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Drug prevention and control of ventilator-associated pneumonia

volume II

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Published in
Frontiers in Pharmacology

ISSN 1664-8714
ISBN 978-2-8325-2686-6
DOI 10.3389/978-2-8325-2686-6

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Drug prevention and control of ventilator-associated pneumonia volume II

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Citation

Zeng, X.-T., Jiang, M., Mao, Z., eds. (2023). *Drug prevention and control of ventilator-associated pneumonia volume II*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2686-6

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frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 29 April 2022
doi: 10.3389/fphar.2022.898630

Using Restricted Cubic Splines to Study the Duration of Antibiotic Use in the Prognosis of Ventilator-Associated Pneumonia

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OPEN ACCESS

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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 17 March 2022
Accepted: 12 April 2022
Published: 29 April 2022

Citation:
Xu Y, Han D, Xu F, Shen S, Zheng X, Wang H and Lyu J (2022) Using Restricted Cubic Splines to Study the Duration of Antibiotic Use in the Prognosis of Ventilator-Associated Pneumonia. Front. Pharmacol. 13:898630. doi: 10.3389/fphar.2022.898630

Background: Ventilator-associated pneumonia (VAP) is the most widespread and life-threatening nosocomial infection in intensive care units (ICUs). The duration of antibiotic use is a good predictor of prognosis in patients with VAP, but the ideal duration of antibiotic therapy for VAP in critically ill patients has not been confirmed. Research is therefore needed into the optimal duration of antibiotic use and its impact on VAP.

Methods: The Medical Information Mart for Intensive Care database included 1,609 patients with VAP. Chi-square or Student's t-tests were used to compare groups, and Cox regression analysis was used to investigate the factors influencing the prognoses of patients with VAP. Nonlinear tests were performed on antibiotic use lasting <7, 7–10, and >10 days. Significant factors were included in the model for sensitivity analysis. For the subgroup analyses, the body mass indexes (BMIs) of patients were separated into BMI <30 kg/m2 and BMI ≥30 kg/m2, with the criterion of statistical significance set at $p < 0.05$. Restricted cubic splines were used to analyze the relationship between antibiotic use duration and mortality risk in patients with VAP.

Results: In patients with VAP, the effects of antibiotic use duration on the outcomes were nonlinear. Antibiotic use for 7–10 days in models 1–3 increased the risk of antibiotic use by 2.6020-, 2.1642-, and 2.3263-fold relative to for >10 days, respectively. The risks in models 1–3 for <7 days were 2.6510-, 1.9933-, and 2.5151-fold higher than those in models with >10 days of antibiotic use, respectively. These results were robust across the analyses.

Abbreviations: APSIII, acute physiology score-III; ALT, alanine aminotransferase; BMI, body mass index; CIMV, continuous invasive mechanical ventilation for less than 96 consecutive hours; CKD, chronic kidney disease; CCI, charlson comorbidity index; CI, confidence interval; DBP, diastolic blood pressure; GC, gastric catheterization; GCS, Glasgow Coma Scale; HR, hazard ratio; ICU, intensive care unit; LODS, logistic organ dysfunction score; MELD, model for end-stage liver; MIMIC-IV, medical information mart for intensive care IV; MIC, minimum inhibitory concentration; MBP, mean blood pressure; PEG, percutaneous (endoscopic) gastrostomy; POUF, performance of urinary filtration; PK/PD, pharmacokinetics and pharmacodynamics; RV, respiratory ventilation for more than 96 consecutive hours; RCS, restricted cubic spline; RBC, red blood cell; RDW, red blood cell distribution width; SCR, serum creatinine; SOFA, sequential organ failure assessment; SAPSII, simplified acute physiology score-II; TT, temporary tracheostomy; VAP, ventilator-associated pneumonia.

Conclusions: The duration of antibiotic treatment had a nonlinear effect on the prognosis of patients with VAP. Antibiotic use durations of <7 days and 7–10 days both presented risks, and the appropriate duration of antibiotic use can ensure the good prognosis of patients with VAP.

Keywords: MIMIC-IV1, ventilator-associated pneumonia, restricted cubic splines, duration of antibiotic use, intensive care unit, mortality, prediction

INTRODUCTION

Ventilator-associated pneumonia (VAP) is one of the most prominent hospital-acquired diseases in intensive care units (ICUs), and has a substantial risk of adverse effects (Xu et al., 2021). The prevalence of VAP is decreasing according to a hospital report from the United States Healthcare Safety Network, with about 10% of patients who require mechanical ventilation being diagnosed with VAP, a rate that has remained steady over the last decade. Each patient incurs additional VAP-related costs of approximately US$ 40,000 (Kalil et al., 2016). In the United States, epidemiological studies have confirmed that the incidence of VAP ranges from 2 to 16 per 1,000 ventilator days (Torres et al., 2017). VAP is recognized as pneumonia that occurs at least 48 h following endotracheal intubation or mechanical ventilation and is marked by infection-related symptoms such as widespread fever, new or progressive pulmonary infiltrations, and changes in the white blood cell count (Grief and Loza, 2018; Galhardo et al., 2020). VAP is associated with difficulty in weaning, prolonged mechanical ventilation and hospitalization times, and increased hospitalization costs for patients, which increase the economic costs to the health care system and consume huge medical resources (Wu et al., 2019; Pozuelo-Carrascosa et al., 2020).

Antibiotics are widely used to treat infectious diseases, and China is one of the world's major producers and consumers of antibiotics. Antibiotics are used by about half of all hospital outpatients in China, according to linked publications, and antibiotic prescriptions account for about half of all medications dispensed by hospitals. The top five antibiotics taken by Chinese in 2013 were cephalexin, amoxicillin, ofloxacin, tetracycline, and norfloxacin (Qiao et al., 2018). Antibiotic resistance is higher in China than in Western countries, and drug-resistant microorganisms are becoming more common (Ding et al., 2019).

Previous reports suggest that infection causes one-third to one-half of all VAP-related deaths, with more fatalities for *Pseudomonas aeruginosa* and *Acinetobacter* species (Garnacho-Montero et al., 2005; Micek et al., 2015; Lima et al., 2020). Antibiotic resistance has also increased in common associated pathogens, and VAP risk is time-dependent, potentially leading to significant time-dependent biases, making it more difficult to empirically determine the duration of antibiotic use for these infections (Torres et al., 2017; Fernando et al., 2020; Bassetti et al.,

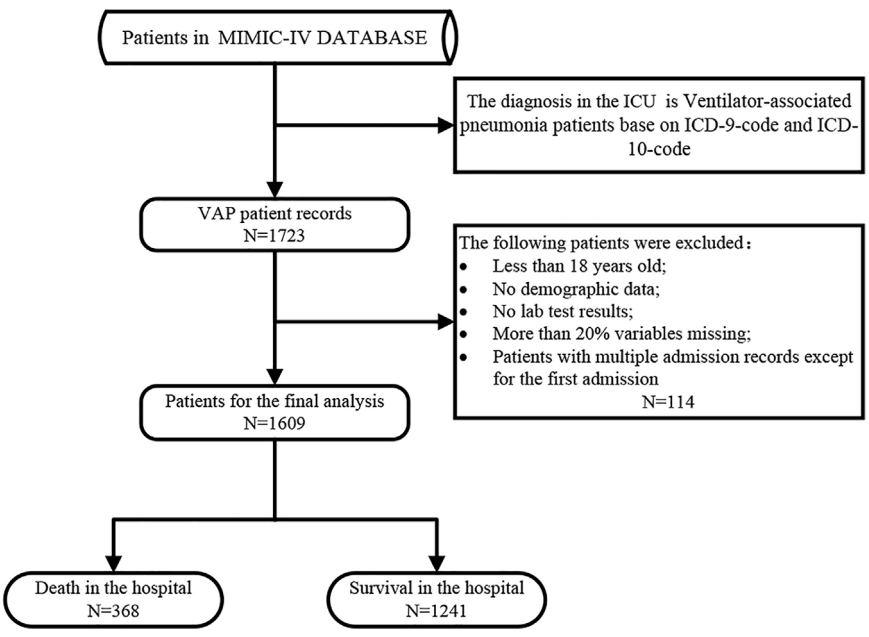

FIGURE 1 | Flow chat of study selection.

TABLE 1 | Baseline characteristics of patients in the study.

Variable		Live	Dead	p-Value
N	—	1,241	368	<0.001
Los_hospital	—	23.00 [16.00, 33.00]	16.00 [10.00, 25.00]	
CKD	No	1,000 (80.6)	259 (70.4)	<0.001
	Yes	241 (19.4)	109 (29.6)	
Norepinephrine	No	659 (53.1)	133 (36.1)	<0.001
	Yes	582 (46.9)	235 (63.9)	
Vasopressin	No	1,021 (82.3)	252 (68.5)	<0.001
	Yes	220 (17.7)	116 (31.5)	
CefazoLIN	No	913 (73.6)	306 (83.2)	<0.001
	Yes	328 (26.4)	62 (16.8)	
Meropenem	No	982 (79.1)	271 (73.6)	0.031
	Yes	259 (20.9)	97 (26.4)	
Vancomycin oral liquid	No	1,122 (90.4)	316 (85.9)	0.017
	Yes	119 (9.6)	52 (14.1)	
Tracheostomy	No	1,105 (89.0)	344 (93.5)	0.016
	Yes	136 (11.0)	24 (6.5)	
CIMV	No	1,068 (86.1)	339 (92.1)	0.003
	Yes	173 (13.9)	29 (7.9)	
Hemodialysis	No	1,131 (91.1)	314 (85.3)	0.002
	Yes	110 (8.9)	54 (14.7)	
Gastric catheterization	No	1,074 (86.5)	345 (93.8)	<0.001
	Yes	167 (13.5)	23 (6.2)	
PEG	No	981 (79.0)	349 (94.8)	<0.001
	Yes	260 (21.0)	19 (5.2)	
POUF	No	1,203 (96.9)	330 (89.7)	<0.001
	Yes	38 (3.1)	38 (10.3)	
RV	No	838 (67.5)	217 (59.0)	0.003
	Yes	403 (32.5)	151 (41.0)	
Temporary tracheostomy	No	947 (76.3)	333 (90.5)	<0.001
	Yes	294 (23.7)	35 (9.5)	
Venous catheterization for renal dialysis	No	1,155 (93.1)	329 (89.4)	0.028
	Yes	86 (6.9)	39 (10.6)	
Antibiotics_day	—	13.00 [7.00, 20.00]	10.00 [5.75, 17.00]	<0.001
APSIII	—	64.00 [48.00, 84.00]	77.50 [59.00, 100.25]	<0.001
Baseexcess	—	0 [−4.00, 2.00]	−1.00 [−5.00, 1.00]	0.029
Totalco2	—	25.00 [22.00, 28.00]	24.00 [21.00, 28.00]	0.012
Charlson_comorbidity_index	—	5.00 [3.00, 7.00]	7.00 [5.00, 8.00]	<0.001
SCR	—	0.60 [0.40, 0.90]	0.80 [0.50, 1.10]	<0.001
GCS	—	9.00 [6.00, 13.00]	7.00 [3.00, 12.00]	<0.001
SOFA	—	8.00 [5.00, 11.00]	9.00 [6.00, 12.00]	<0.001
LODS	—	8.00 [5.00, 10.00]	9.00 [7.00, 12.00]	<0.001
MELD	—	10.00 [10.00, 20.00]	20.00 [10.00, 22.64]	<0.001
SAPSII	—	39.00 [30.00, 49.00]	46.50 [37.00, 55.00]	<0.001
BMI	—	28.44 [24.45, 34.41]	27.15 [23.17, 32.60]	0.001
Urineoutput	—	1,574.00 [915.00,2350.00]	1,146.00 [598.75,2025.00]	<0.001
DBP	—	62 [57, 69]	60 [53, 67]	0.001
MBP	—	78 [71, 86]	76 [69, 84]	0.004
Temperature	—	37.1 [36.8, 37.5]	36.9 [36.6, 37.3]	<0.001
Admission_age	—	63.00 [51.00, 73.00]	70.00 [60.00, 80.00]	<0.001
Hematocrit	—	33.90 [29.00, 39.00]	32.50 [27.90, 37.52]	0.011
Hemoglobin	—	11.20 [9.50, 13.10]	10.70 [9.00, 12.53]	0.004
RBC	—	3.71 [3.13, 4.31]	3.50 [2.99, 4.13]	<0.001
RDW	—	14.40 [13.40, 16.00]	15.00 [13.60, 16.80]	<0.001
ALT	—	1,356.00 [613.00,3024.00]	1,081.50 [516.00,2658.50]	0.031
Platelet_Count	—	886.00 [756.00, 1,024.00]	852.50 [725.00, 996.75]	0.027
Bicarbonate	—	42.00 [39.00, 45.00]	41.00 [39.00, 44.00]	0.010
Basophils	—	3.00 [2.00, 5.00]	3.00 [2.00, 4.00]	0.019

CKD, chronic kidney disease; CIMV, continuous invasive mechanical ventilation for less than 96 consecutive hours; PEG, percutaneous (endoscopic) gastrostomy; POUF, performance of urinary filtration; RV, respiratory ventilation for more than 96 consecutive hours; APSIII, Acute Physiology Score III; SCR, serum creatinine; GCS, glasgow coma scale; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction score; Meld, Model for End-stage Liver Disease; SAPSII, Simplified Acute Physiology Score II; BMI, body mass index; DBP, diastolic blood pressure; MBP, mean blood pressure; RBC, red blood cell; RDW, red blood cell distribution width; ALT, alanine aminotransferase.

TABLE 2 | The results of univariate cox regression analysis.

Variable	HR	95%CI	*p*-Value
Antibiotics_day	0.96	0.96–0.97	<0.001
CKD			
No	Reference	—	
Yes	1.43	1.14–1.79	0.002
Norepinephrine			
No	Reference	—	
Yes	1.45	1.18–1.80	0.001
Vasopressin			
No	Reference	—	
Yes	1.44	1.15–1.79	0.001
CefazoLIN			
No	Reference	—	
Yes	0.52	0.40–0.69	0.001
Tracheostomy			
No	Reference	—	
Yes	0.37	0.24–0.56	<0.001
Gastric catheterization			
No	Reference	—	
Yes	0.29	0.19–0.45	<0.001
PEG			
No	Reference	—	
Yes	0.20	0.13–0.32	<0.001
POUF			
No	Reference	—	
Yes	1.84	1.31–2.57	<0.001
Temporary tracheostomy			
No	Reference	—	
Yes	0.29	0.20–0.41	<0.001
APSIII	1.01	1.01–1.01	<0.001
Charlson_comorbidity_index	1.13	1.09–1.17	<0.001
SCR	1.38	1.24–1.54	<0.001
GCS	0.97	0.95–1.00	0.022
SOFA	1.04	1.01–1.06	0.003
LODS	1.08	1.05–1.11	<0.001
MELD	1.02	1.01–1.03	<0.001
SAPSII	1.02	1.01–1.03	<0.001
BMI	0.98	0.97–0.99	0.003
Urineoutput	1.00	1.00–1.00	<0.001
DBP	0.98	0.97–0.99	<0.001
MBP	0.98	0.97–0.99	0.001
Temperature	0.76	0.67–0.85	<0.001
Admission_age	1.03	1.03–1.04	<0.001
Hemoglobin	0.95	0.92–0.99	0.011
RBC	0.86	0.77–0.96	0.006
RDW	1.05	1.01–1.09	0.007
ALT	1.00	1.00–1.00	0.006
Platelet_Count	1.00	1.00–1.00	<0.001
Bicarbonate	0.93	0.91–0.95	<0.001
Basophils	0.95	0.91–0.98	0.001

CKD, chronic kidney disease; PEG, percutaneous (endoscopic) gastrostomy; POUF, performance of urinary filtration; RV, respiratory ventilation for more than 96 consecutive hours; APSIII, Acute Physiology Score III; SCR, serum creatinine; GCS, glasgow coma scale; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction score; Meld, Model for End-stage Liver Disease; SAPSII, Simplified Acute Physiology Score II; BMI, body mass index; DBP, diastolic blood pressure; MBP, mean blood pressure; RBC, red blood cell; RDW, red blood cell distribution width; ALT, alanine aminotransferase; CI, confidence interval; HR , hazard ratios.

2022). Pathogen diagnosis in patients with VAP typically requires an invasive quantitative culture of the lower respiratory tract using endotracheal aspirates, bronchoalveolar lavage fluid, protective specimen brushes, or semiquantitative noninvasive breath sampling (Arthur et al., 2016). However, due to the stringent growth requirements for detecting pathogens or other aspects of culture methods, important pathogens might not be detected, and it may be difficult to distinguish detected microorganisms from colonizing bacteria and actual pathogens.

While treatment is empirical until definitive results are available, the emergence of multidrug-resistant organisms, particularly in ICUs, necessitates determining the optimal antibiotic use duration (Timsit et al., 2017; Cilloniz et al., 2020; Yoo et al., 2020). In ICU patients, VAP is also the leading cause of antibiotic use (Koulenti et al., 2017), and guidelines recommend that adequate doses should be prescribed to patients with VAP to ensure early, appropriate, broad-spectrum antibiotic treatment, and to optimize the antibacterial treatment effect on patients with VAP by starting at the correct time. Appropriate and adequate treatment has been a consistent factor related to mortality rates (Guidelines for the management of, 2005). However, the importance of immediately administering antibiotics must always be matched against unnecessary hazards from antibiotics such as resistance and secondary infections (Lu et al., 2011).

Obese patients [body mass index (BMI) ≥30 kg/m2] had significantly lower 90-days mortality than nonobese patients (BMI <30 kg/m2) in a 2021 study (Nseir et al., 2021). BMI was also linked to a higher incidence of ICU-acquired infections. In obese patients, continuous antibiotics infusion can optimize the duration of time-dependent antibiotic exposure above the minimum inhibitory concentration (MIC), maximizing apoptosis (De Pascale et al., 2015). In patients with VAP, the optimal duration of antibiotic use could be used as a predictor for swift clinical assessments and prognostic research. It will be important to arrange innovative treatment strategies for patients with VAP if this indicator could effectively predict the probability of negative outcomes. Therefore, a more-thorough and precise insight into the role of an appropriate duration of antibiotic use in the prognosis of patients with VAP is critical.

The purpose of this study was to explore the effect of appropriate timing of antibiotic administration on the survival of VAP patients by applying restricted cubic splines (RCSs) to data in the Medical Information Market for Intensive Care (MIMIC) database.

MATERIALS AND METHODS

Database

The Computational Physiology Laboratory of Massachusetts Institute of Technology, Beth Israel Deacon Medical Center, and Philips Healthcare jointly published the MIMIC-IV database (version 1.0) for the period of 2008–2019.

We received permission from an institutional review board of the Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center for a longitudinal single-center database of clinical information on ICU patients, and data extraction from and access to the database were implemented (Yang et al., 2020). Dr. Yixian Xu, who completed the National Institutes of Health's online training course, extracted the data (certification number: 39194349).

TABLE 3 | Cox regression analyses of the relationship between the duration of antibiotic use and VAP prognosis.

Variable	Model1	Model2	Model3
	HR (95%CI) *p*-value	HR (95%CI) *p*-value	HR (95%CI) *p*-value
>10 days	1	1	1
7–10 days	2.602 (1.894–3.575) <0.001	2.1642 (1.5394–3.0426) <0.001	2.3263 (1.6375–3.3047) <0.001
<7 days	2.651 (1.988–3.536) <0.001	1.9933 (1.4077–2.8226) <0.001	2.5151 (1.7424–3.6307) <0.001

Model 1: univariate.

Model 2: adjust for CKD, norepinephrine, vasopressin, cefazolin, tracheostomy, GC, PEG, POUF, TT, SCR, BMI, urine output, DBP, MBP, antibiotic use duration, temperature, admission age, hemoglobin, RBC, RDW, ALT, platelet count, bicarbonate, and basophils.

Model 3: adjusted forAntibiotics day, CKD, norepinephrine, Vasopressin, CefazoLIN, tracheostomy, GC, PEG, POUF, TT, APSIII, charlson comorbidity index, SCR, GCS, SOFA, LODS, MELD, SAPSII, BMI, urineoutput, DBP, MBP, temperature, Admission age, Hemoglobin, RBC, RDW, ALT, platelet count, Bicarbonate and Basophils.

Patients and Variables

Patients with VAP were identified in the MIMIC-IV database. This study gathered data on the demographics, laboratory tests, medications, vital signs, surgical procedures, and other personal information of patients with VAP (Wu et al., 2021). We extracted the data of 1,609 patients with VAP from the MIMIC-IV database for inclusion in this study. All patients were older than 18 years and had been admitted to the hospital for the first time. **Figure 1** illustrates the exclusion and inclusion criteria of patients in this study.

The following variables were extracted from the records of the patients with VAP in the MIMIC-IV database: 1) Demographic data and vital signs at hospital admission [length of hospital stay, serum creatinine (SCR), antibiotic use days, urine output, mean blood pressure (MBP), diastolic blood pressure (DBP), admission age, temperature, and BMI]; 2) laboratory test data [red blood cell distribution width (RDW), hematocrit, hemoglobin, red blood cell (RBC), alanine aminotransferase (ALT), platelet count, bicarbonate, basophils, base excess, and total CO2]; 3) drugs and surgical operations [norepinephrine, vasopressin, cefazolin, meropenem, vancomycin oral liquid, tracheostomy, continuous invasive mechanical ventilation for less than 96 consecutive hours (CIMV), hemodialysis, gastric catheterization (GC), percutaneous (endoscopic) gastrostomy (PEG), performance of urinary filtration (POUF), respiratory ventilation for more than 96 consecutive hours (RV), temporary tracheostomy (TT), and venous catheterization for renal dialysis; and 4) complications and severity scoring systems [chronic kidney disease (CKD), Charlson Comorbidity Index (CCI), Sequential Organ Failure Assessment (SOFA), Acute Physiology Score-III (APSIII), Simplified Acute Physiology Score-II (SAPSII), Glasgow Coma Scale (GCS), Logistic Organ Dysfunction Score (LODS), and Model for End-stage Liver (MELD)]. The impact of antibiotic use duration on the survival of patients with VAP was investigated. The endpoint for this study was survival at discharge.

Statistical Analysis

Patients were divided into two groups based on whether they survived during their hospitalization. Median and IQR values and mean ± SD values were used to summarize the nonnormally and normally distributed continuous variables, respectively. Student's t-test, the Mann-Whitney *U* test, one-way ANOVA, and the Kruskal-Wallis H test were used to make comparisons of nonnormally distributed statistical data. The Kolmogorov-Smirnov test was used to determine if continuous variables were normally distributed. Categorical variables are expressed as percentages or numbers. We excluded confounding variables and outliers with considerable effects, and applied Chi-square tests to categorical variables and Student's t-tests to continuous variables, with the exceptions of survival status and length of hospital stay.

Before performing the multivariate analysis, a univariate analysis was needed for each factor to determine if the covariance inclusion criteria were met to ascertain variables that affect VAP prognosis. Finally, the duration of antibiotic use was divided into >10, 7–10, and <7 days. A nonlinear test was used to determine whether the duration of antibiotics had a nonlinear effect on VAP prognosis. For subgroup analysis, the patients were divided into nonobese and obese groups.

Cox regression was performed, significant variables were added, three models were constructed, and the sensitivity models were tested. In the Cox regression analysis, model 1 only included the duration of antibiotic use, model 2 included some other variables, and model 3 included all key variables. Restricted cubic splines are good at dealing with the nonlinear relationship between continuous variables and response variables, and they can locate crucial key points. $p < 0.05$ was considered statistically significant. However, using data that had more than 20% missing or data that had missing points, we used the "vim" (Templ et al., 2012) and "MICE" (Zhang, 2016) (R software packages for multiple imputations using Templ's method for exploration and data visualization. Excel and R (ggplot2, RMS package) were used for the statistical analysis.

RESULTS

Patient Baseline Characteristics

Of the 1,609 patients with VAP, 368 died in the hospital. Variables from both groups were displayed and compared, and patients were separated into two groups depending on their survival status. Patients who survived at a median age of 63 (51, 73) days were younger than those who died at 70 (60, 80) years. Surviving patients were less likely to develop complications such as hypertension, chronic obstructive pulmonary disease (COPD), and diabetes than those who died. All variables affected VAP prognosis differently in this study. The baseline

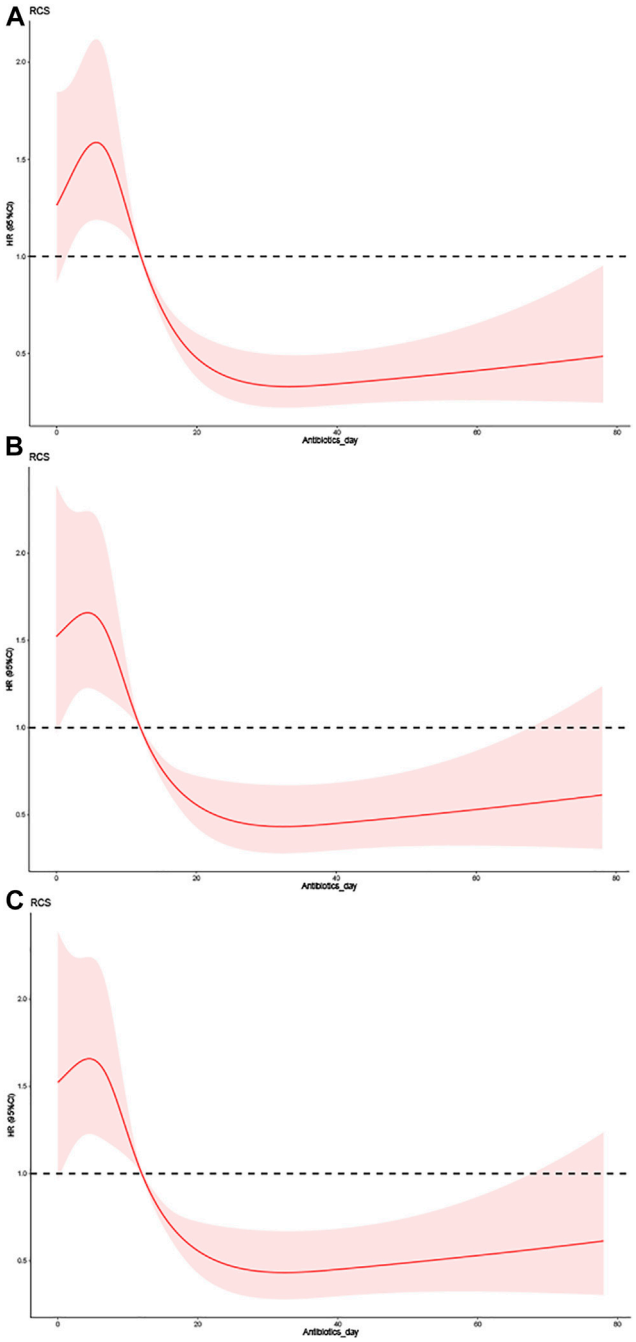

FIGURE 2 | The effect of different doses of the duration of antibiotic use on the prognosis of VAP. **(A)** Univariate. **(B)** Adjusted for CKD, Norepinephrine, Vasopressin, CefazoLIN, Tracheostomy, GC, PEG, POUF, TT, SCR, BMI, Urineoutput, DBP, MBP, Antibiotics day, Temperature, Admission age, Hemoglobin, RBC, RDW, ALT, Platelet Count, Bicarbonate and Basophils. **(C)** Adjusted for Antibiotics day, CKD, Norepinephrine, Vasopressin, CefazoLIN, Tracheostomy, GC, PEG, POUF, TT, APSIII, Charlson comorbidity index, SCR, GCS, SOFA, LODS, MELD, SAPSII, BMI, Urineoutput, DBP, MBP, Temperature, Admission age, Hemoglobin, RBC, RDW, ALT, Platelet Count, Bicarbonate and Basophils.

characteristics, laboratory parameters, and vital signs of survivors and deceased patients during hospitalization are listed in **Table 1**.

Restricted Cubic Splines in the Cox Proportional-Hazards Regression Model

In the univariate Cox regression analysis, model 3 included all variables for which $p < 0.05$. The results indicated that CKD, norepinephrine, vasopressin, cefazolin, tracheostomy, GC, PEG, POUF, TT, antibiotic use duration, APSIII, CCI, SCR, GCS score, SOFA score, LODS, MELD score, SAPSII, BMI, urine output, DBP, MBP, temperature, admission age, hemoglobin, RBC, RDW, ALT, platelet count, bicarbonate, and basophils were prognostic factors for patients with VAP (all $p < 0.05$, **Table 2**).

