. Drafting of the manuscript: AS and MN. Critical revision of the manuscript for important intellectual content: AS, MB, CL, HH, DL, EJ, and MN. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MB was employed by Certe Foundation.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1080007/full#supplementary-material

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Antibiotic use during coronavirus disease 2019 intensive care unit shape multidrug resistance bacteriuria: A Swedish longitudinal prospective study

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Objectives: High frequency of antimicrobial prescription and the nature of prolonged illness in COVID-19 increases risk for complicated bacteriuria and antibiotic resistance. We investigated risk factors for bacteriuria in the ICU and the correlation between antibiotic treatment and persistent bacteria.

Methods: We conducted a prospective longitudinal study with urine from indwelling catheters of 101 ICU patients from Uppsala University Hospital, Sweden. Samples were screened and isolates confirmed with MALDI-TOF and whole genome sequencing. Isolates were analyzed for AMR using broth microdilution. Clinical data were assessed for correlation with bacteriuria.

Results: Length of stay linearly correlated with bacteriuria (R^2 = 0.99, $p \leq 0.0001$). 90% of patients received antibiotics, primarily the beta-lactams (76%) cefotaxime, piperacillin-tazobactam, and meropenem. We found high prevalence of *Enterococcus* (42%) being associated with increased cefotaxime prescription. Antibiotic-susceptible *E. coli* were found to cause bacteriuria despite concurrent antibiotic treatment when found in co-culture with *Enterococcus*.

Conclusion: Longer stays in ICUs increase the risk for bacteriuria in a predictable manner. Likely, high use of cefotaxime drives *Enterococcus* prevalence, which in turn permit co-colonizing Gram-negative bacteria. Our results suggest biofilms in urinary catheters as a reservoir of pathogenic bacteria with the potential to develop and disseminate AMR.

KEYWORDS

UTI, ICU–intensive care unit, COVID-19, MDR–(multidrug resistance), AMR, antibiotic treatment, catheters

Introduction

Urinary tract infections (UTIs) is a major reason for healthcare-associated infections (HAIs) globally (1). In Sweden, UTIs are recognized as the principle cause of HAIs (2). Intensive care unit (ICU) treatment is one of few recognized indications for catheterization, and indwelling catheters are the main source of nosocomial UTIs. Severely ill COVID-19 patients are principally treated in ICUs with associated catheterization, giving this group of patients risk of complicated UTIs, defined by high rates of treatment failure (3). An additional distress is that clinical symptoms of nosocomial UTIs might be concealed by COVID-19-associated damage, potentially increasing the risk of prolonged UTI-related impairment. Alongside systemically used immunosuppressive treatment in this patient group, meta-analyses have revealed that 86% of COVID-19 ICU patients receive antibiotics (4). This raises concerns for atypical infections and multidrug resistance (MDR). Still, data and correlation analysis on antibiotic use, bacterial prevalence, and antimicrobial resistance (AMR) are limited. There is no longitudinal study on AMR development in the COVID-19 ICU cohort.

This prospective study was based on a cohort of COVID-19 patients from the ICU in Uppsala University Hospital, Sweden. We performed longitudinal screening for bacteriuria as well as collected underlying medical, diagnostic, and treatment data. Urinary isolates were consecutively tested for AMR. This study had three aims: to investigate how bacteriuria correlate with length of stay (LOS) and additional clinical variables; how specific treatment correlate to specific colonization patterns; and to explore whether antibiotic-susceptible bacteria persist during treatment. To our knowledge, this is the first report to show how longitudinal antibiotic treatment correlates with bacterial prevalence and AMR in ICU-patients.

Materials and methods

Sample collection and storage

Clinical data were recorded daily and included age, sex, LOS, simplified acute physiology score 3 (SAPS3) at arrival, diabetes, hospitalization outcome, immunosuppressive treatment, antibiotic treatment, and findings from clinical microbiology (clinical routine samples, regular monitoring). All admitted patients received transurethral catheterization in a closed system as part of clinical practice. Urine study samples were collected every Monday, Wednesday and Friday (separate from regular monitoring) aseptically from the catheter into sterile vacutainer tubes and transported cold. Urine study samples were processed within 2 h of collection by aliquoting from vacutainers into cryovials with 10% dimethyl sulfoxide (DMSO) for storage in −80°C. Bacterial isolates from routine samples were not assessed in this study. All samples and data were collected from patients fulfilling the inclusion criteria in any of the intensive care unit facilities at Uppsala University Hospital, Sweden.

Abbreviations: AMR, antimicrobial resistance; COVID-19, coronavirus disease 2019; HAI, healthcare-associated infection/hospital-acquired infection; ICU, intensive care unit; LOS, length of stay; MDR, multidrug resistance; SAPS3, simplified acute physiology score 3; UTI, urinary tract infection.

Species identification and cultivation

In short, urine was plated onto Brilliance^{TM} UTI Clarity^{TM} agar. Significant growth was considered $>10^3$ CFU/ml ($>10^5$ for *Staphylococcus epidermidis*) based on national guidelines for UTIs (**Supplementary Table 1**: includes ECDC UTI comparison). It was in this study assumed that assessment of UTI symptomatology for the cohort might have been compromised. As clinical symptoms could not be assessed, samples are not described in terms of UTIs but instead as bacteriuria/non-bacteriuria. Species were identified using MALDI-TOF and saved frozen in Brain Heart Infusion (BHI, 10% DMSO). Species confirmation and clonality control was performed for a subset of strains with whole-genome sequencing analysis. All cultivation was carried out at 37°C. For a full description, see **Supplementary material**.

Minimum inhibitory concentration testing

Minimum inhibitory concentration (MIC) tests were performed by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) BMD method (V12.0) (5). Experiments were run in biological duplicates in 96-well microtiter plates and bacterial suspensions of 0.5 McFarland standard units were added to each well. Positive (no antibiotic) and negative controls (no bacteria) were added to each plate. Information about procedures, controls and antibiotics are found in **Supplementary Table 2**. The classification of MDR was based on proposed standard definitions, which in short specifies MDR as resistance against minimum three different classes of clinically relevant antibiotics (6).

Data processing and statistical analysis

All analysis was performed in GraphPad Prism v9. In discrete and ratiometric parameters, correlations were investigated with chi-square tests unless otherwise specified. In continuous parameters, correlations were investigated using the Spearman correlation tests, and deviations in means with two-tailed *t*-tests. Significance for linear regression was assessed with the likelihood ratio test and the Wald test, and Gaussian distribution (normality) was measured using the D'Agostino-Pearson normality test. A significant difference was identified at *p*-values smaller than 0.05 with * denoting <0.05, ** <0.01, *** <0.001, and **** <0.0001. Cross-correlation was controlled for death, LOS, SAPS3, and age.

Results

Between Jun 5th, 2020, and Feb 17th, 2021, there were 21,130 recorded COVID-19 cases in Uppsala County, resulting in 151 patients being treated in intensive care. Out of the 151 patients screened, 101 were enrolled in the study. Three patients with a LOS in the ICU of less than 2 days were excluded from the present analysis (**Figure 1**).

Age and SAPS3 were normally distributed (D'Agostino-Pearson, ns) while LOS was not (****) (**Supplementary Figures 1A–C**). Participants had a mean age of 65 [standard deviation (SD): 12.80] and a SAPS3 on arrival of 55 (SD: 9.90). Analysis of frequency

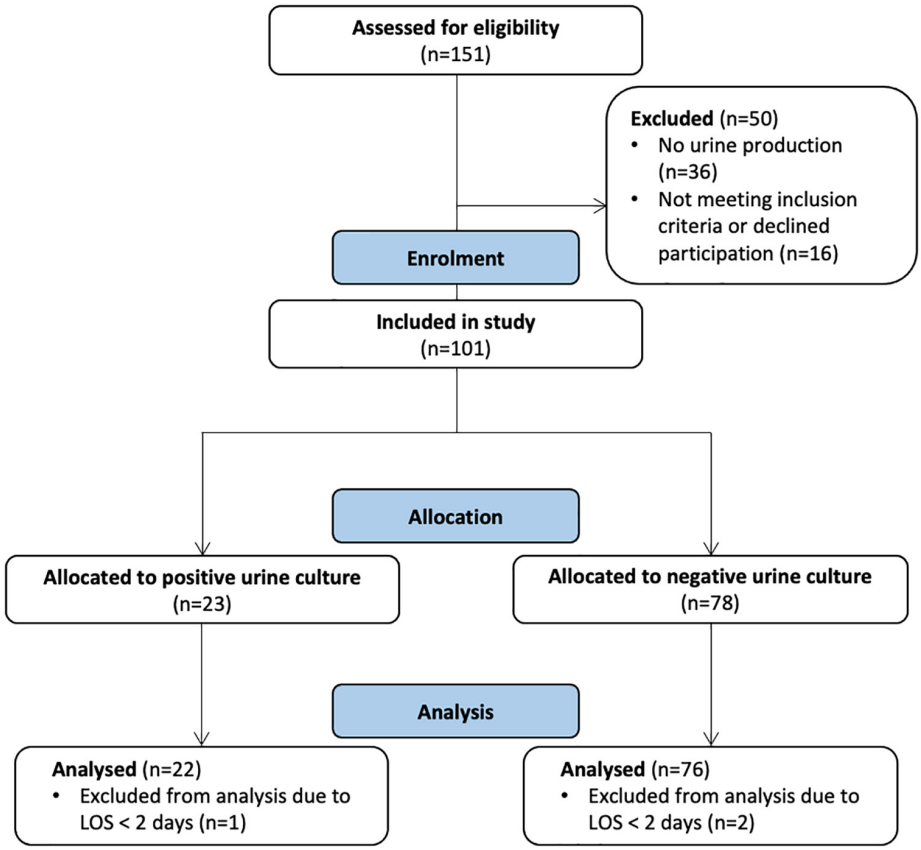


FIGURE 1
CONsolidated Standards of Reporting Trials (CONSORT) flow diagram for the progress of enrollment and allocation based on bacteriuria (urine study samples).

distribution (automatic 5 days binning) of LOS identified two separate groups with a LOS of longer or shorter than 30 days (Supplementary Figure 1C). LOS was divided into groups of shorter (n = 84, x̄: 10.56, SD: 6.56) and longer (n = 13, x̄: 40.85, SD: 7.3) stay. Most participants were men (74%) (Table 1). Diabetes was an underlying disease in 36% of the patients and the overall cohort mortality was 18.4% with no significant difference between bacteriuric and non-bacteriuric patients. 89% (n = 92) received minimum one immunosuppressant, most commonly dexamethasone (69 patients). 90% (n = 90) received at least one antibiotic. Clinical routine samples were positive 78 times across 44 patients (45% of cohort) and our longitudinal urine screen identified 34 potential clones (70 isolates) across 22 patients (23% of cohort). As expected, having any positive clinical routine sample significantly increased the relative risk (RR) of bacteriuria (RR: 3.15**), similarly to not having received any antibiotic (RR: 2.65*) (Table 1). Neither sex, diabetes or immunosuppressive treatment increased the risk of bacteriuria.

No correlation was found between bacteriuria and age or SAPS3, as seen in Figures 2A, B (Spearman's rank correlation), and there were no significant deviations in mean age or SAPS3 of patients with bacteriuria (post-hoc, two-tailed t-test). LOS was significantly different when comparing patients with and without bacteriuria (Spearman/t-test***) (Figure 2C). When comparing shorter and longer LOS, a two-sided chi-square test demonstrated a significant correlation with an increased RR of 2.17 [95% confidence interval (CI 95%): 1.78–4.74] for LOS above 30 days (chi-squared***) (Supplementary Figure 1C). Cumulative frequency of bacteriuria over LOS showed a linear relationship (R^2: 0.99, likelihood/Wald test****) with a slope of 1.91 (CI 95%: 1.78–2.04) (Figure 2D). This relationship indicates that bacteriuria occurred systematically in this setting, and not only as a result of increasing probability over time. Given the 23%-point prevalence in the cohort, the RR of developing bacteriuria increased by 0.44% for each day spent in the ICU. Age, LOS, SAPS3, and death were analyzed for cross-correlation, but only low or non-significant correlation could be observed (Supplementary Figure 1D).

Eighty-one individuals received a total of 253 antibiotic prescriptions (n = 90), not including multiple prescriptions of the same drug within a patient (Figures 3A, B). The most common class was β-lactams (n = 192, 76%), comprising cephalosporins (n = 64), penicillins (n = 49), and carbapenems (n = 32). Nearly all respective treatment consisted of cefotaxime (n = 61), piperacillin-tazobactam (TZP, n = 47) or meropenem (n = 28), and together these three drugs represented 54% of all prescriptions. TZP was prescribed with large dose variation between patients (Figure 3C). Following β-lactams, the most common classes were macrolides, trimethoprim-sulfamethoxazole and linezolid.

Clinical routine samples (all sampling sites) were registered as standard procedure during regular clinical monitoring, while urine study samples (longitudinal urine) were collected separately from clinical routine. In clinical routine samples, 45% (n = 98) were identified with 74 positive bacterial findings (multiple per patient). These were mainly isolated from the respiratory tract (n = 41, 54%)

TABLE 1 Characteristics of enrolled coronavirus disease 2019 (COVID-19) intensive care unit (ICU) patients.

Parameter	Bacteriuria	No bacteriuria	Total	Relative risk	95% CI	*P*-value	Significance
Sex	22	76	98	0.96	0.45–2.23	0.93	ns
Male	16	56	72	–	–	–	–
Female	6	20	26	–	–	–	–
Diabetes	22	76	98	0.68	0.29–1.49	0.35	ns
Yes	6	29	35	–	–	–	–
No	16	47	63	–	–	–	–
Immunosuppressive	22	67	89	0.80	0.35–2.37	0.68	ns
Yes	19	60	79	–	–	–	–
No	3	7	10	–	–	–	–
Deceased (30 days)	22	76	98	0.44	0.12–1.42	0.20	ns
Yes	2	16	18	–	–	–	–
No	20	60	80	–	–	–	–
Bacterial findings in clinical routine samples	22	76	98	3.15	1.40–7.28	0.004	**
Yes	16	29	44	–	–	–	–
No	8	46	54	–	–	–	–
Bacteriuria from clinical routine samples	22	76	98	5.97	3.22–10.6	<0.0001	****
Yes	10	2	12	–	–	–	–
No	12	74	86	–	–	–	–
Antibiotic treatment	22	68	90	2.65	1.16–4.93	0.02	*
No	5	4	9	–	–	–	–
Yes	17	64	81	–	–	–	–

*$P < 0.05$, **$P < 0.01$, and ****$P < 0.0001$.

and blood ($n = 21$, 29%), followed by urine ($n = 12$, 15%) and wound ($n = 2$, 3%). Most cultures belonged to the genus *Staphylococcus* ($n = 28$, 38%), followed by *Enterococcus* ($n = 15$, 21%), *Escherichia* ($n = 10$, 13%) and *Stenotrophomonas* ($n = 8$, 11%) (Figure 4). The majority of findings were Gram-positive ($n = 45$, 63%).

In our study; 70 urine study sample isolates were identified from 22 patients. For better comparison with (mostly cross-sectional) clinical routine samples, each species was only counted once per patient (34 potential clones). The most frequent genus was *Enterococcus* ($n = 15$, 42%), followed by *Staphylococcus* ($n = 6$, 18%) and *Escherichia* ($n = 6$, 18%). Similar to clinical routine samples, the urine study samples were mainly Gram-positive ($n = 22$, 67%) (Figure 4). We re-identified 10/12 of clinical routine urine samples. Our criteria classified significant bacterial growth as a CFU larger than 10^3 (10^5 for *S. epidermidis*), and antibiotic resistance as an MIC at least 2-fold above the clinical breakpoint (EUCAST) for minimum one of the isolates per patient (Supplementary Tables 1, 2). The average day for the first isolate to appear was 15.68 days (SD: 12.35), which tended to be smaller for *Staphylococcus* ($n = 6$, $\bar{x}$: 8.67, SD: 11.72) and larger for *Enterococcus* ($n = 15$, $\bar{x}$: 19.60, SD: 10.87). Mean of *E. coli* first appearance computes similar to *Staphylococcus* ($n = 6$, $\bar{x}$: 10.33, SD: 9.22), but taking distribution into account, *Staphylococcus* generally appeared earlier in colonization than *E. coli*, while *Enterococcus* appeared more constant throughout the days in the ICU (Supplementary Table 1). Multiple patients carried bacteria from the WHO global priority list of AMR pathogens, including two third-generation cephalosporin-resistant *Enterobacteriaceae* (3GCRE, critical) (Figure 4): one MDR *M. morganii* and one ESBL-producing MDR *E. coli*. Importantly, this *E. coli* was the only *Escherichia* isolate successfully colonizing a patient without the presence of a Gram-positive co-colonizer. One *E. faecalis* and all but one *E. faecium* presented high-level tobramycin resistance (HLTR). One *E. faecium* and one *E. durans* were identified with probable vancomycin resistance (Supplementary Figure 2 and Supplementary Table 2). In total, three *E. faecium* strains classified as MDR. A proportion of *Enterococcus* isolates surprisingly demonstrated higher piperacillin (PIP) MIC when adding tazobactam in combination. These results were confirmed with E-tests and 24-hour bioscreen growth experiments (for a subset). One *E. faecium* demonstrated a deviation from the EUCAST screen recommendations with resistance against PIP while being susceptible to ampicillin. This strain was additionally resistant to imipenem and results were confirmed with E-tests (Supplementary Figure 2). No antibiotic resistant *Pseudomonas* was identified, but all isolates showed the typical phenotype "susceptible increased exposure" against aztreonam, ciprofloxacin and ceftazidime. All *Staphylococcus* were resistant against benzylpenicillin (used as penicillinase screen in *S. aureus*), but no MRSA was identified (inferred from cefoxitin screen). One *S. hominis* and one *S. epidermidis* were resistant against cefoxitin, indicating methicillin and complete β-lactam-β-lactamase inhibitor resistance. Troublingly, the same *S. epidermidis*, along