RCSs were used to perform the association analysis between duration of antibiotic use and risk of all-cause mortality from VAP. The RCS test yielded statistically significant findings (**Table 3**). Model 1 included univariate (antibiotic use duration) analyses, as illustrated in **Figure 2A**. The hazard ratios (HRs) of model 1 were 2.6020 (95% confidence interval [CI] = 1.8940– 3.5750, $p < 0.001$) and 2.6510 (95% CI = 1.9880–3.5360, $p < 0.01$) for antibiotic use durations of 7–10 and <7 days, respectively. Model 2 included CKD, norepinephrine, vasopressin, cefazolin, tracheostomy, GC, PEG, POUF, TT, SCR, BMI, urine output, DBP, MBP, antibiotic use duration, temperature, admission age, hemoglobin, RBC, RDW, ALT, platelet count, bicarbonate, and basophils (**Figure 2B**). Model 2 had HRs of 2.1642 (95% CI = 1.5394–3.0426, $p < 0.001$) and 1.9933 (95% CI = 1.4077–2.8226, $p < 0.001$) for antibiotic use durations of 7–10 and <7 days, respectively. As demonstrated in **Figure 2C**, the significant variables from the Cox univariate regression analysis were incorporated into Model 3. Model 3 had HRs of 2.3263 (95% CI = 1.6375–3.3047, $p < 0.001$) and 2.5151 (95% CI = 1.7424–3.6307, $p < 0.001$) for antibiotic use durations of 7–10 and <7 days, respectively. In all models, the prognostic curves for VAP were nonlinear for antibiotic use duration. the antibiotic use duration curve showed a significant change trend at around 12 days (**Figure 2**, all $p < 0.001$). Notably, as the adjustment variable increased, the risk for antibiotic use for ≤10 days was more pronounced, and statistically significant that for >10 days.

Subgroup Analysis of the Restricted Cubic Spline Cox Proportional-Hazards Regression Model

The Cox regression analysis revealed that BMI was a predictive factor for patients with VAP. **Tables 4**, **5** list the baseline characteristics of each subgroup and the Cox regression analysis findings, with the outcomes of both groups being nonlinear ($p < 0.05$). **Figure 3** depicts the nonlinear curve comparison between the two groups. The curve of antibiotic use time was higher in the nonobese than the obese group. This result reflects that patients with VAP and low BMI have a higher

TABLE 4 | Baseline characteristics between different BMI groups.

Variable	—	BMI<30 kg/m2	BMI≥30 kg/m2	*p*-Value
N	—	948	661	0.009
Los_hospital	—	21 [14, 30]	22 [15, 33]	
Status	No	710 (74.9)	531 (80.3)	0.011
	Yes	238 (25.1)	130 (19.7)	
CKD	No	750 (79.1)	509 (77.0)	0.343
	Yes	198 (20.9)	152 (23.0)	
Norepinephrine	No	474 (50.0)	318 (48.1)	0.487
	Yes	474 (50.0)	343 (51.9)	
Vasopressin	No	764 (80.6)	509 (77.0)	0.093
	Yes	184 (19.4)	152 (23.0)	
CefazoLIN	No	711 (75.0)	508 (76.9)	0.427
	Yes	237 (25.0)	153 (23.1)	
Meropenem	No	747 (78.8)	506 (76.6)	0.314
	Yes	201 (21.2)	155 (23.4)	
Vancomycin oral liquid	No	854 (90.1)	584 (88.4)	0.304
	Yes	94 (9.9)	77 (11.6)	
Tracheostomy	No	856 (90.3)	593 (89.7)	0.764
	Yes	92 (9.7)	68 (10.3)	
CIMV	No	815 (86.0)	592 (89.6)	0.039
	Yes	133 (14.0)	69 (10.4)	
Hemodialysis	No	871 (91.9)	574 (86.8)	0.001
	Yes	77 (8.1)	87 (13.2)	
Gastric catheterization	No	830 (87.6)	589 (89.1)	0.383
	Yes	118 (12.4)	72 (10.9)	
PEG	No	766 (80.8)	564 (85.3)	0.022
	Yes	182 (19.2)	97 (14.7)	
POUF	No	915 (96.5)	618 (93.5)	0.007
	Yes	33 (3.5)	43 (6.5)	
RV	No	626 (66.0)	429 (64.9)	0.677
	Yes	322 (34.0)	232 (35.1)	
Temporary tracheostomy	No	760 (80.2)	520 (78.7)	0.502
	Yes	188 (19.8)	141 (21.3)	
Venous catheterization for renal dialysis	No	894 (94.3)	590 (89.3)	<0.001
	Yes	54 (5.7)	71 (10.7)	
Antibiotics_day	>10 days	540 (57.0)	387 (58.5)	0.155
	7–10 days	172 (18.1)	135 (20.4)	
	<7 days	236 (24.9)	139 (21.0)	
APSIII	—	66.00 [49.00, 86.00]	71.00 [52.00, 91.00]	0.011
Baseexcess	—	−1.00 [−5.00, 1.00]	0.00 [−4.00, 2.00]	0.061
Totalco2	—	24.00 [21.00, 28.00]	25.00 [22.00, 29.00]	0.001
Charlson_comorbidity_index	—	6.00 [4.00, 8.00]	6.00 [3.00, 8.00]	0.610
SCR	—	0.60 [0.40, 0.90]	0.70 [0.50, 1.00]	<0.001
GCS	—	8.00 [5.00, 12.00]	9.00 [4.00, 13.00]	0.909
SOFA	—	7.00 [5.00, 11.00]	8.00 [5.00, 12.00]	0.001
LODS	—	8.00 [5.00, 10.00]	8.00 [6.00, 11.00]	0.027
MELD	—	10.00 [10.00, 20.00]	10.00 [10.00, 21.32]	<0.001
SAPSII	—	41.00 [31.00, 51.00]	41.00 [31.00, 50.00]	0.988
Urineoutput	—	1,468.50 [859.50,2292.50]	1,500.00 [814.00,2325.00]	0.818
DBP	—	61 [56, 69]	62 [56, 69]	0.656
MBP	—	77 [71, 85]	78 [71, 86]	0.830
Temperature	—	37.1 [36.7, 37.5]	37.1 [36.8, 37.5]	0.011
Admission_age	—	67.00 [54.00, 78.00]	63.00 [52.00, 72.00]	<0.001
Hematocrit	—	32.70 [28.60, 37.90]	34.70 [29.10, 39.60]	<0.001
Hemoglobin	—	10.90 [9.30, 12.80]	11.30 [9.60, 13.20]	0.005
RBC	—	3.55 [3.04, 4.18]	3.82 [3.17, 4.42]	<0.001
RDW	—	14.40 [13.40, 16.00]	14.60 [13.50, 16.30]	0.014
ALT	—	1,236.50 [586.00,2922.00]	1,335.00 [598.00, 3,137.00]	0.503
Platelet_Count	—	880.00 [749.75,1031.00]	872.00 [755.00, 999.00]	0.384
Bicarbonate	—	41.00 [39.00, 45.00]	42.00 [39.00, 45.00]	0.255
Basophils	—	3.00 [2.00, 4.00]	3.00 [2.00, 4.20]	0.768

TABLE 5 | univariate cox regression results for different BMI groups.

Variable	Low-BMI group (BMI<30 kg/m2)			High-BMI group (BMI≥30 kg/m2)		
	HR	95%CI	*p*-value	HR	95%CI	*p*-value
Antibiotics_day						
>10 days	Reference	—	—	—	—	—
7–10 days	2.53	1.80–3.54	<0.001	2.60	1.69–4.00	0.001
<7 days	2.74	2.04–3.68	<0.001	2.72	1.78–4.14	<0.001
CefazoLIN						
No	Reference	—	—	—	—	—
Yes	0.52	0.37–0.73	<0.001	0.52	0.32–0.83	0.006
Tracheostomy						
No	Reference	—	—	—	—	—
Yes	0.34	0.20–0.58	<0.001	0.41	0.21–0.81	0.010
Gastric catheterization						
No	Reference	—	—	—	—	—
Yes	0.31	0.19–0.50	<0.001	0.24	0.11–0.55	0.001
PEG						
No	Reference	—	—	—	—	—
Yes	0.16	0.09–0.28	<0.001	0.29	0.14–0.63	0.002
POUF						
No	Reference	—	—	—	—	—
Yes	2.02	1.26–3.24	0.004	1.93	1.18–3.14	0.009
Temporary tracheostomy						
No	Reference	—	—	—	—	—
Yes	0.26	0.16–0.41	<0.001	0.34	0.20–0.59	<0.001
APSIII	1.01	1.01–1.01	<0.001	1.01	1.00–1.01	0.005
Charlson_comorbidity_index	1.14	1.1–1.19	<0.001	1.11	1.05–1.18	<0.001
SCR	1.40	1.25–1.57	<0.001	1.38	1.1–1.71	0.005
SOFA	1.04	1.01–1.07	0.006	1.04	1.00–1.08	0.049
LODS	1.08	1.04–1.12	<0.001	1.09	1.03–1.14	0.001
MELD	1.02	1.00–1.03	0.018	1.04	1.02–1.06	<0.001
SAPSII	1.02	1.01–1.02	<0.001	1.03	1.01–1.04	<0.001
Urineoutput	1.00	1.00–1.00	0.003	1.00	1.00–1.00	0.019
Admission_age	1.03	1.02–1.04	<0.001	1.04	1.03–1.05	<0.001
Bicarbonate	0.93	0.91–0.96	<0.001	0.93	0.89–0.97	<0.001

risk from an antibiotic use duration >10 days than those with high BMI. These results were the same in all models.

DISCUSSION

VAP is a primary risk factor for mortality among critically ill patients. Treatment strategies may benefit from objective prognostic assessment tools (International Symposium on Intensive Care, 2016). Due to the obvious severity of VAP and the relative vulnerability of critically ill patients, timely and appropriate antimicrobial therapy is critical for reducing its burden, and infection control is implemented by avoiding prolonged ventilation with adequate sedation and weaning and avoiding endotracheal tubes. Biofilm formation, microaspiration of subglottic secretions, and bacterial translocation from the stomach to the upper respiratory tract and oropharynx are the basis for colonization (Martin-Loeches et al., 2018). Some studies have classified VAP into early-onset and late-onset types based on time of admission, but the bacteriological differences between the two are unclear. As an outcome, antibiotic selection based on pneumonia onset may result in over-and undertreatment of broad-spectrum drugs, so determining the perfect duration of antibiotic use for patients with VAP is critical for ensuring good clinical outcomes (Nair and Niederman, 2015; Torres et al., 2017).

Antibiotic stewardship methods in China include bacterial resistance surveillance at the hospital level, as local antibiotic resistance statistics are crucial for guiding rational antibiotic usage, and the development of antibiotic resistance is closely related to the duration of antibiotic use. In this research, a reliable predictive model for the duration of antibiotic treatment in patients with ventilator-associated pneumonia was developed, which can assist clinicians in making decisions about the prognosis of VAP patients and the optimal duration of antibiotic treatment, improving the patient's chance of survival and reducing the risk of social labor loss cost and national medical resources (Hu et al., 2018; Chen et al., 2021).

Globally, overuse and inappropriate use of antibiotics is widely known as the primary cause of antibiotic resistance, as well as prolonging hospital stays, increasing treatment costs, and mortality associated with infectious diseases, acute upper respiratory infections, and ventilator-related complications. The most prevalent illnesses linked to antibiotic misuse are acute upper respiratory tract infection and ventilator-associated pneumonia. Antibiotic resistance has posed a serious threat to the effectiveness of existing antibiotics and their prescription regimens, affecting people of all

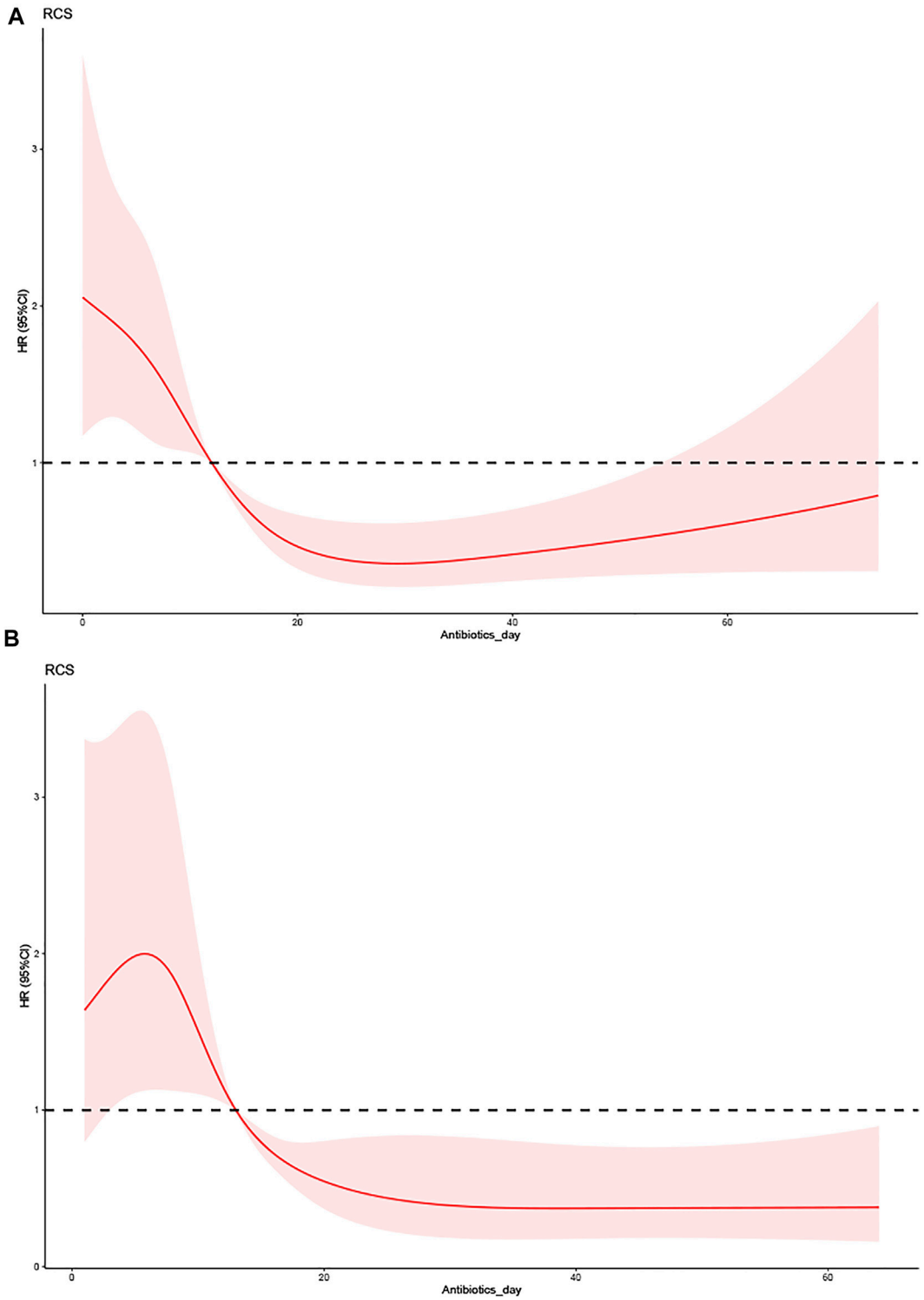

FIGURE 3 | The effect of the duration of antibiotic use on the prognosis of VAP between different BMI groups. **(A)** Low-BMI group (BMI<30 kg/m2). **(B)** High-BMI group (BMI ≥30 kg/m2).

socioeconomic backgrounds in community-acquired and nosocomial infections, as well as high health-care costs that lead to chronic poverty, whether untreated or treated. Excessive risk of morbidity and mortality. Appropriate antibiotic treatment length can effectively prevent drug resistance, better reduce human capital loss, mitigate economic

shocks suffered by poor and developing societies and countries, and lower the risk of medical poverty traps (Ahmad and Khan, 2019; Zhao et al., 2020).

The bactericidal effect of antibiotics has been observed to depend on the time when the MIC is exceeded, and it is recommended to reach a time where the MIC is 100% or even higher. It is reasonable to apply a positive PharmacoKinetics and PharmacoDynamics (PK/PD) target in patients with VAP. In patients with respiratory tract infection, the 30-days survival rate of those receiving long-term β-lactam infusion was dramatically higher than that of patients receiving an intermittent bolus, and antibiotics had a considerable degree of concentration-dependent apoptosis characteristics as well as time-dependent characteristics (Timsit et al., 2017). The optimal duration of antibiotic use should therefore be invoked as a prognostic indicator to detect the clinical outcomes of patients with VAP.

A Cox proportional-hazards regression model fitted with RCS was used in this study to explore the link between the duration of antibiotic use and the risk of all-cause mortality. Compared with a duration of antibiotic use >10 days, patients with VAP and a duration of ≤10 days had a higher mortality risk. The Cox multivariate analysis indicated that the duration of antibiotic use was a prognostic factor for patients with VAP ($p < 0.001$). All prediction curves for antibiotic use time in RCS had similar performances. Based on antibiotic use of >10 days, this study set a long-term antibiotic range within which the risk of VAP was lowest. Notably, the effect of antibiotic use duration on VAP prognosis was nonlinear with statistical significance ($p < 0.001$). When performing sensitivity analyses, all models with antibiotic use ≤10 days also had a higher mortality risk than those with >10 days. For models 1-3, the risks of using antibiotics for 7–10 days were 2.6020-, 2.1642-, and 2.3263-fold higher than for an antibiotic use duration of >10 days, respectively, while the risks of model 1–3 with an antibiotic use duration of <7 days were 2.6020-, 2.1642-, and 2.3263-fold higher, respectively.

In the subgroup analyses, the nonobese and obese groups also had a higher risk from antibiotic use ≤10 days compared with >10 days. According to Pugh et al., the VAP recurrence rate was higher for a short course of 8 days than for a longer course of 12–15 days in patients with VAP from nonfermenting Gram-negative bacilli organisms. This increased recurrence risk did not appear to be linked to increased mortality, the need for prolonged mechanical ventilation, or the time spent in the ICU (Pugh et al., 2015). Insufficient empirical antibiotic treatment has been linked to increased mortality risk (Hanes et al., 2002), and Rhodes et al. found that patients with VAP are a changing population at risk of antibiotic resistance and an underdosing response to altered antibiotic pharmacokinetic characteristics (Rhodes et al., 2018). Initial empirical treatments for VAP should therefore use antibiotics to cover all possible pathogens, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Methicillin-resistant Staphylococcus aureus*, and pathogen identification and drug susceptibility assessments should be carried out as soon as possible. Trials to ensure the adequate coverage and appropriate dose and duration of treatment could reduce the mortality from VAP (Wang et al., 2018; Gore et al., 2020).

De Oliveira noted that across all age groups, obese patients were not more likely to develop pulmonary complications than those with normal BMI (De Oliveira et al., 2017). Wardell et al. found that obesity was not necessarily associated with poor prognoses among critically ill patients (Wardell et al., 2015). This result was consistent with recent research that confirmed the so-called obesity paradox, whereby possibly because adipose tissue appears as an active participant in regulating physiological and pathological processes in hypermetabolic states, adipocytes can not only secrete various hormones and bioactive peptides but also produce and release pro- and anti-inflammatory factors for immune regulation. This may attenuate inflammatory responses during acute disease and improve survival (Fantuzzi, 2005; Torres et al., 2019).

This study explored the relationship between duration of antibiotic use and VAP and performed subgroup analyses to identify a cutoff of approximately 12 days for the prediction curve of antibiotic use duration and quantified the risk across the curve, and also found that obesity was associated with reduced ICU mortality in patients with VAP. Future research should explore other approaches for determining the optimal duration of antibiotic use to help clinicians make better decisions for patients with VAP in ICUs.

Limitations

This study had some limitations. First, the data for this study originated in the MIMIC database. There was a potential risk of bias because most participants in this database were white Americans, which restricts the generalizability of the conclusions. Second, some patient indicators were not completely reported, which resulted in information leakage. Third, the severity of VAP could not be precisely determined. Fourth, the retrospective design of the study meant that some other biases were undoubtedly present. Fifth, patient functional outcomes and post-discharge disposition for ventilator-associated pneumonia were unknown due to a lack of long-term follow-up procedures in the MIMIC-IV database. Despite these limitations, we believe that determining the optimal antibiotic duration can help to better understand the prognosis of patients with VAP in ICUs.

CONCLUSIONS

Considering that patients with VAP are more likely to have unfavorable clinical outcomes, selecting the appropriate antibiotic use duration is critical. VAP patients have a certain risk of death in the ICU, and our study found that the duration of antibiotic use had a nonlinear effect on the prognosis of VAP patients, with the lowest risk of in-hospital mortality in VAP when the duration of antibiotic use was 12 days, which may be a turning point in the prognosis of VAP patients. And so an accurate and reliable prognostic model for the best antibiotic duration to forecast VAP still needs to be developed.

In the furture, we will to keep researching the impact of antibiotic use duration on VAP. In order to develop a predictive model with accurate thresholds, sensitivity, and selectivity, as well as a high level of reliability, to help clinicians make concise management and treatment decisions

for the prognosis of and maximizing the survival chances of patients with VAP.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

YX and DH performed the statistical analysis and data interpretation. JL and HW contributed to the study concept and study design and contributed equally. FX performed literature research and data extraction. SS and XZ were responsible for the quality control of data and algorithms. All authors contributed to the writing of the manuscript and approved the final version.

FUNDING

The study was supported by Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007); National Natural Science Foundation, China (Grant No. 82071340); National foreign expert project of the Ministry of science and technology, China (Grant No. G20200019047).

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ORIGINAL RESEARCH
published: 05 May 2022
doi: 10.3389/fphar.2022.898422

Effects of Gastric Acid Secretion Inhibitors for Ventilator-Associated Pneumonia

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 17 March 2022
Accepted: 20 April 2022
Published: 05 May 2022

Citation:
Li F, Liu H, Zhang L, Huang X, Liu Y, Li B, Xu C, Lyu J and Yin H (2022) Effects of Gastric Acid Secretion Inhibitors for Ventilator-Associated Pneumonia. Front. Pharmacol. 13:898422. doi: 10.3389/fphar.2022.898422

Objective: This study analyzed the association of gastric acid secretion inhibitors (GASIs) [including proton pump inhibitors (PPIs) and histamine 2 receptor antagonists (H2RAs)] with the occurrence of ventilator-associated pneumonia (VAP) and in-hospital mortality in patients who received invasive mechanical ventilation (IMV).

Method: Patients who received IMV and used GASI were included based on records in the MIMIC-IV database. The relationships of GASIs with VAP and the in-hospital mortality were determined using univariate and multivariate logistic regression analyses. Also, the effects of GASIs in some subgroups of the population were further analyzed.

Results: A total of 18,669 patients were enrolled, including 9191 patients on H2RAs only, 6921 patients on PPIs only, and 2557 were on a combination of the two drugs. Applying logistic regression to the univariate and multivariate models revealed that compared with H2RAs, PPIs had no significant effect on the incidence of VAP, and the combination of H2RAs and PPIs was a risk factor for VAP. Compared with H2RAs, univariate logistic regression revealed that, PPIs and combine the two drugs were both risk factors for in-hospital mortality, but multivariate logistic regression showed that they were not significantly associated with in-hospital mortality. In subgroup analysis, there were interaction in different subgroups of age, PCO2, myocardial infarct, congestive heart failure (P for interaction<0.05).

Conclusion: Compared with H2RAs, PPIs did not have a significant association with either VAP or in-hospital mortality; the combination of H2RAs and PPIs was risk factor for VAP, but did not have a significantly associated with in-hospital mortality.

Keywords: ventilator-associated pneumonia, mortality, proton pump inhibitor, histamine 2 receptor antagonist, gastric acid secretion inhibitors

INTRODUCTION

Invasive mechanical ventilation (IMV) is commonly used in critically ill patients in intensive care units (ICUs) to maintain airway patency, prevent aspiration, and improve oxygenation. A critical complication is ventilator-associated pneumonia (VAP). Patients who received IMV had pulmonary infections mostly caused by pathogens present in the hospital environment (Timsit et al., 1000). A meta-analysis estimated that the incidence density of VAP/1000 ventilator days was 15.1%, with higher incidence rates in low-income countries (Bonell et al., 2019). VAP increases hospital stays, mortality, and treatment costs (Koenig and Truwit, 2006; Fadda and Ahmad, 2022).

Patients with IMV are often associated with stress ulcer risk, and preventive application of gastric acid secretion inhibitors (GASIs) can significantly reduce gastrointestinal bleeding (Zhou et al., 2019). The most commonly used drugs are proton pump inhibitors (PPIs) and histamine 2 receptor antagonists (H2RAs). H2RAs were thought to inhibit gastric acid secretion by blocking histamine receptors, PPIs work by inhibiting the final stages of gastric acid production (Toews et al., 2018). On the one hand, these drugs can improve the pH gastric juice and allow excessive bacterial growth (Buendgens et al., 2016); on the other hand, they can reduce reflux, and prevent bacteria movement to the pharynx and lungs. The purpose of this study was to determine the effects of two different GASIs on VAP incidence and in-hospital mortality among patients with IMV.

METHODS

Data Source

The Beth Israel Deaconess Medical Center (BIDMC), Massachusetts General Hospital, and the Massachusetts Institute of Technology jointly developed the Medical Information Mart for Intensive Care (MIMIC) in 2003. The project was funded by the National Institutes of Health (Yang et al., 2020). The MIMIC-IV database provides information on more than 70,000 patients admitted to the ICU of BIDMC from 2008 to 2019, and it is freely available to researchers. All patients were re-identification under the Safe harbor provisions of and Health Insurance Portability and Accountability Act, thereby waiving the need to obtain informed consent (Wu et al., 2021).

Patient Population

Patients were searched for in the MIMIC-IV database. The inclusion criteria identified 25,031 patients who used IMV. The exclusion criteria were 1) not the first ICU admission (2,495 patients), 2) ICU stay of less than 24 h (1379 patients), 3) >5% missing data (141 patients), and 4) not used PPIs or H2RAs (2444 patients).

Variable Selection and Outcome

Baseline characteristics during ICU admission were obtained using Structured Query Language, and if there were multiple values, the worst values for that period were recorded. Information was collected on demographic characteristics (year of admission, sex, body weight, and age), disease severity [Acute Physiology Score III (APSIII), Sequential Organ Failure Assessment scores (SOFA), laboratory examination results white blood cells (WBC), hemoglobin (HB), platelets (PLT)], [partial pressure of carbon dioxide (PCO2), partial pressure of oxygen (PO2), lactate, anion gap, blood urea nitrogen (BUN), blood sugar (GLU), international normalized ratio (INR), and total bilirubin (TBIL)], commodities (myocardial infarction, congestive heart failure, peptic ulcer disease, diabetes, cerebrovascular disease, renal disease, liver disease, and sepsis), treatment regimens [renal replacement therapy (RRT) vasopressors use, PPIs, H2RAs, and IMV duration], hospital length of stay (LOS), and ICU LOS. PPIs included esomeprazole, lansoprazole, omeprazole, and pantoprazole; and H2RAs included famotidine, ranitidine, and cimetidine.

End points were a VAP diagnosis and all-cause in-hospital mortality. According to the standard VAP diagnostic criteria of the Centers for Disease Control and Prevention, the specific diagnostic criteria used in this study are listed in **Table 1** (American Thoracic Society and Infectious Diseases Society of America, 2005; Klompas et al., 2012).

Statistical Analysis

Data were collated and outliers were deleted. If a variable had >20% missing data, it was discarded; otherwise, the 10-degree interpolation method was applied. Categorical data were expressed as frequencies or percentages. Continuous variables were expressed as medians and interquartile ranges. Chi-square and Kruskal–Wallis H tests were used to identify significant differences between the groups.

Logistic regression was used to analyze the relationships of PPIs, H2RAs, and the combination of PPIs and H2RAs with VAP and in-hospital mortality. These results were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). The logistic regression presupposes a linear relationship between the continuous independent variables and logit(P), so we first performed the boxTidwell test (Brescia et al., 2021).*p* values for the variables age, weight, PLT, AG, INR, and TBIL were all greater than 0.05 (**Supplementary Table S1**) consistent with a linear relationship with logit(P), and the remaining continuous variables were converted to categorical variables based on clinical significance or inter-quartile spacing. Univariate and multivariate models were established for each end point. In the multivariate model, we adjusted for patients' general information age, sex, weight; disease severity score APSIII, SOFA score; routine hematological tests WBC, HB, PLT; patients' liver and kidney functions: BUN, TBIL, INR; indicators reflecting patients' internal environment PO2, PCO2, anion gap, GLU, lactate and patients' major comorbidities myocardial infarction, congestive heart failure, cerebrovascular disease, peptic ulcer disease, liver disease, diabetes, renal disease, sepsis were adjusted. In addition, whether patients were on RRT after ICU admission, vasopressors use, and IMV duration were also used for adjustment. The variance inflation factor showed no multicollinearity between these variables (**Supplementary Table S2**).