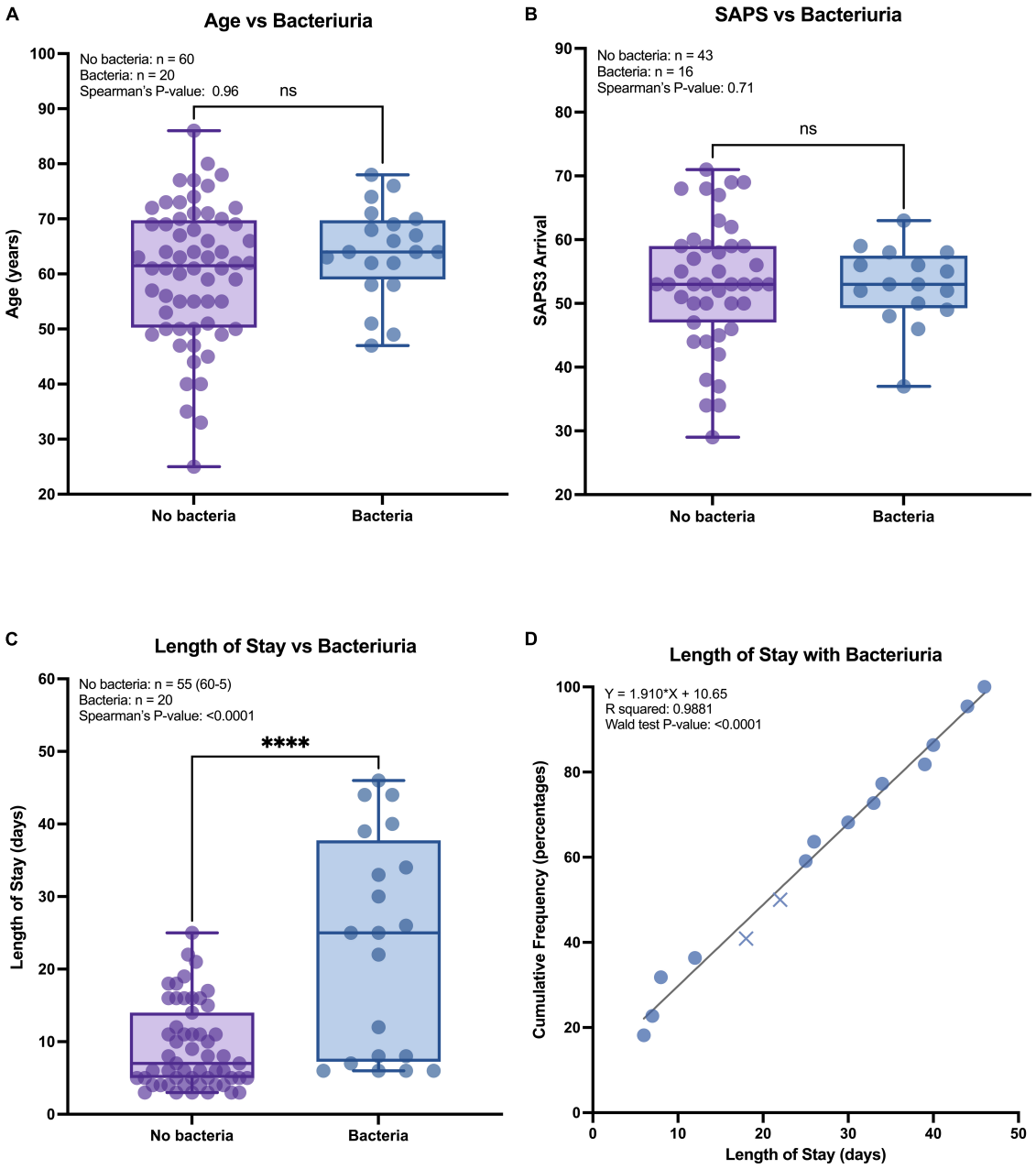


FIGURE 2
Continuous parameters and risk of bacteriuria. **(A–C)** All patients were grouped by urine colonization, no (purple) and yes (blue), compared against three continuous parameters. The comparison was measured with a two-tailed *t*-test (illustration) and Spearman's rank correlation (top left text). ****$P < 0.0001$. **(D)** Patients with urine study samples were sorted by cumulative frequency against the length of stay (LOS) and tested against a linear regression model (significance measured with the likelihood test and Wald test). Pattern indicate patients that survived (circle) and patients that died (cross).

with a second isolate of the same species, demonstrated "potential vancomycin impaired clinical response" (VAN, Supplementary Table 1). The same MDR *S. epidermidis* co-colonized the patient with susceptible *E. coli*. Apart from *S. epidermidis*, an additional MDR was classified in *S. capitis*.

Given the high prevalence of *Enterococcus* (Figure 4), and the high *Enterococcus* tolerance against the most prescribed treatment (Figure 3A), we decided to investigate the correlation between antibiotic prescription and bacteriuria. Treatment with MEM or CTX was found to correlate with *E. faecium* colonization (Figure 5A). To account for possible biases in prescription and isolate number, we calculated relative prevalence for every strain against those three antibiotics (isolates divided by number of prescriptions). The relative prevalence for *E. faecium* was confirmed again to be significantly higher during MEM and CTX than other strains (chi square test, Figure 5B). There was a clear increase in usage of CTX and MEM during the COVID pandemic when comparing to the pre-pandemic (Figure 5C) (Swedish eHealth Agency and Strama: prescription data in Uppsala County, Inpatient Care, ICU).

Investigating *Enterococcus* colonization further, we quantified co-colonization events. We observed that all but one MDR *E. coli* co-colonized with Gram-positive bacteria, four out of five with

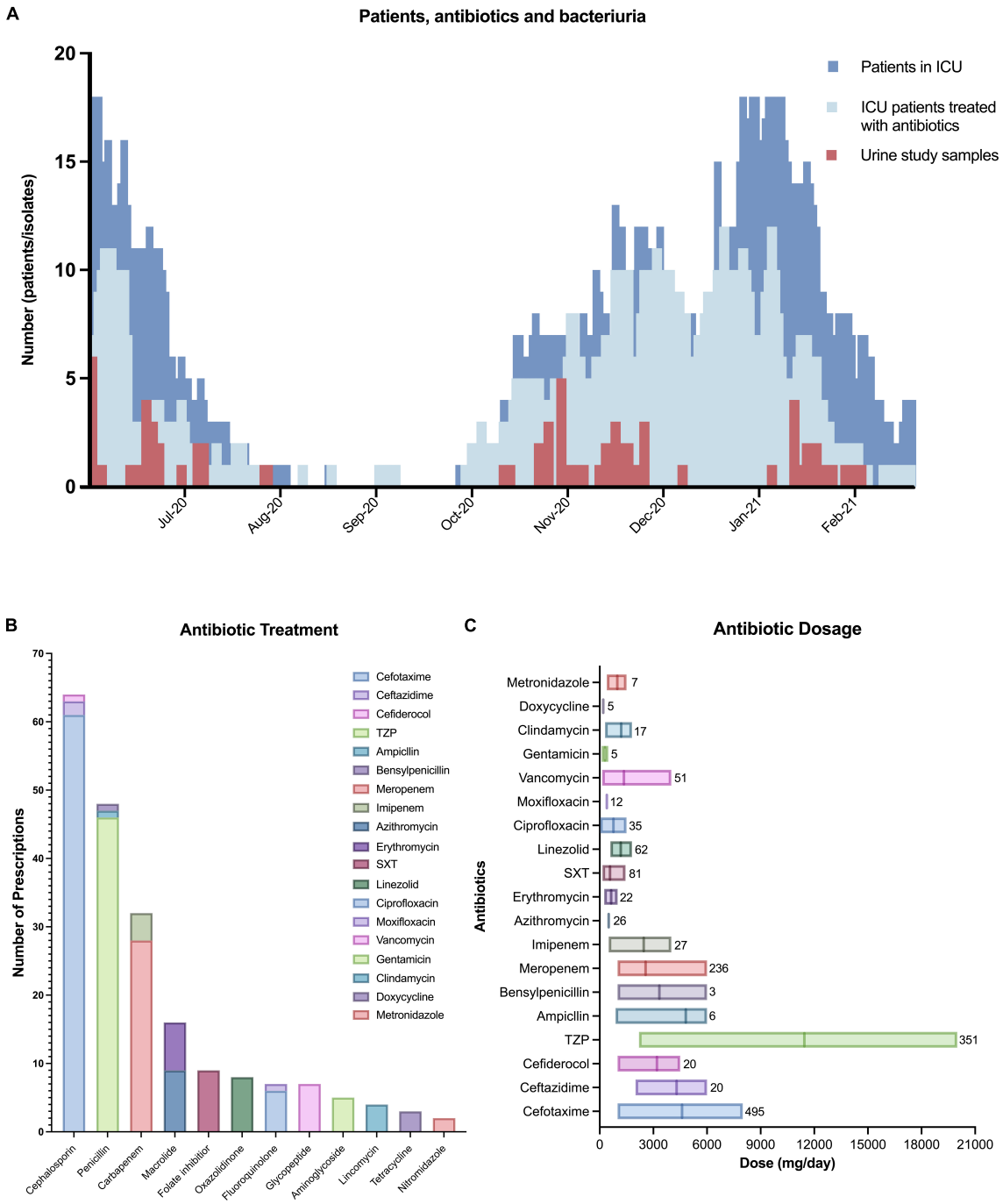


FIGURE 3
Antibiotic treatment. **(A)** Epidemiological overview of all patients admitted to the Uppsala university hospital intensive care unit (dark blue) according to the Swedish Intensive Care Registry (SIR), the number of ICU patients in our cohort receiving at least one antibiotic (bright blue), and the number of urine study samples identified (red). **(B)** The number of prescribed antibiotics where each antibiotic is counted maximum once per patient. TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole. **(C)** The dose of antibiotic prescriptions with the internal line marking the mean and the box number indicates the total number of prescription events.

Enterococcus. Only one of these enterococcal co-colonizations occurred with antibiotic-resistant *E. faecium*, while the remaining occurred with antibiotic-susceptible *E. faecalis* (Supplementary Figure 3). To better understand this association between antibiotic prescription and *Enterococcus-Escherichia* colonization over time, we constructed two patient-specific timelines of the patient with resistant *E. faecium* and a patient with susceptible *E. faecalis* (Figure 6).

Figure 6A illustrates patient A who stayed 34 days in the ICU and received early administration of cefotaxime and meropenem. Four days after meropenem, betamethasone administration started, and we identified 10^3 CFU/ml of *E. durans* in urine. Three days later, *E. durans* was replaced with 10^5 CFU/ml of *E. faecium* (clonal, novel ST127) and Enteroaggregative *E. coli* (EAEC clonal, ST69). The patient was prescribed TZP against which *E. faecium* was *in vitro* resistant. *E. coli* showed resistance against only PIP

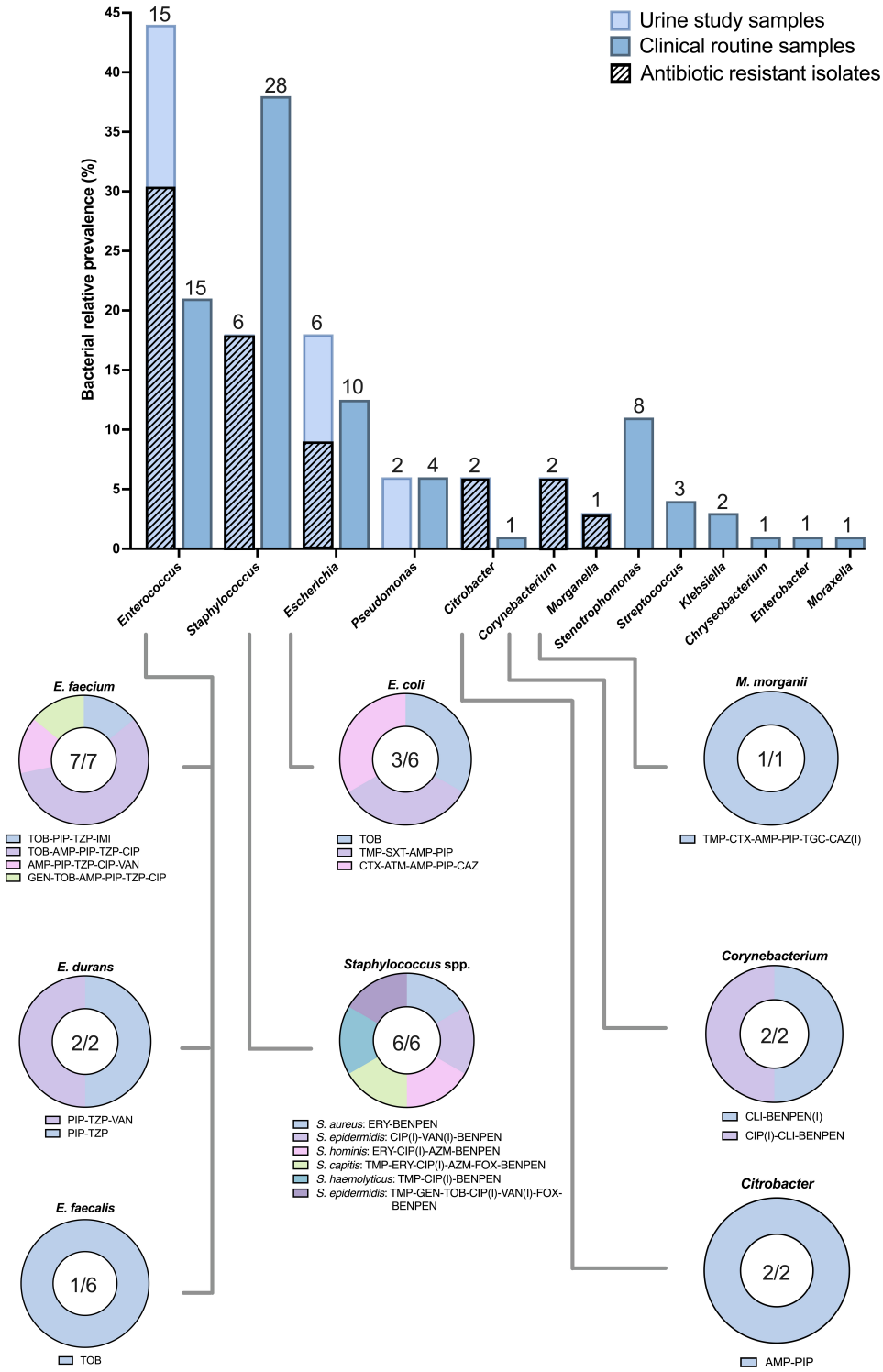


FIGURE 4
Relative prevalence and antibiotic resistant isolates. The relative prevalence of identified bacteria in urine study samples (bright blue) and any positive clinical routine sample (dark blue). The number above the bar shows the number of bacteria where each species is counted maximum once per patient. Diagonal patterns (bright blue bars) indicate resistance against at least one tested antibiotic. The lower panel illustrates identified resistant phenotypes, with the number within the circle graph showing the number of resistant bacteria in relation to the total number identified. Abbreviations for antibiotics can be found in Supplementary Table 1.

but remained during TZP treatment. *E. faecium* and *E. coli* both demonstrated a 2-fold MIC increase against PIP and TZP during active treatment (8-fold increase for TZP in *E. coli*). The MIC for TZP measured in *E. faecium* was consistently twice as high compared to PIP, as previously noted. *E. faecium* were ampicillin-susceptible PIP/TZP/IMI-resistant, despite ampicillin being used by EUCAST for inferred resistance against PIP (Supplementary Table 2). The increase seen for tobramycin marks a change from non-HLTR to

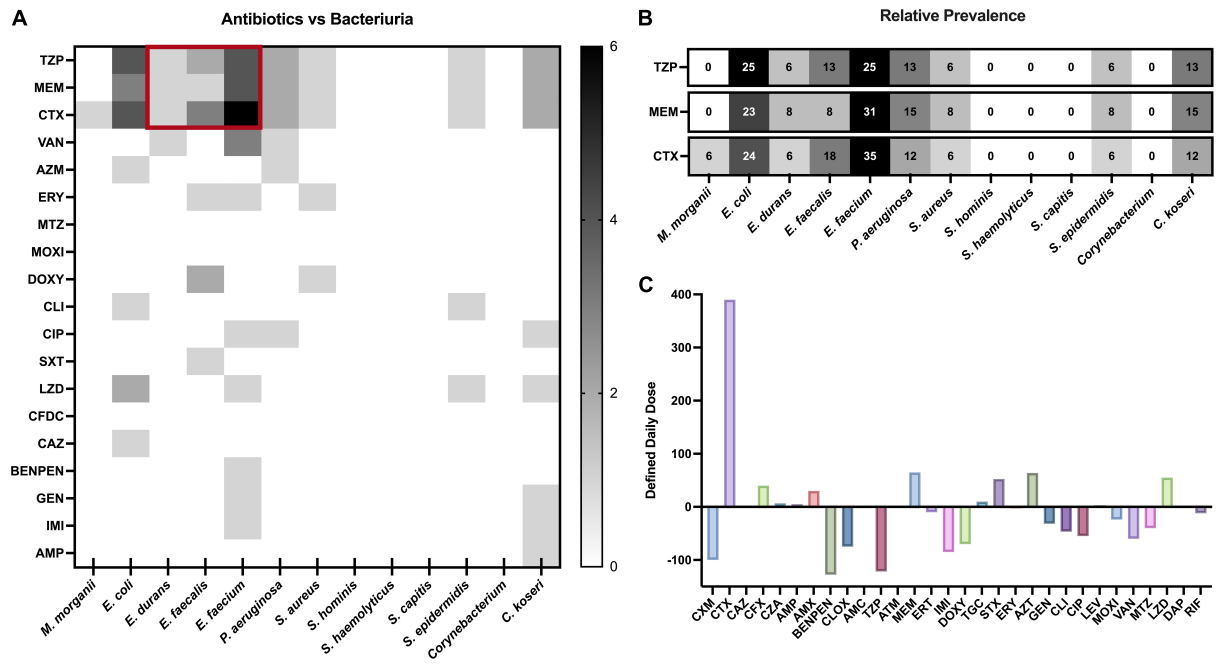


FIGURE 5
Relationship between antibiotic use and bacteriuria. **(A)** Heatmap correlation between bacteriuria and antibiotic treatment illustrated in numbers. Abbreviations for antibiotics can be found in Supplementary Table 1. **(B)** Correlation heatmap showing the proportion within a species that was exposed to a specific antibiotic. Analysis pool based on that the patient received at least one antibiotic (any) and was colonized by minimum one species (any). Numbers represent the percentage of isolates exposed to the given antibiotic (TZP/MEM/CTX). The percentage for any given antibiotic can be above 100% as isolates from different species were occasionally co-colonizing during the same treatment. **(C)** The difference in antibiotic prescription in the Uppsala ICU from 2019 to 2020 (pre-pandemic to pandemic) given in defined daily dose. Positive values indicate prescription increase while negative values indicate prescription decrease.