The relationships of PPIs, H2RAs, and the combination of PPIs and H2RAs with VAP were assessed using subgroup

TABLE 1 | Centers for Disease Control and Prevention's clinical surveillance definition for VAP.

Category	Detailed Description
Radiologic criteria (two or more serial radiographs with at least one of the following)	1. New or progressive and persistent infiltrate 2. Consolidation 3. Cavitation
Systemic criteria (at least one)	1. Fever (>38 °C or > 100.4°F) 2. Leukopenia (<4000 WBC/mm3) or leukocytosis (≥12,000 WBC/mm3) 3. For adults ≥70 years old, altered mental status with no other recognized cause
Pulmonary criteria (at least two)	1. New onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements 2. Worsening gas exchange (e.g., desaturations, increased oxygen requirements, or increased ventilator demand) 3. New onset or worsening cough, or dyspnea, or tachypnea 4. Rales or bronchial breath sounds

All patients on ventilation more than 48 h.

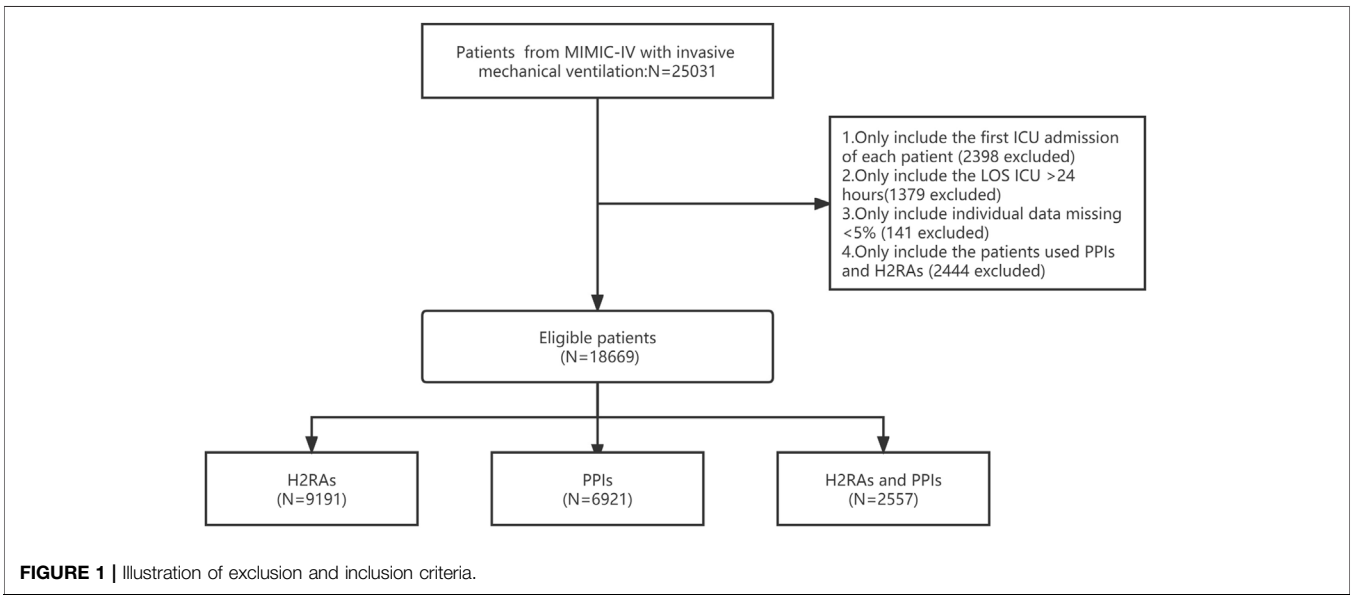

FIGURE 1 | Illustration of exclusion and inclusion criteria.

analyses. The subgroups were classified according to age, sex, APSIII, SOFA score, PO2, PCO2, HB, WBC, PLT, anion gap, BUN, INR, body weight, GLU, TBIL, lactate, myocardial infarct, congestive heart failure, cerebrovascular disease, peptic ulcer disease, liver disease, diabetes, renal disease, sepsis, RRT, vasopressors use, and IMV duration.

R software (http://www.R-project.org) and SPSS software (version 27.0, IBM, United States) were used for all statistical analyses. All probability values were bilateral, and $p < 0.05$ was considered indicative of statistical significance.

RESULTS

Clinical Characteristics

This study enrolled 18,669 ICU patients with IMV and GASIs (see flowchart in **Figure 1**). There were 9191 patients in the H2RAs group, 6,921 patients in the PPIs group, 2,557 patients in the combination of PPIs and H2RAs group. H2RAs group were more male ($p < 0.05$). PPIs group were older ($p < 0.05$). The combination of PPIs and H2RAs group had higher APSIII, SOFA scores; more vasopressors and RRT use; longer IMV duration, hospital LOS and ICU LOS ($p < 0.05$). In comorbidity, patients in the three groups were most complicated with sepsis (67.7%, 76.2% and 84.8%, respectively).More detailed characteristics of the remaining participants stratified by different GASIs can be seen in **Table 2**.

The distribution of GASIs in different years was shown in **Figure 2**. The proportion of H2RAs increased year by year, and the comparison among all groups was significant, and the highest in 2017–2019 group (55.93%) ($p < 0.05$). The proportion of PPIs decreased year by year, except that there was no statistical difference between 2011–2013 group and 2014–2016 group ($p > 0.05$), other groups had statistical differences, the lowest in 2017–2019 group (31.00%) ($p < 0.05$). There was no statistical difference in the combination of PPIs and H2RAs among all groups ($p > 0.05$).

TABLE 2 | Characteristics of patients with GASIs.

	H2RAs	PPIs	H2RAs and PPIs	*p*
N	9191	6921	2557	—
Age (year)	66.00 (54.00, 76.00)	67.00 (56.00, 77.00)	66.00 (55.00, 77.00)	<0.001
Sex	—	—	—	<0.001
Male	5634 (61.3)	4009 (57.9)	1465 (57.3)	—
Female	3557 (38.7)	2912 (42.1)	1092 (42.7)	—
Weight (kg)	82.40 (69.60, 98.05)	80.97 (68.30, 96.70)	83.28 (70.20, 98.53)	<0.001
Year of admission	—	—	—	<0.001
2008–2010	2805 (30.5)	2680 (38.7)	943 (36.9)	—
2011–2013	2092 (22.8)	1597 (23.1)	571 (22.3)	—
2014–2016	2322 (25.2)	1551 (22.4)	582 (22.8)	—
2017–2019	1972 (21.5)	1093 (15.8)	461 (18.0)	—
APSIII	—	—	—	<0.001
<58	6225 (67.7)	3496 (50.5)	994 (38.9)	—
58–82	1848 (20.1)	1822 (26.3)	763 (29.8)	—
>82	1118 (12.2)	1603 (23.2)	800 (31.3)	—
SOFA	—	—	—	<0.001
<6	3489 (38.0)	1858 (26.8)	446 (17.4)	—
6–9	3910 (42.5)	2519 (36.4)	911 (35.6)	—
>9	1792 (19.5)	2544 (36.8)	1200 (46.9)	—
Vasopressors	—	—	—	<0.001
No	8178 (89.0)	5719 (82.6)	1882 (73.6)	—
Yes	1013 (11.0)	1202 (17.4)	675 (26.4)	—
RRT	—	—	—	<0.001
No	8842 (96.2)	6171 (89.2)	2148 (84.0)	—
Yes	349 (3.8)	750 (10.8)	409 (16.0)	—
IMV duration (hour)	—	—	—	<0.001
<96	6694 (72.8)	4495 (64.9)	1012 (39.6)	—
≥96	2497 (27.2)	2426 (35.1)	1545 (60.4)	—
WBC(K/uL)	—	—	—	<0.001
<10	993 (10.8)	1025 (14.8)	191 (7.5)	—
≥10	8198 (89.2)	5896 (85.2)	2366 (92.5)	—
HB(g/L)	—	—	—	<0.001
<120	8624 (93.8)	6692 (96.7)	2487 (97.3)	—
≥120	567 (6.2)	229 (3.3)	70 (2.7)	—
PLT (K/uL)	132.00 (99.00, 179.00)	123.00 (73.00, 181.00)	114.00 (62.00, 171.00)	<0.001
INR	1.40 (1.20, 1.80)	1.50 (1.30, 2.30)	1.60 (1.30, 2.70)	<0.001
Anion gap (mmol/L)	17.00 (15.00, 19.00)	18.00 (15.00, 22.00)	19.00 (16.00, 23.00)	<0.001
TBIL (mg/dL)	0.60 (0.40, 1.10)	0.80 (0.50, 2.10)	0.90 (0.50, 2.30)	<0.001
PO2(mmHg)	—	—	—	<0.001
<60	3135 (34.1)	3591 (51.9)	1562 (61.1)	—
≥60	6056 (65.9)	3330 (48.1)	995 (38.9)	—
PCO2(mmHg)	—	—	—	<0.001
<50	4907 (53.4)	3327 (48.1)	883 (34.5)	—
≥50	4284 (46.6)	3594 (51.9)	1674 (65.5)	—
PH	—	—	—	<0.001
<7.35	6263 (68.1)	4978 (71.9)	2074 (81.1)	—
≥7.35	2928 (31.9)	1943 (28.1)	483 (18.9)	—
Lactate (mmol/l)	—	—	—	<0.001
<3.3	6396 (69.6)	4528 (65.4)	1338 (52.3)	—
≥3.3	2795 (30.4)	2393 (34.6)	1219 (47.7)	—
BUN(mg/dl)	—	—	—	<0.001
<20	2544 (27.7)	1228 (17.7)	343 (13.4)	—
≥20	6647 (72.3)	5693 (82.3)	2214 (86.6)	—
Glucose (mg/dl)	—	—	—	<0.001
<200	6075 (66.1)	3912 (56.5)	1192 (46.6)	—
≥200	3116 (33.9)	3009 (43.5)	1365 (53.4)	—
Myocardial infarct	—	—	—	0.074
No	7495 (81.5)	5570 (80.5)	2041 (79.8)	—
Yes	1696 (18.5)	1351 (19.5)	516 (20.2)	—
Congestive heart failure	—	—	—	<0.001
No	6862 (74.7)	4650 (67.2)	1697 (66.4)	—
Yes	2329 (25.3)	2271 (32.8)	860 (33.6)	—
Cerebrovascular disease	—	—	—	<0.001
No	7398 (80.5)	6042 (87.3)	2053 (80.3)	—

(Continued on following page)

TABLE 2 | (*Continued*) Characteristics of patients with GASIs.

	H2RAs	PPIs	H2RAs and PPIs	*p*
Yes	1793 (19.5)	879 (12.7)	504 (19.7)	—
Peptic ulcer disease	—	—	—	<0.001
No	9132 (99.4)	6469 (93.5)	2468 (96.5)	—
Yes	59 (0.6)	452 (6.5)	89 (3.5)	—
Liver disease	—	—	—	<0.001
No	8480 (92.3)	5417 (78.3)	2143 (83.8)	—
Yes	711 (7.7)	1504 (21.7)	414 (16.2)	—
Diabetes	—	—	—	<0.001
No	6544 (71.2)	4653 (67.2)	1742 (68.1)	—
Yes	2647 (28.8)	2268 (32.8)	815 (31.9)	—
Renal disease	—	—	—	<0.001
No	7683 (83.6)	5211 (75.3)	1926 (75.3)	—
Yes	1508 (16.4)	1710 (24.7)	631 (24.7)	—
Sepsis	—	—	—	<0.001
No	2966 (32.3)	1644 (23.8)	388 (15.2)	—
Yes	6225 (67.7)	5277 (76.2)	2169 (84.8)	—
VAP	—	—	—	<0.001
No	8590 (93.5)	6432 (92.9)	2132 (83.4)	—
Yes	601 (6.5)	489 (7.1)	425 (16.6)	—
In-hospital mortality	—	—	—	<0.001
No	7904 (86.0)	5383 (77.8)	1886 (73.8)	—
Yes	1287 (14.0)	1538 (22.2)	671 (26.2)	—
LOS ICU (day)	3.46 (2.03, 7.04)	4.04 (2.21, 7.92)	7.75 (3.71, 15.08)	<0.001
LOS hospital (day)	8.87 (5.62, 14.88)	9.88 (5.95, 16.82)	15.95 (8.80, 26.96)	<0.001

p < *0.05 means significant difference.*

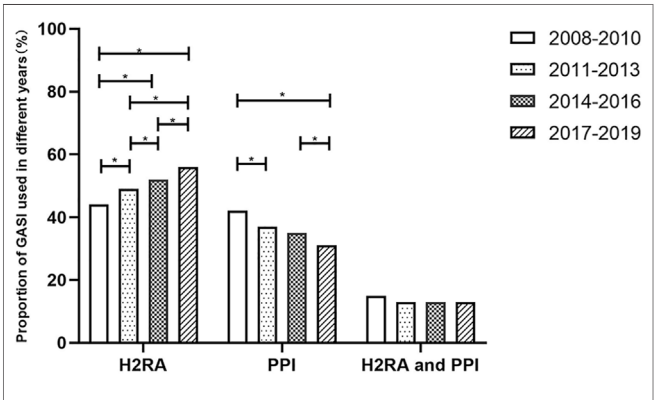

FIGURE 2 | Comparison of distribution proportion between GASIs in different years.

Relationships of GASIs With VAP and In-Hospital Mortality

Applying logistic regression revealed that compared with H2RAs group, the combination of PPIs and H2RAs group was a risk factor for VAP in the univariate and multivariate models ($p < 0.05$), whereas PPIs group was not significantly associated with VAP ($p > 0.05$). Compared with H2RAs, univariate logistic regression revealed that, PPIs and the combination of H2RAs and PPIs were both risk factors for in-hospital mortality, but multivariate logistic regression revealed that PPIs and the combination of H2RAs and PPIs were not significantly associated with in-hospital mortality ($p > 0.05$) (**Table 3**).

Subgroup Analysis

Subgroup analyses were performed on various covariate, most of which presented no obvious interaction (**Table 4**, $p > 0.05$). There were interactions in different subgroups of age, PCO2, myocardial infarct, congestive heart failure (P for interaction<0.05). PPIs was a protect factor for VAP in the subgroup of age <65 years, APSIII <58, PCO2<50 mmHg, IMV duration≥96 h, without used vasopressors, without myocardial infarct, without congestive heart failure, without liver disease, without diabetes, without renal disease. ($p < 0.05$).

DISCUSSION

The patients who received IMV in the ICU had severe diseases that were often accompanied by stress ulcers, resulting in inadequate nutritional supplies, and further digestive tract hemorrhage, gastrointestinal perforation, and life-threatening conditions in serious cases. Stress ulcer treatment guidelines for gastrointestinal bleeding prevention in critically ill patients recommend using PPIs (or H2RAs as a reasonable alternative), but sucralfate is not recommended (Ye et al., 2020). Previous research has linked stress ulcer medication use to an increased VAP risk. Our current study showed that 18,669 patients had received GASIs while on IMV therapy, and 1,515 (8.12%) of them developed VAP, which is a relatively high rate. Moreover, we found that the utilization rate of H2RAs increased year by year compared with PPIs, and the proportion of patients using only

TABLE 3 | Logistic regression analysis of GASIs to VAP and in hospital mortality for both models.

—		Univariate Model		Multivariate Medol	
		OR [CI(95%)]	*p* Value	OR [CI(95%)]	*p* Value
VAP	H2RAs	reference	—	reference	—
	PPIs	1.087 [0.960-1.229]	0.188	0.883 [0.769-1.014]	0.08
	H2RAs and PPIs	2.849 [2.493-3.253]	<0.001	1.476 [1.273-1.711]	<0.001
In-hospital mortality	H2RAs	reference	—	reference	—
	PPIs	1.755 [1.617-1.904]	<0.001	1.105 [0.997-1.224]	0.06
	H2RAs and PPIs	2.185 [1.965-2.429]	<0.001	0.903 [0.792-1.028]	0.12

OR, odds ratio; CI: confidence interval. p < *0.05 means significant difference.*
Multivariate logistic regression, variables: age, sex, weight, APSIII, SOFA, WBC, HB, PLT, PCO2, PO2, BUN, INR, TBIL, lactate, glucose, anion gap, congestive heart failure, cerebrovascular disease, peptic ulcer disease, myocardial infarct, liver disease, diabetes, renal disease, sepsis, vasopressors, RRT, IMV, duration.

H2RAs reached 55.93% from 2017 to 2019. To investigate the prognostic impact of specific GASIs, this study investigated the effect of PPI, H2RAs and their combination on VAP and in-hospital mortality. The results showed that compared to H2RAs, PPIs was found no significant effect on VAP or in-hospital mortality, but their combination was risk factors for VAP whereas no significant effect on in-hospital mortality.

Aspiration is an important factor in hospital acquired pneumonia (HAP) and VAP pathogenesis. By inhibiting acid production, PPIs and H2RAs increase nosocomial pathogen colonization in the oropharynx and trachea, while delaying gastric emptying, greatly increasing the risk of infection from aspiration (Tablan et al., 2004). The flora analysis of VAP also supported this, and the pathogenic bacteria of patients who received PPIs and H2RAs were mostly *pseudomonas*, Gram-negative bacteria, and methicillin-resistant *Staphylococcus aureus*, which may be caused by gastric alkalinization (Grindlinger et al., 2016). In critically ill patients, there was no difference in pneumonia incidence between H2RAs and PPIs, but PPIs significantly reduced GIB incidence (Deliwala et al., 2021), and H2RAs had a lower cost and higher survival rate (MacLaren and Campbell, 2014). Both of them have advantages and disadvantages. However, some studies have suggested the presence of a difference in VAP incidence between the two GASIs. Miano et al. reported that pantoprazole was associated with a higher HAP incidence than was ranitidine (Miano et al., 2009). A meta-analysis also found that PPIs are more effective than H2RAs, but may also increase the risk of VAP (Alquraini et al., 2017). Zhou et al. believed that for high-risk critically ill patients, PPIs and H2RAs can significantly reduce gastrointestinal bleeding, with both potentially increasing the pneumonia incidence (Zhou et al., 2019). Other studies had shown that there was no difference in the effect of PPI on VAP compared with H2RAs, but PPI had a better anti-gastrointestinal bleeding effect (Arriola et al., 2016). Some studies had pointed out that PPIs and H2RAs had no effect on the risk of death or extubation in critically ill patients (Li et al., 2020). The effect of PPIs and H2RAs on VAP, the results of previous studies were inconsistent.

In this study, PPIs compared with H2RAs had no difference in VAP and in hospital mortality. The combination of PPIs and H2RAs would lead to a higher incidence of VAP, but did not have a beneficial effect on in hospital mortality, which reminds us not to use the two drugs to inhibit gastric acid secretion at the same time. We hypothesized that the combination of the two drugs meant that the dose was increased, the inhibition of gastric acid secretion was stronger, and the gastric alkalization was more serious. On the one hand, the incidence of VAP was increased, and on the other hand, the treatment effect of stress ulcer was better, thus having the impact on mortality. Further confirmation is certainly needed.

This study found that PPIs compared with H2RAs was a protect factor for VAP in subgroup of age<65 years, APSIII <58, PCO2<50 mmHg, without used vasopressors, without myocardial infarct, without congestive heart failure, without liver disease, without diabetes, without renal disease. The effect of PPIs on VAP was more beneficial in mild, young patients without chronic heart, renal and liver disease. Therefore, we still suggest that more consideration should be given to the use of PPIs after the evaluation of patients with IMV, and PPIs was also the preferred drug for stress ulcer in the guidelines. Studies have shown that compared with the using H2RAs, PPIs prophylaxis was the most effective preventive strategy in patients at high risk of developing stress ulcer bleeding (Barkun et al., 2013). However, in recent years, the proportion of H2RAs had increased rapidly, and the reasons affecting doctors' decision-making need to be further discussed.

In clinical practice, it is necessary to measure VAP incidence and the efficiency of stress ulcer prevention and treatment, and select appropriate drugs according to the situation of each patient. In this study, the combination of PPIs and H2RAs is not recommended, which increases the risk of VAP. In patients with less severe disease, younger and with fewer comorbidities, PPIs was associated with a lower incidence of VAP compared to H2RAs. However, no matter how GASIs was selected, it had no significant impact on in hospital mortality.

Limitations

This study analyzed a big-data sample spanning 12 years, but there were some limitations. First, it uses a retrospective single-center design, which has some uncontrolled confounding bias. Second, there was no comparison of the effects of the drugs on gastrointestinal bleeding to determine their therapeutic effects. Third, logistic regression presupposes a linear relationship between continuous independent variables and logit(P), but in some machine learning methods such as ensemble modeling,

TABLE 4 | Subgroup analysis of the associations between GASIs and VAP.

	H2RAs		PPIs		H2RAs and PPIs		P For Interaction
			OR [CI(95%)]	*p* Value	OR [CI(95%)]	*p* Value	
Age (years)	—	—	—	—	—	—	0.048
<65	8522	reference	0.730 (0.597–0.892)	0.026	1.299 (1.050–1.604)	<0.001	—
≥65	10147	reference	1.046 (0.862–1.269)	0.650	1.702 (1.380–2.097)	<0.001	—
Gender	—	—	—	—	—	—	0.782
Male	11108	reference	0.900 (0.755–1.072)	0.237	1.420 (1.173–1.717)	<0.001	—
Female	7561	reference	0.862 (0.686–1.081)	0.200	1.608 (1.266–2.038)	<0.001	—
SOFA	—	—	—	—	—	—	0.156
<6	5793	reference	0.720 (0.504–1.017)	0.066	1.422 (0.929–2.135)	0.097	—
6–9	7340	reference	0.831 (0.667–1.033)	0.097	1.496 (1.182–1.888)	0.001	—
>9	5536	reference	1.053 (0.845–1.314)	0.648	1.601 (1.275–2.013)	<0.001	—
Vasopressors	—	—	—	—	—	—	0.092
NO	15779	reference	0.839 (0.718–0.979)	0.026	1.375 (1.158–1.631)	<0.001	—
YES	2890	reference	1.127 (0.816–1.564)	0.470	1.861 (1.356–2.568)	<0.001	—
CRRT	—	—	—	—	—	—	0.896
NO	17161	reference	0.905 (0.779–1.050)	0.187	1.506 (1.281–1.768)	<0.001	—
YES	1508	reference	0.809 (0.547–1.204)	0.291	1.364 (0.914–2.049)	0.131	—
Sepsis	—	—	—	—	—	—	0.051
NO	4998	reference	0.953 (0.452–1.957)	0.897	3.337 (1.551–6.968)	0.002	—
YES	13671	reference	0.873 (0.758–1.005)	0.060	1.426 (1.226–1.658)	<0.001	—
Myocardial infarct	—	—	—	—	—	—	0.001
NO	15106	reference	0.810 (0.694–0.945)	0.008	1.518 (1.290–1.786)	<0.001	—
YES	3563	reference	1.231 (0.891–1.706)	0.210	1.376 (0.950–1.989)	0.090	—
Congestive heart failure	—	—	—	—	—	—	0.047
NO	13209	reference	0.792 (0.666–0.942)	0.009	1.475 (1.231–1.766)	<0.001	—
YES	5460	reference	1.091 (0.863–1.382)	0.468	1.517 (1.164–1.976)	0.002	—
Cerebrovascular disease	—	—	—	—	—	—	0.282
NO	15493	reference	0.914 (0.781–1.069)	0.260	1.474 (1.239–1.752)	<0.001	—
YES	3176	reference	0.800 (0.589–1.081)	0.150	1.542 (1.153–2.057)	0.003	—
Peptic ulcer disease	—	—	—	—	—	—	0.061
NO	18069	reference	0.885 (0.769–1.018)	0.087	1.454 (1.250–1.689)	<0.001	—
YES	600	reference	3.036 (0.712–22.018)	0.187	7.474 (1.681–55.389)	0.019	—
Liver disease	—	—	—	—	—	—	0.272
NO	16040	reference	0.845 (0.725–0.983)	0.030	1.470 (1.251–1.726)	<0.001	—
YES	2629	reference	1.182 (0.828–1.703)	0.363	1.762 (1.177–2.651)	0.006	—
Diabetes	—	—	—	—	—	—	0.163
NO	12939	reference	0.820 (0.694–0.969)	0.020	1.378 (1.152–1.646)	<0.001	—
YES	5730	reference	1.040 (0.811–1.335)	0.755	1.763 (1.346–2.308)	<0.001	—
Renal disease	—	—	—	—	—	—	0.063
NO	14820	reference	0.825 (0.703–0.967)	0.018	1.473 (1.245–1.741)	<0.001	—
YES	3849	reference	1.173 (0.878–1.573)	0.284	1.652 (1.192–2.289)	0.003	—
APSIII	—	—	—	—	—	—	0.132
<58	10715	reference	0.771 (0.605–0.979)	0.034	1.719 (1.319–2.228)	<0.001	—
58–82	4433	reference	0.972 (0.768–1.228)	0.810	1.485 (1.155–1.907)	0.002	—
>82	3521	reference	0.888 (0.689–1.146)	0.359	1.322 (1.016–1.721)	0.038	—
WBC (K/uL)	—	—	—	—	—	—	0.472
<10	2209	reference	0.625 (0.360–1.078)	0.092	0.950 (0.427–1.976)	0.895	—
≥10	16460	reference	0.899 (0.779–1.037)	0.144	1.501 (1.289–1.746)	<0.001	—
HB (g/L)	—	—	—	—	—	—	0.902
<120	17803	reference	0.886 (0.771–1.018)	0.089	1.480 (1.275–1.718)	<0.001	—
≥120	866	reference	0.594 (0.135–2.195)	0.456	1.694 (0.275–7.966)	0.530	—
PCO2 (mmHg)	—	—	—	—	—	—	0.004
<50	9117	reference	0.662 (0.518–0.842)	0.001	1.445 (1.106–1.879)	0.006	—
≥50	9552	reference	1.027 (0.865–1.219)	0.758	1.524 (1.272–1.825)	<0.001	—
PO2 (mmHg)	—	—	—	—	—	—	0.497
<60	8288	reference	0.924 (0.782–1.093)	0.359	1.508 (1.261–1.804)	<0.001	—
≥60	10381	reference	0.796 (0.616–1.022)	0.077	1.433 (1.088–1.874)	0.009	—
Lactate (mmol/L)	—	—	—	—	—	—	0.189
<3.3	12262	reference	0.879 (0.741–1.042)	0.138	1.356 (1.112–1.650)	0.002	—
≥3.3	6407	reference	0.921 (0.724–1.171)	0.501	1.618 (1.281–2.045)	<0.001	—
Glucose (mg/dl)	—	—	—	—	—	—	0.879
<200	11179	reference	0.905 (0.734–1.114)	0.347	1.481 (1.171–1.868)	0.001	—
≥200	7490	reference	0.862 (0.716–1.037)	0.116	1.475 (1.215–1.789)	<0.001	—
BUN (mg/dl)	—	—	—	—	—	—	0.944

(Continued on following page)

TABLE 4 | (*Continued*) Subgroup analysis of the associations between GASIs and VAP.

	H2RAs		PPIs		H2RAs and PPIs		P For Interaction
			OR [CI(95%)]	*p* Value	OR [CI(95%)]	*p* Value	
<20	4115	reference	0.871 (0.557–1.340)	0.534	1.324 (0.780–2.187)	0.285	—
≥20	14554	reference	0.884 (0.764–1.023)	0.098	1.485 (1.271–1.735)	<0.001	—
IMV duration (hour)	—	—	—	—	—	—	0.196
<96	12201	reference	0.942 (0.722–1.227)	0.661	1.780 (1.256–2.485)	0.001	—
≥96	6468	reference	0.846 (0.719–0.994)	0.043	1.403 (1.190–1.654)	<0.001	—

OR, odds ratio; CI: confidence interval. p < *0.05 means significant difference.*
Multivariate logistic regression, variables: age, sex, weight, APSIII, SOFA, WBC, HB, PLT, PCO2, PO2, BUN, INR, TBIL, lactate, glucose, anion gap, congestive heart failure, cerebrovascular disease, peptic ulcer disease, myocardial infarct, liver disease, diabetes, renal disease, sepsis, vasopressors, RRT, IMV, duration.

non-linearity can be handled automatically without prior specification, and the application of this method can be tried in future research (Zhang et al., 2022). Finally, due to some limitations in the database, the total dose and duration of the drug could not be accurately calculated in this study, and these factors could be taken into account in the design of future studies.

CONCLUSION

Compared with H2RAs, PPIs did not have a significant association with either VAP or in-hospital mortality; the combination of H2RAs and PPIs was risk factor for VAP, but did not have a significantly associated with in-hospital mortality.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The data were available on the MIMIC-IV website at https://mimic-iv.mit.edu.

AUTHOR CONTRIBUTIONS

FL, HL and LZ contributed equally to this work. FL extracted the data from the MIMIC-IV database. HL and LZ participated in data analysis and interpretation. FL and LZ wrote the first draft of the paper. XH and YL planned the analyses, and BL and CX guided the literature review. HY and JL conceptualized the research aims. All author approves the final manuscript.