HLTR phenotype. To verify the results of ampicillin, imipenem PIP/TZP and tobramycin, BMDs were rerun in conjunction with E-tests, confirming these observations. The tobramycin phenotype indicated heteroresistance when confirmed with E-tests by growth of individual colonies within the zone of clearance.

Figure 6B illustrates patient B who stayed 40 days and received early cefotaxime and TZP treatment, including one day with both drugs simultaneously. Three weeks into intensive care, we identified 10^4 CFU/ml of *E. faecalis* and Uropathogenic *E. coli* (UPEC). Uncorrelated to our findings, the patient was restarted on TZP treatment that same day, suggestively suppressing colonization in agreement with *in vitro* susceptibility. Interestingly, *E. faecalis* (clone, ST16) re-emerged at 10^4 CFU/ml soon after stopping treatment, followed 2 days later by the same clonal UPEC (ST10309). The patient received a change of urinary catheter and an administration of cefotaxime. Following that intervention, *E. coli* was no longer found while cephalosporin-tolerant *E. faecalis* remained at a lower concentration (10^2 CFU/ml) that fluctuated over the last week of intensive care. The second appearance of the strains came with higher MICs for aminoglycosides, including an above clinical breakpoint level for *E. coli*.

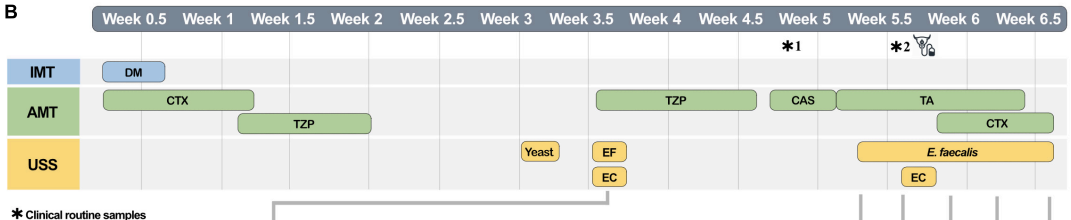
Discussion

To our knowledge, this is the first longitudinal report of bacteriuria and antimicrobial resistance in COVID-19 ICU patients, and the first large-scale epidemiological surveillance of bacteriuria in a Swedish ICU. This study is also the first to show how antibiotic treatment correlates with prevalence of *Enterococcus*, and how co-colonizers can behave in patients *via* patient timelines.

Twenty-three percent of patients experienced bacteriuria with an increased risk of 2.17 when staying more than 30 days. Bacteriuria occurred more frequently in patients surviving intensive care, most likely due to survivors' bias, with increased risk of colonization by longer LOS. Bacteriuria against LOS showed a linear regression, implying a systematic and potentially preventable occurrence in ICU practice (Figure 2D). The estimated daily risk in our study is lower (0.42%) than previous reports, ranging between 2% and 6% (7–9). A partial explanation to this difference might come from that 90% of our patients received antibiotic treatment, but other reasons include differences in classification of bacteriuria. The Uppsala ICU averaged 2.8 different antibiotics per patient (253/90), similar to the earliest reports of COVID-19 from Wuhan (4, 10). 71% (64/90) of patients received third-generation cephalosporins and 36% (32/90) carbapenems (Figure 3B), both recognized as broad spectrum antibiotics against Gram-negative bacteria. While not receiving antibiotics significantly correlated with bacteriuria (Table 1), our study also illustrates that most bacteria had resistance against at least one tested antibiotic, and that multiple bacteria classified as MDR (Figure 4). Discrepancies in reporting and definitions of resistance remains a concern for comparison of global AMR data (11).

In 45% of our patients, bacteria were detected in clinical routine samples (Table 1). These outcomes are approximately twice as high as HAIs reported from other COVID-19 ICU cohorts, although reliable and comparable data are scarce (12, 13). *Enterococcus* spp. and *Staphylococcus* spp. were most prevalent in both urine study samples and clinical routine samples (Figure 4). The high number

	10^3 CFU/ml	10^5 CFU/ml	10^5 CFU/ml	10^5 CFU/ml	10^5 CFU/ml	10^3 CFU/ml	10^3 CFU/ml
	E. durans 1	*E. faecium* 1	*E. coli* 1	*E. faecium* 2	*E. coli* 2	*E. faecium* 3	*E. coli* 3
Gentamicin	< 8	< 8	0.5	< 8	0.5	< 8	1
Tobramycin	< 8	128	1	128	1	**256**	1
Ampicillin	2	4	**> 128**	4	**> 128**	4	**> 128**
Piperacillin	**16**	**16**	**> 256**	**32**	**> 256**	**32**	**> 256**
TZP	**32**	**32**	0.5	**64**	4	**64**	4
Imipenem	< 4	**64**	< 4	**24**	< 4	**6**	< 4
Tigecycline	0.06	0.03	0.5	0.03	0.5	0.06	0.5
Ciprofloxacin	0.25	1	< 0.03	2	< 0.03	2	< 0.03
Vancomycin	0.5	1	NA	1	NA	2	NA
Linezolid	2	2	NA	2	NA	4	NA
Trimethoprim	NA	NA	**> 128**	NA	**> 128**	NA	**> 128**
SXT	NA	NA	**64**	NA	**64**	NA	**64**
Cefotaxime	NA	NA	< 0.125	NA	< 0.125	NA	< 0.125
Ceftazidime	NA	NA	< 0.25	NA	< 0.25	NA	< 0.25
Meropenem	NA	NA	< 0.5	NA	< 0.5	NA	< 0.5
Aztreonam	NA	NA	< 0.25	NA	< 0.25	NA	< 0.25



	10^4 CFU/ml	10^4 CFU/ml
	E. faecalis 1	*E. coli* 1
Gentamicin	16	1
Tobramycin	< 8	2
Ampicillin	2	4
Piperacillin	4	0.5
TZP	8	0.5
Imipenem	< 4	< 4
Tigecycline	0.25	0.0625
Ciprofloxacin	0.5	0.03125
Vancomycin	1	NA
Linezolid	2	NA
Trimethoprim	NA	< 0.25
SXT	NA	< 0.25
Cefotaxime	NA	< 1.25
Ceftazidime	NA	< 0.25
Meropenem	NA	< 0.5
Aztreonam	NA	< 0.25

	10^4 CFU/ml	10^4 CFU/ml	10^5 CFU/ml	10^2 CFU/ml	10^4 CFU/ml	10^3 CFU/ml
	E. faecalis 2	*E. coli* 2	*E. faecalis* 3	*E. faecalis* 4	*E. faecalis* 5	*E. faecalis* 6
Gentamicin	16	2	16	16	16	16
Tobramycin	16	**4**	16	16	16	16
Ampicillin	2	4	4	1	1	1
Piperacillin	4	0.5	8	4	4	8
TZP	8	0.5	8	8	8	8
Imipenem	< 4	< 4	< 4	< 4	< 4	< 4
Tigecycline	0.25	0.0625	0.25	0.25	0.25	0.25
Ciprofloxacin	0.5	0.03125	< 0.25	< 0.25	< 0.25	< 0.25
Vancomycin	1	NA	1	4	1	1
Linezolid	2	NA	2	4	1	2
Trimethoprim	NA	< 0.25	NA	NA	NA	NA
SXT	NA	< 0.25	NA	NA	NA	NA
Cefotaxime	NA	< 1.25	NA	NA	NA	NA
Ceftazidime	NA	< 0.25	NA	NA	NA	NA
Meropenem	NA	< 0.5	NA	NA	NA	NA
Aztreonam	NA	< 0.25	NA	NA	NA	NA

FIGURE 6
Individual patient timelines. *Clinical routine samples. Timeline of patient A **(A)** and patient B **(B)** at the intensive care unit stays is illustrated in half-weeks. The timeline shows immunomodulatory treatment (IMT, blue), with betamethasone (BET) and dexamethasone (DM), antimicrobial treatment (AMT, green) with caspofungin (CAS), triazole (TA), cefotaxime (CTX), meropenem (MEM), and piperacillin-tazobactam (TZP), and urine study samples (USS, yellow). Stars above the timeline indicate clinical routine samples, and the catheter symbol indicates a change of urinary catheter. The table under the timeline shows the identified minimum inhibitory concentration, with resistance according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) marked in bold red. In figure **(B)** *E. faecalis* and *E. coli* have been abbreviated EF and EC, respectively.

of *Enterococcus* is surprising. A large study from the US has demonstrated how *Enterococcus* have consistently been the second most isolated urinary bacteria, after *E. coli*, from catheterized ICU patients, irrespective of decade (1990–2007) and symptomatology (14). The European Centre for Disease Prevention and Control (ECDC) did in two surveillance reports, years 2008–2012 and 2017, show that *Enterococcus* was the second most reported ICU-based UTI in the EU as well, again only following *E. coli* (15, 16). Unlike numerous EU members, Sweden has not adopted the definitions suggested by the ECDC, leading to absence in global statistics (2, 15–17). Regional healthcare instead recognizes clinical definitions of UTIs, characterized by diagnostics on symptomatology, urine

dipsticks and C-reactive protein levels, occasionally aided by medical imaging (18). Urine cultivation is only performed for species determination and AMR-profiling on clinical indication of a UTI, and thresholds for significance (colony forming units) is determined by factors such as disease severity, sampling local, sampling method and bacterial pathogenicity group (19, 20). While practical definitions might suffice in treating individual patients, the absence of a definition can confine on equal care and antibiotic stewardship programs, while also preventing comparative representation (18). Swedish authorities have asserted that results from occasional national surveillance have been in accordance with neighboring European countries (17). Local authorities did in 2019 however, recognize UTIs as the main cause of Swedish HAIs, accounting for a staggering 60.8%, founded on marker-based journal-evaluations (2). The same report estimated the direct mortality of nosocomial UTIs to 0.4%, but indirect mortality to 4.8% (mainly due to secondary sepsis), and also showed that only having a UTI compared to not having any HAI increased inpatient care with 7.5 days. The estimated cost for Swedish inpatients corresponds to approx. 1000 EUR/day, thus putting the projected additional cost per UTI-patient to 7500 EUR.

Our study found few *E. coli*, but also few *P. aeruginosa* and a complete absence of other common Gram-negative UTI pathogens, such as *Klebsiella* and *Proteus* in urine. Immunosuppressives are known to increase risk for infection, and while the β-lactam-heavy treatment might have prevented most Gram-negative bacteria from colonizing, the regimen has little effect on more tolerant Gram-positives. Internal cephalosporin-carbapenem tolerance among *Enterococcus* spp. is well-described (21), and *in vitro* MIC results confirmed that all *E. faecium* isolates were penicillin-aminoglycoside tolerant (**Figure 4**). While the overall antibiotic use went down in the ICU compared to 2019 (pre-pandemic), we illustrate here how the specific use of meropenem, and particularly cefotaxime, radically increased in 2020 (**Figure 5C**). Moreover, we demonstrate how the prevalence of *Enterococcus* in urine coincided with treatment of meropenem and cefotaxime (**Figures 5A, B**). These factors considered; we can conclude that the high use of these β-lactams likely contributed to the proportionally elevated prevalence of *Enterococcus* in the ICU.

E. coli bacteriuria mainly occurred in concurrence with Gram-positive colonizers. While it would be intriguing to suggest that drug resistant *Enterococcus* might protect susceptible *E. coli*, our study suggests a more complex picture (**Supplementary Figure 3**). Two *E. coli* were separately isolated with MDR *E. faecium* and *S. epidermidis*, but three isolates were found alongside antibiotic susceptible *E. faecalis*. Antibiotic susceptibility in *E. faecalis* did however, not affect the strains' ability to colonize/survive. Apparent from our timeline of patient B was the agreement between *in vitro* susceptibility against TZP, and bacteriuria clearance of both *E. faecalis* and co-colonizing *E. coli* (**Figure 6B**). Importantly, the same clonal *E. faecalis* (confirmed by WGS) reappeared a full 12 days later, only to be followed by the same clonal *E. coli*. Previous molecular studies *in vitro* have shown how *Enterococcus* promote infection of *E. coli* through biofilm formation, increased virulence, and suppression of the immune system (22–24). Cases reporting *Enterococcus* spp. preceding *E. coli in vivo* are rare. Uropathogens in biofilms are known to endure with minimal metabolic activity, especially on urinary catheters. Virulent UTI bacteria, such as UPEC, have moreover been shown to adhere in extracellular matrix, inside cells, or deeper tissue layers (25). *Enterococcus* is known to act as a pioneer-species for polymicrobial colonization of catheters *in vitro* (26), and importantly in the case of patient B, the catheter had not been changed. When the catheter later was exchanged in combination with cefotaxime, *E. coli* was cleared and *E. faecalis* demonstrated a 10^3 CFU/ml-drop. The *E. coli* had *in vitro* susceptibility but *E. faecalis* are intrinsically resistant while still experiencing the CFU-reduction, suggesting biofilm on the catheter (**Figure 6B**). Only 1/15 *Enterococcus* isolates started appearing before 7 days of catheterization, signifying that adequate time is needed to establish colonization (**Supplementary Table 1**). Virulence and persistence mechanisms for these strains would require further genetic and molecular investigations out of scope for the present study.

Isolates from patient A demonstrated a MIC-increase against PIP and TZP during antibiotic treatment (**Figure 6A**). Rapid changes in AMR have previously been explained by heteroresistance (27), phase variation, gene amplification, plasmid copy number increase, or epigenetic modifications (28). Notably, EUCAST and the Clinical Laboratory Standards Institute (CLSI) indicate ampicillin for inferred resistance against PIP, yet our strains of *E. faecium* demonstrated PIP-TZP and imipenem resistance while being ampicillin-susceptible. This has previously been reported in *E. faecalis*, but to the best of our knowledge not in *E. faecium* (29, 30). That the addition of tazobactam escalates the MIC against PIP is concerning, especially given the broad use of this combination. Molecular studies are needed to elucidate underlying mechanisms for resistance in these strains.

Our study brings attention to several limitations when assessing nosocomial UTIs. Not having a global consensus when defining these infections converts a concern when reviewing previous studies and meta reports, where a discord in significance thresholds and distinguishment between diagnosis and microbial findings make comparative conclusions challenging. In concord with previous studies, we too want to highlight the risk for hidden UTI statistics during systemic inflammation and kidney injury, where primary diagnostic criteria might be masked (7, 31). We also recognize that our study has limitations. Our investigation did not allow for follow-up on colonization and treatment before or after ICU stay, hence we cannot rule out pre/post ICU antibiotics and bacteriuria. Cultivation did not allow for detection of anaerobic bacteria and might have reduced transient gene- or plasmid amplification events in relation to antibiotic resistance. As our permit allowed for non-invasive sample collection, we could not assess microbial growth in patients experiencing anuria.

In conclusion, we identified LOS as a predisposing factor for bacteriuria in Swedish COVID-19 ICU patients. We detected MDR bacteria defined as "critical" or of high concern on the WHO priority list (32, 33). High-level use of β-lactams, especially cefotaxime, likely contributed to a disproportionally high prevalence of Gram-positive colonizers and MDR bacteria, mostly *Enterococcus*. The ability of *E. coli* to cause bacteriuria despite effective antibiotic treatment, when found in co-culture with cephalosporin-tolerant *Enterococcus*, highlights the role of biofilm in urinary catheters as a reservoir of pathogenic bacteria with the potential to develop and disseminate AMR. We want to stress that AMR and healthcare-associated UTIs increase healthcare costs and constitute persistent risks for patients, and that polymicrobial biofilms in catheters probably are more common and complicated than what the categories of UTI diagnostics might imply. This study provides new insight into the role of ICU stay and antibiotic use in shaping bacteriuria, and how colonization permits polymicrobial communities of susceptible but pathogenic bacteria to remain during treatment.

Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

Data found in this research are part of the PronMed study approved by the National Ethical Review Agency [Dnr 2017/043 (with amendments 2020-01623, 2020-02719, 2020-05730, and 2021-01469) and 2022-00526-01)] and listed at ClinicalTrials.gov (NCT03720860). Informed consent was obtained from the patient or next of kin. The Declaration of Helsinki and its subsequent revisions were followed. Adult ICU patients admitted between the June 5, 2020 and February 17, 2021 with reverse-transcription polymerase chain reaction (RT-PCR) positive nasopharyngeal swabs were prospectively recruited to the study. Exclusion criteria were pregnancy, current breastfeeding, and age under 18. End of follow-up was defined to the end of ICU treatment.

Author contributions

PK, HW, and JJ: conceptualization and writing—original draft. PK, ED, JP, MH, and RF: data curation. PK, JP, NF-K, HW, and JJ: formal analysis. HW, MH, RF, and JJ: funding acquisition. PK, JP, HW, and JJ: investigation. PK, HW, JJ, MH, RF, and ML: methodology. HW, JJ, MH, and RF: project administration. HW and JJ: validation. JP, ED, NF-K, MH, RF, and ML: writing—review and editing. All authors contributed to the interpretation of results and critical review of the manuscript and had access to the data, except for identifiable clinical data to which access was restricted to those acquiring and analyzing it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1087446/full#supplementary-material

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Incidence and clinical outcomes of bacterial superinfections in critically ill patients with COVID-19

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Background: Bacterial superinfection is not uncommon in critically ill patients with coronavirus disease (COVID-19) pneumonia requiring intensive care unit (ICU) treatment. However, there is still a lack of evidence related to bacterial superinfection and their clinical significance in critically ill patients with COVID-19. Therefore, we assessed the incidence of bacterial superinfections and their effects on clinical outcomes in critically ill patients with COVID-19.

Materials and methods: This single-center retrospective cohort study analyzed critically ill patients with COVID-19 admitted to the ICU at a tertiary academic hospital between February 2020 and December 2021. We reviewed data including patient demographics, clinical and microbiological characteristics, and outcomes.

Results: During the study period, 106 patients (median [IQR] age, 67 [58–75] years) were included, of which 32 (30%) were diagnosed with bacterial superinfections. Of these, 12 cases (38%) were associated with multidrug-resistant pathogens. *Klebsiella aerogenes* (6 cases [19%]) and *Klebsiella pneumoniae* (6 cases [19%]) were the most common pathogens associated with superinfections. The median time to bacterial superinfection was 13 (IQR, 9–20) days after ICU admission. Patients with bacterial superinfections had significantly fewer ventilator-free days on day 28 (0 [IQR, 0–0] days) than those without bacterial superinfections (19 [IQR, 0–22] days) (p<0.001). Patients with bacterial superinfections had a longer ICU length of stay (32 [IQR, 9–53] days) than those without bacterial superinfections (11 [IQR, 7–18] days) (p <0.001). Additionally, they had a longer hospital length of stay after ICU admission (39 [IQR, 18–62] days) than those without bacterial superinfections (18 [IQR, 12–37] days) (p =0.001). There were no differences in ICU mortality or in-hospital mortality between the two groups. In the multivariable analysis, higher SAPS II score (OR, 2.697; 95% CI, 1.086–6.695) and thrombocytopenia (OR, 3.318; 95% CI, 1.355–8.123) were identified as risk factors for development of bacterial superinfection.