FUNDING

This work was supported by The National Social Science Foundation of China (grant/award no. 16BGL183), The National Natural Science Foundation of China (No. 82072232; 81871585), The Natural Science Foundation of Guangdong Province (No. 2018A030313058), Technology and Innovation Commission of Guangzhou Science, China (No.201804010308).

ACKNOWLEDGMENTS

We would like to thank the Massachusetts Institute of Technology, the Beth Israel Deaconess Medical Center for the MIMIC project, and Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007), Guangzhou, Guangdong, China.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.898422/full#supplementary-material

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frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 20 May 2022
doi: 10.3389/fphar.2022.903378

Management of Ventilator-Associated Pneumonia: Quality Assessment of Clinical Practice Guidelines and Variations in Recommendations on Drug Therapy for Prevention and Treatment

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Purpose: To assess the quality of clinical practice guidelines (CPGs) related to drug therapy for prevention and control of ventilator-associated pneumonia (VAP) and compare the differences and similarities between recommendations.

Methods: Electronic databases (including PubMed, Cochrane library, Embase, Web of Science), guideline development organizations, and professional societies were searched to identify CPGs for VAP from 20 January 2012 to 20 January 2022. The Appraisal of Guidelines Research & Evaluation (AGREE) II instrument was used to evaluate the quality of the guidelines. The recommendations on drug therapy for prevention and treatment for each guideline were extracted, and then a descriptive synthesis was performed to analyze the scope/topic, and consistency of the recommendations.

Results: Thirteen CPGs were included. The median score and interquartile range (IQR) in each domain are shown below: scope and purpose 72.22% (63.89%,83.33%); stakeholder involvement 44.44% (38.89%,52.78%); rigor of development 43.75% (31.25%,57.29%); clarity and presentation 94.44% (77.78%,94.44%); applicability 20.83 (8.34%,33.34%) and editorial independence 50% (33.33%,66.67%). We extracted 21 recommendations on drug therapy for prevention of VAP and 51 recommendations on drugs used for treatment. Some controversies remained among the included guidelines.

Conclusion: There is considerable variability in the development processes and reporting of VAP guidelines. Despite many similarities, the recommendations still had some inconsistencies in the details. For the prevention and treatment of VAP, local microbial epidemiology and antibiotic sensitivity must be considered, and recommendations should be regularly revised as new evidence emerges.

OPEN ACCESS

Edited by:
Xian-Tao Zeng,
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *24 March 2022*
Accepted: *29 April 2022*
Published: *20 May 2022*

Citation:
Li H-Y, Wang H-S, Wang Y-L, Wang J, Huo X-C and Zhao Q (2022) Management of Ventilator-Associated Pneumonia: Quality Assessment of Clinical Practice Guidelines and Variations in Recommendations on Drug Therapy for Prevention and Treatment. Front. Pharmacol. 13:903378. doi: 10.3389/fphar.2022.903378

Keywords: ventilator-associated pneumonia, drug prevention and treatment, clinical practice guideline, AGREE II, recommendation

1 INTRODUCTION

1.1 A Basic Introduction to Ventilator Associated Pneumonia

Ventilator-associated pneumonia (VAP) is a special type of nosocomial infection typified by pulmonary parenchymal inflammation, which usually occurs 48 h after artificial airway or mechanical ventilation (Infectious disease group RmboCMA 2018). VAP is believed to be an important cause of healthcare-associated infections, resulting in increased morbidity and mortality, it is one of the most frequently occurring infections in the intensive care unit (ICU) (Sangale et al., 2021). Despite the rapid development of critical care medicine, the incidence rate and mortality of VAP remain high. VAP is reported to affect 5–40% of patients receiving invasive mechanical ventilation for more than 2 days, with large variations depending upon the country, ICU type, and criteria used to identify VAP (American Thoracic 2005; Seguin et al., 2014). The data from the International Nosocomial Infection Control Consortium (INICC) confirmed that the incidence of VAP was 14.1/1000 mechanical ventilator-days, and the mortality was 36.6% (Rosenthal et al., 2020). VAP can also prolong hospitalization and intubation times, increase the use of antibiotics, affect the prognosis of severely ill patients, and increase medical expenses (Kollef et al., 2012; Álvarez-Lerma and Sánchez García 2018; Papazian et al., 2020). Therefore, curbing VAP has become the most urgent problem facing medical institutions. Microbiological tools have currently made progress, but the epidemiology and diagnostic criteria of VAP are still controversial, which complicates the interpretation of prevention, treatment, and outcome research (Nair and Niederman 2015; Timsit et al., 2017).

1.2 Antimicrobial Resistance

Antimicrobial resistance is not only a global crisis, but also a global problem occupying the attention of both governments and society. The Antibiotic Resistance Global Report on Surveillance issued by the World Health Organization (WHO) in April 2014 (WHO 2014). It is reported that in the Americas, *Escherichia coli* has high resistance to the third generation cephalosporins and fluoroquinolones, and *Klebsiella pneumoniae* has strong and widespread resistance to the third generation cephalosporins, Methicillin resistant *Staphylococcus aureus* (MRSA) was present in up to 90% of patients in some parts of the region. In Europe, *Klebsiella pneumoniae* is highly resistant to the third generation cephalosporins, MRSA was present in up to 60% of patients in some parts of the region (WHO 2014). At present, in China, the overall prevalence of MRSA remains at about 35%, the proportion of *Escherichia coli* resistant to third-generation cephalosporins is still more than 55%, the proportion of *Pseudomonas aeruginosa* resistant to carbapenems remains around 20%, and the proportion of *Acinetobacter baumannii* resistant to carbapenems is on the rise, at nearly 60% (Zhang et al., 2016). In 2001, WHO published the "WHO global strategy for containment of antibiotic resistance", to address the problem of bacterial drug resistance. This put forward global action suggestions to deal with antibiotic resistance (WHO 2001). The UK announced its "5-year antibiotic resistance strategy 2013 to 2018" in 2013 (Affairs DoHfEFaR 2013). In 2016, China issued the "national action plan to curb bacterial drug resistance (2016–2020)", requiring all large medical institutions to attach great importance to the clinical application of antibiotics and improve their management strategies (Ministry of education 2016).

At the same time, countries have implemented clinical guidelines to further standardize the medication use by professionals and the public. The National Institute for health and Care Excellence (NICE), a British Government institution, has formulated evidence-based clinical medication guidelines for antibiotics to guide the rational use of antibiotics and increase the clinical management of antibiotic use. At the same time, the Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI) in British released the clinical guidelines with the theme of "Start Smart-then Focus" (Ashiru-Oredope et al., 2012). China has issued regulations and normative documents such as the "Administrative Measures for the Clinical Application of Antibiotics" to guide the use of antibiotics, but it is necessary to clarify the relevant supervision needed to ensure that the guidelines do play a normative and guiding role (Author Anonymous 2013).

1.3 Objective of the Study

Clinical practice guidelines (CPGs) are systematically constructed recommendations formulated to aid decision-making among medical professionals, which provide evidence-based recommendations for clinical practitioners and other healthcare professionals about the management of patients with diseases or other clinical conditions (Rosenfeld et al., 2013; Vandvik et al., 2013). They help to improve the quality of medical treatment and patients' prognosis (Woolf et al., 1999). To standardize the prevention, diagnosis, and treatment of VAP, many national and international organizations have developed the relevant CPGs. The prevention and treatment of VAP in different countries is based on its pathogenic characteristics and antimicrobial sensitivity, which is significant and important for guiding empirical treatment (Torres et al., 2017; Chou et al., 2018; Infectious disease group RmboCMA 2018; Leone et al., 2018).

To date, there is still uncertainty regarding VAP management (Nair and Niederman 2015). Many CPGs have been developed by different organizations to change the empirical management of VAP. Increased production of CPGs is accompanied by growing concern about variations in quality and recommendations. External validation and prospective evaluation of guidelines are therefore necessary. So we have performed a comprehensive review of guidelines related to drug therapy for prevention and treatment of VAP to assess their methodological

quality using the Appraisal of Guidelines for Research & Evaluation (AGREE) II (Ma et al., 2020) instrument and compared the differences between them, to provide a reference for the prevention and treatment of VAP and further promote rational drug use.

2 MATERIALS AND METHODS

2.1 Guideline Identification

Relevant guidelines were identified through computerized searches of PubMed, Cochrane library, Embase, Web of Science using a combination of text free terms and their corresponding Mesh terms, as well as three major Chinese academic databases. The search strategy is showed in **Supplementary File S1**. The important professional society websites regarding critical care medicine and infection were also searched for VAP guidelines, **Supplementary File S2** lists the important websites with potential VAP guidelines. In addition, we checked the references of included guidelines and consulted experts in the field.

All guidelines related to drug therapy for prevention or treatment of VAP published in English or Chinese from 20 January 2012 to 20 January 2022 were included. Documents were considered guidelines if they met the following criteria: (Infectious disease group RmboCMA, 2018): A guideline should have a clear recommendation on drug therapy for prevention or treatment of VAP for adults and contain all related supporting materials and documents. (Sangale et al., 2021). Evidence-based guidelines. The guidelines report on search strategies, literature quality or data extraction, and classify the level of evidence (LOE) and the strength of recommendation (SOR). (Seguin et al., 2014). If the guidelines had updated versions, only the most recent version was included.

Exclusion criteria: Single-author overviews, editorials, letters to the editor, textbook-like publications, short summaries, documents without clear recommendations, and secondary publications (including versions translated from other languages) were excluded. If a guideline only applied to children, patients with immunodeficiency or COVID-19, it was also excluded.

2.2 Quality Assessment

CPGs were evaluated independently by four assessors from different backgrounds, including one ICU expert (H-SW), two pharmacists (H.-YL, JW), and one methodologist (X-CH). All assessors have extensive experience in evaluating CPGs using the AGREE II instrument. AGREE II consists of 23 key items organized into six domains (Ma et al., 2020). The scope and purpose domain includes the main objectives of the CPG, the target population and health questions; the stakeholder involvement domain concerns the extent to which the CPG was developed by the appropriate stakeholders and represents the opinions of its intended users; the rigor of development domain focuses on the procedure for synthesizing and gathering evidence and the methods used to formulate the recommendations; the clarity of presentation domain focuses on whether recommendations are specific and clear, different options for addressing the condition or health issue are clearly presented, and key recommendations are easily identifiable; the applicability domain assesses processes related to guideline dissemination and implementation, such as additional materials, organizational facilitators and barriers, monitoring or audit and cost implications; the editorial independence domain is concerned with whether the interests or views of the funding body have influenced the forming of the final recommendations and whether the competing interests of all guideline developers have been recorded, addressed and reported. The score for each domain is obtained by summing up all the scores of the individual items in one domain and then standardizing using the following formula: (obtained score - minimum possible score)/(maximum possible score - minimum possible score). The standardized scores ranged from 0 to 100%, a score of 60% average was chosen to establish the proportion of guidelines that scored points above this level in every domain.

2.3 Data Collection

We developed a draft data extraction form which included document characteristics (e.g., year of publication, country/region, version, development organization and team) and methodological features of the guideline (e.g., multidisciplinary cooperation, sources of evidence, criteria for selecting the evidence, grading method, methodology used to formulate the recommendations, stakeholder involvement, funding, and disclosure of conflicts of interest). Consistent with the scope of this study, we also tabulated the information on drug therapy for prevention and treatment of VAP, which we used to determine if the recommendations only applied to VAP, as is the case of some recommendations, or if they were pertinent to VAP, however, all evidence supporting the recommendations came from VAP-related research.

2.4 Data Statistical Analysis

A descriptive analysis was performed by calculating each domain score and scaled domain score. Agreement among the four assessors was calculated by the intraclass correlation coefficient (ICC) with 95% confidence interval (CI) for each domain. According to the scale proposed by Fleiss, the degree of agreement between 0.00 and 0.40 was deemed poor, 0.41 to 0.75 was fair to good, and 0.75 to 1.00 was excellent (Everitt and Fleiss 1981). Statistical analyses were conducted using SPSS 23.0.

3 RESULTS

3.1 Search Results and Baseline Characteristics

A total of 3572 relevant documents were obtained in the initial examination, and 13 guidelines were finally included according to the inclusion and exclusion criteria (Gupta et al., 2012; Association CcmboCM 2013; Klompas et al., 2014; Mehta et al., 2014; Álvarez Lerma et al., 2014; Kalil et al., 2016;

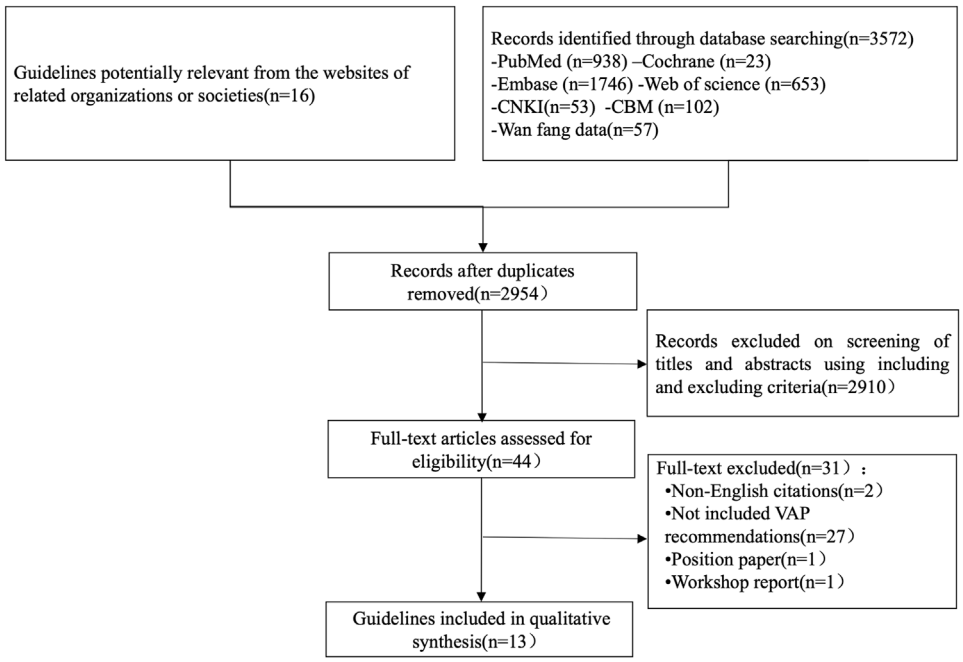

FIGURE 1 | Flow chart of the identification process of CPGs for VAP.

Mikasa et al., 2016; Torres et al., 2017; Chou et al., 2018; Infectious disease group RmboCMA 2018; Leone et al., 2018; Collins et al., 2020; Association SoCRDoCM 2021). The flow chart is shown in **Figure 1**.

For each guideline finally included, we systematically collected all relevant information and data. All guidelines were evidence-based. Five guidelines were updated versions (Klompas et al., 2014; Kalil et al., 2016; Mikasa et al., 2016; Chou et al., 2018; Infectious disease group RmboCMA 2018). The general characteristics of the included guidelines are listed in **Table 1**.

3.2 Quality Assessment

Four assessors independently assessed the 13 guidelines with an ICC value of 0.82 (95% CI = 0.73–0.87), which indicated a high level of reliability among assessors. The quality of the guidelines varied greatly, from fulfilling most of the AGREE criteria to only fulfilling an unsatisfactory number of items. Across all guidelines, none of them had high scores for all domains, and the assessors assigned the highest score to the domain of "clarity of presentation" and the lowest score to "applicability". The ERS 2017 guideline (Torres et al., 2017) ranked highest in overall quality, whereas the CMA 2018 guideline (Infectious disease group RmboCMA 2018) ranked the lowest. (**Table 2**; **Figure 2**). **Supplementary File S3** shows the important methodology for guideline development of included CPGs.

3.2.1 Scope and Purpose

The median score and interquartile range (IQR) of this domain was 72.22% (63.89%,83.33%), The highest score in this domain was 83.33% (Kalil et al., 2016; Torres et al., 2017; Infectious disease group RmboCMA 2018; Collins et al., 2020), and the lowest score was 61.11% (Gupta et al., 2012; Association CcmboCM 2013; Álvarez Lerma et al., 2014). The overall score in this field is high. All guidelines had clear overall objectives. The main problem is that the description of the target population is not clear, only four guidelines (Kalil et al., 2016; Torres et al., 2017; Infectious disease group RmboCMA 2018; Collins et al., 2020) specifically described the target population.

3.2.2 Stakeholder Involvement

The median score and IQR of the stakeholder involvement domain was 44.44% (38.89%,52.78%), The highest score in this domain was 77.78% (Kalil et al., 2016; Torres et al., 2017), and the lowest score was 27.78% (Álvarez Lerma et al., 2014). Two guidelines (15.38%) scored over 60% (Kalil et al., 2016; Torres et al., 2017). Five guidelines included methodologists in evidence synthesis and guideline development (Association CcmboCM 2013; Klompas et al., 2014; Kalil et al., 2016; Torres et al., 2017; Collins et al., 2020). No guideline reported the involvement of patients or patient representatives, but the ERS 2017 guideline did provid a suggested interpretation of recommendations by the targeted stakeholders including patients, clinicians, and health policy makers (Torres et al., 2017).

3.2.3 Rigor of Development

The median score and IQR of the rigor of development domain was 43.75% (31.25%,57.29%). The highest score in this domain was 79.17% (Torres et al., 2017), and the lowest score was 16.67%

TABLE 1 | General characteristics of the included guidelines.

Guideline	Country	Developing Organization	Target Population	Theme of Recommendations	Version
Qiu, HB 2021 (Association SoCRDoCM, 2021)	China	SCRD of CMA	Patients with mechanical ventilation	VAP treatment	First Version
Collins, T. 2020 (Collins et al., 2020)	British	BACCN	Critically ill adult patients	VAP prevention	First Version
Chou, C.C. 2018 (Chou et al., 2018)	Taiwan, China	IDST/TSPCCM	CAP, HAP, VAP, HCAP in adults and pediatric pneumonia	VAP prevention VAP treatment	Updated
Qu, JM 2018 (1)	China	IDG of RMBCMA	Non-immunocompromised patients with HAP/VAP over 18	VAP prevention VAP treatment	Updated
Lenoe, M. 2018 (Leone et al., 2018)	France	SFAR/SRLF	HAP/VAP (including COPD, neutropenia, post-operative, and pediatrics)	VAP treatment	First Version
Torres, A. 2017 (Torres et al., 2017)	Europe	ERS/ESICM/ ESCMID/ALAT	Adult patients with HAP and VAP, does not apply to patients with primary and secondary immune deficiency	VAP prevention VAP treatment	First Version
Mikasa, K. 2016 (Mikasa et al., 2016)	Japan	JAID/JSC	Patients with respiratory infectious diseases in Japan and covered all such diseases in adults and children	VAP treatment	Updated
Kalil, A.C. 2016 (Kalil et al., 2016)	America	IDSA/ATS	Non-immunocompromised patients with HAP/VAP	VAP treatment	Updated
Mehta, Y. 2014 (Mehta et al., 2014)	India	ISCCM	Patients at risk of nosocomial infections	VAP prevention	First Version
Klompas, M. 2014 (Klompas et al., 2014)	America	SHEA/IDSA/AHA/ APIC	VAP	VAP prevention	Updated
Alvarez-Lerma, F. 2014 (Álvarez Lerma et al., 2014)	Spain	SSICM/SSICN	VAP	VAP prevention	First version
Li, YM 2013 (Association CcmboCM, 2013)	China	CCMCMA	VAP	VAP prevention VAP treatment	First Version
Gupta, D. 2012 (Gupta et al., 2012)	India	ICS and NCCP	VAP/HAP in adults	VAP prevention VAP treatment	First Version

HAP: Hospital-acquired Pneumonia; VAP: Ventilator-associated Pneumonia; SCRD, of CMA: Subgroup of Critical Respiratory Diseases of Chinese Medical Association; BACCN: British Association of Critical Care Nurses; ISCCM: Indian Society of Critical Care Medicine; IDST: Infectious Diseases Society of Taiwan; TSPCCM: Taiwan Society of Pulmonary and Critical Care Medicine; IDG, of RMBCMA: Infectious disease group, Respiratory medicine branch of Chinese Medical Association; SFAR: French Society of Anesthesia and Intensive Care Medicine; SRLF: French Society of Intensive Care; ERS: European Respiratory Society; ERSESICM: European Society of Intensive Care Medicine; ESCMID: European Society of Clinical Microbiology and Infectious Diseases; ALAT: Latin American Thoracic Association; ICU: Intensive Care Unit; COPD: Chronic obstructive Pulmonary Disease; JAID: Japanese Association for Infectious Diseases; JSC: Japanese Society of Chemotherapy; IDSA: Infectious Diseases Society of America; ATS: American Thoracic Society; HAIs: Hospital-acquired Infections; SSICM: The Spanish Societies of Intensive Care Medicine; SSICN: ISCCM: The Spanish Societies of Intensive Care Nurses Indian Society of Critical Care Medicine; AHA: American Hospital Association; APICA: association for Professionals in Infection Control and Epidemiology; CCMCMA: Critical care medicine branch of Chinese Medical Association. ICS, and NCCP: Indian Chest Society and National College of Chest Physicians.

(Mehta et al., 2014). Only two guidelines (15.38%) scored over 60% (Gupta et al., 2012; Torres et al., 2017). The overall score in this field is low because of a lack of systematic methods for reporting the searching or evaluation of evidence.

3.2.4 Clarity of the Presentation Domain

The median score and IQR of the clarity of the presentation domain was 94.44% (77.78%,94.44%). The highest score in this domain was 94.44% (Gupta et al., 2012; Association CcmboCM 2013; Klompas et al., 2014; Álvarez Lerma et al., 2014; Kalil et al., 2016; Mikasa et al., 2016; Torres et al., 2017), and the lowest score was 66.67% (Chou et al., 2018). All guidelines scored over 60%. The overall score in this field is the highest in six fields, and the quality of methodology is the best. All guidelines clearly describe each item in this field.

3.2.5 Applicability Domain

The median score and IQR of the clarity of the applicability domain was 20.83% (8.33%,33.34%), which is the lowest score of all items. The highest score in this domain was 50.00% (Gupta et al., 2012; Kalil et al., 2016), and the lowest score was 4.17% (Association CcmboCM 2013; Mehta et al., 2014; Association SoCRDoCM 2021). No guideline scored over 60%. Most guidelines do not consider potential obstacles to implementation. The ERS 2017 guideline provided pocket guidelines in the supplementary files and added "implementation considerations" for every recommendation in the pocket guidelines (Torres et al., 2017).

3.2.6 Editorial Independence Domain

The median score and IQR of the editorial independence domain was 50% (33.3%, 66.67%). The highest score in this domain was 83.33% (Gupta et al., 2012; Álvarez Lerma et al., 2014; Kalil et al., 2016), and the lowest score was 0% (Association CcmboCM 2013; Infectious disease group RmboCMA 2018). Three CPG (23.08%) scored over 60% (Gupta et al., 2012; Álvarez Lerma et al., 2014; Kalil et al., 2016). Six guidelines (Gupta et al., 2012; Mehta et al., 2014; Álvarez Lerma et al., 2014; Kalil et al., 2016; Chou et al., 2018; Collins et al., 2020) stated that the sponsor's views had no impact on the recommendation, and five guidelines (Association CcmboCM 2013; Mehta et al., 2014; Chou et al., 2018; Infectious disease group RmboCMA 2018; Collins et al., 2020) did not mention the conflict of interest for the members of the formulation team.

TABLE 2 | AGREE II Domain scores for included guidelines.

Guideline	Scope and Purpose (%)	Stakeholder Involvement (%)	Rigor of Development (%)	Clarity of Presentation (%)	Applicability (%)	Editorial Independence (%)	Mean Score (%)
Qiu, HB 2021 (Association SoCRDoCM, 2021)	72.22	33.33	52.08	83.33	4.17	50.00	49.19
Collins, T. 2020 (Collins et al., 2020)	83.33	50.00	43.75	77.78	20.83	33.33	51.50
Chou, C.C. 2018 (Chou et al., 2018)	66.67	44.44	37.50	66.67	16.67	33.33	44.21
Qu, JM 2018 (Infectious disease group RmboCMA, 2018)	83.33	38.89	20.83	72.22	16.67	0	38.66
Lenoe, M. 2018 (Leone et al., 2018)	72.22	50.00	37.50	77.78	12.50	50.00	52.78
Torres, A. 2017 (Torres et al., 2017)	83.33	77.78	79.17	94.44	41.67	50.00	71.06
Mikasa, K. 2016 (Mikasa et al., 2016)	72.22	44.44	35.42	94.44	25.00	50.00	53.59
Kalil, A.C. 2016 (Kalil et al., 2016)	83.33	77.78	68.75	94.44	50.00	83.33	74.54
Mehta, Y. 2014 (Mehta et al., 2014)	66.67	44.44	16.67	83.33	4.17	33.33	41.44
Klompas, M. 2014 (Klompas et al., 2014)	66.67	55.56	27.08	94.44	20.83	50.00	52.43
Alvarez-Lerma, F. 2014 (Álvarez Lerma et al., 2014)	61.11	27.78	50.00	94.44	20.83	83.33	56.25
Li, YM 2013 (Association CcmboCM, 2013)	61.11	44.44	43.75	94.44	4.17	0	41.32
Gupta, D. 2012 (Gupta et al., 2012)	61.11	38.89	62.50	94.44	50.00	83.33	65.05
Median score Interquartile range (IQR)	72.22 (63.89,83.33)	44.44 (38.89,52.78)	43.75 (31.25,57.29)	94.44 (77.78,94.44)	20.83 (8.34,33.34)	50.00 (33.33,66.67)	52.43 (42.83,60.65)

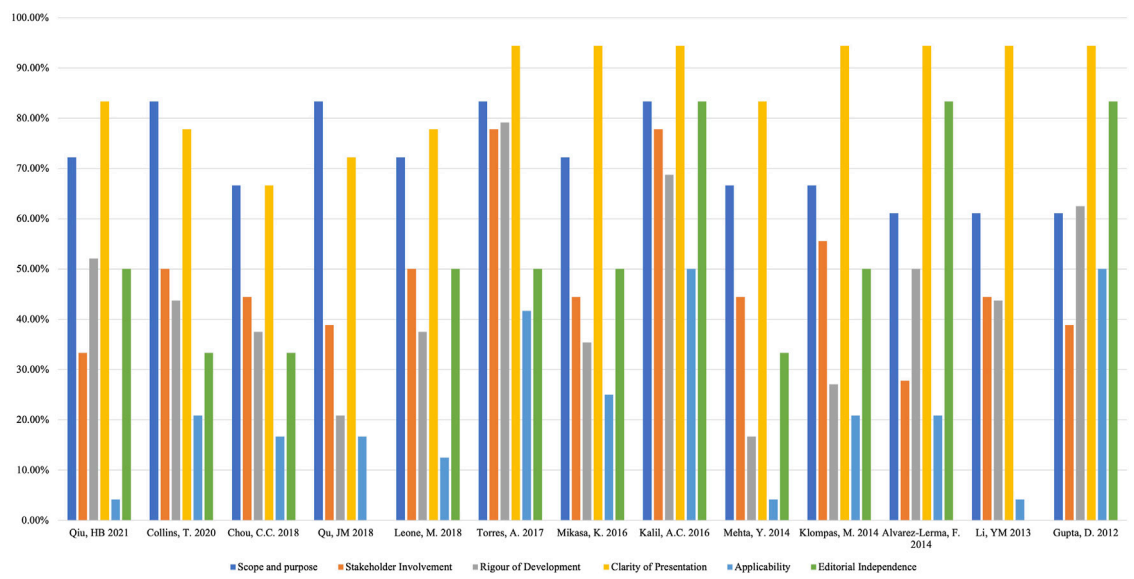

FIGURE 2 | AGREE II Domain scores for included guidelines.

TABLE 3 | Grading system of evidence and recommendation.