Conclusion: In critically ill patients with COVID-19, bacterial superinfections were common, and more than one-third of the bacterial superinfection cases were caused by multidrug-resistant pathogens. As patients with bacterial superinfections had worse clinical outcomes, the development of bacterial superinfections should be actively monitored.

KEYWORDS

incidence, intensive care units, outcome, SARS-CoV-2, superinfection, COVID-19, thrombocytopenia

1. Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection pandemic has lasted for more than 2 years since 2020. Over 500 million confirmed cases have been reported worldwide, and over six million deaths have been recorded (1). Because the SARS-CoV-2 infection often leads to the development of acute respiratory distress syndrome (ARDS), critically ill patients with severe coronavirus disease (COVID-19) pneumonia often require prolonged mechanical ventilation (2–4). One recent randomized controlled trial showed that early administration of dexamethasone in ARDS reduced the duration of mechanical ventilation and overall mortality (5). Moreover, the use of dexamethasone in COVID-19 ARDS has become commonplace, as it was found to lower mortality risk in patients requiring oxygen therapy and mechanical ventilation (6). Unfortunately, however, a study conducted by Bernard et al. (7) showed that the use of high-dose glucocorticoids was associated with an increased risk of secondary bacterial infections in patients with ARDS. In addition, the SARS-CoV-2 has been reported to cause immune dysregulation through increase of neutrophil-lymphocyte-ratio, and T lymphopenia (8). Thus, with the increased use of glucocorticoids compounded with a dysregulated host immune response caused by the SARS-CoV-2, secondary infections in critically ill patients with COVID-19 have become a major concern.

Studies of previous viral pandemics have showed that additional bacterial infections were associated with increased mortality, higher rates of respiratory distress, and more frequent ICU admissions (9, 10). As such, a better understanding of bacterial superinfections in COVID-19 is needed to improve patient outcomes. However, current literature on bacterial superinfections in COVID-19 is scarce, and most studies have focused on patients with mild-to-moderate illness severity rather than critically ill patients admitted to the ICU (11–15). Although the current guidelines recommend empirical therapy with antimicrobials in patients with severe COVID-19 pneumonia, there is a lack of evidence related to bacterial superinfection including multidrug-resistant (MDR) pathogen infections, and their clinical significance (16, 17).

In this study, we hypothesized that critically ill patients with COVID-19 who develop bacterial superinfection are at increased risk for worse clinical outcomes. Here, we assessed the incidence of bacterial superinfection, including new episodes of pneumonia, urinary tract infection (UTI), bacteremia, or other infections, and those of multidrug-resistant pathogens and determined their effects on clinical outcomes in critically ill patients with severe COVID-19 pneumonia who require ICU admission.

2. Materials and methods

2.1. Study design and patients

This single-center, retrospective cohort study analyzed critically ill patients with severe COVID-19 pneumonia who were admitted to a 12-bed disaster ICU at Seoul National University Hospital, a tertiary academic hospital in South Korea that served as a nationally designated hospital for patients with severe and critical COVID-19, between February 2020 and December 2021.

Adult patients (aged ≥18 years) who were diagnosed with COVID-19 through reverse transcription-polymerase chain reaction assay and were admitted to the disaster ICU due to severe COVID-19 pneumonia were included and followed up until the time of hospital discharge or death. According to the World Health Organization guidelines for COVID-19, severe COVID-19 pneumonia was defined as the presence of at least one of the following: oxygen saturation < 90% in room air or signs of severe respiratory distress (accessory muscle use, inability to complete full sentences, or respiratory rate > 30 breaths per minute) (13). We excluded patients who had confirmed bacterial infections within 6 months prior to ICU admission or within 48 h after ICU admission, completed a Physician Orders for Life-Sustaining Treatment (POLST, including do-not-intubate orders) form, stayed in the ICU for less than 48 h, or were transferred from an overseas hospital. The Institutional Review Board (IRB) of Seoul National University Hospital waived the requirement for written informed consent and approved this study (approval number: IRB-H-2106-213-1,231).

2.2. Data collection

We reviewed the following data of all the patients in our database: demographic characteristics, comorbidities, Charlson comorbidity index, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, Sequential Organ Failure Assessment (SOFA) score, Simplified Acute Physiology Score II (SAPS II) score, site of sample collection and method (sputum culture, endotracheal aspirates, bronchoscopic washing, bronchoalveolar lavage, urine culture, blood culture, or other cultures), bacterial species, antibiotic susceptibility, and clinical outcomes. During the study period, all patients included in this study underwent systematic screening for colonization by MDR bacteria (nasal methicillin-resistant *Staphylococcus aureus*, sputum carbapenem-resistant *Acinetobacter baumannii*, rectal vancomycin-resistant *Enterococci*, and rectal carbapenem-resistant *Enterobacteriaceae*) on ICU admission. These data were also reviewed. Based on the definition used in previous studies, an immunocompromised condition was defined as a diagnosis of primary immunodeficiency disorder, a diagnosis of human immunodeficiency virus (HIV) infection or acquired immune deficiency syndrome (AIDS), solid organ/hematopoietic stem cell transplant recipients, and receipt of any chemotherapy or immunosuppressants, including corticosteroids (prednisolone ≥20 mg/day, or an equivalent dose of other corticosteroids, for 2 weeks or longer) in the 6 months prior to COVID-19 diagnosis (18–20). Although the definition of ARDS under high-flow nasal oxygen (HFNO) is unclear and there is a difference in the PaO_2:FiO_2 ratio compared to when the mechanical ventilator is applied (21), we used an expanded definition of ARDS as follows (22): PaO_2:FiO_2 ratio ≤ 300 mmHg, patients treated with HFNO of at least 30 L/min or with a positive end-expiratory pressure ≥ 5 cm of water, and bilateral infiltrates documented by chest radiography or a computed tomography scan.

2.3. Bacterial superinfection and multidrug-resistant pathogens

A blood culture was obtained within 24 h of ICU admission, and thereafter, microbiologic samples were obtained according to the discretion of the attending intensivists. Bacterial superinfection was defined as clinical deterioration and the presence of bacteria identified in the lower respiratory tract (sputum, endotracheal aspirates, bronchoscopic washing, or bronchoalveolar lavage) urine culture, blood culture, or other culture samples (e.g., pleural effusion, ascitic fluid) after 48 h of ICU admission. MDR pathogens were defined as bacteria that are resistant to three or more types (one or more of each type) of antibiotics with different structures (different mechanisms of action) (23, 24).

2.4. Statistical analysis

Continuous variables were reported as medians and interquartile ranges (IQR), and categorical variables were expressed as counts and percentages. Between-group differences in baseline characteristics were assessed using the chi-square test or Fisher's exact test for qualitative variables and Student *t*-test or Mann–Whitney *U* test for quantitative variables. Univariable and multivariable logistic regression analyses were used to identify the risk factors for bacterial superinfection in patients with severe COVID-19 pneumonia. Independent variables were selected based on biological plausibility and associations in the scientific literature (15, 25, 26). All the variables with a *p*-value of <0.20 in the univariable analysis were included in the multivariable stepwise backward logistic regression model to avoid model overfitting (27). In addition, we generated a receiver-operating characteristic (ROC) curve and estimated the area under the curve (AUROC) to determine the predictive value and optimal cut-off values of variables with a value of p of less than 0.05 in the multivariable logistic regression analysis of risk factors for the development of bacterial superinfection. The optimal cut-off values were determined based on Youden's index, which maximizes the sum of the sensitivity and specificity. The results were presented as odds ratios (OR) with 95% confidence intervals (CI). All the analyses were two-tailed, and *p*-values less than 0.05 were considered significant. IBM SPSS Statistics (version 25.0 for Windows; IBM, Armonk, NY, United States) was used for all the statistical analyses.

3. Results

3.1. Patient characteristics

Of the 120 patients assessed for eligibility, 14 patients were excluded for the following reasons: (1) five patients had confirmed bacterial infections within 6 months prior to ICU admission or within 48 h after ICU admission, (2) six patients completed a POLST (including do-not-intubate orders) form, (3) one patient stayed in the ICU for less than 48 h, and (4) two patients were transferred from an overseas ICU. A total of 106 patients were included in this study, of which 32 (30%) were diagnosed with bacterial superinfections (bacterial superinfection group), and 74 (70%) were without bacterial superinfections (COVID-only group) (Figure 1).

Table 1 shows the demographic and clinical characteristics at the baseline. The median age was 67 (IQR, 58–75) years, 65% of the patients were men, the median body mass index was 24.2 (IQR, 22.6–25.8) kg/m^2, 52% had hypertension, and 34% had diabetes mellitus. The demographic and clinical characteristics of the patients at baseline were comparable between the two groups, except for the platelet count, SOFA score, and SAPS II score. The bacterial superinfection group had significantly lower platelet counts ($p = 0.005$) and higher SOFA ($p = 0.008$) and SAPS II scores ($p = 0.011$) than those of the COVID-only group.

3.2. Interventions and clinical outcomes

Table 2 summarizes the interventions required during ICU stay and the clinical outcomes according to the presence of bacterial superinfection. ARDS was identified in 89 of 106 (84%) critically ill patients with severe COVID-19 pneumonia: 91% (29 of 32 patients)

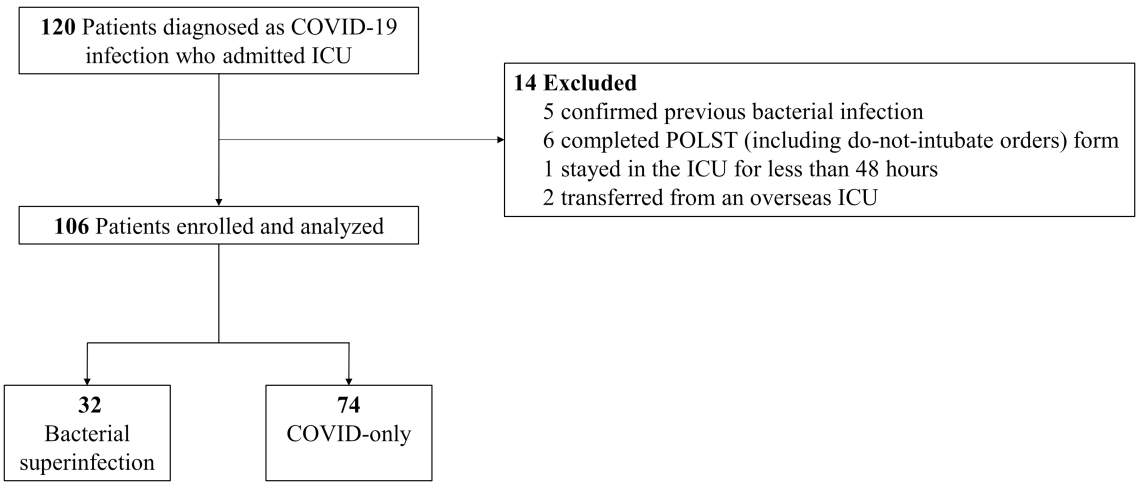

FIGURE 1
Flow diagram of the study population. POLST, portable orders for life-sustaining treatment; ICU, intensive care unit; COVID-19, coronavirus disease.

TABLE 1 Baseline and clinical characteristics of critically ill patients with COVID-19.

Variables	Total (n=106)	Bacterial superinfection (n =32)	COVID-only (n =74)	p−value
Age, years	67 (58–75)	67 (61–72)	68 (58–76)	0.508
Male, *n* (%)	69 (65)	24 (75)	45 (61)	0.159
BMI, kg/m^2	24.2 (22.6–25.8)	24.3 (22.7–27.1)	24.2 (22.6–25.5)	0.529
Comorbidities, *n* (%)				
Hypertension	55 (52)	14 (44)	41(55)	0.270
Diabetes mellitus	36 (34)	11 (34)	25 (34)	0.953
Heart failure	1 (1)	0 (0)	1 (1)	>0.999
Chronic liver disease	1 (1)	0 (0)	1 (1)	>0.999
Chronic kidney disease	11 (10)	4 (13)	7 (10)	0.637
Chronic obstructive pulmonary disease	5 (5)	3 (9)	2 (3)	0.160
Immunocompromised	7 (7)	2 (6)	5 (7)	>0.999
Charlson comorbidity index	3 (2–4)	3 (2–4)	3 (2–4)	0.735
Upon ICU admission				
APACHE II score	13 (10–21)	17 (11–22)	12 (9–20)	0.099
SOFA score	5 (3–10)	9 (5–12)	4 (3–8)	0.008
SAPS II score	33 (23–46)	40 (28–57)	31 (21–41)	0.011
Screening test, *n* (%)				
Nasal MRSA	3 (3)	2 (6)	1 (1)	0.203
Sputum CRAB	0 (0)	0 (0)	0 (0)	
Rectal VRE	4 (4)	1 (3)	3 (4)	>0.999
Rectal CRE	2 (2)	0 (0)	2 (3)	>0.999
Laboratory data				
WBC, 10^3/μL	7.9 (5.4–10.7)	8.3 (6.5–12.7)	7.4 (5.3–10.3)	0.366
Leukocytes count, 10^3/μL	6.9 (4.5–9.2)	7.4 (5.9–11.0)	6.4 (4.4–8.8)	0.396
Lymphocytes count, 10^3/μL	0.7 (0.5–0.9)	0.6 (0.3–0.9)	0.6 (0.4–0.8)	0.981
Platelets count, 10^3/μL	195 (134–255)	135 (98–193)	220 (149–276)	0.005
Fibrinogen, mg/dL	436 (354–502)	432 (323–489)	447 (364–518)	0.069
Lactic acid, mmol/L	1.4 (1.1–1.9)	1.4 (1.2–1.9)	1.4 (1.0–2.0)	0.547
CRP, mg/dL	7.9 (4.2–17.3)	9.4 (3.7–18.3)	7.4 (4.2–15.8)	0.575
Procalcitonin, ng/mL	0.2 (0.1–0.5)	0.2 (0.1–0.7)	0.2 (0.1–0.5)	0.918
LDH, IU/L	478 (346–593)	494 (349–608)	472 (341–569)	0.890

Values are presented as number (%), median (interquartile range). LOS, length of stay; ICU, intensive care unit; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; SAPS II, Simplified Acute Physiology Score II; MRSA, methicillin-resistant *Staphylococcus aureus*; CRAB, carbapenem-resistant *Acinetobacter baumannii*; VRE, vancomycin-resistant *Enterococci*; CRE, carbapenem-resistant Enterobacteriaceae; CRP, C-reactive protein; LDH, lactate dehydrogenase.

in the bacterial superinfection group and 81% (60 of 74 patients) in the COVID-only group (p =0.219). There were no significant differences in the interventions required during ICU stay between the two groups, such as HFNO, mechanical ventilator, extracorporeal membrane oxygenator, renal replacement therapy, prone positioning, nitric oxide use, and vasopressor use.

The median time to bacterial superinfection was 13 (IQR, 9–20) days after ICU admission. Among the patients who received mechanical ventilator treatment during ICU stay (27 [84%] patients in the bacterial superinfection group and 51 [69%] patients in the COVID-only group), ventilator-free days at 28 days were significantly lower in the bacterial superinfection group than those in the COVID-only group: 0 (IQR, 0–0) days versus 19 (IQR, 0–22) days (p<0.001) (Table 2). Moreover, the ICU length of stay was significantly longer in the bacterial superinfection group than that in the COVID-only group: 32 (IQR, 9–53) days versus 11 (IQR, 7–18) days (p<0.001). Additionally, the length of hospital stay after ICU admission was significantly longer in the bacterial superinfection group than that in the COVID-only group: 39 (IQR, 18–62) days versus 18 (IQR, 12–37) days (p =0.001). ICU mortality, in-hospital mortality, and 28-day mortality were higher in the bacterial superinfection group, but these differences were not statistically significant.

TABLE 2 Interventions and clinical outcomes in critically ill patients with COVID-19.

Variables	Total (n=106)	Bacterial superinfection (n =32)	COVID-only (n =74)	p-value
Acute respiratory distress syndrome, *n* (%)*	89 (84)	29 (91)	60 (81)	0.219
Life support treatment during ICU stay, *n* (%)				
High-flow nasal oxygen	101 (95)	30 (94)	71 (96)	0.637
Mechanical ventilation	78 (74)	27 (84)	51 (69)	0.098
Extracorporeal membrane oxygenator	8 (8)	4 (13)	4 (5)	0.239
Renal replacement therapy	12 (11)	6 (19)	6 (8)	0.178
Prone positioning	74 (70)	24 (75)	50 (68)	0.444
Inhaled nitric oxide	16 (15)	8 (25)	8 (11)	0.078
Vasopressor	80 (76)	28 (88)	52 (70)	0.058
Clinical outcomes				
Hospital length of stay after ICU admission, days (IQR)	22 (13–45)	39 (18–62)	18 (12–37)	0.001
ICU length of stay, days (IQR)	12 (7–32)	32 (9–53)	11 (7–18)	<0.001
Ventilator-free days at day 28, days (IQR)**	15 (0–21)	0 (0–0)	19 (0–22)	<0.001
ICU mortality, *n* (%)	20 (19)	7 (22)	13 (18)	0.603
In-hospital mortality, *n* (%)	22 (21)	8 (25)	14 (19)	0.478
28-day mortality, *n* (%)	9 (9)	4 (13)	5 (7)	0.446

*Acute respiratory distress syndrome definition: (1) PaO_2:FiO_2 ratio ≤ 300 mmHg, (2) patients treated with HFNO of at least 30 l/min or with a positive end-expiratory pressure ≥ 5 cm of water, and (3) bilateral infiltrates documented by chest radiography or computed tomography scan. **Calculated for patients receiving mechanical ventilator treatment (27 for bacterial superinfection group and 51 for COVID-only group). ICU, intensive care unit, IQR, interquartile range.

3.3. Microbiological results and risk factors of bacterial superinfection

Of the 32 bacterial superinfections, 12 (38%) were caused by MDR pathogens. Gram-positive and Gram-negative bacteria were responsible for 12 and 20 cases, respectively, of superinfection, of which 7 (58%) and 5 (25%), respectively, were caused by MDR pathogens. Table 3 shows the types of bacteria that caused bacterial superinfections and the MDR status according to the source of infection. Of the 23 cases of lower respiratory tract infections, 5 (22%) were caused by MDR pathogens. Of the 7 cases of bloodstream infections, 5 (71%) were caused by MDR pathogens. Catheter-associated urinary tract infections were caused only by MDR pathogens. The most common pathogens associated with bacterial superinfections were *Klebsiella aerogenes* (6 cases [19%]) and *Klebsiella pneumoniae* (6 cases [19%]) (Figure 2).

In the univariable analysis, a high SOFA score (OR, 1.132; 95% CI, 1.022–1.254; p = 0.018), or SAPS II score (OR, 1.031; 95% CI, 1.006–1.056; p = 0.016), and a low platelet count (OR, 0.992; 95% CI, 0.987–0.998; p = 0.006) were identified as risk factors for the development of bacterial superinfection (Table 4). In the multivariable analysis model 1, platelet count, APACHE II, and SAPS II scores were treated as continuous variables. The SAPS II score (OR, 1.065; 95% CI, 1.013–1.121; p = 0.014) and platelet count (OR, 0.993; 95% CI, 0.987–0.998; p = 0.011) were identified as risk factors for the development of bacterial superinfection (Table 4). We generated an ROC curve and estimated the AUROC to determine the predictive value and optimal cut-off value of SAPS II and platelet count for the development of bacterial superinfection. Using a cut-off value of 42, the SAPS II score predicted bacterial superinfection with a sensitivity of 50.0% and specificity of 75.7% with an AUROC of 0.647 (95% CI, 0.536–0.759; p < 0.001). Using a cut-off value of 172,000/μL, platelet count predicted bacterial superinfection with a sensitivity of 71.9% and specificity of 68.9% with an AUROC of 0.696 (95% CI, 0.577–0.816; p < 0.001). In the multivariable analysis model 2, platelet count, APACHE II, and SAPS II scores were treated as categorical variables. Based on the results of the ROC curve analysis and biological plausibility, a SAPS II score of 42, an APACHE II score of 11, and a platelet count of 150,000/μL were set as the cut-off values in the present study. The SAPS II score (OR, 2.697; 95% CI, 1.086–6.695; p = 0.032) and platelet count (OR, 3.318; 95% CI, 1.355–8.123; p = 0.012) were identified as risk factors for the development of bacterial superinfection.

3.4. Thrombocytopenia and clinical outcomes

Further sensitivity analysis was performed by dividing the patients into two groups according to their risk factors for the development of bacterial superinfection: a high-risk group with a SAPS II score ≥ 42 or thrombocytopenia (platelet count <150,000/μL), and a low-risk group with a SAPS II score < 42 and without thrombocytopenia (Table 5). The incidence of bacterial superinfection was significantly higher in the high-risk group than in the low-risk group: 49.1% (26 of 53 patients) versus 11.3% (6 of 53 patients), respectively (p < 0.001). Of the 32 cases of bacterial superinfection, 12 (38%) were caused by MDR pathogens and were observed only in the high-risk group. Among the patients who received mechanical ventilator treatment

TABLE 3 Source of superinfection and microbiology in critically ill patients with COVID-19.

Bacteria		Lower respiratory tract infection		Bloodstream infection		Catheter-associated urinary tract infection		Total
		Non-MDR	MDR	Non-MDR	MDR	Non-MDR	MDR	
G(+)	*Staphylococcus aureus*	1	2	1	0	0	0	4
	Staphylococcus epidermidis	0	0	0	3	0	0	3
	Streptococcus pneumoniae	2	0	0	0	0	0	2
	Staphylococcus haemolyticus	0	0	0	2	0	0	2
	Corynebacterium species	0	0	1	0	0	0	1
G(−)	*Klebsiella aerogenes*	5	1	0	0	0	0	6
	Klebsiella pneumoniae	5	0	0	0	0	1	6
	Stenotrophomonas maltophilia	3	0	0	0	0	0	3
	Escherichia coli	0	1	0	0	0	1	2
	Pseudomonas aeruginosa	2	0	0	0	0	0	2
	Acinetobacter baumannii	0	1	0	0	0	0	1
		18	5	2	5	0	2	32

MDR, multidrug-resistant; G(+), gram positive; G(−), gram negative.

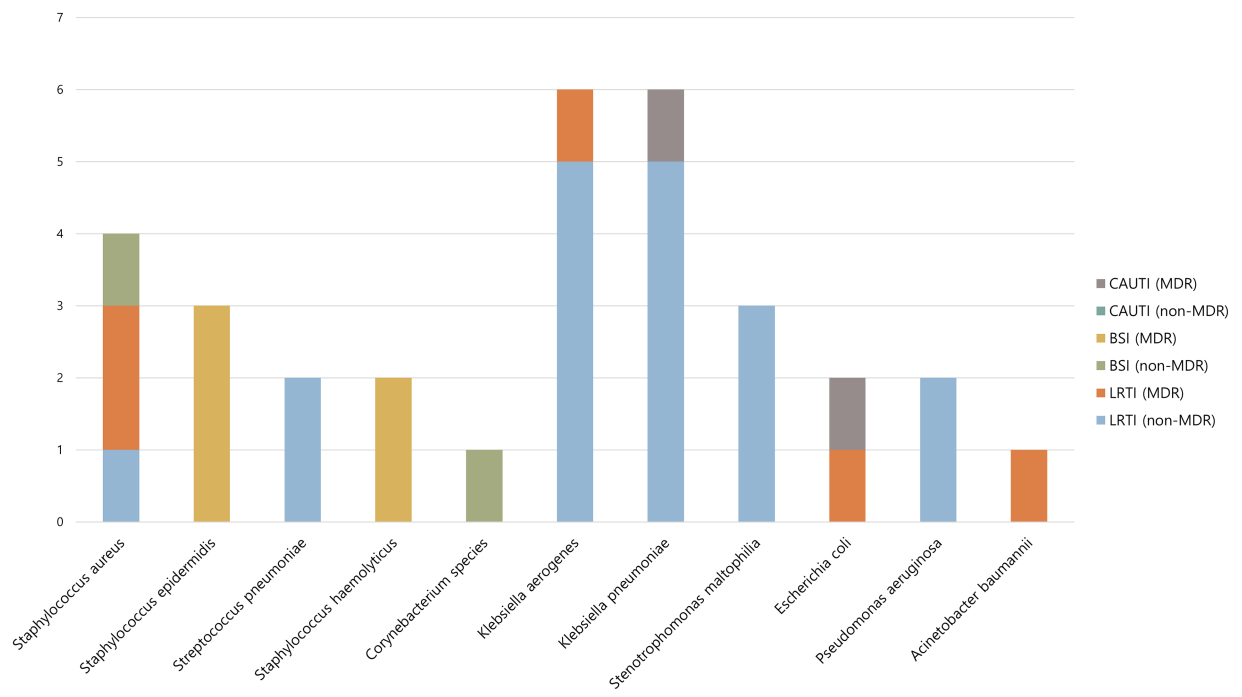

FIGURE 2
Causative microorganism of bacterial superinfection according to site of infection and MDR status. MDR, multidrug-resistant; CAUTI, catheter-associated urinary tract infection; BSI, bloodstream infection; LRTI, lower respiratory tract infection.

during their ICU stay (45 [85%] patients in the high-risk group and 33 [62%] patients in the low-risk group), the number of ventilator-free days at day 28 were significantly lower in the high-risk group than in the low-risk group: 0 (IQR, 0–18) days versus 21 (IQR, 18–23) days ($p < 0.001$). Moreover, the length of ICU stay was significantly longer in the high-risk group than in the low-risk group: 24 (IQR, 10–46) days versus 10 (IQR, 5–14) days ($p < 0.001$). Additionally, the length of hospital stay after ICU admission was significantly longer in the high-risk group than in the low-risk group: 33 (IQR, 15–64) days versus 16 (IQR, 11–28) days ($p < 0.001$). The ICU mortality, in-hospital mortality, and 28-day mortality rates were significantly higher in the high-risk group than in the low-risk group (Table 5).

TABLE 4 Risk factors for the development of bacterial superinfection in critically ill patients with COVID-19.

	Univariable		Model 1*		Model 2**	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age	1.011 (0.979–1.044)	0.504				
Male sex	1.933 (0.766–4.882)	0.163				
Charlson comorbidity index	1.041 (0.827–1.310)	0.907				
Immunocompromised	0.920 (0.169–5.010)	0.923				
APACHE II score	1.046 (0.991–1.103)	0.103	0.912 (0.817–1.018)	0.100		
SOFA score	1.132 (1.022–1.254)	0.018				
SAPS II score	1.031 (1.006–1.056)	0.016	1.065 (1.013–1.121)	0.014	2.697 (1.086–6.695)	0.032
Neutrophil count	1.000 (1.000–1.000)	0.395				
Lymphocyte count	1.000 (0.999–1.001)	0.981				
Platelet count	0.992 (0.987–0.998)	0.006	0.993 (0.987–0.998)	0.011	3.318 (1.355–8.123)	0.012
Fibrinogen	0.996 (0.992–1.000)	0.073				

*In model 1, platelet count, APACHE II, and SAPS II scores were treated as continuous variables. All the variables with a *p*-value of <0.20 in the univariable analysis were included in the multivariable stepwise backward logistic regression model. **In model 2, APACHE II score (APACHE II ≥11), SAPS II score (SAPS II ≥42), and platelet count (platelet count <150,000/mm^3) were treated as categorical variables. All the variables with a *p*-value of <0.20 in the univariable analysis were included in the multivariable stepwise backward logistic regression model. OR, odds ratio; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Assessment; SAPS II, Simplified Acute Physiology Score II.

TABLE 5 Bacterial superinfection and clinical outcomes in critically ill patients with COVID-19 according to their SAPS II score and platelet count.

	High-risk*	Low-risk*	*p*-value
	(*n* =53)	(*n* =53)	
Bacterial superinfection, *n* (%)	26 (49)	6 (11)	<0.001
MDR pathogen superinfection, *n* (%)	12 (23)	0 (0)	<0.001
Treatment outcomes			
Hospital length of stay after ICU admission, days (IQR)	33 (15–64)	16 (11–28)	<0.001
ICU length of stay, days (IQR)	24 (10–46)	10 (5–14)	<0.001
Ventilator-free days at day 28, days (IQR)**	0 (0–18)	21 (18–23)	<0.001
ICU mortality, *n* (%)	16 (30)	4 (8)	0.003
In-hospital mortality, *n* (%)	18 (34)	4 (8)	0.001
28-day mortality, *n* (%)	8 (15)	1 (2)	0.015

*The high-risk group was defined as patients with SAPS II ≥42 or thrombocytopenia (platelets <150,000/μL), and the low-risk group was defined as patients without both conditions. **Calculated for patients receiving mechanical ventilator treatment (45 for high-risk group and 33 for low-risk group). COVID-19, coronavirus disease; SAPS II, Simplified Acute Physiology Score II; MDR, multidrug-resistant; ICU, intensive care unit; IQR, interquartile range.

4. Discussion

In this retrospective cohort study of critically ill patients with severe COVID-19 pneumonia, there were 32 cases of infections with bacterial pathogens, newly confirmed after ICU admission. More than one-third of the cases were associated with MDR pathogens, with *Klebsiella aerogenes* and *Klebsiella pneumoniae* being the most common pathogens. Patients with bacterial superinfections had worse clinical outcomes, including fewer ventilator-free days, longer ICU stay, and longer hospital stay after ICU admission. However, there were no statically significant differences in ICU and in-hospital mortality between patients with and without bacterial superinfections. A higher SAPS II score and thrombocytopenia were independent risk factors for the development of bacterial superinfection. Moreover, patients with such risk factors had a significantly higher incidence of bacterial superinfection and worse clinical outcomes than those without risk factors. Notably, bacterial superinfection caused by MDR pathogens occurred only in patients with risk factors.

Our results are consistent with those of previous studies that reported the bacterial superinfection rate as 9–59% (11, 12, 15, 25, 26, 28, 29). Of these, three studies were conducted on ICU patients, and it is judged that the incidence rate has varied due to differences in the definition of superinfection. A study conducted in Iran used only sputum and tracheal aspirates without bronchoscopic examination when acquiring respiratory specimens, and reported a superinfection incidence rate of 12% (29). In the study conducted in Spain, the incidence rate of superinfection was reported as 41% including fungal superinfection (25). Finally, a study conducted in the United States reported a ventilator-associated pneumonia incidence rate of 44% using multiplex PCR in addition to quantitative culture with bronchoalveolar lavage specimens (28). In particular, Bardi et al. (25) reported that the median time to superinfection was 9 (IQR, 5–11)

days and Pickens et al. (28) reported that the average time to ventilator-associated pneumonia was 10.8 days, which are similar to our results. Therefore, the development of bacterial superinfections should be carefully monitored in critically ill patients with severe COVID-19 pneumonia who require hospitalization for more than 1 week.

In the present study, patients with bacterial superinfections had significantly fewer ventilator-free days than those without bacterial superinfections. Moreover, as the liberation from mechanical ventilation was delayed, the ICU length of stay was significantly greater in patients with bacterial superinfections than in those without bacterial superinfections. Additionally, the aforementioned study by Bardi et al. (25) reported that bacterial superinfections in the ICU were associated with an increase in ICU length of stay and mortality. Although our results were not statistically significant, ICU mortality was numerically higher in the patients with bacterial superinfections than that in those without bacterial superinfections. Our study may have been underpowered to detect a clinically important difference in mortality. To date, studies analyzing the risk of bacterial superinfections in critically ill patients with severe COVID-19 pneumonia admitted to the ICU are rare. Previous studies have reported that low lymphocyte count at baseline, diabetes, APACHE II score, use of interleukin-6 receptor antagonists, use of corticosteroids, and ICU length of stay were risk factors for the development of bacterial superinfections (15, 25, 26). In the univariable analysis of our study, SOFA score, SAPS II score, and platelet count were found to be risk factors for the development of bacterial superinfection. In the multivariable analysis, the SAPS II score and platelet count were identified as independent risk factors, regardless of whether these variables were treated as categorical or continuous variables in the analysis. Moreover, patients with such risk factors had a significantly higher incidence of bacterial superinfection and worse clinical outcomes than those without risk factors. The results of this study suggest that thrombocytopenia and high SAPS II score are associated with an increased risk of bacterial superinfection and worse clinical outcomes.

There are several possible mechanisms whereby thrombocytopenia may occur in patients with COVID-19. First, SARS-CoV-2 can directly infect bone marrow, which may reduce platelet production (30, 31). Second, megakaryocytes dynamically release platelets during pulmonary circulation (32), and in patients with lung consolidation due to COVID-19 pneumonia, the damaged pulmonary capillary bed causes megakaryocyte rupture and prevents platelet release. Third, damaged lung tissue and pulmonary endothelial cells activate platelets in lung tissue and increase platelet consumption by creating microthrombi (30). In addition, thrombocytopenia can occur in patients with COVID-19 for various other reasons such as decreased thrombopoietin (TPO) production as a result of parenchymal liver injury, immune thrombocytopenic purpura (ITP), heparin-induced thrombocytopenia (HIT), hemophagocytic syndrome, and drug-induced myelosuppression (30, 33, 34). In this study, 89 patients (84%) developed ARDS, and some of these patients may have developed thrombocytopenia as a result of decreased platelet release from the pulmonary circulation and increased platelet consumption due to microthrombi.

Recent studies have reported that thrombocytopenia is related to COVID-19 patients' worse laboratory and clinical outcomes (35–38). To our knowledge, this is the first report of an association between thrombocytopenia and secondary bacterial infection in patients with COVID-19. Thrombocytopenia caused by COVID-19 has been confirmed to increase inflammatory markers, and the incidence of disseminated intravascular coagulation (DIC), ARDS, ICU admission, and mortality (36, 38). Thrombocytopenia can reportedly be used as a prognostic indicator of severity and mortality of COVID-19 (35, 37). There are several plausible explanations of the mechanism whereby thrombocytopenia leads to poor clinical outcomes. The first is immunothrombosis. According to previous studies, the platelets of patients with COVID-19 tend to aggregate with T cells, monocytes, and neutrophils, causing immunothrombosis (39–41). In patients with COVID-19, this immune-mediated thrombosis can occur in multiple organs, including the lungs, and is closely related to disease severity and mortality (42). Second, thrombocytopenia increases the permeability of the systemic and pulmonary vessels, which can contribute to the progression of sepsis and ARDS (43, 44). The last explanation is that platelets act as a defense mechanism in the immune response and serve as effector cells. This last explanation may be the mechanism whereby thrombocytopenia acted as a risk factor for bacterial superinfection in our study. Traditionally, platelets have been thought to act only on hemostasis; however, several recent studies have revealed the inflammatory and immune capabilities of platelets. Platelets contain several pro-inflammatory and anti-inflammatory molecules and interact with various types of immune cells by secreting them (45–47). For example, platelets can recruit leukocytes by recognizing intravascular pathogens using functional pattern recognition receptors such as toll-like receptors (TLRs) located on the surface and secreting various chemokines (48). TLR4-dependent platelet–neutrophil interaction is responsible for the removal of intravascular bacteria by forming neutrophil extracellular traps (NETs) during Gram-negative bacterial infections (49). Additionally, in the murine model, platelet glycoprotein Ib (GPIb), also known as CD42, recognizes vascular pathogens and presents them to macrophages and dendritic cells (50). The mechanisms whereby platelets are responsible for innate and adaptive immunity is a topic of ongoing research (51). In summary, when critically ill patients with COVID-19 develop thrombocytopenia, the risk of secondary bacterial infection increases because the number of platelets available for defense against bacterial infection decreases. Further studies are required to understand the underlying pathophysiological mechanisms better.

The key findings of this study are as follows: (1) Bacterial superinfection occurred frequently in critically ill patients with COVID-19 pneumonia, leading to worse clinical outcomes. (2) Infections caused by MDR pathogens occurred frequently during ICU stay, even in patients with no evidence of colonization by MDR bacteria on ICU admission. (3) To the best of our knowledge, this study is the first to report that thrombocytopenia, a poor prognostic factor in COVID-19, is also associated with secondary bacterial infection and worse clinical outcomes. Moreover, bacterial superinfections caused by MDR pathogens occurred only in patients with higher SAPS II scores and thrombocytopenia.