Guideline	Grading System Used	Description of Evidence	Description of Recommendation
Qiu, HB 2021 (Association SoCRDoCM, 2021)	GRADE	High; Moderate; Low; Very low	Strong; Weak
Collins, T. 2020 (Collins et al., 2020)	GRADE	High (1); Moderate (2); Low (3); Very low (4)	Strong; Moderate; Weak
Chou, C.C. 2018 (Chou et al., 2018)	GRADE	High [A]; Moderate [B]; Low [C]; Very low [D]	Strong [1]; Weak [2]
Qu, JM 2018 (Infectious disease group RmboCMA, 2018)	Self-defined	High(I); Moderate (II); Low (III)	Strong(A); Moderate(B); Weak(C)
Lenoe, M. 2018 (Leone et al., 2018)	GRADE	Strong; Moderate; Weak; Very weak	GRADE 1+; GRADE 1-; GRADE 2+; GRADE 2-
Torres, A. 2017 (Torres et al., 2017)	GRADE	High; Moderate; Low; Very low	Strong; Weak
Mikasa, K. 2016 (Mikasa et al., 2016)	Self-defined	I (Randomized comparative study); II (Non-randomized comparative study); III (Case report); IV (Specialist's opinion)	A (strongly recommended); B (general recommendation), C (comprehensive evaluation by the attending physician)
Kalil, A.C. 2016 (Kalil et al., 2016)	GRADE	High; Moderate; Low; Very low	Strong; Weak
Mehta, Y. 2014 (Mehta et al., 2014)	GRADE	High (A) to very low (C)	Strong (grade 1); weak (grade 2)
Klompas, M. 2014 (Klompas et al., 2014)	GRADE and Canadian Task Force on Preventive Health Care	High(I); Moderate (II); Low (III)	Basic practices; Special approaches; Generally not Recommended; No recommendation
Alvarez-Lerma, F. 2014 (Álvarez Lerma et al., 2014)	GRADE	High; Moderate; Low; Very low	Strong; Weak
Li, YM 2013 (Association CcmboCM, 2013)	GRADE	High(A); moderate(B); low(C); very low(D)	Strong (1); Weak (2)
Gupta, D. 2012 (Gupta et al., 2012)	Modified GRADE system	Level 1; Level 2; Level 3; Useful practice point	GRADE A; GRADE B

GRADE: Grading of Recommendations Assessment, Development and Evaluation.

3.3 Quality Assessment Comparison of Guidelines Developed With and Without GRADE System

All guidelines reported explicit grading for the strength of the recommendations. Nine guidelines reported that they used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool for evaluating the quality of evidence and forming the final recommendations (Association CcmboCM 2013; Mehta et al., 2014; Álvarez Lerma et al., 2014; Kalil et al., 2016; Torres et al., 2017; Chou et al., 2018; Leone et al., 2018; Collins et al., 2020; Association SoCRDoCM 2021). Two guidelines (Mikasa et al., 2016; Infectious disease group RmboCMA, 2018)used a self-defined grading system, and one guideline (Klompas et al., 2014) used a combined grading system incorporating GRADE and the Canadian Task Force on Preventive Health Care and one guideline (34) use a modified GRADE system. (**Table 3**). We compared the quality of the guidelines developed with and without GRADE system. SPSS 23.0 software was used for statistical analysis, and $p < 0.05$ was considered a statistically significant difference. The results showed that there was no significant difference in the six domains of AGREE II. See **Supplementary File S4** for details.

3.4 Recommendations on Drug Therapy for Prevention of VAP

We extracted 21 recommendations regarding drug therapy for prevention of VAP from 13 guidelines, including 5 strong recommendations, 5 moderate recommendations, 5 weak recommendation, 3 special approaches, 2 definitively not recommended, and one no formal recommendation. VAP has specific risk factors and pathogenesis. The recommendations that resulted from interpretation of the evidence varied among guidelines. **Supplementary File S5** shows the recommendations on drug therapy for prevention, and **Table 4** describes the chronological trend of recommendations on drug therapy for prevention of VAP.

3.4.1 Enteral Nutrition

There were 3 recommendations related to enteral nutrition for the prevention of VAP. Two Chinese guidelines (Association CcmboCM 2013; Infectious disease group RmboCMA 2018) recommended that early enteral nutrition is superior to parenteral nutrition as it can promote intestinal peristalsis, help to maintain the integrity of intestinal mucosal structure and barrier function, reduce pathogen colonization and bacterial translocation. The SHEA 2014 guideline (Klompas et al., 2014) did not recommend parenteral nutrition for VAP prevention, because it will reduce neither the incidence

TABLE 4 | Chronological trend of recommendations on drug therapy for prevention of VAP.

Guidelines	Enteral Nutrition (SOR/LOE)	SOD (SOR/LOE)	SDD (SOR/LOR)	Chlorhexidine (SOR/LOE)	Probiotics (SOR/LOE)	Ulcer Prophylaxis (SOR/LOE)	Aerosol Inhalation (SOR/LOE)
Collins, T. 2020 (Collins et al., 2020)	−−	−−	−−	Moderate/High	−−	−−	−−
Qu, JM 2018 (Infectious disease group RmboCMA 2018)	Moderate/ Moderate	Moderate/ Moderate	Moderate/ Moderate	Strong/Moderate	Moderate/ Moderate	Moderate/ Moderate	−−
Torres, A. 2017 (Torres et al., 2017)	−−	Weak/Low	−−	No formal recommendation	−−	−−	−−
Mehta, Y. 2014 (Mehta et al., 2014)	−−	−−	−−	Strong/High	−−	−−	−−
Klompas, M. 2014 (Klompas et al., 2014)	−/Moderate	−/High	−−	−/Moderate	−/Moderate	−/Moderate	−−
Alvarez-Lerma, F. 2014 (Álvarez Lerma et al., 2014)	−−	Strong/High	Strong/High	Strong/Moderate	−−	−−	−−
Li, YM 2013 (Association CcmboCM 2013)	Weak/Moderate	Weak/ Moderate	Weak/ Moderate	Strong/Low	Weak/Moderate	−−	Weak/Low

Strongly recommended Moderate recommended Weakly recommended; Recommended (not have the SOR) Strongly not recommended Moderate not recommended Weakly not recommended; Not recommended (not have the SOR, or no formal recommendation).SDD: selective digestive decontamination; SOD: selective oral decontamination; SOR: Strength of recommendation; LOE: Level of evidence.

of VAP nor the duration of mechanical ventilation, hospital stay or mortality.

3.4.2 Selective Oral Decontamination (SOD) or Selective Digestive Decontamination (SDD)

5 guidelines (Association CcmboCM 2013; Klompas et al., 2014; Álvarez Lerma et al., 2014; Torres et al., 2017; Infectious disease group RmboCMA 2018) made the suggestions for the use of SOD or SDD. The CMA 2013 guideline (Association CcmboCM 2013) and the SSICM 2014 guideline (Álvarez Lerma et al., 2014) recommend the use of SOD or SDD to prevent VAP, because it can decrease the rate of VAP mortality, although it had no effect on the time of mortality or length of mechanical ventilation. The SHEA 2014 guideline (Klompas et al., 2014) and the ERS 2017 guideline (Torres et al., 2017) advocated the use of SOD and avoidance of SDD, because most studies were conducted in countries or settings with low levels of antibiotic resistance, effectiveness of SOD or SDD in settings with high levels of antibiotic resistance has not been systematically assessed. Also, the potential effects of antibiotic use on antimicrobial resistant infections are inconclusive. The CMA 2018 guideline (1) did not provide explicit recommendations and only stated that SDD may increase the risk of drug-resistant bacterial infections, but there were no long-term follow-up studies; therefore, the Chinese guideline stressed the cautious use of SOD or SDD after weighing the advantages and disadvantages.

3.4.3 Chlorhexidine

7 guidelines (Association CcmboCM 2013; Klompas et al., 2014; Mehta et al., 2014; Álvarez Lerma et al., 2014; Torres et al., 2017; Infectious disease group RmboCMA 2018; Collins et al., 2020) related to antiseptic oral rinse or the use of chlorhexidine. Four guidelines (Association CcmboCM 2013; Klompas et al., 2014; Mehta et al., 2014; Álvarez Lerma et al., 2014) published between 2013 and 2014 recommend the use of chlorhexidine for oral care, there is evidence that its use as a gargle may help to reduce the risk of VAP; The ERS 2017 guideline (Torres et al., 2017) decided not to issue a recommendation on the use of chlorhexidine until more safety data have become available, due to the unclear balance between a potential reduction in pneumonia rate and a potential increase in mortality. Also, the BACCN 2020 guideline (Collins et al., 2020) recommend that using an antiseptic oral rinse after brushing can help reduce the risk of VAP but may increase the mortality risk, further studies found that the use of chlorhexidine to prevent VAP was effective in cardiothoracic ICU, but it was unclear in the non-cardiothoracic ICU population, they advise caution with the routine use of chlorhexidine as part of an oral care program. It can be seen that in contrast to with other countries, the current China guidelines recommend the prophylactic use of chlorhexidine.

3.4.4 Prophylactic Probiotics

3 guidelines discussed the use of prophylactic probiotics. Two Chinese guidelines (Association CcmboCM 2013; Infectious disease group RmboCMA 2018) do not recommended that probiotics be routinely given for prevention; the SHEA 2014 guideline (Klompas et al., 2014) recommended administering prophylactic probiotics.

3.4.5 Stress Ulcer Prophylaxis

Two guidelines (Klompas et al., 2014; Infectious disease group RmboCMA 2018) have recommendations on stress ulcer prophylaxis, both the Chinese guideline (Infectious disease group RmboCMA 2018) and the SHEA 2014 guideline (Klompas et al., 2014) state they are definitively not recommended for VAP prevention: interventions with good-quality evidence suggesting that they neither lower VAP rates nor decrease duration of mechanical ventilation, length of stay, or mortality.

TABLE 5 | Chronological trend of recommendations on drug treatment of VAP.

Guideline	Empiric Treatment Recommendation (SOR/LOE)	Aerosolized Antibiotics Recommendation (SOR/LOE)	Duration of Antibiotic Therapy (SOR/LOE)
Qiu, HB 2021 (Association SoCRDoCM 2021)	——	For VAP/HAP patients infected with multidrug-resistant gram-negative bacteria, systemic antibiotics combined with aerosol inhalation antibiotics can be considered to improve the cure rate of pneumonia and the clearance rate of respiratory bacteria (Weak/Low)	——
Qu, JM 2018 (Infectious disease group RmboCMA 2018)	For HAP/VAP patients with risk factors of MDR *Pseudomonas aeruginosa* and other MDR gram-negative bacilli infection or high risk of death, the use of two different types of antibiotics in combination is recommended; For patients with HAP/VAP who are not critical/have no risk factors for MDR infection, a single antibiotic can be used in empirical treatment (Strong/Low)	——	——
Lenoe, M. 2018 (Leone et al., 2018)	——	The administration of nebulized colimycin (sodium colistimethate) and/or aminoglycosides is suggested in documented HAP due multidrug-resistant Gram-negative bacilli documented pneumonia established as sensitive to colimycin and/or aminoglycoside, when no other antibiotics can be used (based on the results of susceptibility testing) *Data are only available for VAP (GRADE 2+)	The antibiotic treatment for HAP for longer than 7 days is not recommended, including for non-fermenting Gram-negative bacilli, apart from specific situations (immunosuppression, empyema, necrotizing or abscessed pneumonia) * Data are only available for VAP (GRADE 1-)
Torres, A. 2017 (Torres et al., 2017)	It is recommended that empiric treatment regimens be informed by the local distribution of pathogens associated with VAP and their antimicrobial susceptibilities. (See **Supplementary File S6** for details)	——	Using a 7–8-days course of antibiotic therapy is suggested in patients with VAP without immunodeficiency, cystic fibrosis, empyema, lung abscess, cavitation, or necrotizing pneumonia and with a good clinical response to therapy (Weak recommendation, moderate quality of evidence)
Kalil, A.C. 2016 (Kalil et al., 2016)	It is recommended that empiric treatment regimens be informed by the local distribution of pathogens associated with VAP and their antimicrobial susceptibilities. (See **Supplementary File S6** for details)	Both inhaled and systemic antibiotics, rather than systemic antibiotics alone are suggested for patients with VAP due to gram-negative bacilli that are susceptible to only aminoglycosides or polymyxins (colistin or polymyxin B) (Weak recommendation, very low-quality evidence)	For patients with VAP, a 7-days course of antimicrobial therapy rather than a longer duration is recommended (Strong recommendation, moderate-quality evidence)
Li, YM 2013 (Association CcmboCM 2013)	The initial empirical anti-infective treatment of VAP patients is usually single drug anti-infective treatment with appropriate antibacterial spectrum; If the pathogen is multi drug resistant, the combination treatment of antibiotics can be selected (1B)	For pulmonary infection caused by multidrug-resistant non fermenting bacteria, when the effect of systemic anti infection treatment is poor, combined aerosol inhalation of aminoglycosides or polymyxin and other drugs can be considered (1C)	VAP anti infection course is generally 7–10 days. If the patient has poor clinical response, multi drug resistant bacterial infection or immune function defect, the treatment time can be appropriately prolonged (1B)
Gupta, D. 2012 (Gupta et al., 2012)	There is no evidence to suggest that combination therapy is superior to monotherapy (1A)	Aerosolized antibiotics (colistin and tobramycin) may be a useful adjunct to intravenous antibiotics in the treatment of MDR pathogens where toxicity is a concern and should not be used as monotherapy but should be used concomitantly with intravenous antibiotics (2A)	In patients with VAP due to *Pseudomonas*, *Acinetobacter*, and MRSA, a longer duration (14 days) of antibiotic course is recommended; In other patients with VAP who are clinically improving, a 7-days course of antibiotics is recommended (1A)

HAP: Hospital-acquired Pneumonia; VAP: Ventilator-associated Pneumonia; MRSA: Methicillin-resistant Staphylococcus aureus*; MDR: Multidrug resistance; SOR: Strength of recommendation; LOE: Level of evidence.*

3.4.6 Aerosol Inhalation of Antibiotics

The CMA 2013 guideline (Association CcmboCM 2013) recommended that Patients on mechanical ventilation should not routinely use aerosol inhalation of antibiotics to prevent VAP.

3.5 Recommendations on Drug Treatment for VAP

We extracted 51 recommendations regarding drug treatment for VAP from 8 guidelines (Gupta et al., 2012; Association CcmboCM 2013; Kalil et al., 2016; Mikasa et al., 2016; Torres et al., 2017; Infectious disease group RmboCMA 2018; Leone et al., 2018; Association SoCRDoCM 2021), including 28 strong recommendations, 22 weak recommendations, and one strongly not recommended. These recommendations included empirical antibiotics for VAP, etiological treatment, and the length of a course of antibiotic therapy. **Supplementary File S6** shows the recommendations on drug treatment, and **Table 5** describes the chronological trend of recommendations on drug treatment of VAP.

3.5.1 Empirical Antibiotics

We extracted 21 recommendations regarding empiric therapy for VAP from 5 guidelines (Gupta et al., 2012; Association CcmboCM 2013; Kalil et al., 2016; Torres et al., 2017; Infectious disease group RmboCMA 2018). For empiric therapy, all guidelines recommended that the empirical treatment plan should be determined according to the local distribution of pathogens associated with VAP and their antimicrobial susceptibilities, drug resistance rates vary widely between countries, regions, and hospitals. Two Chinese guidelines (Association CcmboCM 2013; Infectious disease group RmboCMA 2018), the ERS 2017 guideline (Torres et al., 2017) and the IDSA 2016 guideline (Kalil et al., 2016) recommended that the empirical antibiotic treatment usually adopts appropriate antibacterial spectrum single drug treatment. Narrow-spectrum antibiotics (ertapenem, ceftriaxone, cefotaxime, moxifloxacin, or levofloxacin) were suggested for patients with low risk of multidrug resistance (MDR) infection and early-onset VAP (Torres et al., 2017). But the NCCP 2012 guideline (Gupta et al., 2012) recommend combination therapy due to the high prevalence rates of MDR pathogens in late-onset VAP aiming to maximize the chances of appropriateness of the initial regimen.

If the pathogen is considered empirically to be multidrug resistant bacteria, antibiotic combination treatment can be selected. The IDSA 2016 guideline (Kalil et al., 2016) gave indications for empiric dual gram-negative and MRSA therapy and recommended vancomycin or linezolid as empirical antibiotics for MRSA treatment. Combination therapy and antibiotics for MRSA treatment were suggested by the CMA 2018 guideline when a high risk of MDR exists, and empirical agents and antibiotics are listed in a table in the guidelines, but unfortunately, no recommendations on strength was supplied in the guideline text (Infectious disease group RmboCMA 2018).

3.5.2 Etiological Treatment

In our study, about 15 recommendations relate to the etiological treatment of VAP. If the infecting pathogen is identified, the corresponding antimicrobial treatment plan (narrow-spectrum or broad-spectrum, single drug or combination) should be formulated with reference to the results of *in vitro* drug sensitivity tests. The IDSA 2016 guideline (Kalil et al., 2016), CMA 2018 guideline (Infectious disease group RmboCMA 2018) and IDST 2018 guideline (Chou et al., 2018) give detailed treatment plans for the etiological treatment of VAP, but the CMA 2018 guideline and the IDST 2018 guideline did not give any recommendation on strength.

Acinetobacter Baumann was found to be susceptible to sulbactam (SBT) and ampicillin (ABPC) which was recommended as a first-choice drug for respiratory infectious diseases (not VAP alone) in the JAID (Mikasa et al., 2016) guideline. The CMA 2018 guideline also recommended the use of SBT and ABPC, but for *Acinetobacter Baumann* infection (extensively-drug resistant or pan drug resistant), a combined regimen (sulbactam combined with polymyxin, tigecycline, or doxycycline) should be used. The IDSA 2016 guideline cautioned against the use of tigecycline in patients with hospital-acquired pneumonia (HAP) or VAP caused by *Acinetobacter* species, however they did not recommend any specific drug for *Acinetobacter* Baumann. This was based on evidence synthesis which indicated that the dose currently recommended on the label of tigecycline worsened clinical outcomes compared with several other therapies. The panel's strong caution against tigecycline, despite low-quality evidence, was intended to emphasize the importance of avoiding potentially harmful therapies, particularly when alternative choices exist. This is in sharp contrast to the Chinese guidelines.

3.5.3 Duration of Antibiotic Treatment

Five guidelines (Gupta et al., 2012; Association CcmboCM 2013; Kalil et al., 2016; Torres et al., 2017; Leone et al., 2018) covering 7 recommendations relate to the duration of antibiotic treatment. In the IDSA 2016 guideline (Kalil et al., 2016) and the SFAR 2018 guideline (Leone et al., 2018), a 7-days course of antimicrobial treatment rather than one of longer duration was strongly recommended. In the ERS 2017 guideline (Torres et al., 2017), a weak recommendation was given to using a 7–8-days course of antibiotic therapy. The CMA 2013 guideline (Association CcmboCM 2013) and the NCCP 2012 guideline (Gupta et al., 2012)points out that generally the normal length for a course of anti-infective drugs for VAP is 7–10 days. If the patient has poor clinical response, multidrug resistant bacterial infection or deficient immune function, the treatment time can be appropriately prolonged. We noted that none of the recommendations were based on evidence ranked as "strong quality", even though they were both derived from evidence in many systematic reviews or meta-analyses. The IDSA 2016 guideline reported the advantages of a short-course regimen, which decreases antibiotic exposure and antibiotic resistance without increasing mortality or recurrent disease, and the decreased antibiotic exposure almost certainly reduces costs

and side effects (Kalil et al., 2016). Longer courses may still be appropriate in some circumstances where the patient has a delayed clinical response. However, different patients can have treatment courses of variable length. Many guidelines suggest that we should consider the factors of both the host and the pathogen and make a comprehensive individualized judgment in combination with the clinical reaction and laboratory examination results.

3.5.4 Aerosol Inhalation Antibiotic Therapy

Five guidelines (Gupta et al., 2012; Association CcmboCM 2013; Kalil et al., 2016; Leone et al., 2018; Association SoCRDoCM 2021) relate to the Aerosol inhalation antibiotic therapy. Both the Chinese guidelines (Association CcmboCM 2013; Association SoCRDoCM 2021) and other guidelines recommended that, for VAP patients infected with multidrug-resistant gram-negative bacteria, systemic antibiotics combined with aerosol inhalation antibiotics can be considered to improve the cure rate of pneumonia and the clearance rate of respiratory bacteria. It is necessary to make a comprehensive assessment of the dose, administration mode, adverse reactions and other factors associated with inhaled antibiotics in order to weigh the advantages and disadvantages.

4 DISCUSSION

4.1 The Quality of Guidelines Needs to Be Improved in the Future

Since October 2016, the National Health Commission of the People's Republic of China has initiated work to construct a national clinical practice guideline database aiming to promote CPG development, dissemination, and implementation in China. CPGs aim to formulate specific, explicit recommendations that, if properly adopted in clinical settings, will produce better outcomes for patients, and promote cost-effective practices (WHO 2018). The preponderance of CPGs developed by various organizations on similar or the same topic has been increasing throughout the world. All guidelines included in this study were evidence-based. The results show that the quality of the guidelines assessed was generally modest but varied between different organizations. The IDSA, SFAR and international ERS guidelines tended to have higher scores than others.

The methodologist plays a key role in guideline development meetings by helping the guideline development group formulate recommendations informed by the evidence in a transparent and explicit manner (WHO 2018). Over the past few years, we have seen a substantial increase in the number of guideline groups introducing and using the GRADE tool and in the number of options available within GRADE (Shekelle 2018). Usually, different guidelines used different scales or systems to evaluate or rate the quality and strength of evidence and recommendations (Harpole et al., 2003). In this study, although most of the included guidelines stated that they had used the GRADE system to assess and rate evidence quality and recommendation level, the assessors did not think that all guidelines used the GRADE system correctly. GRADE is based on the belief that recommendations should be based on a systematic review of the scientific literature guided by specific questions relating to the intervention, exposure, or approach under consideration (WHO 2018). GRADE evidence profiles should generally be part of the final report of the systematic review and contain an assessment of the evidence quality and a summary of findings across studies for each critical and important outcome and every key question (Holger Schünemann 2013). The guideline development group used evidence summaries as the basis for group discussions and to formulate recommendations.

The low score in the field of applicability indicates that the guideline expert group regards the development and implementation of the guideline as a separate issue and does not pay enough attention to the potential obstacles in its promotion and dissemination (Alonso-Coello et al., 2010). When reporting guidelines, the guideline team should provide tools such as charts applying recommendations to practice to facilitate implementation (WHO 2018).

Guideline recommendations should be based on the balance between the estimated costs of the interventions or services and their expected benefits compared with an alternative (Álvarez Lerma et al., 2014). Although formally assessing the cost effectiveness of an intervention, service or program can help decision-makers ensure that maximum gain is achieved from limited resources, economic evaluation evidence has rarely been cited in the guidelines we included. Unfortunately, drug recommendations for VAP were seldom based on systematic economic evaluation. Prices of different antimicrobial agents vary widely; cost-utility analysis is needed for a rational recommendation. Economic evaluation should start during guideline scoping and development of guideline questions especially those concerned with economic outcomes.

4.2 Recommendation Changes and Trends of Drug Therapy for Prevention

At present, VAP prevention measures focus on the pathogenesis to reduce the occurrence of VAP and improve the prognosis of patients. The recommendations for preventing VAP for these guidelines include non-drug prevention and drug prevention. Non-drug prevention mainly includes semi-recumbent position; use of new endotracheal intubation and subglottic secretion drainage; reducing the use of invasive ventilation, shortening the time of invasive ventilation, and limiting the use of narcotic drugs (Li Bassi et al., 2017; Infectious disease group RmboCMA 2018).

For drug prevention of VAP, we found that some disparities remain among the included guidelines. When there are substantial differences in major recommendations of guidelines, patients and clinical practitioners may question the validity which may then lead to poor adherence and implementation (Shekelle 2018). VAP drug prevention forms a special genre, it is challenging to explain the VAP prevention literature, because many measures have been reported to reduce the incidence rate of VAP, but the limitations of its diagnostic criteria make it difficult to identify the true effectiveness of preventative strategies.

At present, there are still many disputes about the specific scheme of selective purification and its clinical application in areas with different antibiotic resistance levels (Wittekamp et al., 2018; Hurley 2020; Rommes et al., 2020). Although several Randomized Controlled Trial (RCT) studies show that selective purification will not lead to the increase of antibiotic resistance rate, many ICU centers around the world are still cautious about its clinical application (de Smet et al., 2009; Oostdijk et al., 2014; Wittekamp et al., 2018). However, another SDD trial (clinicaltrials.gov NCT02389036) is being conducted in Canada, the UK and Australia (countries with moderate or above antibiotic resistance) which is conducting a concurrent cohort study alongside a randomized trial to assess the impact of SDD on antibiotic resistance patterns. Therefore, the results of this program will provide us with more information about the use of SDD in this population (Francis et al., 2014).

Recent studies have also questioned the efficacy and safety of oral chlorhexidine. Although there is evidence that its use as a gargle may help to reduce the risk of VAP, but this is unclear in the non-cardiothoracic ICU population (Houston et al., 2002; Segers et al., 2006; Labeau et al., 2011; Cuccio et al., 2012; Hua et al., 2020). Some studies have reported that oral care with chlorhexidine may increase mortality, possibly because some patients may inhale some preservatives that cause acute lung injury (Price et al., 2014; Klompas et al., 2016; Klompas 2017; Deschepper et al., 2018; Harris et al., 2018). A recent randomized trial show that in mechanically ventilated ICU patients, no benefit was observed for de-adoption of chlorhexidine and implementation of an oral care bundle on ICU mortality or time to discharge but may have improved oral health (Dale et al., 2021).

The application of probiotics in patients with mechanical ventilation is still controversial. Two recent meta-analysis have shown that the application of probiotics helped to prevent VAP without impacting the length of ICU stays or mortality (Su et al., 2020; Zhao et al., 2021). However, another recent meta-analysis has reached the opposite conclusion (Batra et al., 2020). By analyzing the above research, we found that the main problem is the difference of inclusion criteria. Probiotics may be an attractive intervention in the prevention of ventilator-associated pneumonia in adult hospitalized patients. However, the certainty of the evidence on its cost-effectiveness is very low. Future randomized controlled trials of probiotics should include cost data to inform bedside practice, clinical guidelines, and medical policies (Lau et al., 2020).

4.3 Recommendation Changes and Trends of Drug Therapy for Treatment

The treatment of VAP includes two aspects: first, empirical treatment, which needs to consider the severity of the patient's disease, suspected pathogens and MDR risk factors; second, etiological treatment. Indeed, some evidence used by those guidelines was derived from the same trials or systematic reviews, meaning that they are based on shared evidence. Variations could be the result of differences in data interpretation or the indication of available resources, while actual recommendations could still be tailored to local and cultural contexts. Nonetheless, one needs to be cautious when considering guidelines for local use and should make sure that the clinical data and evidence are in harmony with the clinical judgment.

The pathogens found in VAP patients are mainly Gram-negative bacteria (Abd-Elmonsef et al., 2018). Antibiotics active against Gram-negative bacteria should be preferred in the early stage of infections with unknown bacterial. Currently, we need to avoid the excessive use of antibiotics as their inappropriate application will increase the mortality. When treating VAP, it is also necessary to avoid the application of broad-spectrum antibiotics as this will induce drug resistance. Antimicrobial flora and resistance patterns can vary considerably between and within countries, regions, hospitals, ICUs in a hospital, and specimen sources. In China, It is reported that the isolation rate of *acinetobacter* baumannii in the VAP pathogen spectrum was as high as 35.7–50.0%, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and the isolation rate of *Acinetobacter* Baumann has been reportedly increasing year by year (Infectious disease group RmboCMA 2018). By the 2014–2019 Chinese Antimicrobial Resistance Surveillance of Nosocomial Infection, the detection rates of MRSA, Methicillin Resistant Coagulase-Negative Staphylococci (MRCNs) and vancomycin resistant enterococci had decreased, but the drug resistance of *Acinetobacter* Baumann to various antibiotics was at a serious level (Network Nbrm 2021). However, surveillance studies in the United States suggested that the organisms most associated with VAP have been *S. aureus* (approximately 20–30% of isolates), *P. aeruginosa* (approximately 10–20% of isolates), enteric gram-negative bacilli (approximately 20–40% of isolates), and *Acinetobacter* Bahmani (approximately 5–10% of isolates) (Sievert et al., 2013). Understanding the distribution and drug resistance of local pathogens in VAP patients is helpful to formulate a scientific empirical treatment plan, which is very important in reducing the mortality of VAP patients and delaying the occurrence of bacterial drug resistance.

Antimicrobial stewardship is an important issue; however, the approach to manage it differs considerably in different countries. Currently more and more attention has been paid to the deterioration of VAP-associated death rates in China. The use of antibiotics in China faces severe challenges, and in recent years the National Health Commission of the People's Republic of China has continued to issue notices on further strengthening the management of the clinical application of antimicrobial drugs to curb bacterial resistance (Commission GOotnhaFP 2017; Commission GOoNHaH GOoNHaH 2020). The aim is to improve the level of diagnosis and treatment of infectious diseases, improve testing, promote the accurate use of antibacterial drugs, and rely on information construction to help the scientific management of antibacterial drugs. Through the use of these different measures, the level of scientific management of antibacterial drugs will be continuously improved.