However, our study had several limitations. First, it was performed at a single tertiary academic hospital. Therefore, our results may not necessarily be generalizable to other hospital settings. Second, given the retrospective nature of this study, some inadequate or missing data may have affected the outcomes. Finally, although fungal and cytomegalovirus co-infections are known to occur commonly in patients with COVID-19 pneumonia, our center did not routinely screen for them, and serum beta-d-glucan, serum galactomannan, and CMV antigenemia in the blood were not investigated. Therefore,

we focused on the results regarding the presence of bacterial superinfections and MDR pathogens.

5. Conclusion

Bacterial superinfections were common in critically ill patients with severe COVID-19 pneumonia, and more than one-third of these infections were caused by MDR pathogens. Moreover, patients with bacterial superinfections had worse clinical outcomes, including fewer ventilator-free days and longer ICU stays and hospital stays after ICU admission than those without bacterial superinfections. Higher SAPS II scores and thrombocytopenia were independent risk factors for the development of bacterial superinfection. Patients with these risk factors had a significantly higher incidence of bacterial superinfection and worse clinical outcomes than those without these risk factors. Because critically ill patients with severe COVID-19 pneumonia often require prolonged mechanical ventilation, they should be actively monitored for the development of bacterial superinfections.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional review boards of Seoul National University Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SY, JL, S-ML, and HL: conceptualization, methodology, and writing – review and editing. SY and HL: data curation. SY: formal analysis. HL: investigation. HL: project administration. SY: writing – original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Delayed MSC therapy enhances resolution of organized pneumonia induced by antibiotic resistant *Klebsiella pneumoniae* infection

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Introduction: Mesenchymal stromal cells (MSC) are a promising therapeutic for pneumonia-induced sepsis. Here we sought to determine the efficacy of delayed administration of naïve and activated bone marrow (BM), adipose (AD), and umbilical cord (UC) derived MSCs in organized antibiotic resistant *Klebsiella* pneumosepsis.

Methods: Human BM-, AD-, and UC-MSCs were isolated and expanded and used either in the naïve state or following cytokine pre-activation. The effect of MSC tissue source and activation status was assessed first *in vitro*. Subsequent experiments assessed therapeutic potential as a delayed therapy at 48h post infection of rodents with *Klebsiella pneumoniae*, with efficacy assessed at 120h.

Results: BM-, AD-, and UC-MSCs accelerated epithelial healing, increased phagocytosis, and reduced ROS-induced epithelial injury *in vitro*, with AD-MSCs less effective, and naïve MSCs more effective than pre-activated MSCs. Delayed MSC administration in pre-clinical organized *Klebsiella* pneumosepsis had no effect on physiologic indices, but enhanced resolution of structural lung injury. Delayed therapy with pre-activated MSCs reduced mRNA concentrations of fibrotic factors. Naïve MSC treatment reduced key circulating cell proportions and increased bacterial killing capacity in the lungs whereas pre-activated MSCs enhanced the phagocytic index of pulmonary white cells.

Discussion: Delayed MSC therapy enhanced resolution of lung injury induced by antibiotic resistant *Klebsiella* infection and favorably modulated immune cell profile. Overall, AD-MSCs were less effective than either UC- or BM-MSCs, while naïve MSCs had a more favorable effect profile compared to pre-activated MSCs.

KEYWORDS

mesenchymal stromal cells, sepsis, immune tolerance, pneumonia, fibrosis, rodent animal models, peripheral blood mononuclear cells

1. Introduction

Sepsis is defined as a dysfunctional immune response to infection, and it accounts for 20% of total mortality worldwide (1) with community-acquired bacterial pneumonia having the highest incidence rate among sepsis related infections (2). The most recent European Prevalence of Infection in Intensive Care study (EPIC III) shows that the most prevalent pathogens associated with increased mortality were hospital-acquired anti-biotic resistant strains of Enterococcus, Klebsiella, and Acinetobacter (3).

Sepsis can be divided into three phases, early, transition, and late sepsis, with each phase having distinct immunopathologies (4). Early-phase sepsis is characterized by a predominantly hyperinflammatory response, with increased cytokine production and inflammatory cell infiltration leading to increased capillary permeability, host tissue damage, end-organ damage, and a mortality rate of 10–15% (5). The transitionary phase is crucial in the resolution of sepsis as this is where the inflammation is becomes regulated and repair commences, with resulting changes in the immune cell profile including increased M2 macrophages (6), increased regulatory T cells (Tregs) (7), and reduction in natural killer (NK) cells and mature neutrophils (8). During this phase, the patient will either return to immune homeostasis, having controlled the infection with minimal cellular functional abnormalities, or, where infection persists, will enter what is known as late-phase sepsis; characterized by immunosuppression (9) and immune cell tolerance and exhaustion (10–12). It has been reported that up to 70% of all sepsis related deaths occur in the late phase of sepsis (13). Due to persistent exposure to injurious stimulation, these tolerant cells are unable to respond to further signals, therefore reducing the patient's ability to combat secondary infections which occur in 39% of sepsis cases (14). In short, the later the phase of sepsis, the more difficult it is to combat using conventional methods.

Mesenchymal stromal cells (MSCs) are currently in clinical testing for several diseases and clinical syndromes due to their immunomodulatory capacity (15), pro-reparative functions (16), immune-evasive mechanisms (17), anti-microbial effects (18), and their efficacy in early-phase models of acute pneumonia sepsis [reviewed in (19)]. However, due to the pathology of sepsis and the necessary criteria, such as the SOFA score, that patients need to fall under to be considered 'sepsis', it can delay the administration of therapeutics which also contributes to the associated high mortality rate (20). The use of a freshly expanded and, or autologous MSC therapy could further contribute to the delay in administration. MSCs do have the potential to be administered as an off the shelf (cryopreserved) therapy from allogenic donors which would speed up the process (21), however a few factors such as determining an optimal tissue source of MSCs and dosing regimen, remains an open discussion in the field of regenerative medicine.

Bone marrow (BM) remains the most widely used MSC source because this was the first described source. Adipose (AD) and umbilical cord (UC) sources are becoming more widely studied with differing advantages of each source. All three cell types display surface markers characteristic of MSCs, as laid out by ISCT (22) with an exception being needed of the negative marker CD34 for adipose MSCs (23). The other standards they conform to are plastic adherence and multi-lineage differentiation potential.

This study aimed to identify the optimal tissue source of both naïve and cytokine pre-activated MSCs to enhance the resolution of late-phase organized antibiotic resistant Klebsiella pneumosepsis. The aim was to mimic the more clinically relevant situation when therapies are applied late in the evolution of the infection process.

2. Materials and methods

2.1. MSC culture and preparation

Bone marrow MSCs (BM-MSCs) and adipose MSCs from lipoaspirate (AD-MSC) were isolated from healthy volunteers at the Clinical Research Facility, University Hospital Galway, using standard isolation methods (22). Umbilical cord MSCs (UC-MSCs) were isolated from the perivascular tissues of healthy cords by Tissue Regeneration Therapeutics Ltd. (TRT Ltd., Toronto, Canada) and shipped at passages 1–3 (24, 25). The method of isolation, preparation and culture of these MSCs is detailed in online supplement.

MSCs were pre-activated at passage 3 using using cytomix (IL-1β (50 ng/mL), TNF-α (50 ng/mL) and IFN-ɣ (50 ng/mL)) (Immunotools Ltd., Friesoythe, Germany) for 24 h. MSCs in culture were freshly harvested, were washed twice in PBS before being typsinised and pelleted. Following two further washes in PBS, cells were counted and checked for viability using Trypan blue exclusion dye (Sigma) and administered within 1 h of harvest. Cell suspensions were over 85% viable in both naïve and pre-activated preparations. A viability of 70% was the lowest limit of viability that would be accepted.

Conditioned media (CM) was collected by replacing cytomix containing media with serum free media for a further 24 h. Cells were cryopreserved at passage 2 and characterized at passage 3 using flow cytometry (Supplementary Figure S1).

2.2. *In vitro* analyses of MSC function

2.2.1. Nuclear factor-κB activation assay

An A549 pulmonary epithelial cell line incorporating a stably transfected κB-luciferase reporter construct (Thermo Fisher, Waltham, MA, United States) was grown in 96 well plates. Cell monolayers were injured using cytomix, or sham (vehicle) injury, then treated with CM from BM, UC, and AD-MSCs (with and without cytomix pre-activation), or vehicle control. Cells were assayed for luciferase content at 24 h using the OneGlo™ luciferase substrate assay (Promega, Madison, WI, United States) as an indicator of NF-κB activation and inflammation.

2.2.2. Cell metabolic assessment of viability

MTT assays were performed to assess cell metabolic function as an index of viability using 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Aldrich Ltd., Wicklow, Ireland) reconstituted in culture medium (5 mg/mL) to evaluate cell viability and proliferation. Bronchial epithelial cell line (BEAS2B; ATCC) monolayers were subjected to oxidative injury using hydrogen peroxide (H_2O_2; Sigma) 8 mM, then treated with CM from BM, UC, and AD-MSCs (with and without cytomix pre-activation), or vehicle for 4 h. After treatment, cells were washed with PBS, followed by incubation with MTT reagent for 3 h at 37°C in a humidified cell

culture incubator. Cells were lysed and the formazan solubilized using dimethyl sulfoxide (DMSO, Sigma) and absorbance readings were measured using the Varioskan™ Flash microplate reader (Thermo Fisher Ltd.) at 595 nm wavelength. The degree of cell viability was presented as a percentage relative to uninjured control.

2.2.3. Inflammatory cytokine production and phagocytic index

The THP-1 monocyte-like cell line (ATCC) was used to generate a macrophage-like monolayer via 25 nM PMA exposure for 48 h with a subsequent 24 h of rest. Cells were exposed to either 100 ng/mL of *E. coli* lipopolysaccharide (LPS), or sham (vehicle), then treated with CM from BM, UC, and AD-MSCs (with and without cytomix pre-activation), or vehicle. TNF-α production was measured by ELISA (R&D Systems, UK). To measure the phagocytic capacity of these cells Zymosan A FITC BioParticles™ (Thermo Fisher Ltd.) were opsonised with human serum for 1 h before being added to the cells for 40 min. The cell monolayer was washed twice with DPBS before being fixed in 4% PFA for 10 min. The cells were washed twice and kept in DPBS until analysis using the Cytation 1 (BioTek Instruments, Inc.).

2.2.4. Wound healing assay

Single linear wounds were created in confluent A549 cell monolayers with a 1,000 µL pipette tip in a 24-well plate, as previously described (26). Monolayers were randomized to incubation in CM from BM, UC, and AD-MSCs (with and without cytomix pre-activation), or vehicle, and the extent of wound closure measured 24 h later using the Cytation 1 (BioTek Instruments, Inc.).

2.2.5. Neutrophil apoptosis assay

The HL-60 neutrophil-like cell line was exposed to 1.5% DMSO for 6 days to induce differentiation into polymorphonuclear (PMN) cells. Differentiated cells were exposed to 600 µM H_2O_2 for 4 h to induce apoptosis. Concurrently, cells were treated with CM from BM, UC, and AD-MSCs, with and without cytomix pre-activation, or vehicle. Levels of apoptosis were determined using FITC Annexin V and PI (Biolegend, San Diego, CA, United States) on the BD Accuri™ C6 Flow Cytometer and expressed as a percentage of total cells.

2.3. Ethics statement

All animal work was approved by the Animal Care Research Ethics Committee of the National University of Ireland, Galway and conducted under license from the Health Products Regulatory Authority, Ireland (Licence number AE19125/P067). Specific-pathogen-free adult male Sprague Dawley (CD) rats (Envigo, UK) weighing between 350 and 450 g were used in all experiments.

2.4. *Klebsiella pneumoniae*-induced lung injury

In all groups, animals were administered pre-operative analgesia (Bupaq 0.03 mg/kg; Chanelle, Galway, Ireland) 1 h prior to anesthesia using isoflurane (Iso-Vet; Chanelle). The animals were then orotracheally intubated under direct vision with a 14G catheter (BD Insyte®; BD Biosciences). A bolus of 0.5×10^9 CFU of clinically isolated, multidrug resistant, *K. pneumoniae* (Supplementary Table S1) in a 300 µL suspension was instilled followed by a bolus of air and the animals were allowed to recover from anesthesia as previously described (27) before proceeding to treatment at 48 h post inoculation. Results were collected 120 h post inoculation (Image 1).

2.5. Experimental design

2.5.1. Series 1: naïve MSCs in long-term sepsis

To ascertain the optimal tissue source of MSCs in our long-term sepsis model, 48 animals were entered into series 1 and received 0.5×10^9 CFU *K. pneumoniae* intratracheally at time zero. Forty-eight hours after pneumonia induction, animals received either (i) Vehicle, (ii) 1×10^7 BM-MSC/kg, (iii) 1×10^7 UC-MSC/kg, or (iv) 1×10^7 AD-MSC/kg IV. Animals were monitored for a further 72 h before parameters were assessed at 120 h post pneumonia induction.

2.5.2. Series 2: pre-activated MSCs in long term sepsis

To determine whether an additional effect of MSC pre-activation would be evident in our model, MSCs from the three tissue sources were exposed to cytomix as described for 24 h immediately prior to trypsinisation. Forty-eight hours after pneumonia induction, animals were randomized to receive (i) Vehicle, (ii) 1×10^7 cytomix pre-activated BM-MSC/kg, (iii) 1×10^7 cytomix pre-activated UC-MSC/kg, or (iv) 1×10^7 cytomix pre-activated AD-MSC/kg IV ($n = 12$ per group). Animals were monitored for a further 72 h before parameters were assessed at 120 h post infection induction.

2.5.3. Assessment of injury and recovery

At 120 h post pneumonia induction, animals were anesthetised with subcutaneous ketamine (75 mg.kg^{-1} Ketalar™; Pfizer, Cork, Ireland) and medetomidine (0.5 mg.kg^{-1} Dormidor™; Vetoquinol Ltd., Buckingham, UK). After confirmation of depth of anesthesia by paw pinch, IV access was obtained via tail vein using a 22G cannula (BD Insyte) and secured. Surgical tracheostomy was performed, using a 12G tracheostomy tube and intra-arterial access was gained by siting a 22G cannula in the right carotid artery for blood sampling and fluid administration. Anaesthesia was maintained with IV alfaxalone (2 mg.kg^{-1} Alfaxan™; Vetoquinol Ltd.) and paralysis with IV cisatracurium besylate (0.5 mg.kg^{-1} Tracrium™; GlaxoSmithKline PLC., London, UK). Protective mechanical ventilation (V_t 7 mL/kg, FiO_2 0.21) was commenced. Animals were ventilated for 15 min before static lung compliance was measured, following which inspired gas was changed to FiO_2 1.0 and ventilation proceeded for a further 7 min. Arterial blood gas analysis was performed after both ventilation stages as previously described (28).

2.5.4. Bacterial load

At the end of the procedure, animals were sacrificed by exsanguination under anesthetic overdose and blood retained for PBMC isolation, CFU counts, and plasma collection. Bronchoalveolar lavage (BAL) fluids were collected from the lungs for cytokine profiles and bacterial load measurements. Blood and BAL were plated onto UTI agar plates (Fannin Ltd., Galway, Ireland) and incubated overnight at 37°C. Total colony number of *K. pneumoniae* were counted.

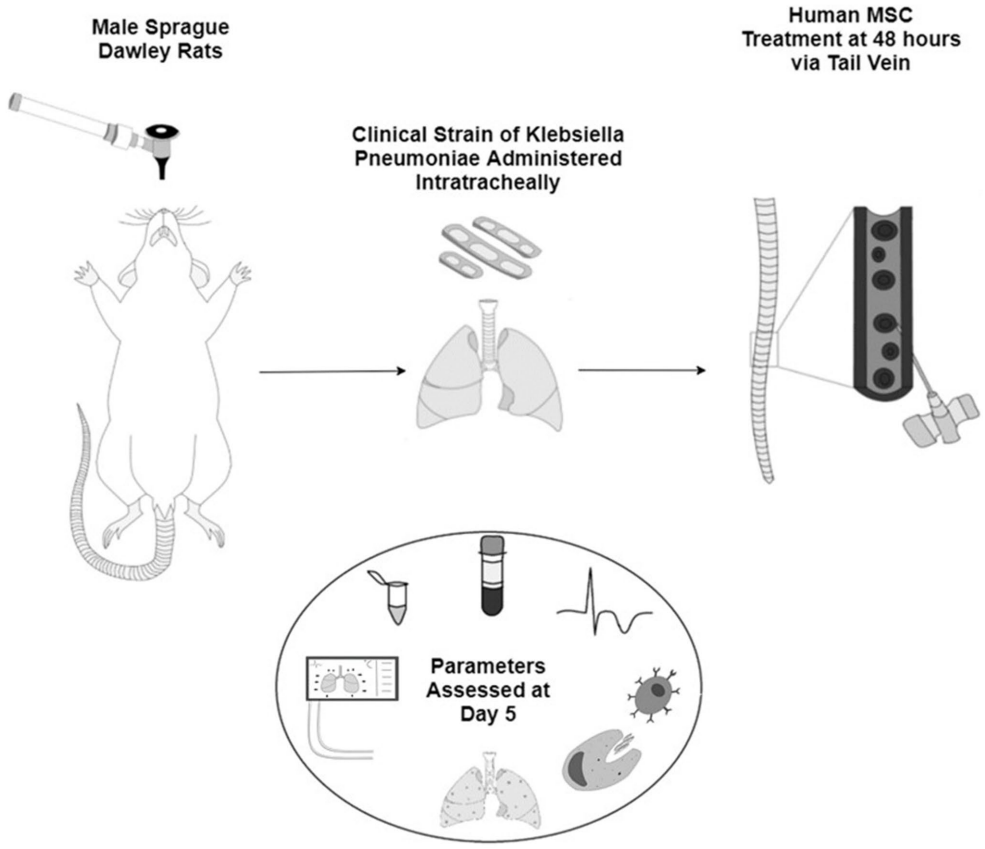

IMAGE 1
Schematic representation of the animal model of sepsis with male Sprague Dawley rats inoculated with a clinical strain of *Klebsiella Pneumoniae* intratracheally. After 48 h the animals are given either vehicle or one of the three sources of human MSCs IV. All pre- and post-mortem parameters are assessed at day 5 of the study.

2.5.5. Real-time PCR

Post BAL collection, lung tissue was minced and stored at −80°C for quantitative PCR. Following RNA extraction using Tri Reagent (Sigma), cDNA was synthesized with 1 μg of RNA using the Improm-II Reverse transcription kit (Promega) according to manufacturer's instructions. qPCR was performed using a StepOnePlus™ Real-Time PCR System (Thermo Fisher) with a fast SYBR™ Green master mix (Thermo Fisher) and ultrapure water including the primers (Supplementary Table S2) at 10 pmol /well of a MicroAmp® Fast Optical 96-well Reaction Plate (Applied Biosystems). Annealing temperature was set to 60°C with 40 cycles of amplification. RNA expression was normalized to GAPDH expression and analysed using the ΔΔCt method.