VAP will continue to be the main infection in ICU in the next decade (Xie et al., 2019). This paper evaluates the quality of VAP guidelines and summarizes and analyzes the drugs recommended for prevention and treatment. The differences in drugs for

prevention and treatment of VAP in different countries have been compared. At the same time, according to the publication time of different guidelines, the changing trend of VAP prevention and treatment across the years has been summarized. We hope that the present study contributes to the prevention and control of VAP. Therefore, for the prevention of VAP, appropriate antibiotics should be selected according to the characteristics of drug resistance in different countries. In addition, due to the increase of antibiotic resistance, the development of new drugs against MDR pathogens is also very urgent.

4.4 Strengths and Limitations

Our study possesses several strengths. First, our research team consisted of methodologists with full experience in the development and assessment of CPGs and clinical experts and obtained consensus from all appraisers to ensure the reliability of our conclusions. Second, we conducted a thorough systematic literature search. Third, we extracted and compared recommendations for drug use for VAP.

Nevertheless, several limitations should be noted. First, we only assessed guidelines published in the English and Chinese languages and on some important professional society websites, which may not represent all the guidelines for VAP. Guidelines published in other ways (i.e., books, booklets, other websites, or health institution documents) may have been omitted, which may have introduced bias into our assessment. Second, we attached relatively more weight to the quality of guideline development than to whether the recommendations were feasible in our specific practice environments or matched a particular clinical practice We extracted recommendations on drug therapy for VAP prevention and treatment; however, many guidelines were developed for both HAP and VAP, and their recommendations did not differentiate between the two mostly because some guidelines considered VAP to be a special type of HAP, and primary studies included a mix of HAP and VAP samples. In this study, we recognized that patients with HAP and VAP belong to 2 distinct groups and extracted the recommendations that only applied to VAP or recommendations that were described as pertinent to VAP/HAP with evidence indicating the recommendation came from VAP-related research.

5 CONCLUSION

In conclusion, there is considerable variability in the development process and reporting of VAP guidelines, although the principles for guideline development have been described. The experience of the organization and experts in assessing evidence and developing guidelines may explain higher scores for some items. There were substantial differences in some recommendations of VAP guidelines. For the prevention and treatment of VAP, local microbial epidemiology and antibiotic sensitivity must be considered, along with economic issues. The most effective clinical practice guidelines should incorporate the current best evidence and place these in the context of local patterns of drug resistance. Recommendations should be regularly revised as new evidence emerges.

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.

AUTHOR CONTRIBUTIONS

H-YL, H-SW, and QZ designed the research, analyzed the data, and wrote the draft manuscript. H-YL, Y-LW and JW performed literature search, extracted the recommendations. H-YL, H-SW, Y-LW and X-CH evaluated CPGs independently, reviewed the manuscript, and provided critical scientific input. H-YL, H-SW, QZ and X-CH provided critical scientific input. All authors made contribution to this article, read, and approved the final version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.903378/full#supplementary-material

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frontiers | Frontiers in Pharmacology

OPINION
published: 31 May 2022
doi: 10.3389/fphar.2022.921307

Adverse Drug Reactions Caused by Antimicrobials Treatment for Ventilator-Associated Pneumonia

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Keywords: ventilator-associated pneumonia, antimicrobials, adverse drug reactions, ICU therapy, medication use, drug interaction

INTRODUCTION

Ventilator-associated pneumonia (VAP) develops in intensive care units (ICU) patients who have been mechanically ventilated for at least 48 h, and antimicrobials are an important treatment in clinical management. About 50% of the antimicrobials in ICUs are used for the treatment of VAP, of which the use rate of unnecessary, inappropriate or suboptimal antimicrobials accounts for 30%–60% (Luyt et al., 2014), so the antimicrobial treatment of VAP patients is still a major clinical difficulty. For patients with VAP, inadequate treatment and delay in administering of effective antibacterial drugs are both associated with increased mortality (Muscedere et al., 2012; Swanson and Wells, 2013), and long-term use of broad-spectrum antimicrobials can increase the incidence of adverse drug reactions (ADR) (Fagon et al., 2000) and antimicrobial resistance. Therefore, enhancing management of antimicrobials will be beneficial to reduce the occurrence of ADR, improve the effectiveness of antibacterial drugs treatment, and curb the spreading of drug resistance. This article mainly introduces the cases of ADR caused by antimicrobials treatment of VAP, in order to provide a reference for the safe, effective and rational use of antibacterial drugs in clinical practice.

CLINICAL CHARACTERISTICS OF VAP AND SELECTION OF ANTIMICROBIALS

There are many pathogenic species of VAP, and the main pathogens include Gram-positive and Gram-negative bacteria: *Staphylococcus aureus* (including methicillin-resistant *Staphylococcus aureus*, MRSA), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (Huang et al., 2018; Luyt et al., 2018). The antimicrobial treatment with VAP is generally divided into two steps: one For patients with an early diagnosis of VAP, antimicrobials should be used empirically by most clinicians. Generally, it is based on the local distribution of pathogens, risk factors for MDR pathogen infection, etc.; two Once the patient's microbiological examination results are obtained, a corresponding treatment plan should be formulated according to the results of the susceptibilities, and the treatment should be adjusted to narrow-spectrum antimicrobials on time (Luyt et al., 2014).

At present, there are many kinds of antimicrobials for VAP. Ceftazidime, cefepime, piperacillin/tazobactam, levofloxacin can be used for patients without risk factors of multidrug-resistant (MDR) pathogens. New β-lactam antimicrobials (ceftazidime-avibactam) and carbapenems (imipenem/meropenem) are available for the empirical treatment of MDR/extensive drug resistance (XDR) pathogens of VAP. Vancomycin or linezolid should be added if MRSA infection is suspected or present. In conclusion, if there is no risk of MDR infection, the antimicrobials which recommended

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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *15 April 2022*
Accepted: *09 May 2022*
Published: *31 May 2022*

Citation:
Shen S and Hou N (2022) Adverse Drug Reactions Caused by Antimicrobials Treatment for Ventilator-Associated Pneumonia. Front. Pharmacol. 13:921307. doi: 10.3389/fphar.2022.921307

are relatively narrow-spectrum. If there is a risk of MDR infection, broad-spectrum antimicrobials with anti-pseudomonas effect are recommended, and antimicrobials against MRSA should be added when necessary.

The duration of antimicrobial treatment has always been the focus of clinical attention. The evidence that short-term antimicrobial therapy reduces drug resistance, but the recurrence of drug withdrawal is rare, the advantages of short-term antimicrobial therapy outweigh the disadvantages. Short course of treatment (7 days) for VAP patients rather than 8–15 days is recommended by American guidelines (Kalil et al., 2016). AMMI guidelines indicate that more prolonged periods of time (14 days) was proposed to be used on MDR infection or *Pseudomonas aeruginosa* infection (Rotstein et al., 2008).

CASES OF ADR CAUSED BY VAP ANTIMICROBIALS TREATMENT

VAP is one of the most common ICU-acquired infections and has a high risk of death. Most patients have complicated conditions. Antimicrobial treatment is the cornerstone of VAP treatment, especially for MDR and XDR infections. Articles published within the last 10 years to 1 April 2022 were searched through Pubmed databases. We used 'Ventilator-associated pneumonia' as the search terms. The search was restricted to case reports. The database search produced 178 articles. After browsing the abstract of the articles, four were reviewed for the final inclusion according to the theme of the article.

Case 1 Vancomycin-Induced Thrombocytopenia

A 52-year-old man was admitted to ICU with hypertension and secondary pulmonary edema, requiring endotracheal intubation due to acute hypoxemic respiratory failure, and acute kidney injury. He developed ventilator-associated pneumonia during treatment. But no related pathogens were cultured, so vancomycin was given to empirically treat VAP. Three days later, platelets dropped sharply from 172×10^9/L to 3×10^9/L. A presumptive diagnosis of vancomycin induced thrombocytopenia (VIT) was made. All medications were discontinued and methylprednisolone (500 mg/day) and intravenous immune globulin were prescribed. The patient's platelet count returned to normal after 18 days.Vancomycin-dependent antiplatelet antibodies was identified in patient serum by flow cytometry. But VIT was not completely understood. The antibody bind platelets only in the presence of vancomycin had been described *in vitro* experiments. So the vancomycin-induced thrombocytopenia increased the risk of bleeding. In addition, it took at least 6 days to develop VIT after initial exposure to vancomycin, and an average of 8 days to reach platelet nadir, but the interval between re-exposure to vancomycin was significantly shorter. Since medication of vancomycin had been stopped, 18 days later, the patient's platelets returned to normal, this is probably caused by the reduction of vancomycin clearance due to acute renal injury. It is suggested that the slow speed of drug clearance should be considered during medication for patients with renal function injury, which avoid serious consequences caused by drug accumulation (Abdalhadi et al., 2020).

Case 2 Piperacillin/Tazobactam-Induced Platelet Dysfunction

A 73-year-old male patient was in a coma from an out-of-hospital cardiac arrest. After resuscitation, he was admitted to the cardiac intensive care unit (CCU) with mechanical ventilation. On hospital day 5 (HD5), he was diagnosed with *Pseudomonas aeruginosa* ventilator-associated pneumonia with sepsis. The glomerular filtration rate (GFR) was 29 mL/min/1.73 m^2. Piperacillin/tazobactam (TZP) was given 3×4.5 g daily. On HD 8, the patient developed bleeding symptoms. On HD 14, due to further deterioration of renal function, TZP was adjusted to 3×2.25 g daily. TZP was stopped because it might be the cause of platelet dysfunction. After blood transfusion treatment, the condition was relieved. Although platelet counts and coagulation tests were normal during treatment, platelet function tests showed severely impaired ADP-dependent platelet aggregation. The reason is most likely the toxicity of TZP, which is exacerbated by drug accumulation during the progression of renal failure. It suggests that regular monitoring of renal function is very important (Skoric et al., 2020).

Case 3 Ciprofloxacin-Induced Rhabdomyolysis

A 64-year-old male patient was intubated and given mechanical ventilation for subacute non-ST elevation myocardial infarction (NSTEMI) and pulmonary edema. Empirical antimicrobial treatment was started due to the increase of C-reactive protein (CRP). A travel history in a country at risk of carbapenem-resistant *Enterobacteriaceae* (CRE), and the drug susceptibility test showed low sensitivity to carbapenems and sensitivity to ciprofloxacin, so ciprofloxacin 400 mg q8h was given. Creatine kinase (CK) levels increased to 4,981 U/l Ciprofloxacin was suspected as causative for rhabdomyolysis and it was discontinued immediately. The patient's creatine kinase levels began to increase significantly after increasing the dose of ciprofloxacin, possibly due to fluoroquinolone-induced rhabdomyolysis. This report highlights the global spread of carbapenem-resistant Enterobacteriaceae. Clinicians need to be aware that some common antimicrobials cause ADR. For example, these drugs may lead to rhabdomyolysis (Grisold et al., 2013).

Case 4 Cefepime Associated With Phenytoin-Induced Stevens-Johnson Syndrome

A 49-year-old man with sepsis and renal failure suffered from a generalized status epilepticus during treatment of VAP with cefepime, then phenytoin was added to treatment. Cefepime

was discontinued due to its epileptogenic potential, after 24 h under treatment with phenytoin, the patient developed macular rash on the trunk and lower extremities. This symptom is consistent with Stevens-Johnson syndrome, there was drug interaction probably between cefepime and phenytoin. The cause of drug interaction might be the immune response triggered by a reaction between water nucleophilic groups of phenytoin and carbonyl group of the cefepime, forming cephalosporic acid which leads to cephalosporin derived proteins. At the same time, due to the renal damage of the patient, the complete elimination time of cefepime from the body is prolonged, which induces the coexistence of the two drugs in the body, and causes pharmacological interaction. In addition, cephalosporins themselves may also contribute to these clinical manifestations. It can be seen that doctors and pharmacists need pay attention to the interaction between drugs during treatment (Marco-Del Río et al., 2017).

Naranjo's algorithm or Drug Interaction Probability Scale (DIPS) was used to assess the causal relationship between the suspected drug and drug reaction. According to this system, the ADR in the four reports could be categorized as probable (ADR probability score 6–8). Among them, 2 (case 3 and case 4)has causality evaluation records in the original literature.

At present, most antimicrobials need to be excreted through the kidney, and some of them have nephrotoxicity. In the above cases, the three patients (case 1, case 2 and case4) were accompanied by renal function injury in the ADR, which may lead to accumulation of drugs and prolong its' time to eliminate from the body, increase toxic and side effects of drugs. These may be would cause increase the possibility of ADR. Based on the complexity of VAP and the underlying diseases of the patients, careful selection of antibacterial is required for special populations. With the increased resistance of pathogenic microorganisms to antimicrobial drugs, some commonly antimicrobials have been reintroduced. It is that seems to have good effective on VAP. But the ADR of common antimicrobials may be ignored, thus increasing the occurrence of adverse reactions (case 3). In addition to the inherent toxic and side effects of drugs, the treatment is frequently accompanied by a variety of drugs in the case of complex conditions, which drug interactions may be occur inevitably. In case 4, phenytoin is a drug that must be closely monitored. It is a great inductor of hepatic microsomal enzymes and can reduce the plasmatic levels of other drugs. Therefore, the possibility of pharmacokinetic interaction need to be considered when combined with other drugs. VAP with MDR or XDR, the combined use of multiple antibacterial drugs can be considered. But the combined use of drugs may change the pharmacokinetic characteristics, especially when multiple drugs are used together and the patient's liver and kidney functions are damaged, which may increase the occurrence of ADR. In this case, we should pay close attention to the changes in the corresponding indicators and monitor the medication timely. For patients with other complicated conditions, drug susceptibility testing, evidence-based medicine, and pharmacokinetic theory can be combined with clinical diagnosis to judge the risk and benefit of the patient. In addition, the study found that antimicrobial peptides provide a new alternative, which is a potential therapeutic approach to overcome antibiotic resistance, improve killing efficacy and reduce side effects (Zhang et al., 2021). At the same time, we need to be aware the relevant mechanisms of antimicrobial resistance. According to the Shen's report, model conjugative plasmids can help us understand the spread of antimicrobial resistance, which will be beneficial to ensure the therapeutic effect and safety of antimicrobial in clinical application (Shen et al., 2022). In conclusion,optimal antimicrobial therapy for VAP treatment remains inidentified. Clinicians and clinical pharmacists should take new strategy approach to old drugs and make effective utilization of existing resources to reduce the possible antimicrobial resistance.

This paper summarizes and analyzes the adverse reactions related to the clinical antimicrobial treatment of VAP in the retrieved cases, reminds doctors and clinical pharmacists to pay attention to adverse antibacterials reaction and monitoring the patient during medication use, therapeutic regimen of VAP need to be supervised closely in clinical practice.

AUTHOR CONTRIBUTIONS

NH proposed the study concept and design; SS collated the literature; SS performed the formal analysis; SS wrote the manuscript; NH was responsible for review and supervision. All authors have read and agree to the published version of the manuscript.

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Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor X-TZ declared a past co-authorship with the author NH.

frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 08 June 2022
doi: 10.3389/fphar.2022.903593

Shema Oral Liquid Ameliorates the Severity of LPS-Induced COPD *via* Regulating DNMT1

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Background: Chronic obstructive pulmonary disease (COPD) is the most common respiratory disease with high morbidity and mortality. Shema oral liquid (Shema) is a traditional Chinese medicine (TCM) approved for the treatment of respiratory diseases. Clinical applications have shown that Shema has antitussive, expectorant, and anti-asthmatic effects, but its definite efficacy to COPD is still unclear. This study aimed to explore the therapeutic capacity and potential mechanism of Shema in treatment of COPD.

Methods: Network pharmacology was used to investigated the possible pharmacological mechanism of Shema against COPD. A rat model of lipopolysaccharide (LPS)-induced COPD was established to determine pulmonary ventilatory function, serum inflammatory cytokines, and pulmonary pathological change. Subsequently, tandem mass tag (TMT)-based quantitative proteomics was used to further reveal the therapeutic targets related with Shema against COPD. Western blot was finally performed to validate the expression of targeted proteins screened by proteomics research.

Results: Network pharmacology analysis indicated that Shema against COPD mainly inhibited the inflammation and affected the immune system. The animal experiment demonstrated that Shema treatment protected the lung tissue from LPS induced injury, inhibited the levels of serum inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, and improved the respiratory ventilatory function by upregulating forced expiratory volume in 0.1 s (FEV0.1), FEV0.3, forced vital capacity (FVC), and the ratios of FEV0.1 (0.3)/FVC. Proteomic analysis and western blot both proved that Shema inhibited the expression of DNA methyltransferase 1 (DNMT1) in the lung tissue.

Conclusion: The therapeutic mechanism of Shema in treatment of COPD may involve inhibiting inflammatory response, improving pulmonary ventilatory function, and alleviating LPS-induced lung injury through regulating the expression of DNMT1. This study also shed

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Edited by:
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *24 March 2022*
Accepted: *17 May 2022*
Published: *08 June 2022*

Citation:
Zhang F, Guo F, Liu Y, Zhang Y, Li D and Yang H (2022) Shema Oral Liquid Ameliorates the Severity of LPS-Induced COPD via Regulating DNMT1. Front. Pharmacol. 13:903593. doi: 10.3389/fphar.2022.903593

Abbreviations: COPD, chronic obstructive pulmonary disease; DNMT, DNA methyltransferase; DEPs, differentially expressed proteins; ELISA, enzyme-linked immunosorbent assay; FEV0.1, forced expiratory volume in 0.1 s; FEV0.3, forced expiratory volume in 0.3 s; FEV, forced expiratory volume; FVC, forced vital capacity; GO, gene ontology; HE, hematoxylin-eosin; IL, interleukin; KEGG, kyoto encyclopedia of genes and genomes; LPS, lipopolysaccharide; PPI, protein-protein interaction; ROS, reactive oxygen species; SD, standard deviation; Shema, Shema oral liquid; TMT, tandem mass tag; TCM, traditional Chinese medicine; TNF, tumor necrosis factor.

light on the development of therapeutic strategies in treating COPD by intervening DNMT-related pathways.

Keywords: chronic obstructive pulmonary disease, shema oral liquid, network pharmacology, quantitative proteomics, lipopolysaccharide, DNMT1

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a prevalent and chronic respiratory disease characterized by expiratory airflow limitation, chronic systemic inflammation, airway remodeling and lung emphysema, leading to a progressive decline in pulmonary function (Wang et al., 2018). COPD is a severe global public health challenge, and it is also one of the leading causes of death worldwide (Halpin et al., 2017). The pathogenesis of COPD includes inflammatory reaction (Ghobadi et al., 2017), protease-antiprotease imbalance (Mohan et al., 2016), oxidative stress (Hikichi et al., 2019), muscle dysfunction and lung microbiome (Huang et al., 2014; Barreiro and Jaitovich, 2018). Several types of drugs such as antibiotics, steroids, immunomodulators, antitussives, expectorants, bronchodilators, and leukotriene receptor antagonists are frequently used to suppress the symptoms of COPD, but they have limited clinical efficacy (Jeffery, 2001). Thus, deeper researches focusing on increasing our understanding of pathogenic mechanism and developing novel therapies are urgently needed.

Traditional Chinese medicine (TCM) has been widely used for centuries in China. Their proven efficacy, minimal side effects and low cost are considered valuable as alternative or complementary medicines for health care and disease treatment worldwide. A traditional Chinese prescription Shema oral liquid (Shema) comes from the records including "Shegan Mahuang Decoction" in the Synopsis of Golden Chamber and "Maxing Shigan Decoction" in the Treatise on Febrile Diseases. The formula consists of ten types of TCMs: *Ephedra Herba* (Ma-Huang), *Arisaematis Rhizoma* (Dan-Nan-Xing, mixed with oxgall), *Morus alba* L. (Sang-Bai-Pi), *Belamcandae Rhizome* (She-Gan), *Raphani Semen* (Lai-Fu-Zi), *Amygdalus Communis* Vas (Xing-Ren), *Cynanchum glaucescems* (Bai-Qian), *Scutellariae Radix* (Huang-Qin), *Schisandrae Chinensis Fructus* (Wu-Wei-Zi), and Gypsum Fibrosum (Shi-Gao), which has been approved for the treatment of respiratory diseases. Clinical applications have indicated that Shema has antitussive, expectorant, and anti-asthmatic effects (Cheng, 2021). However, the precise efficacy and mechanism of Shema for the treatment of COPD is unclear.

Here, we used network pharmacology to predict the possible pharmacological mechanism of Shema in treatment of COPD, which could provide the theoretical basis for the mechanistic study. Next, we established a lipopolysaccharide (LPS)-induced COPD rat model and confirmed the molecular targets concluded from the network pharmacology analysis. Then quantitative proteomics and western blot were performed to further illustrate the potential therapeutic targets of Shema against COPD. Therefore, our study provided the definite investigation of clarifying the efficacy and mechanism of Shema against COPD.

MATERIALS AND METHODS

Network Pharmacology Analysis of Shema in Treatment of COPD

The chemical ingredients of Shema were obtained from TCMID database (http://www.megabionet.org/tcmid/) (Xue et al., 2012). The molecular targets of ingredients from Shema were mainly collected from BATMAN-TCM database (http://bionet.ncpsb.org/batman-tcm/). The major source of COPD-related disease targets was acquired from two databases including Human Phenotype Ontology (HPO, https://hpo.jax.org/app/) and DisGeNET (http://www.disgenet.org/). The potential targets of Shema and the disease targets of COPD were combined and imported to STRING database (http://www.string-db.org/) to generate a protein-protein interaction (PPI) network (Szklarczyk et al., 2017). The PPI network was created by Cytoscape software (version 3.7.2, Boston, MA, United States). In order to confirm the possible pharmacologic effect of Shema in treatment of COPD, David database (version 6.8, https://david.ncifcrf.gov/) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of genes and Genomes (KEGG) enrichment analysis.

Experimental Animals

Male Sprague-Dawley rats (180–220 g) were obtained from Beijing HFK Bioscience Co. Ltd., China (license number was SCXK (Jing) 2016-0004). All experiments were designed and handled in accordance with the local ethical guidelines for animal care and usage. Before the experiment, rats were adapted to the feeding environment for 3 days.

Drugs

Shema is composed of ten different TCMs as followings: Ma-Huang, Dan-Nan-Xing, Sang-Bai-Pi, She-Gan, Lai-Fu-Zi, Xing-Ren, Bai-Qian, Huang-Qin, Wu-Wei-Zi, and Shi-Gao (weight ratio 3: 3: 5: 5: 4: 5: 5: 5: 3: 10). The herbal extract was prepared by Hainan Zhongshenghemei Biopharmaceutical Co. Ltd., China. The extract was dissolved in distilled water. The clinical oral dose of Shema was 0.5 ml/kg/day (60 kg, 10 ml/time, t.i.d.), which was equivalent to the gavage dose of 3.125 ml/kg/day for the rats (200 g). Therefore, we set 1.0, 3.0, and 6.0 ml/kg/day as the gavage doses in our research. Dexamethasone tablets (0.75 mg/Tablet) were obtained from Guangdong South Land Pharmaceutical Co. Ltd., China.

COPD Animal Model Construction

The rat model of COPD was constructed as previously reported (Vernooy et al., 2002). The rats were anesthetized with 1% pentobarbital sodium (40 mg/kg, Sigma, MA, United States) intraperitoneally, followed by a tracheal injection of 0.2 ml LPS (1.0 μg/μL, in 0.9% saline, from *Escherichia coli* 055: B5, solarbio,

Beijing, China) through an endotracheal tube (rat 14G type, Zhongyanboji, Beijing, China) twice a week. According to the gavage doses, the rats were randomly divided into six groups (n = 12 per group): control group (Control), COPD model group (COPD), Shema low-dose group (Shema 1.0 ml/kg), Shema medium-dose group (Shema 3.0 ml/kg), Shema high-dose group (Shema 6.0 ml/kg), and dexamethasone group (DXMS 2.0 mg/kg). The rats in Shema or DXMS treated groups were administered intragastrically with Shema or dexamethasone once daily for 21 days continuously. The rats in the control group were injected with the same volume of saline twice a week, and administered with distilled water for 21 days. On the 22nd day, we monitored the pulmonary ventilatory function of the rats, and then sacrificed them to do various experiments.

Pulmonary Ventilatory Function Measurement

After intraperitoneal anesthesia by 1% pentobarbital sodium (40 mg/kg), six rats from all groups were randomly selected for the measurement of pulmonary ventilatory function. The subcutaneous tissue was separated to expose the trachea, and then endotracheal intubation was performed. The rat was connected with a pulmonary functionality test system (AniRes2005 analytic system, Beijing Bestlab High-Tech, China) to measure forced expiratory volume in 0.1 s (FEV0.1), forced expiratory volume in 0.3 s (FEV0.3) and forced vital capacity (FVC).

Pulmonary Histopathologic Examination

The left lung was removed immediately and fixed in 4% paraformaldehyde (Solarbio, Beijing, China). The tissue was embedded in paraffin, cut into 5 μm thick slices, and then dyed with hematoxylin-eosin (HE). The histological change of the slide was observed under the light microscopy (Olympus, Tokyo, Japan). Five random photographs of each slide were obtained. The histological score was evaluated *via* the following indicators: 1) pulmonary emphysema; 2) alveolar edema; 3) inflammatory cell infiltration; 4) alveolar hemorrhage; 5) destroyed alveolar. Each item was scored as follows: 0 for normal, 1 for slight, 2 for moderate, and 3 for severe (Sun et al., 2011). The accumulated scores were regarded as the pulmonary histological score of the sample.

Serum Inflammatory Cytokines Detection

Peripheral blood was obtained from the retro-orbital venous plexus on the 11th and 21st day, respectively, followed by the centrifugation to collect the supernatant. The serum levels of interleukin (IL)-1β, IL-6, IL-13, and tumor necrosis factor (TNF)-α were detected by enzyme-linked immunosorbent assay (ELISA) kits (Huamei, Wuhan, China). All the procedures were completed according to the strict instruction of the manufacturer.

TMT-Based Quantitative Proteomic Analysis

Nine biological replicates (n = 3/group) were identified from the control group, COPD group, and Shema 6.0 ml/kg group. Proteins from the middle lobe of the right lung tissue were extracted by lysis solution (containing 2% SDS and 7 M urea) mixed with 1× protease inhibitor cocktail (Sigma, MA, United States). The protein sample was centrifuged at 13,000 g for 15 min at 4°C, and the supernatant was gained. The BCA protein assay kit (Pierce, CA, United States) was used to measure the concentration of the protein sample. After a series of treatments, the sample was finally labeled with a TMT10 plex Isobaric Label Reagent Set (Thermo Scientific, MA, United States). The TMT-labeled peptide sample was separated using the Ultimate 3000 HPLC operating system (Thermo Scientific, MA, United States). The fractionated samples were centrifuged, condensed, and then dried by a vacuum concentrator. Subsequently, the samples were redissolved in 0.1% formic acid (in aqueous solution) and analyzed with an EASY-nLC1200 Orbitrap Elite (Thermo Scientific, CA, United States). The differentially expressed proteins (DEPs) were regarded as significantly expressed proteins based on fold change >1.20 or <0.80 as well as p-value < 0.05.

Western Blot

Tissue lysate from the middle lobe of the right lung tissue was prepared using ice-cold RIPA lysis buffer and protease inhibitors (Solarbio, Beijing, China). Samples were crushed by the ultrasonic wave (Xinzhi, Ningbo, China), and then centrifuged at 13,000 g for 15 min (4°C). The concentration of each protein sample was determined by a BCA protein assay kit (Pierce, CA, United States). Next, 40 μg protein was separated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto a PVDF membrane (Millipore, Darmstadt, Germany). The membrane was blocked with a diluted primary antibody DNMT1 (1:1000, cat. no. 5032, Cell Signaling Technology, MA, United States) overnight at 4°C. β-actin (1:1000, cat. no. 4970, Cell Signaling Technology, MA, United States) served as an internal reference. After incubation with a secondary antibody, the protein band was examined with an enhanced chemiluminescence agent (Millipore, MA, United States).

Statistical Analysis

SPSS 20.0 software was used for statistical analysis. Data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and Student's t-test were used for comparisons. In proteomics research Scaffold software was employed for data analysis, and Mann-Whitney test was used to compare differences between two groups. Values with $p < 0.05$ were accepted as statistically significant.