2.5.6. Lung histology and stereology

The intact left lung was isolated and fixed using 4% PFA, and the extent of histologic lung damage was determined using quantitative stereological techniques as previously described (29) in addition to the SlideScan tool from GitHub.[1]

2.5.7. Pulmonary white cell analysis

Total white cells and differential cell counts were performed on BAL fluid using cytospin columns and diff-quik staining (Fisher Sci, Ireland). The proportions of neutrophils, monocytes and other leukocytes were quantified. The phagocytic index of adherent BAL white cells was determined by visualizing ingestion of opsonized FITC-labeled zymosan particles (Thermo Fisher) using fluorescence microscopy (Cytataion 1, Biotek Ltd). Cells with >2 ingested particles were considered to be phagocytosing. The bacterial killing potential of the cells was determined by the production of ROS in the phagolysosome using the nitroblue tetrozolium (NBT) assay. Cells were exposed to 5 ng/mL NBT for 1 h and ROS production in the phagolysosome observed using light microscopy.

2.5.8. Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells were isolated from the whole blood of the experimental animals using histopaque 1077 (Sigma). Briefly, whole blood was diluted 1:1 with phosphate buffered saline (PBS) (Sigma-Aldrich) and layered over Histopaque-1077. The sample was then centrifuged at 400 × g for 30 min. The PBMC layer was removed and washed twice in Hanks Balanced Salt Solution (HBSS) (Sigma-Aldrich). After the final wash step the PBMCs were resuspended in PBS and viability assessed using Trypan Blue

1 https://github.com/CMasterson/ScanSlide2.git

(Sigma-Aldrich). The PBMCs were then either stained for flow cytometry or plated at 8×10^4 cells/well in a 96 well plate and exposed to 100 ng/mL LPS or vehicle for 24 h to determine their reactivity to an endotoxin stimulus. Media was collected and TNF-α and IL-6 secretion levels analysed by ELISA (R&D/Biotechne, Minneapolis, MN, United States) according to manufactures guidelines.

2.5.9. Cell isolation from lung tissue

Rat lung tissue was minced and digested using collagenase IV (200 U/mL; Thermo Fisher Scientific) and DNAse 1 (200 U/mL; Millipore Sigma) at 37°C for 2 h with regular agitation. Single cell suspensions of digested lung were prepared by passing through a 40 µm cell strainer (Thermo Fisher Scientific) and centrifuging at 400 × g for 5 min. The cell pellet was then resuspended in 3 mL of 1X Red Cell Lysis Solution (Milteny Biotec) and incubated for 2 min at room temperature. The solution stopped by dilution with T-cell media and centrifuged at 400 × g for 5 min. The supernatant was discarded, and the cell pellet resuspended in 10 mL of PBS and counted.

2.5.10. Antibody staining for flow cytometry

For flow cytometry, 10^6 PBMCs or single cell lung digestions were stained with the relevant antibodies (Miltenyi Biotec and Thermo Fisher Scientific) and dead-cell exclusion dye DRAQ7 (BioLegend, San Diego, CA, United States) at their most optimal staining concentration predetermined by titration experiments. For FOXP3 staining, cells were fixed and permeabilised with FOXP3/Transcription Factor Staining Buffer Kit (Thermo Fisher Scientific) as per the manufacturer's instructions. Briefly, single cell suspensions were prepared in azide/serum free PBS and washed twice. 1 µL of Ghost Dye Red 780 Viability Dye (Tonbo Biosciences, San Diego, CA, United States) was used to stain cells in 1 mL PBS. The PBMCs were then washed twice with flow cytometry stain buffer, stained, and incubated for 30 min on ice. The cells were then washed two more times and 1 mL of Fixation/Permeabilization buffer was added to each tube and pulse vortexed. The samples were incubated for at least 30 min. Next, 2 mL of 1X Permeabilization buffer was added, and samples were centrifuged for 5 min @ 400 × g at RT two times. The PBMCs were then blocked with an anti-CD32 antibody for 10 min before staining with FOXP3. The cells were incubated for 30 min at RT and protected from light before being washed two more times with 1X permeabilization buffer and finally resuspended in FACS buffer and analysed on the FACS Canto II Flow Cytometer (BD Biosciences). The cell population was gated on with doublet and dead cell exclusion occurring before data analysis (Supplementary Figure S2). Flow cytometry data was analysed using FlowJo analysis software v10.1 (BD Biosciences).

2.6. Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism 8.0.1 software. The results are presented as the mean ± standard deviation (SD). Unpaired, two tailed student *T*-Tests (Mann–Whitney test) were used to compare relative changes in smaller datasets with a significance threshold of $p < 0.01$ to account for multiple comparisons within the sample. All data sets were subjected to Shapiro–Wilk test to test for normal distribution. Normally distributed data was analysed using parametric 1-way ANOVA with multiple comparisons and Dunnett's statistical hypothesis testing. Data not normally distributed was analysed using non-parametric Kruskal-Wallis analysis correcting for multiple comparisons using Dunn's test.

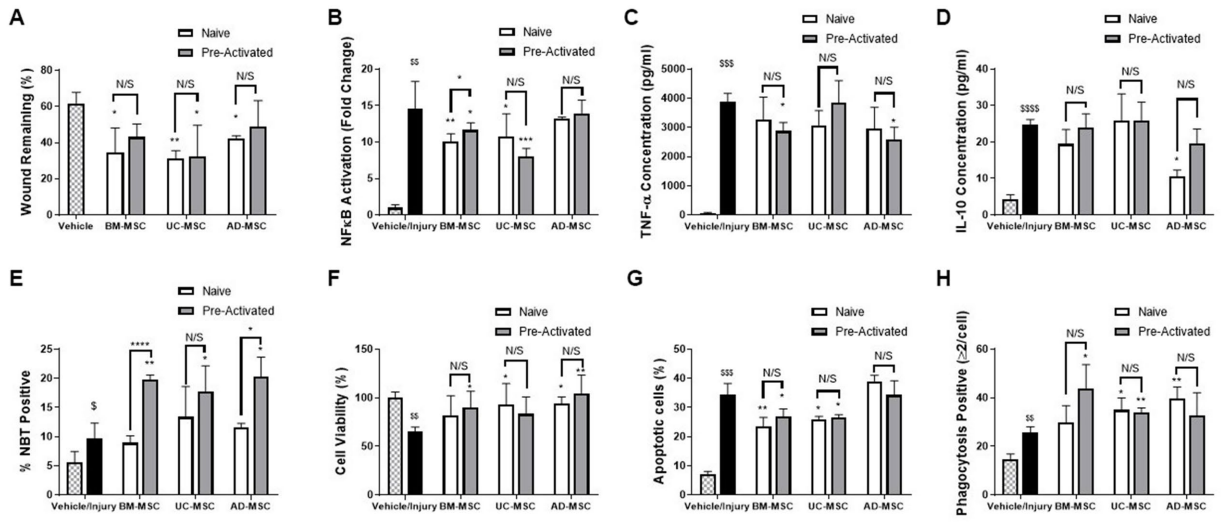

FIGURE 1
In vitro assessment of naïve and pre-activated MSCs from Bone marry, adipose tissue and umbilical cord. MSC-CM significantly reduced the wound size in A549 cell monolayers **(A)** compared to control and; decreased the inflammatory response to injury as shown by NF-κB activation in A549 cells **(B)**, MSC-CM significantly reduced the production of inflammatory TNF-α from endotoxin stimulated THP-1 monocyte./macrophage cells **(C)** Ad-MSC-CM, but not other MSC-CM, reduced change IL-10 secretion from endotoxin stimulated THP-1 cells **(D)**, while THP-1 NBT production was increased by pre-activated, but not naïve MSC-CM **(E)**. MSC-CM increased cell viability in BEAS2B cells when exposed to a ROS injury **(F)**. MSC-CM also significantly reduced the apoptosis induced by H2O2 exposure in HL-60s **(G)** and significantly increased the phagocytosis of THP-1 cells **(H)** (N=4–6, Graphs representative of 3 independent experiments, bars represent mean+SD, *$p \leq 0.05$ vs. injury, **$p \leq 0.01$ vs. injury, ***$p \leq 0.001$ vs. injury, ****$p \leq 0.0001$ vs. injury. $^{\$}p \leq 0.05$ vs. vehicle, $^{\$\$}p \leq 0.01$ vs. vehicle, $^{\$\$\$}p \leq 0.001$ vs. vehicle, $^{\$\$\$\$}p \leq 0.0001$ vs. vehicle).

3. Results

3.1. Impact of MSC tissue source and activation status on mechanisms of action

The three tissue sources of MSC-CM were compared using a panel of *in vitro* functional assays and demonstrated a varying profile of efficacy. Naïve BM-, UC-, and AD-MSC CM significantly improved wound closure of pulmonary epithelial cell monolayers subjected to scratch wound. Cytomix licensing reduced wound healing efficacy in all the MSC types (Figure 1A). Naïve BM- and UC-, but not AD-MSCs, were effective in reducing NF-κB mediated inflammation in pulmonary epithelial cells and cytomix pre-activation further reduced NF-κB in UC-MSCs only (Figure 1B). TNF-α secretion in endotoxin stimulated monocyte/macrophage immune cells was increased following injury, which was decreased in the presence of preactivated BM- and AD-MSCs (Figure 1C). Naïve AD-MSCs- but no other cell therapy—significantly reduced IL-10 secretion in monocyte/macrophage immune cells (Figure 1D). Monocyte/macrophage ROS production was significantly enhanced when exposed to pre-activated—but not naïve—MSC-CM compared to immune cells exposed to injury (Figure 1E). Naïve UC-MSCs, pre-activated BM-MSCs, and both naïve and pre-activated AD-MSCs improved lung epithelial cell viability after a severe H_2O_2 injury, while pre-activation did not further increase efficacy (Figure 1F). Both naïve and pre-activated BM- and UC-MSC CM significantly reduced the rate of apoptosis in neutrophil-like HL-60 cells when exposed to H_2O_2 and pre-activation did not significantly alter this (Figure 1G). Naïve UC- and AD- along with pre-activated BM- and UC-MSC CM significantly improved macrophage-like THP-1 phagocytosis (Figure 1H) and pre-activation did not significantly alter this. Supplementary Table S3 summarizes the performance of the different cells across this panel of *in vitro* assays.

3.2. MSC therapy enhances resolution of structural lung injury

Klebsiella pneumonia infection resulted in increased alveolar wall thickening, cell infiltration to the alveolar space, and atelectasis, and reduced airspace fraction at 120 h (Figures 2A and 2E). The airspace fraction of the lung tissue was increased in animals treated

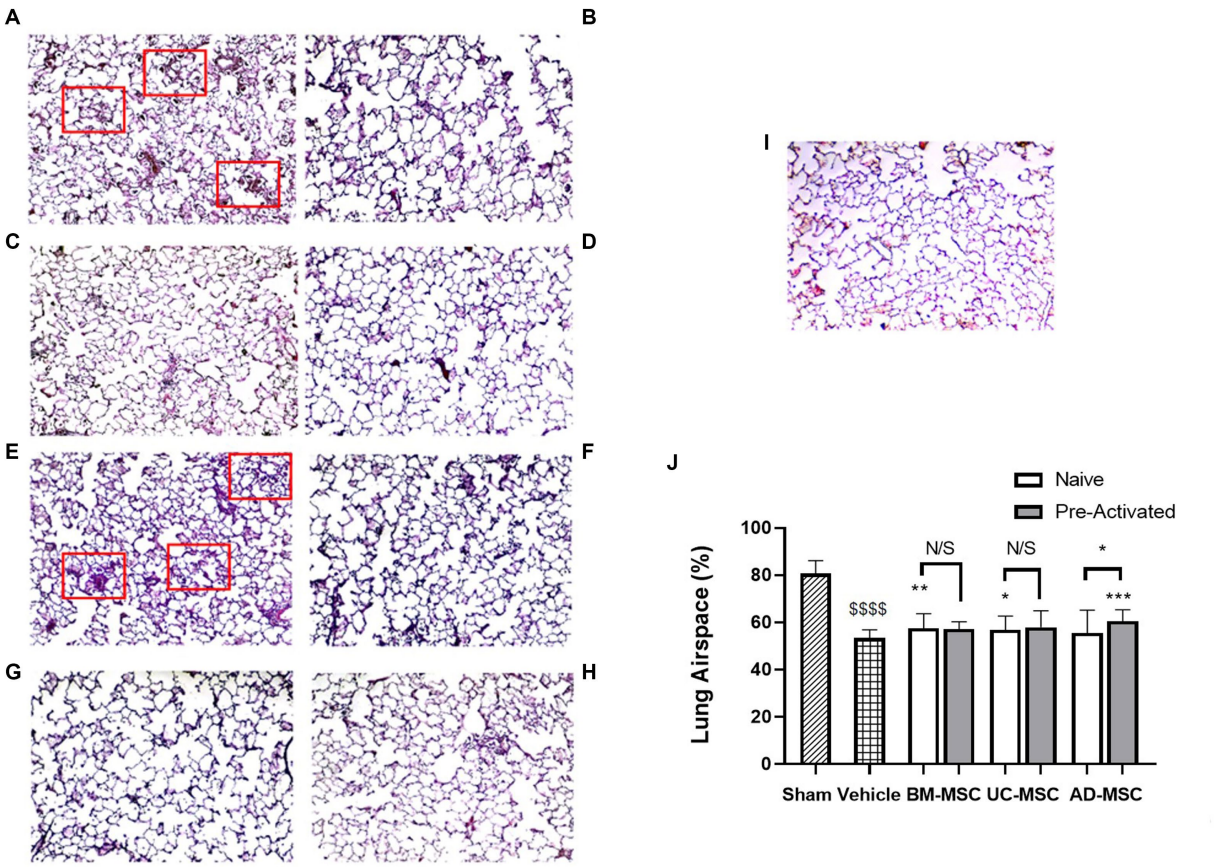

FIGURE 2
MSC therapy reduced Klebsiella pneumonia induced histological lung injury. At 5days post klebsiella infection, there was evident loss of airspace due to alveolar thickening, atelectasis, and infiltration **(A)** compared to sham animals **(I)**. Administration of naïve BM- **(B)** or UC-MSCs **(C)**, but not naïve AD-MSCs **(D)** increased lung airspace compared to vehicle treated **(A)** animals. In contrast, preactivated pre-activated BM- **(F)**, UC- **(G)**, or AD- **(H)** MSCs did not restore airspace compared to vehicle treated **(E)** animals. A quantitative analysis is presented in **(J)**. Red boxes highlight areas of significant alveolar infiltration and injury in the vehicle groups (N=10–12 animals per group, bars represent mean+SD, *$p \leq 0.05$ vs. vehicle, **$p \leq 0.01$ vs. vehicle, ***$p \leq 0.001$ vs. vehicle, ****$p \leq 0.0001$ vs. vehicle; $^{\$\$\$\$}p \leq 0.0001$ vs. sham).

with naïve BM- and UC-MSCs but not AD-MSCs compared to vehicle control (Figures 2A–D), as assessed quantitatively (Figure 2J). Conversely, pre-activated AD-MSCs—but not BM-MSCs or UC-MSCs restored lung airspace (Figures 2E–H). Quantitative analysis revealed the reduction in lung airspace resulting from *K. pneumoniae* infection, and the potential for naïve BM- and UC-MSCs, and activated AD-MSCs, to attenuate the lung airspace reduction (Figures 2J,I).

Established Klebsiella pneumonia increased mRNA concentrations of Collagen I and VI and ICAM 1 and lung myeloperoxidase (Figure 3). Naïve MSCs did not alter mRNA levels of collagen I or collagen VI compared to vehicle (Figures 3A,B). In contrast, pre-activated UC-MSCs decreased collagen VI, and both pre-activated BM- and UC-MSCs decreased collagen I (Figures 3A,B). Naïve BM-MSCs significantly reduced the levels of ICAM mRNA compared to vehicle, whereas the other naïve or pre-activated MSCs had no effect (Figure 3C). There were no changes in myeloperoxidase (MPO) levels compared to vehicle treatment with any MSC type (Figure 3D). At 120 h post infection, the bacterial load in the lungs were low overall, and numerically lower with MSC therapy, but these differences were not statistically different (Supplementary Figure S3).

3.3. MSC therapy modulates the pulmonary and systemic immune response

Established Klebsiella pneumonia increased neutrophils and monocytes in the BAL fluid in vehicle treated animals compared to sham (Figures 4A,B). In naïve MSC treated animals there was no significant increase in BAL neutrophils or monocytes compared to Sham. In contrast, pre-activated MSCs significantly increased BAL neutrophils and monocytes compared to Sham. There was no significant change between vehicle and MSC treated animals for the number of neutrophil and monocytes in the BAL fluid (Figures 4A,B). Adherent BAL cells isolated from naïve MSC-treated animals had improved ROS production but did not have an improved phagocytic index compared to vehicle (Figures 4C,D). In contrast, BAL cell ROS production was not enhanced after pre-activated MSC treatment (Figure 4C) but animals treated with pre-activated BM-MSCs MSCs had an improved phagocytic index compared to vehicle (Figure 4D).

Klebsiella pneumonia increased the percentage of monocytes in the peripheral blood of vehicle treated animals, and this increase was attenuated by naïve BM- and AD-MSC cell treatment (Figure 5A). Naïve UC- and AD-MSCs—but not the other cell types—significantly increased the proportion of Tregs compared to vehicle treated and

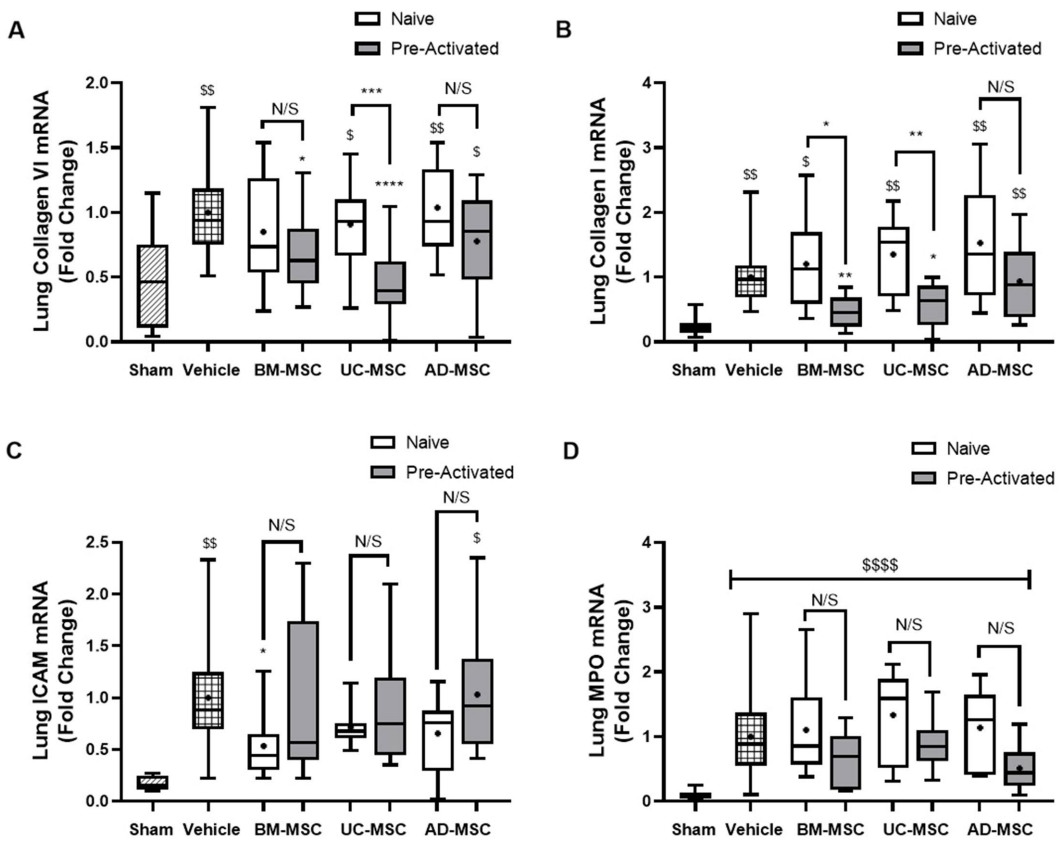

FIGURE 3
Effect of MSC therapy on Klebsiella pneumonia induced collagen, ICAM-1 and MPO mRNA concentrations. Naïve MSC administration at 48h to long term models of pulmonary sepsis does not significantly lower the mRNA levels of collagen VI and I in the lung tissue **(A,B)** while pre-activated BM- and UC-MSCs significantly decreased the expression of collagen I & VI compared to vehicle **(A,B)**. Only naïve BM-MSCs significantly decrease the expression of ICAM-1 compared to vehicle control **(C)** and there were no changes in expression of ICAM compared to vehicle control for pre-activated MSC treatments **(C)**. MPO mRNA levels remained unchanged compared to vehicle control for naïve and pre-activated MSC treatment groups **(D)** (N=4–6 per group, bars represent mean fold change from sham + SD, *$p \leq 0.05$ vs. vehicle, **$p \leq 0.01$ vs. vehicle, ***$p \leq 0.001$ vs. vehicle, ****$p \leq 0.0001$ vs. vehicle. $^{\$}p \leq 0.05$ vs. sham, $^{\$\$}p \leq 0.01$ vs. sham, $^{\$\$\$}p \leq 0.001$ vs. sham, $^{\$\$\$\$}p \leq 0.0001$ vs. sham).

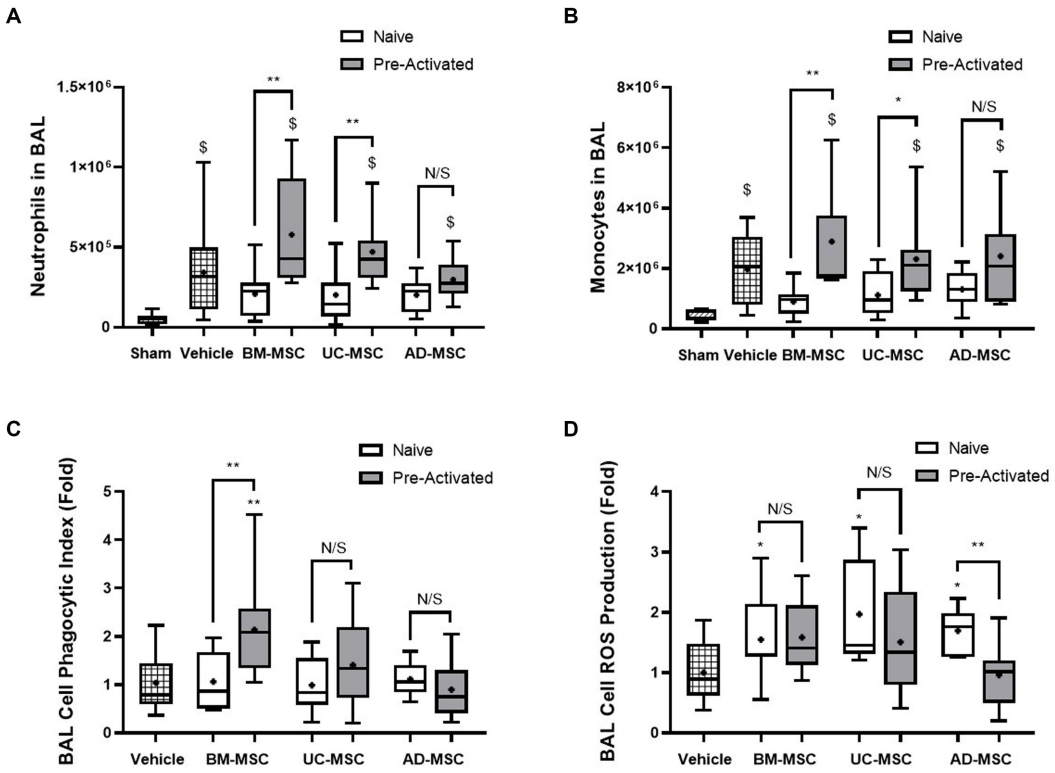

FIGURE 4
Effects of MSC therapy on Klebsiella pneumonia infection induced alveolar fluid neutrophils and monocytes. The total BAL neutrophil **(A)** and monocyte counts **(B)** were elevated compared to sham but not significantly different in naïve cell treated groups compared to vehicle control. Pre-activated MSC administration retained significantly higher levels of neutrophils **(A)** and monocytes **(B)** compared to sham. Following naïve MSC administration, ROS production in adherent BAL cells was significantly increased **(C)**, whereas the phagocytic index remained unchanged between groups. Pre-activated MSCs did not significantly affect ROS production **(C)**. The administration of pre-activated BM-MSCs resulted in increased phagocytic index of adherent BAL monocytes/macrophages compared to vehicle control **(D)** (N=10–12 per group, box plot lines=median, error bars=min-max, bars represent mean+SD, *$p \leq 0.05$ vs. vehicle, **$p \leq 0.01$ vs. vehicle, ***$p \leq 0.001$ vs. vehicle, ****$p \leq 0.0001$ vs. vehicle. $^{\$}p \leq 0.05$ vs. sham, $^{\$\$}p \leq 0.01$ vs. sham, $^{\$\$\$}p \leq 0.001$ vs. sham, $^{\$\$\$\$}p \leq 0.0001$ vs. sham).

sham animals (Figure 5B). The increase in NK cell proportions in vehicle treated animals was attenuated by naïve and pre-activated BM-MSC therapy, but not by the other cell types (Figure 5C). There were no significant changes in the classical and non-classical subtypes of monocytes across all treatment groups (data not shown), nor in the ratio of $CD4^+$ helper and $CD8^+$ cytotoxic T cell populations among all animal groups (Figure 5D).

4. Discussion

Mesenchymal stromal cell therapy for critical illnesses such as acute respiratory distress syndrome and sepsis has progressed from positive preclinical experiments to clinical trials using a variety of human tissue sources, isolation techniques and expansion protocols. These pre-clinical investigations have largely focused on on early-phase sepsis with MSC administration typically occurring pre-symptomatic or early in the disease time course (19).

However, patients generally present later in the course of their severe infection, and so studies that model these later phases are required.

The optimal MSC source for clinical translation also remains unclear. While BM is the most used and therefore the most understood MSC, they only comprise 0.001–0.01% of bone marrow cells and tend to undergo early senescence, and bone marrow harvest is an invasive procedure (30). AD-MSCs are also accessible from healthy donors, typically requiring liposuction, which is also an invasive procedure. However, AD-MSCs occur in much higher frequencies, up to 500 times more than BM (31) and proliferate longer than BM (30). UC-MSCs are acquired from the most immature source of the three allowing for greater proliferation compared to the other sources (32), reduced telomere shortening, and less chances of being environmentally altered due to previous infections (33). Previous reports, though limited, suggest that the immunosuppressive properties and proliferative capacity of AD- and UC-MSCs may be better than BM-MSCs (34). Molecular mechanisms underlying enhanced immunosuppressive effects may include enhanced secretion of the cytokines IL-10 and TGFβ (35). However, given the inconsistencies in the literature we developed a series of *in vitro* assays to directly compare these three MSC cell sources.

Our initial *in vitro* assessment gave great promise to the effects of MSCs on tissue healing and innate cell functionality, demonstrating the varying profile of MSC functions depending on their tissue source and their naïve vs. cytokine pre-activated state. We pre-activated MSCs with a cytokine mix, because this has previously proven an

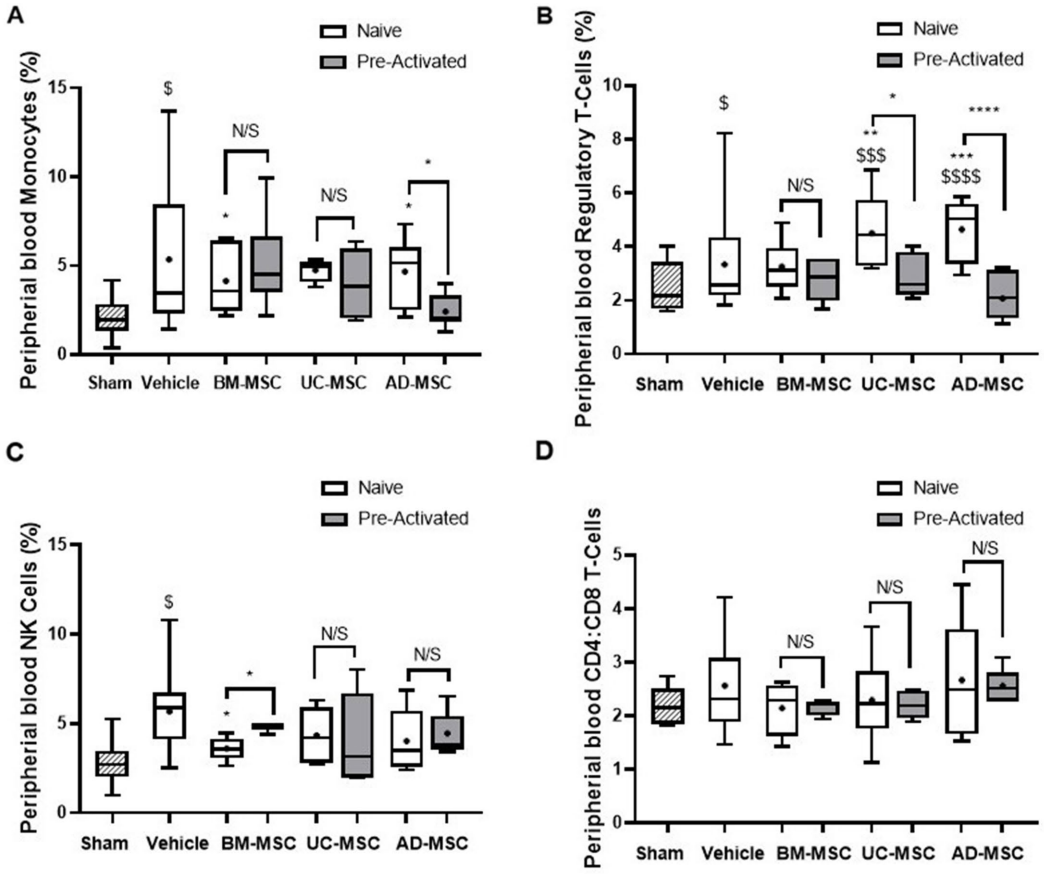

FIGURE 5
Effects of MSC therapy partially altered peripheral blood white cell profile. Naïve MSC therapy attenuated the increase in monocytes seen in the vehicle treated animals, this was not seen in the pre-activated MSC treated animals **(A)**. There was a significant increase in suppressive CD4+ T-regs in the naïve vehicle group compared to sham that was not seen in the pre-activated treatment groups **(B)**. MSCs therapy does not modulate NK **(C)** or $CD4^+$:$CD8^+$ T cell ratios **(D)** in the peripheral blood mononuclear cells (N=5–7 per group, box plot lines=median, error bars=min-max, bars represent mean+SD, *$p \leq 0.05$ vs. vehicle, **$p \leq 0.01$ vs. vehicle, ***$p \leq 0.001$ vs. vehicle, ****$p \leq 0.0001$ vs. vehicle. $^{\$}p \leq 0.05$ vs. sham, $^{\$\$}p \leq 0.01$ vs. sham, $^{\$\$\$}p \leq 0.001$ vs. sham, $^{\$\$\$\$}p \leq 0.0001$ vs. sham).

effective enhancer of therapy in preclinical models (36). Overall, our findings in the *in vitro* assays suggest that BM- and UC-MSC performed comparably in the majority of assays, out-performing AD-MSCs, while preactivated BM-MSCs performed best.

To address these translational gaps, we developed a later phase pneumosepsis model utilizing an antibiotic resistant bacterial pathogen with a relatively extended duration of infection and injury to identify the optimal tissue source of both naïve and cytokine pre-activated MSCs to aid resolution of this more established pneumosepsis. We wished to determine whether the previously demonstrated protective role of MSCs on lung function during earlier phases of lung infection, as shown by Masterson et al. (27), would translate to this later phase pneumosepsis model.

Our findings in the later phase Klebsiella pneumonia infection model demonstrate the greater potential for delayed therapy with naïve MSC therapy, compared to pre-activated MSC therapy, to facilitate restoration of lung structure, increasing lung airspace. Our pre-clinical studies further supported our *in vitro* findings, with AD-MSCs generally less effective than either UC- or BM-MSCs. Overall, the MSCs were less effective than when administered in earlier phase pneumonia models (27), which is perhaps not unexpected. Studies have shown that MSCs can preserve or restore the epithelial and endothelial barrier in the lungs by reducing inflammation, improving tissue healing, increasing local cell survival, and enhancing autophagy (37). This may have occurred here however; the majority of physiological parameters had returned to healthy control levels at this late phase of infection, and so an effect after the administration of MSCs cannot be seen.

Further *ex vivo* analysis demonstrated an increased ROS production in BAL macrophage/monocyte cells, in animals administered any of the naïve MSC types, as previously demonstrated (38). This contrasts with our *in vitro* experiments where naïve MSCs showed only slight increases in ROS production. BAL cell phagocytosis, previously shown to be enhanced in inflammatory ARDS (39), was not affected by naïve MSC therapy during the late phase. Again, this contrasted with *in vitro* exposure of various immune and lung cell types to naïve MSC-derived medium, where phagocytosis was increased, epithelial wound closure accelerated, and cell protection observed during hydrogen peroxide injury. The fact that the *in vitro* studies were conducted using the MSC secretome only, while the *in vivo* studies used the whole cells, and the fact the *in vitro* models are of human (rather than animal) origin, may at least partially may explain these differences.

In other analyses, peripheral blood monocytes were decreased with naïve BM- and AD-MSC therapy, while the increase in NK cells was attenuated by naïve and pre-activated BM-MSC therapies. In addition, Tregs were increased with naïve UC- and AD-MSCs, but not with the other cell types. However, as these proportion changes were small and there was no translation to an effect in *in vivo* physiology, it is difficult to ascertain the significance of this. The effect of the injury on circulating PBMC proportions was lost when using pre-activated cells. Again, this would point to the current standard MSC therapeutics likely being ineffective in later phase sepsis, suggesting the focus should remain on delivery of MSC therapy as early as possible in the disease time course to prevent both mortality in early phase and progression to later phase sepsis.

In healthy lungs there is a balance between degradation and synthesis of collagen that is disrupted during fibrosis. Gene expression analysis also revealed differences between animals receiving naïve and pre-activated MSCs. The levels of mRNA which would indicate the development or progression of fibrosis and inflammation were analysed in the lung tissues of animals in all groups. The levels would indicate that while there was not a substantial amount of collagen mRNA, it was significantly increased compared to sham in the case of collagen I and VI. Expression of both collagen types was decreased in animals receiving pre-activated BM- and UC-MSCs, but not naïve MSCs from any source. Levels of ICAM were reduced in tissues from animals treated with naïve BM-MSCs and largely unchanged in groups administered other cell types and pre-activated cells. While pro-collagen has to be cleaved before it is incorporated into lung tissue (40), these findings suggest that the potential for fibrosis may be reduced by treatment with pre-activated BM- and UC- MSCs.

Given the trend for loss of effect after pre-activation in this study, and its promise in other studies would indicate that this may not be the optimal pre-activation strategy for this stage of pulmonary sepsis or for late administration time-points. Cumulatively, BM-MSCs without pre-activation had the greatest impact overall, however due to a lack of translation in physiological parameters, further investigation will be needed. An alternative animal model, different dosing regimens, and another pre-activation strategy should be considered.

5. Conclusion

Our findings in the later phase Klebsiella pneumonia infection model demonstrate the potential for delayed therapy with naïve—but not pre-activated—MSC therapy to facilitate restoration of lung structure, increasing lung airspace. While the effects of MSC were less marked than those seen in earlier phase pneumonia, this is not unexpected given the established nature of this injury. Overall, our findings in the *in vitro* assays, and in our model of delayed MSC treatment of Klebsiella induced pneumonia suggested that AD-MSCs were less effective than either UC- or BM-MSCs. In addition, the naïve MSCs appear to have a more favorable profile of effect when compared to the pre-activated MSCs. Taken together, these are important insights into the likely effectiveness of this advanced therapeutic medicinal product and will inform future drug development and clinical trial inclusion parameters.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Animal Care Research Ethics Committee of the National University of Ireland, Galway.

Author contributions

DO'T and JL: conceptualization, supervision, project administration, and funding acquisition. CM and SH: methodology. CM: validation. DB and CM: formal analysis, writing—original draft preparation. CM, DB, JB, and SH: investigation and data curation. DO'T, CM, and JL: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1132749/full#supplementary-material

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