RESULTS

PPI Network Construction and Functional Enrichment Analysis of Shema Against COPD

355 chemical compounds and 482 putative targets of Shema were obtained, and 668 molecular targets of COPD were collected (**Supplementary Table S1**). There were 69 overlapping genes between Shema targets and COPD targets (**Figure 1A**, **Supplementary Table S2**). The potential targets of Shema and

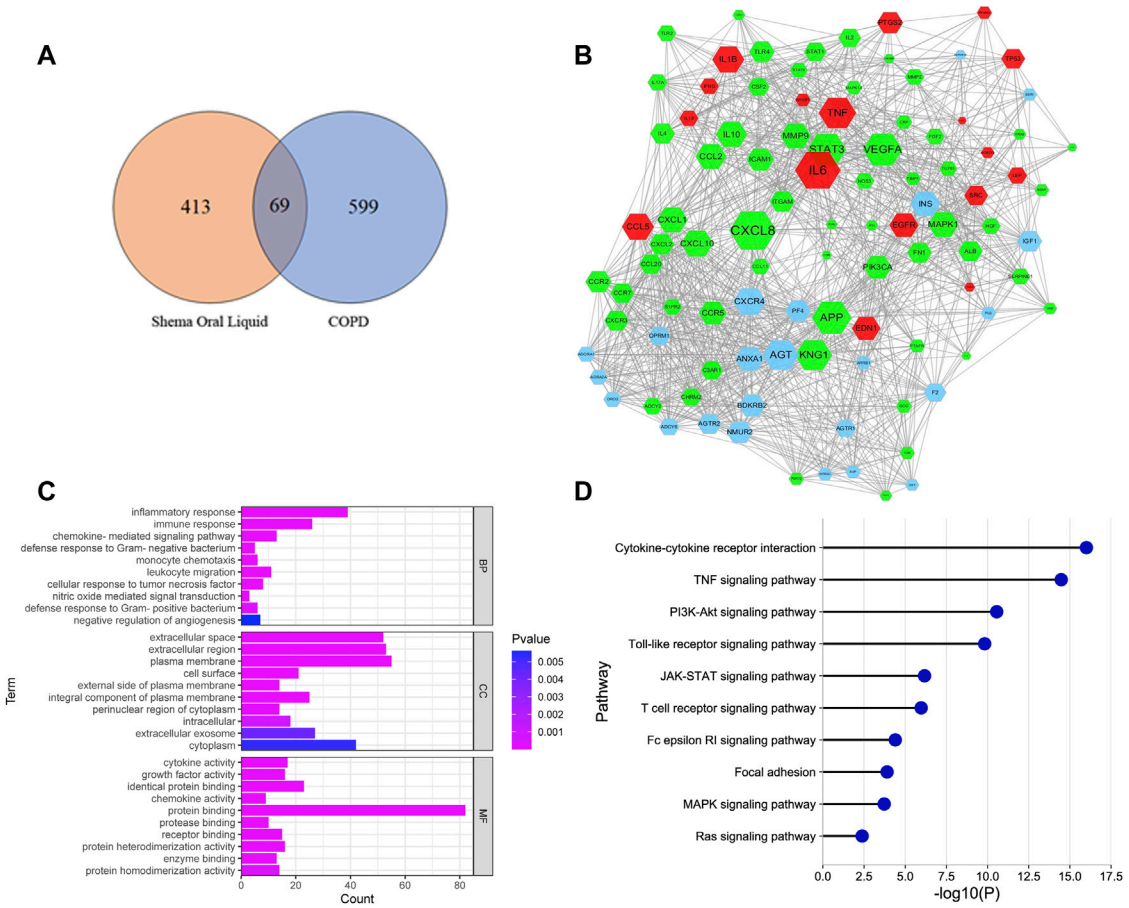

FIGURE 1 | Network pharmacology analysis of Shema in treatment of COPD. **(A)** Venn diagram between the drug targets of Shema and the disease targets of COPD. **(B)** PPI network constructed on the basis of Shema targets and COPD targets. Blue hexagon nodes represented the targets of Shema. Green hexagon nodes represented the targets of COPD. Red hexagon nodes represented the overlapping targets between Shema and COPD. The interaction with confidence score >0.8 was selected. **(C)** GO enrichment analysis of the candidate targets for Shema against COPD including BP, CC and MF terms. **(D)** KEGG pathway enrichment analysis of the candidate targets for Shema against COPD.

the disease targets of COPD were uploaded to STRING database to construct a PPI network, and protein interaction data with high confidence of a score >0.8 was selected (**Figure 1B**). **Figure 1B** showed that inflammatory cytokines such as IL-1β, IL-6, IL-13, and TNF-α were centrally located in the PPI network, suggesting that these molecular targets may be related with the pathogenesis of COPD and the treatment of Shema.

GO analysis showed that the most significantly enriched categories in biology process (BP) term were correlated with chemokine mediated signaling pathway, nitric oxide-mediated signal transduction, monocyte chemotaxis, negative regulation of angiogenesis, inflammatory response, cellular response to tumor necrosis factor, immune response, and defense response to Gram-positive/negative bacterium (**Figure 1C**). KEGG analysis showed that the signaling pathways involved in Shema against COPD were mainly associated with cytokine-cytokine receptor interaction, focal adhesion, Ras, TNF, MAPK, PI3K-Akt, JAK-STAT, T cell receptor, Toll-like receptor, and Fc epsilon RI signaling pathways (**Figure 1D**).

Shema Enhanced the Pulmonary Ventilatory Functionality

The parameters involving FEV0.1, FEV0.3, and FVC were measured to observe the impairment of pulmonary ventilatory function after Shema treatment. The values of FEV0.1, FEV0.3, and FVC in the COPD group were significantly decreased compared with the control group, while these values in Shema 3.0 and 6.0 groups were significantly elevated compared with the COPD group. Moreover, the ratios of FEV0.1/FVC and FEV0.3/FVC in the COPD group were significantly downregulated compared with the control group, but these two ratios in Shema 6.0 group were significantly increased compared with the COPD group (**Figure 2A**).

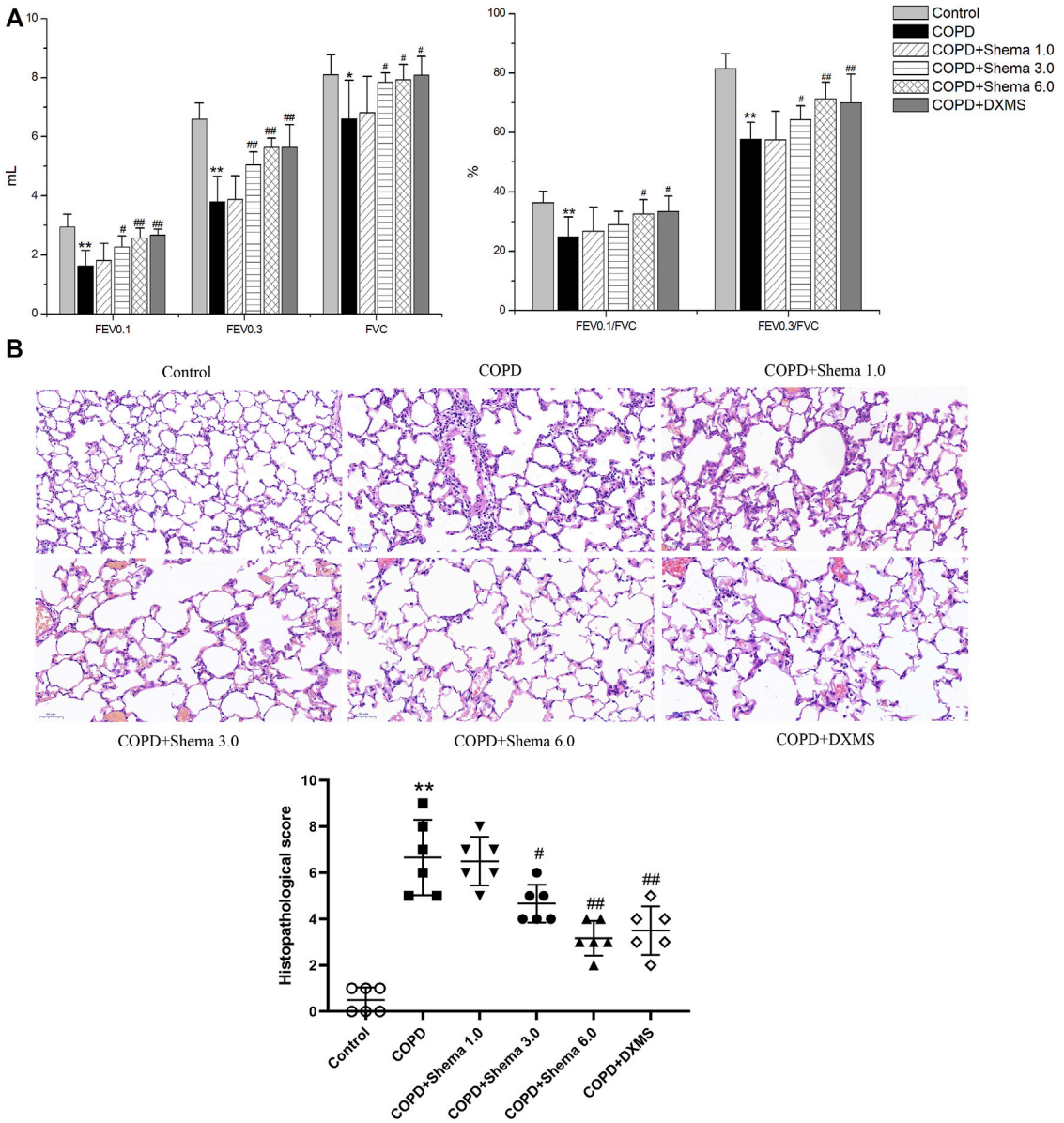

FIGURE 2 | (Continued).

Shema Alleviated the Lung Tissue Injury Induced by LPS

The structures of the bronchiole and pulmonary alveolus were normal in the control group. However, the alveolar wall and septum was thickened, the alveolar structure was even disordered, extensive inflammatory cells were infiltrated in the airway wall, and pulmonary edema was observed in the COPD group. The inflammatory cell infiltration was decreased, and the alveolar fluid exudation was reduced in the Shema 3.0 and 6.0 groups. The histological score in the COPD group was significantly increased compared with the control group. Meanwhile, the histopathological scores in the Shema 3.0 and 6.0 groups were dramatically declined compared with the COPD group (**Figure 2B**).

Shema Reduced the Levels of Serum Inflammatory Cytokines

To identify whether the inflammatory cytokines concluded from network pharmacology results were involved in the therapeutic targets of Shema treatment, these cytokines in the serum were detected by ELISA test. Compared with the control group, the levels of IL-1β and TNF-α in the COPD group were markedly increased on the 11th day, and the levels

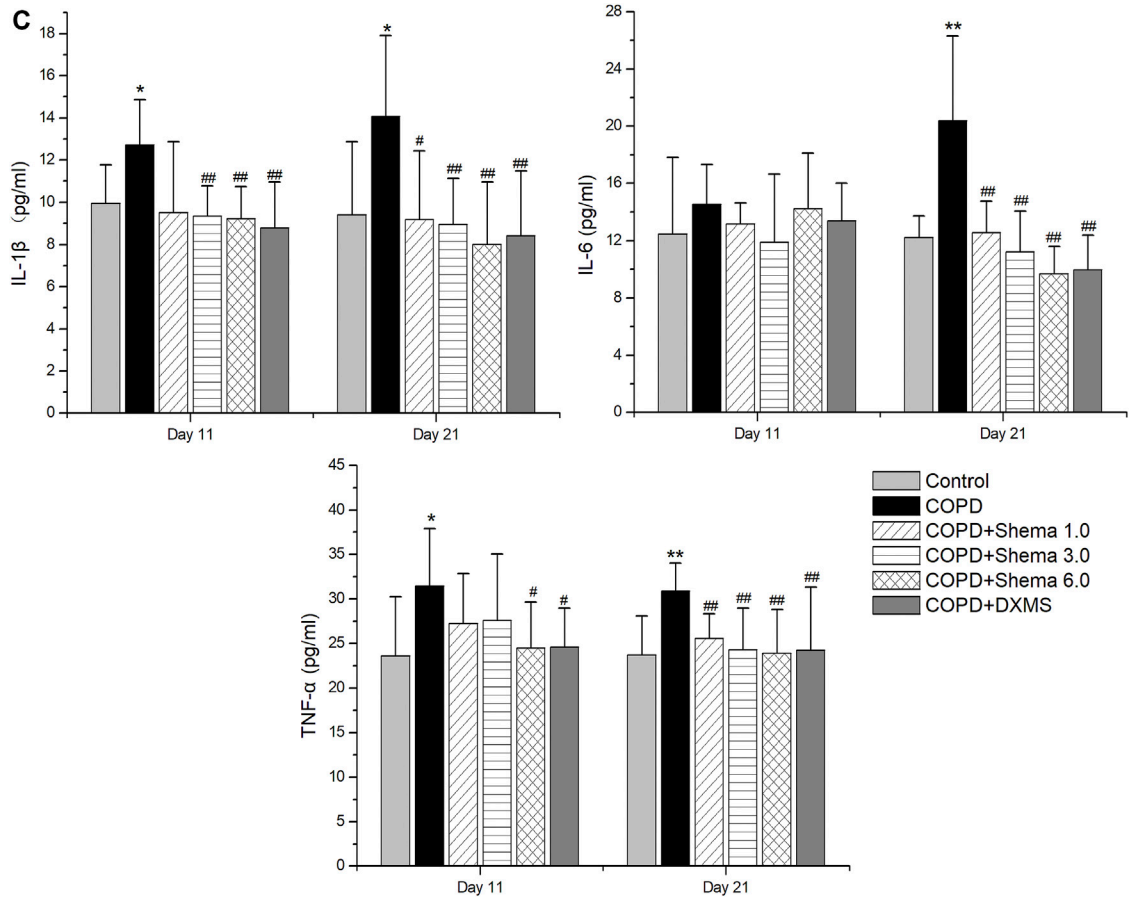

FIGURE 2 | (Continued).**(A)** Effect of Shema on pulmonary ventilatory function of COPD rats examined by a pulmonary function test system including FEV0.1, FEV0.3, FVC, FEV0.1/FVC, and FEV0.3/FVC $n = 6$. **(B)** Effect of Shema on pulmonary pathological changes of COPD rats determined by HE staining involving pathological features of lung tissues (scale bar: 50 μm, magnification: ×200) and histological scores of lung tissues $n = 6$. **(C)** Effect of Shema on serum levels of inflammatory factors including IL-1β, IL-6, and TNF-α on the 11th and 21st days, determined by ELISA test $n = 7$. Data represented as mean ± SD. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. control group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ vs. COPD group (a) (b).

of IL-1β, IL-6, and TNF-α in the COPD group were increased on the 21st day. Compared with the COPD group, the level of TNF-α in the Shema 6.0 group was decreased, and the levels of IL-1β in both Shema 3.0 and 6.0 group were decreased on the 11th day; the levels of IL-1β, IL-6, and TNF-α in Shema-treated groups were decreased on the 21st day, in a certain dose-dependent manner (**Figure 2C**). Nevertheless, our results showed that the levels of IL-13 had no significant differences on the 11th and 21st days.

Proteomic Analysis of Shema-Related DEPs Indicated the Therapeutic Targets of Shema Against COPD

Using the TMT-labeled quantitative proteomic analysis, we finished high throughput screening of protein expressions, and 6,033 proteins were identified. Our results showed that 192 and 426 proteins in the Shema 6.0 group were significantly upregulated and downregulated respectively, compared with the COPD group. A total of 185 DEPs including 131 upregulated and 54 downregulated DEPs in the COPD group were identified, compared with the control group. There were 106 overlapping DEPs in these two comparisons (**Figure 3A**).

Subsequently, a PPI network containing 106 overlapping DEPs was constructed to further investigate the potential targets of Shema against COPD (**Figure 3B**). GO analysis was used to elucidate the biological significance of these DEPs on the basis of their BP, cellular component (CC), and molecular function (MF). BP category of GO analysis included oxidation-reduction process, cell-cell adhesion, negative regulation of apoptotic process, response to lipopolysaccharide, response to hypoxia, innate immune response, integrin-mediated signaling pathway, DNA replication, cellular response to interleukin-1, and negative regulation of interleukin-6 production (**Figure 3C**). KEGG enrichment analysis indicated

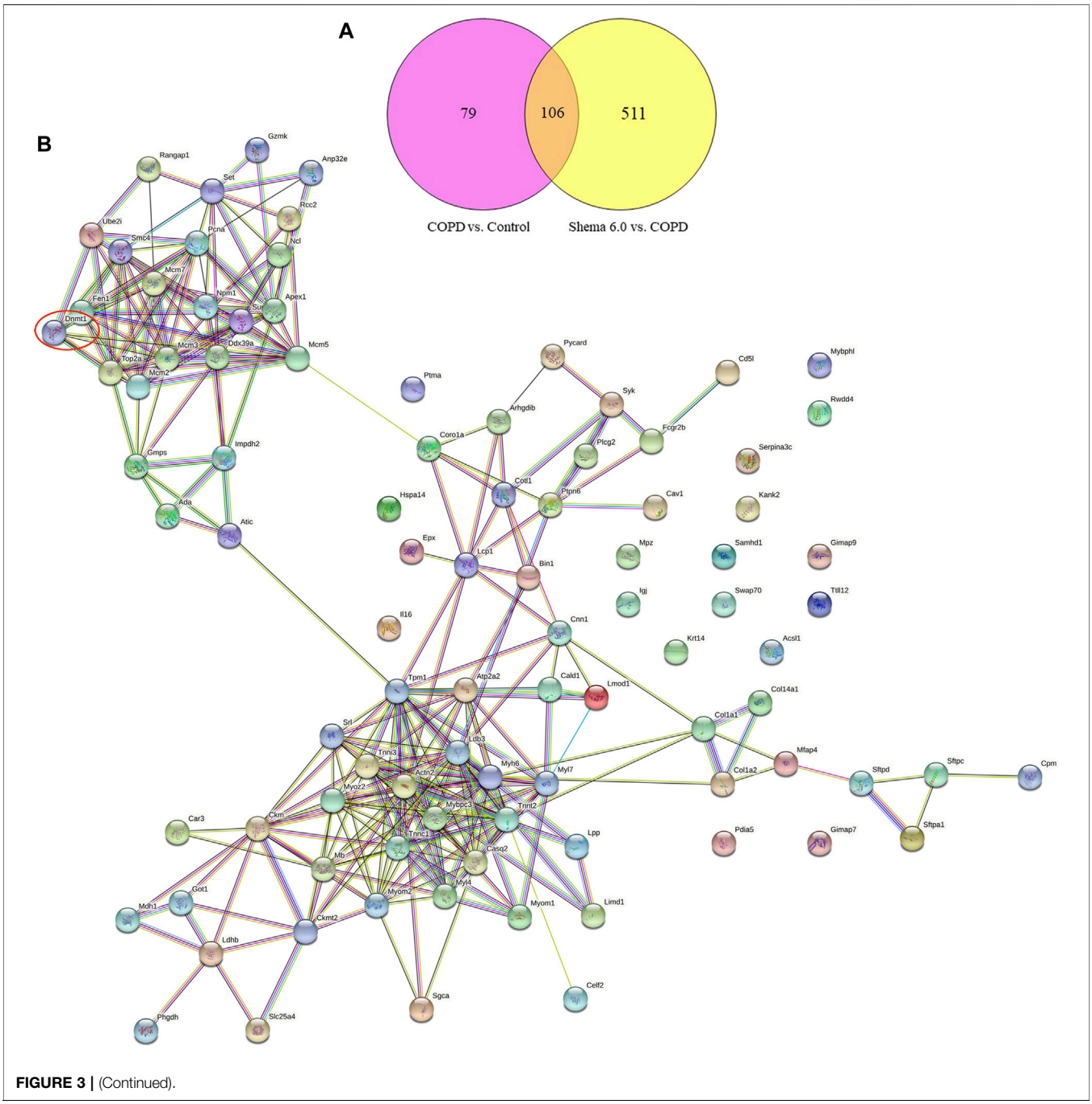

FIGURE 3 | (Continued).

that the signaling pathways of Shema against COPD were mainly related with oxidative phosphorylation, focal adhesion, DNA replication, regulation of actin cytoskeleton, Fc gamma R-mediated phagocytosis, B cell receptor signaling pathway, PPAR signaling pathway, ECM-receptor interaction, Fc epsilon RI signaling pathway, and fatty acid degradation (**Figure 3D**).

According to network analysis, one reliable and functional protein DNA methyltransferase 1 (DNMT1), was identified and analyzed. DNMT1 was one of the downregulated DEPs in the Shema-treated group. DNMT1 is reported to be a key maintenance methylase in mammal DNMTs family (Bayarsaihan, 2011). DNMTs-mediated aberrant methylation is linked to the pathogenesis and development of COPD (Adcock et al., 2011). DNMT1 expression in the lung tissue from COPD patients was higher than that in the lung tissue from non-smokers (Zeng et al., 2020).

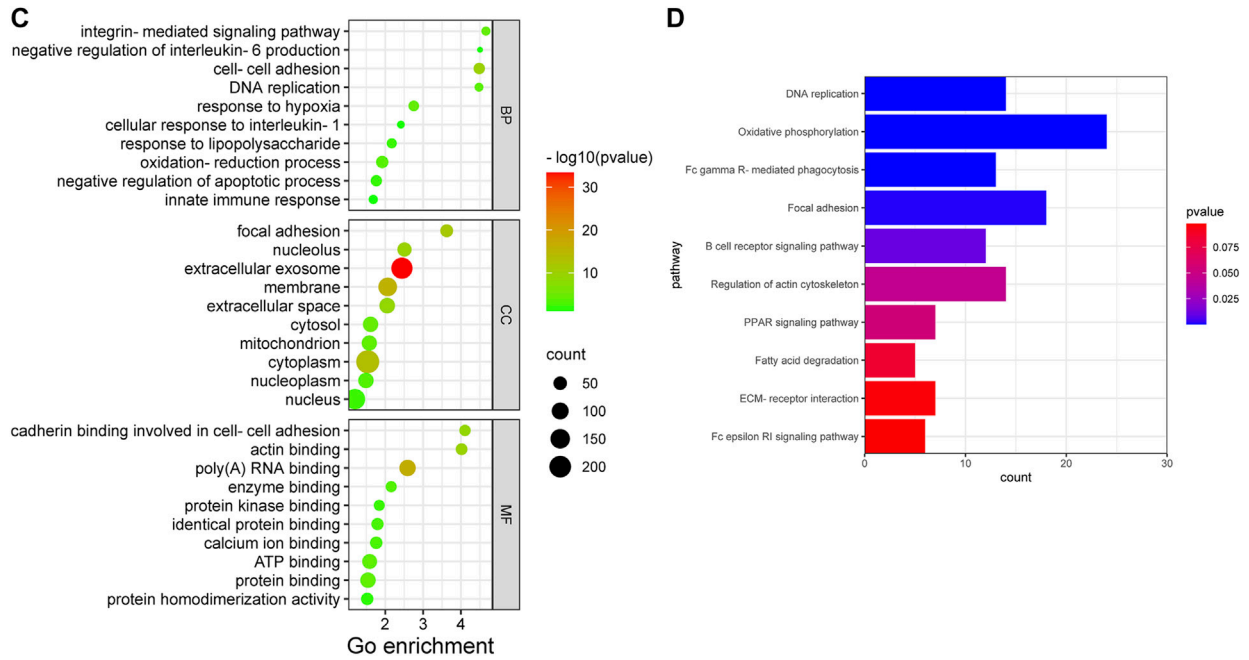

FIGURE 3 | (Continued).

We also constructed another PPI network of the DEPs by STRING database to simulate the relationships of protein interactions after Shema intervention. The protein interactions with confidence score >0.6 were reserved. Eighteen sub-clusters were extracted by the MCODE plug-in of Cytoscape software to identify highly interconnected subnetworks. The proteins in the subnetwork 2, 4, 8, 9, and 15 had the known targets of COPD, associated with chronic inflammation, pulmonary oxidation, and the imbalance of protease-antiprotease, as previously reported. The proteins in subnetwork 17 contained the direct target of Shema such as DNMT1, which was the same crucial protein contained in the PPI network of the 106 overlapping DEPs (**Figure 3E**). Finally, based on the above two kinds of network analysis, DNMT1 may be the important molecular target of Shema in treatment of COPD, and thus it was chosen to be verified by western blot.

Shema Regulated the Expression of DNMT1 *via* Western Blot Validation

The relative content of the key functional protein DNMT1 was examined by western blot, in order to validate the expression identified by the quantitative proteomic analysis. Our data showed that the expression of DNMT1 in the COPD group was markedly upregulated compared with the control group. Additionally, compared with the COPD group, the expression of DNMT1 was significantly decreased in the Shema 6.0 group (**Figure 4**). The change in the expression of DNMT1 protein was consistent with the trend of the quantitative proteomic result.

DISCUSSION

Our research explored the protective effect and mechanism of Shema against COPD using network pharmacology. PPI network showed that inflammatory factors, such as IL-1β, IL-6, IL-13, and TNF-α, were the most critical targets contained in the pathogenesis of COPD and the therapeutic targets of Shema. GO enrichment analysis in BP category was mainly related with immune response, inflammatory reaction and defense response to bacterium. Previous studies have shown that normal immune response can prevent organs from further infection or injury during the process of inflammation. However, chronic lung inflammation such as COPD is often accompanied by immune dysfunction, leading to extensive lung damage and airway remodeling (Bhat et al., 2015). Both innate and adaptive immune systems are known to participate in the development of chronic infection in COPD (Brusselle et al., 2011).

Reports showed that IL-1-like cytokines were elevated in the lung tissue of the COPD patient, indicating the significance of an inflammasome in the pathological process of COPD (Rycroft et al., 2012). The activated pro-inflammatory caspases such as caspase 1 caused the cleavage of proinflammatory factors and the generation of IL-1β and IL-18 (Rovina et al., 2013). The serum levels of IL-6, IL-8, and TNF-α in acutely exacerbated COPD patients were increased, which may threaten pulmonary function (Lin et al., 2019). Furthermore, published studies have suggested that the release of inflammatory cytokines involving IL-1β, IL-6, and TNF-α participated in activating p38 MAPK and JNK signaling pathways in chronic lung diseases (Barnes et al., 2019). Our results showed that Shema suppressed the serum

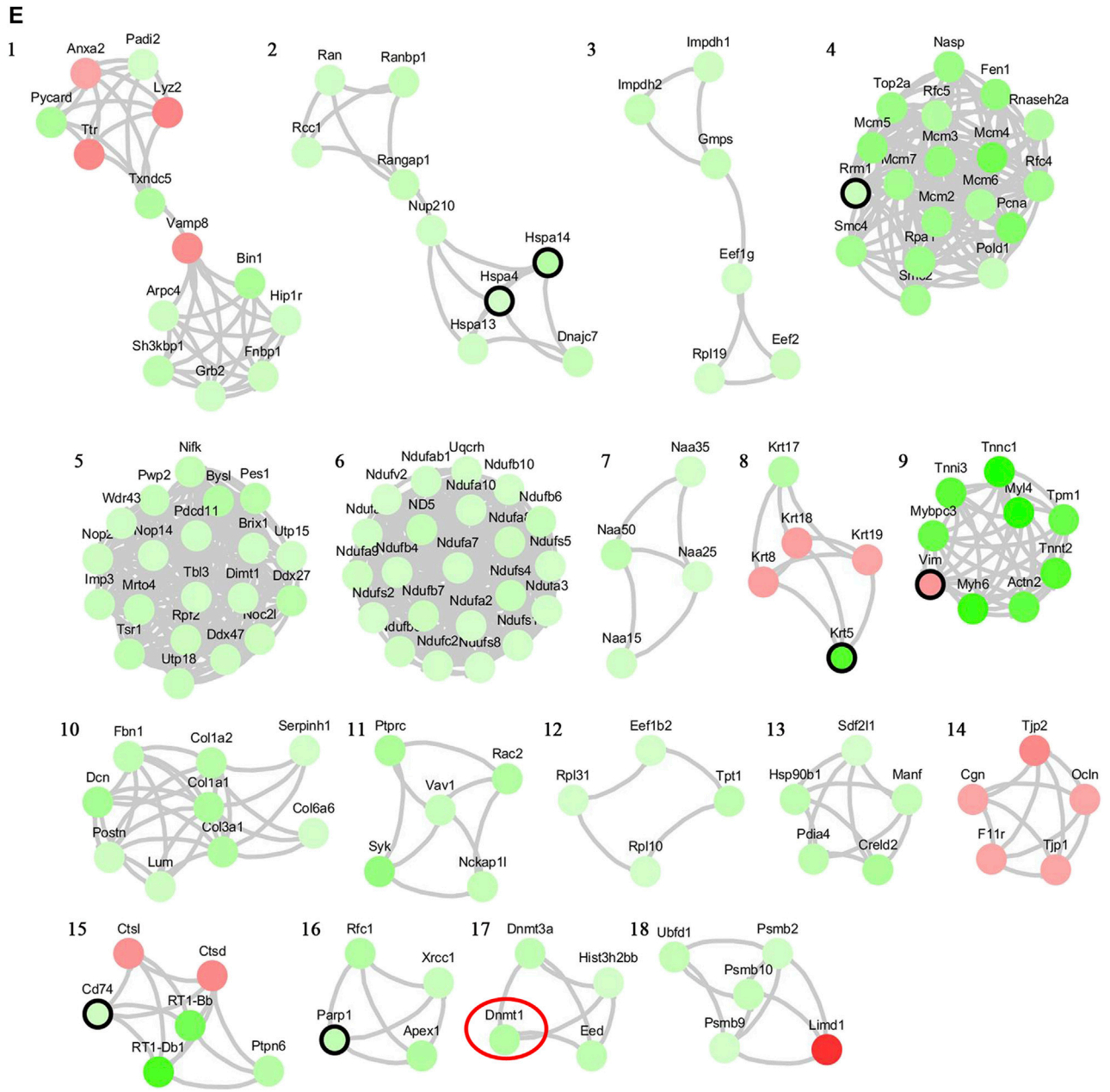

FIGURE 3 | (Continued).Functional analysis of Shema-regulated proteins in lung tissue detected by quantitative proteomics. **(A)** Venn diagram showed that there were 106 overlapping DEPs between COPD vs. control and Shema 6.0 vs. COPD. **(B)** PPI network was constructed according to the 106 overlapping DEPs. There were only 95 DEPs in the network, because 11 proteins did not have corresponding gene symbols. **(C)** GO analysis revealed the top 10 significant enrichment terms in BP, CC, and MF, respectively. **(D)** KEGG analysis demonstrated the top 10 enrichment pathways. **(E)** PPI network showed the direct and potential targets of Shema for the treatment of COPD. The nodes with the black border were the reported targets of COPD. Green nodes represented downregulated proteins, and red nodes represented upregulated proteins(a) (b) (c).

levels of inflammatory cytokines including IL-1β, IL-6, and TNF-α, which implied that Shema exerted a protective effect *via* anti-inflammation. At the same time, these data had partially verified the results concluded from network pharmacology.

Patients with COPD have declined lung functions and irreversible pulmonary limitations, as diagnosed by a ratio of FEV1/FVC <0.70 (Vestbo et al., 2013). According to the declined results of FEV0.1, FEV0.3, FVC, FEV0.1/FVC, and FEV0.3/FVC, the pulmonary ventilatory function typically dropped in COPD rats. Shema treatment significantly increased these values, indicating that Shema may improve the lung ventilative function of COPD

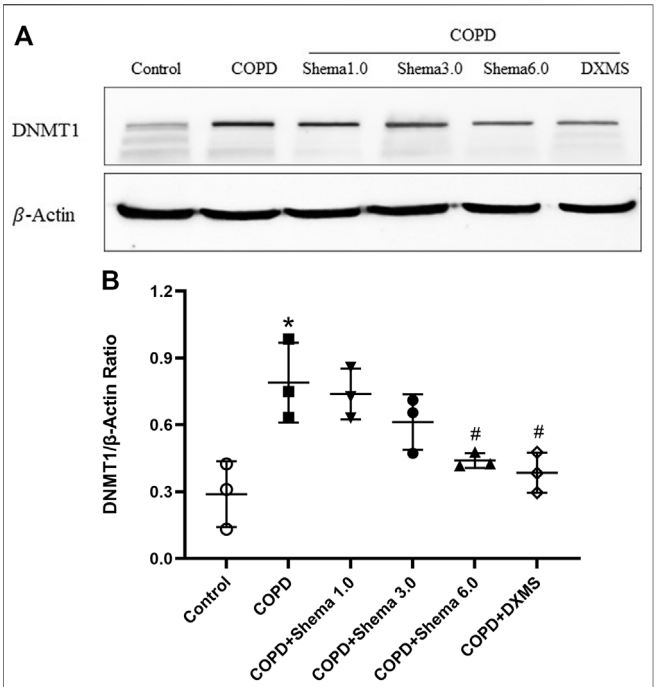

FIGURE 4 | Western blot validation of DNMT1expression in the lung tissue among different groups. **(A)** Typical images of DNMT1. **(B)** Statistical quantification showing the relative expression of DNMT1. Data represented as mean ± SD, $n = 3$. $^{*}p < 0.05$ vs. control group; $^{\#}p < 0.05$ vs. COPD group.

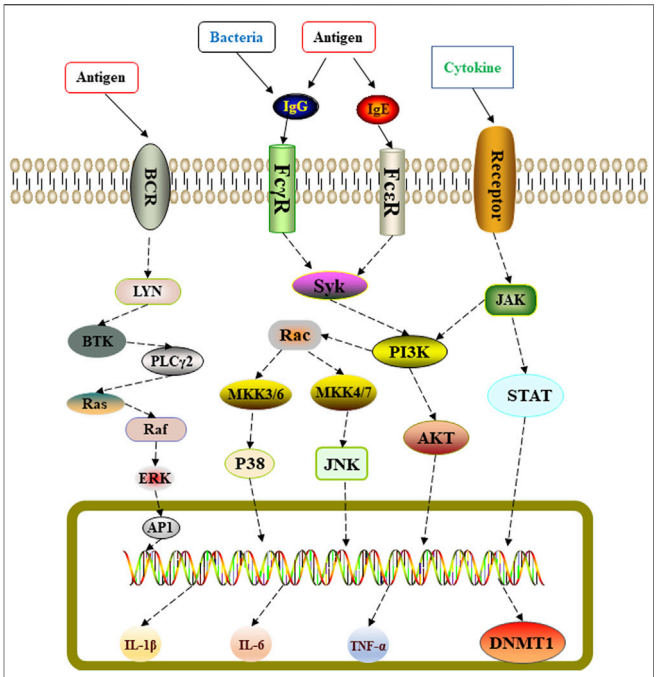

FIGURE 5 | Schematic representation of Shema-mediated protective effect against LPS-induced lung injury in COPD rats.

rats. Furthermore, pathological results also revealed that Shema attenuated the infiltration of inflammatory cell, the exudation of alveolar fluid and the thickening of pulmonary interstitial, and thus protected the lung tissue from LPS-induced injury.

The proteomic profile of the lung tissue was investigated to further explore the potential targets related with Shema against COPD. A total of 106 overlapping DEPs were identified, which may be the possible therapeutic targets of Shema. Go enrichment analysis indicated that the potential regulatory mechanism of Shema treatment was mainly related with inflammation, immunity response, apoptotic process and DNA replication. Two kinds of PPI network analysis demonstrated that DNMT1 may be very important as the candidate target. Western blot result displayed that Shema markedly downregulated the expression of DNMT1 in the lung tissue of COPD rats, consistent with proteomic result.

Human DNMTs family has five members: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L (Lyko, 2018). There traditional roles of DNMT1, DNMT3A, and DNMT3B are to establish and maintain DNA methylation patterns, which are highly disrupted and associated with expression changes in the small airways of COPD patients (Vucic et al., 2014). The upstream region of the Klotho promoter containing rich CpG islands is the common target for DNMTs. The Klotho protein is a transmembrane protein highly expressed in the kidney, choroid plexus, and neuron, playing a critical role in aging, inflammation, oxidative stress, calcium-phosphate metabolism, and cognitive process (Zhou et al., 2021). Recent research data have suggested that Klotho expression was decreased in blood monocytes, alveolar macrophages and respiratory epithelial cells of COPD patients or mouse models (Gao et al., 2015; Kureya et al., 2016). The expression of Klotho had an important influence on lung inflammation, and may have therapeutic implication in COPD treatment (Li et al., 2015). The treatment of cigarette smoke extract *in vitro* enhanced the levels of DNMTs (DNMT1, DNMT3A, and DNMT3B) and the methylation of Klotho promoter, ultimately leading to activate Notch signaling pathway. The inhibitions of DNMTs and Notch pathway suppressed inflammatory response and cellular apoptosis in a cell model of COPD (Qiu et al., 2018). Cigarette smoke extract treatment also promoted the expression of DNMT3A in dendritic cells of COPD mouse models, and DNMT3A regulated Th17/Treg cell balance *via* c-Jun/AIF1 axis (Huang et al., 2021). Our study further illustrated that DNMT1 was significantly increased in the lung tissue of COPD rats, which may generate novel mechanistic insight into therapeutic targets for the treatment of COPD.

In summary, we used network pharmacology to predict the potential molecular targets of Shema in treatment of COPD (**Figure 5**). Experimental research on LPS induced COPD rats proved that Shema strengthened pulmonary ventilatory function, suppressed the serum levels of inflammatory cytokines, and alleviated the pulmonary injury through regulating the

expression of DNMT1. Shema mainly exerted the anti-inflammatory activity and lung-protective action involved in the protective effect on COPD. Our study shed light on the development of therapeutic strategies in treating COPD by intervening DNMT-related signaling pathways.

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 animal study was reviewed and approved by the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

AUTHOR CONTRIBUTIONS

HY conceived and designed the study. FZ and YL conducted most of the experiments with assistance from YZ and DL. FG completed the analysis of network pharmacology and quantitative proteomics. FZ wrote the manuscript. All authors agree with the submission of this manuscript.

FUNDING

This research was funded by the National Major Science and Technology Project (No. 2019ZX09201005-001-005).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.903593/full#supplementary-material

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The reviewer LB declared a shared affiliation with the author FZ to the handling editor at the time of review.

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ORIGINAL RESEARCH
published: 08 June 2022
doi: 10.3389/fphar.2022.868920

The Association Between Bronchoscopy and the Prognoses of Patients With Ventilator-Associated Pneumonia in Intensive Care Units: A Retrospective Study Based on the MIMIC-IV Database

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Background: In intensive care units (ICUs), the morbidity and mortality of ventilator-associated pneumonia (VAP) are relatively high, and this condition also increases medical expenses for mechanically ventilated patients, which will seriously affect the prognoses of critically ill patients. The purpose of this study was to determine the impact of bronchoscopy on the prognosis of patients with VAP undergoing invasive mechanical ventilation (IMV).

Methods: This was a retrospective study based on patients with VAP from the Medical Information Mart for Intensive Care IV database. The outcomes were ICU and in-hospital mortality. Patients were divided based on whether or not they had undergone bronchoscopy during IMV. Kaplan-Meier (KM) survival curves and Cox proportional-hazards regression models were used to analyze the association between groups and outcomes. Propensity score matching (PSM) and propensity score based inverse probability of treatment weighting (IPTW) were used to further verify the stability of the results. The effect of bronchoscopy on prognosis was further analyzed by causal mediation analysis (CMA).

Results: This study enrolled 1,560 patients with VAP: 1,355 in the no-bronchoscopy group and 205 in the bronchoscopy group. The KM survival curve indicated a significant difference in survival probability between the two groups. The survival probabilities in both the ICU and hospital were significantly higher in the bronchoscopy group than in the no bronchoscopy group. After adjusting all covariates as confounding factors in the Cox model, the HRs (95% CI) for ICU and in-hospital mortality in the bronchoscopy group were 0.33 (0.20–0.55) and 0.40 (0.26–0.60), respectively, indicating that the risks of ICU and in-hospital mortality were 0.67 and 0.60 lower than in the no-bronchoscopy group. The same trend was obtained after using PSM and IPTW. CMA showed that delta-red blood cell

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Edited by:
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 03 February 2022
Accepted: 06 May 2022
Published: 08 June 2022

Citation:
Zhang L, Li S, Yuan S, Lu X, Li J, Liu Y, Huang T, Lyu J and Yin H (2022) The Association Between Bronchoscopy and the Prognoses of Patients With Ventilator-Associated Pneumonia in Intensive Care Units: A Retrospective Study Based on the MIMIC-IV Database. Front. Pharmacol. 13:868920. doi: 10.3389/fphar.2022.868920

distribution width (RDW) mediated 8 and 7% of the beneficial effects of bronchoscopy in ICU mortality and in-hospital mortality.

Conclusion: Bronchoscopy during IMV was associated with reducing the risk of ICU and in-hospital mortality in patients with VAP in ICUs, and this beneficial effect was partially mediated by changes in RDW levels.

Keywords: ICU, ventilator-associated pneumonia, bronchoscopy, mortality, causal mediation analysis

INTRODUCTION

Ventilator-associated pneumonia (VAP), defined as infection of the lung parenchyma in patients after at least 48 h of exposure to invasive mechanical ventilation (IMV), (Papazian et al., 2020), is one of the most common infectious diseases in intensive care units (ICUs) (Hunter, 2012), affecting up to 40% of patients on mechanical ventilators (Spalding et al., 2017). Various previous studies found that VAP was associated with longer IMV durations and ICU stays, and also increased antimicrobial use (Hayashi et al., 2013). In some developed countries, VAP was also found to increase the average hospitalization cost of patients by approximately US$ 40,000 (Zimlichman et al., 2013). The mortality rate of patients with VAP may exceed 50% (Ruiz et al., 2000); the results of 58 randomized studies on VAP indicated that the estimated attributable mortality rate was 9% (range 3–17%) (Melsen et al., 2011). VAP has high morbidity and mortality, and also increases the medical expenses of mechanically ventilated patients, which will seriously affect the prognoses of critically ill patients.

Bronchoscopy has been widely used for clinical diagnoses and treatment of respiratory diseases. Airway examination and transbronchial biopsy have greatly improved the diagnosis rates of pulmonary inflammatory (Baselski and Wunderink, 1994; Su et al., 2020) and substantial lung (Navani et al., 2011) diseases, and can also help improve the treatment effect (Pedro et al., 2019). However, currently there is no unified conclusion on whether bronchoscopy can improve the prognoses of patients with VAP. This study used the large public Medical Information Mart for Intensive Care (MIMIC)-IV database as a basis to determine the impact of bronchoscopy on the prognoses of patients with VAP in the ICU.

METHODS

Data Source and Population

The MIMIC-IV is a large, free, and open database; the latest version is MIMIC-IV (version 1.0) (Johnson et al., 2021), which contains comprehensive information on approximately 250,000 patients hospitalized from 2008 to 2019, and provides strong data support for clinical studies (Yang et al., 2020; Wu et al., 2021). The database was approved by the Massachusetts Institute of Technology (Cambridge, Mass.) and the Beth Israel Deaconess Medical Center (Boston, Mass.), and consent was obtained for collection of the original data (Zhang et al., 2021). The database also anonymizes patient information, and so informed consent did not need to be obtained. The researchers needed to complete corresponding courses and obtain certificates to access and extract data from this database.

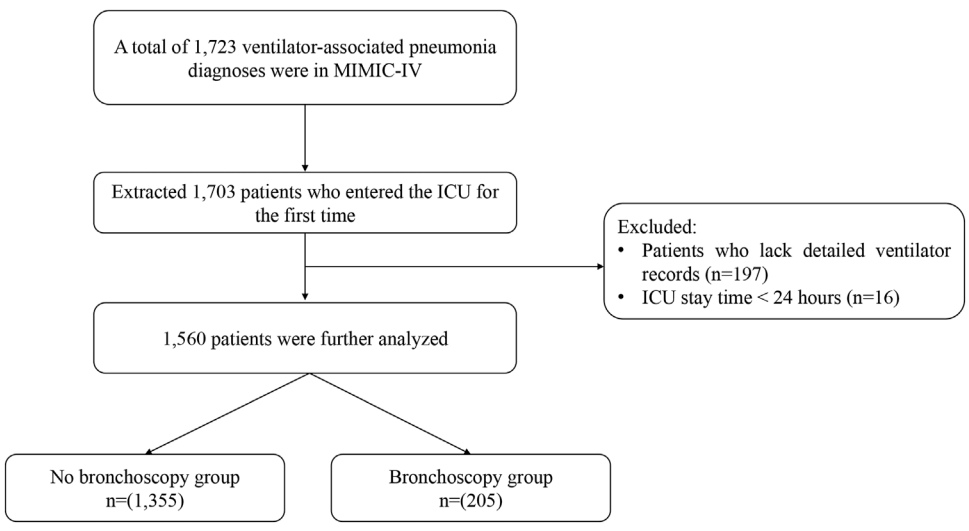

FIGURE 1 | Inclusion and exclusion flowchart of the study.

TABLE 1 | Baseline characteristics of original population.

	Original Population			PSM Population		
	No Bronchoscopy Group	Bronchoscopy Group	*p*-Value	No Bronchoscopy Group	Bronchoscopy Group	*p*-Value
N	1,355	205		195	195	
Age (year)	64.00 (53.00, 75.00)	62.00 (50.00, 74.00)	0.302	66.00 (53.50, 76.00)	63.00 (50.00, 75.50)	0.546
Gender (%)			0.432			0.915
Male	850 (62.7)	135 (65.9)		129 (66.2)	127 (65.1)	
Female	505 (37.3)	70 (34.1)		66 (33.8)	68 (34.9)	
Ethnicity (%)			0.177			0.139
White	772 (57.0)	130 (63.4)		105 (53.8)	123 (63.1)	
Black	154 (11.4)	17 (8.3)		17 (8.7)	17 (8.7)	
Others	429 (31.7)	58 (28.3)		73 (37.4)	55 (28.2)	
BMI	28.25 (24.25, 33.69)	28.44 (25.60, 34.66)	0.184	29.01 (24.39, 35.15)	28.40 (25.55, 34.61)	0.844
APSIII	69.00 (51.00, 90.00)	70.00 (56.00, 91.00)	0.286	71.00 (57.00, 94.50)	71.00 (56.00, 91.00)	0.395
First care unit (%)			0.001			0.426
MICU/SICU	1,007 (74.3)	174 (84.9)		157 (80.5)	164 (84.1)	
Others	348 (25.7)	31 (15.1)		38 (19.5)	31 (15.9)	
Duration of IMV (hour)	136.00 (60.50, 253.50)	145.00 (59.00, 286.00)	0.332	127.00 (64.50, 243.50)	145.00 (56.50, 287.00)	0.306
Vasopressors (%)	518 (38.2)	63 (30.7)	0.046	61 (31.3)	60 (30.8)	1.000
CRRT (%)	43 (3.2)	5 (2.4)	0.726	5 (2.6)	5 (2.6)	1.000
Comorbidities						
Sepsis (%)	1,308 (96.5)	200 (97.6)	0.578	192 (98.5)	190 (97.4)	0.721
Myocardial infarct (%)	257 (19.0)	24 (11.7)	0.015	34 (17.4)	24 (12.3)	0.200
Congestive heart failure (%)	453 (33.4)	59 (28.8)	0.214	63 (32.3)	55 (28.2)	0.440
Hypertension (%)	625 (46.1)	101 (49.3)	0.444	93 (47.7)	97 (49.7)	0.761
Cerebrovascular disease (%)	346 (25.5)	47 (22.9)	0.474	54 (27.7)	46 (23.6)	0.417
Chronic pulmonary disease (%)	388 (28.6)	70 (34.1)	0.125	62 (31.8)	66 (33.8)	0.746
Liver disease (%)	255 (18.8)	33 (16.1)	0.401	41 (21.0)	33 (16.9)	0.366
Renal disease (%)	308 (22.7)	37 (18.0)	0.157	38 (19.5)	36 (18.5)	0.897
Diabetes (%)	430 (31.7)	48 (23.4)	0.02	52 (26.7)	45 (23.1)	0.482
Malignancy (%)	127 (9.4)	23 (11.2)	0.478	21 (10.8)	22 (11.3)	1.000
Vital signs						
Mean heart rate (min-1)	86.54 (74.81, 99.31)	87.09 (75.58, 98.89)	0.701	84.37 (73.52, 97.26)	87.37 (75.65, 99.66)	0.202
Mean Mbp (mmHg)	77.64 (70.94, 85.10)	76.16 (71.04, 84.00)	0.545	75.88 (69.81, 82.98)	76.16 (70.98, 83.57)	0.309
Mean respiratory rate (min-1)	19.71 (17.39, 22.67)	19.73 (17.34, 22.76)	0.646	20.12 (17.44, 23.03)	19.73 (17.40, 22.83)	0.450
Mean temperature (°C)	37.07 (36.69, 37.45)	36.95 (36.60, 37.37)	0.107	37.07 (36.67, 37.42)	37.00 (36.60, 37.37)	0.392
Mean SpO2 (%)	97.93 (96.19, 99.11)	97.63 (95.96, 98.81)	0.069	97.86 (96.15, 98.97)	97.64 (96.04, 98.78)	0.438
Urineoutput (ml)	1,490.00 (859.00, 2315.00)	1,620.00 (915.00, 2285.00)	0.811	1,425.00 (819.50, 2132.50)	1,620.00 (902.50, 2292.50)	0.317
Microbiology (%)			0.120			0.058
Unclear	413 (30.5)	53 (25.9)		55 (28.2)	46 (23.6)	
Gram positive	251 (18.5)	39 (19.0)		23 (11.8)	37 (19.0)	
Gram negative	531 (39.2)	96 (46.8)		88 (45.1)	95 (48.7)	
Others	160 (11.8)	17 (8.3)		29 (14.9)	17 (8.7)	
Laboratory tests						
WBC (k/uL)	11.80 (8.60, 16.40)	12.20 (9.40, 16.10)	0.464	11.80 (8.40, 16.90)	12.30 (9.50, 16.30)	0.498
Neutrophils (%)	81.00 (74.00, 86.50)	84.00 (76.70, 88.50)	<0.001	83.00 (76.05, 88.10)	84.00 (76.15, 88.25)	0.625
Lymphocytes (%)	9.20 (5.70, 14.70)	8.60 (5.40, 13.00)	0.171	9.80 (5.60, 15.00)	8.70 (5.40, 13.10)	0.310
Basophils (%)	0.20 (0.00, 0.40)	0.20 (0.00, 0.30)	0.557	0.20 (0.00, 0.40)	0.20 (0.00, 0.30)	0.356
Eosinophils (%)	0.60 (0.00, 1.80)	0.60 (0.10, 1.40)	0.891	0.40 (0.00, 1.50)	0.60 (0.10, 1.40)	0.171
Monocytes (%)	5.50 (3.60, 8.00)	4.60 (3.00, 6.00)	<0.001	4.60 (3.00, 6.00)	4.60 (3.00, 6.00)	0.936
Hemoglobin (g/dl)	10.60 (8.80, 12.50)	10.50 (9.40, 12.30)	0.282	11.00 (9.30, 12.65)	10.50 (9.40, 12.30)	0.543
MCH (pg)	30.20 (28.60, 31.65)	30.50 (29.10, 31.70)	0.107	30.70 (29.40, 31.80)	30.40 (29.05, 31.70)	0.322
MCHC (%)	32.70 (31.50, 33.80)	33.20 (31.80, 34.40)	<0.001	33.30 (32.10, 34.20)	33.20 (31.85, 34.40)	0.786
RDW (%)	14.60 (13.55, 16.30)	14.60 (13.60, 16.10)	0.737	14.60 (13.50, 15.90)	14.50 (13.60, 16.00)	0.849
Platelet (k/uL)	189.00 (133.50, 251.00)	204.00 (138.00, 277.00)	0.096	186.00 (146.50, 250.00)	204.00 (136.50, 275.50)	0.391
Anion Gap (mEq/L)	15.00 (12.00, 18.00)	14.00 (12.00, 17.00)	0.114	15.00 (12.00, 17.00)	14.00 (12.00, 17.00)	0.432
Bicarbonate (mEq/L)	22.00 (19.00, 25.00)	24.00 (21.00, 27.00)	<0.001	24.00 (21.00, 27.50)	24.00 (21.00, 27.00)	1.000
Total calcium (mEq/L)	8.20 (7.70, 8.80)	8.30 (7.80, 8.90)	0.062	8.40 (7.80, 8.90)	8.30 (7.80, 8.90)	0.791
Free calcium (mEq/L)	1.11 (1.04, 1.16)	1.11 (1.05, 1.17)	0.099	1.11 (1.05, 1.16)	1.11 (1.05, 1.17)	0.546
Magnesium (mEq/L)	1.90 (1.70, 2.20)	2.00 (1.80, 2.20)	0.105	1.90 (1.80, 2.20)	2.00 (1.80, 2.20)	0.694
Phosphate (mEq/L)	3.60 (2.90, 4.50)	3.70 (3.10, 4.60)	0.121	3.50 (2.90, 4.70)	3.60 (3.05, 4.55)	0.390
Sodium (mEq/L)	139.00 (136.00, 143.00)	140.00 (136.00, 142.00)	0.763	139.00 (137.00, 143.00)	140.00 (136.00, 142.00)	0.903
Potassium (mEq/L)	4.10 (3.70, 4.60)	4.10 (3.70, 4.50)	0.712	4.10 (3.70, 4.60)	4.10 (3.70, 4.50)	0.876

(Continued on following page)

TABLE 1 | (*Continued*) Baseline characteristics of original population.

	Original Population			PSM Population		
	No Bronchoscopy Group	Bronchoscopy Group	*p*-Value	No Bronchoscopy Group	Bronchoscopy Group	*p*-Value
PaCO2 (mmHg)	41.00 (35.00, 50.00)	43.00 (37.00, 55.00)	0.004	43.00 (36.50, 51.50)	43.00 (37.00, 54.50)	0.981
PaO2 (mmHg)	106.00 (65.00, 192.00)	122.00 (84.00, 209.00)	0.006	127.00 (81.00, 218.00)	122.00 (84.00, 210.00)	0.956
Lactate (g/dl)	1.70 (1.10, 2.70)	1.50 (1.00, 2.70)	0.061	1.60 (1.15, 2.60)	1.50 (1.00, 2.70)	0.266
Creatinine (g/dl)	1.00 (0.80, 1.70)	1.00 (0.70, 1.60)	0.161	1.00 (0.80, 1.65)	1.00 (0.70, 1.60)	0.174
Glucose (mg/dl)	139.00 (111.00, 181.00)	131.00 (109.00, 168.00)	0.058	136.00 (111.00, 167.50)	133.00 (110.00, 168.00)	0.600
INR	1.30 (1.10, 1.50)	1.20 (1.10, 1.40)	0.217	1.30 (1.10, 1.50)	1.20 (1.10, 1.40)	0.208
PTT (s)	30.60 (26.90, 38.00)	30.80 (27.30, 37.70)	0.688	31.60 (27.20, 39.60)	30.80 (27.35, 37.60)	0.404
ALT (iu/L)	30.00 (17.00, 61.00)	34.00 (18.00, 67.00)	0.16	28.00 (17.00, 63.50)	34.00 (18.00, 69.50)	0.325
AP (iu/L)	78.00 (57.00, 114.00)	81.00 (54.00, 114.00)	0.911	76.00 (57.00, 108.50)	81.00 (54.50, 115.00)	0.692
Total bilirubin (mg/dl)	0.60 (0.40, 1.10)	0.60 (0.40, 1.10)	0.821	0.60 (0.40, 1.00)	0.60 (0.40, 1.10)	0.964
Albumin (g/dl)	2.90 (2.50, 3.30)	2.90 (2.60, 3.50)	0.309	3.00 (2.60, 3.40)	2.90 (2.60, 3.50)	0.334
Delta-WBC	-1.00 (-4.55, 2.60)	-1.20 (-5.20, 2.40)	0.488	-1.20 (-4.62, 1.83)	-1.15 (-5.40, 2.63)	0.934
Delta-RDW	0.50 (-0.10, 1.70)	0.30 (-0.30, 1.30)	0.046	0.60 (0.00, 1.50)	0.35 (-0.30, 1.40)	0.353
Delta-platelet	58.50 (-9.00, 176.25)	106.00 (-0.50, 208.50)	0.048	64.00 (-1.00, 177.00)	104.00 (-6.50, 208.75)	0.192

ICU patients diagnosed with VAP in the MIMIC-IV database were included. For patients admitted to the ICU more than once, only information on the first admission was obtained. Patients without a detailed record of ventilator use or who stayed in the ICU for less than 24 h were excluded. Patients were divided based on whether or not they had undergone bronchoscopy during IMV.

Data Extraction

Data with less than 20% missing values in the database were extracted using Structured Query Language. The included demographic information were age, sex, body mass index (BMI), and race. Other information included the first care unit, disease severity [Acute Physiology Score III (APSIII)], interventional therapy [vasopressor use, continuous renal replacement therapy (CRRT) use, and duration of IMV], major comorbidities [sepsis, myocardial infarction (MI), congestive heart failure (CHF), hypertension, cerebrovascular disease (CD), chronic pulmonary disease (CPD), liver disease (LD), renal disease (RD), diabetes, and malignancy]; microbiology; results of the first laboratory tests after ICU admission [white blood cells (WBC), neutrophils, lymphocytes, basophils, eosinophils, monocytes, red blood cells (RBC), hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelets, anion gap (AG), bicarbonate, calcium total, calcium free, magnesium phosphate, chloride, sodium, potassium, base excess (BE), calculated total CO_2, pH, $PaCO_2$, PaO_2, lactate, creatinine, blood urea nitrogen (BUN), glucose, international normalized ratio (INR), prothrombin time (PT), partial thromboplastin time (PTT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), lactate dehydrogenase (LDH), total bilirubin, and albumin], and vital signs within 24 h of ICU admission [mean heart rate (mHR), mean value of mean arterial pressure (mMAP), mean respiratory rate (mRR), mean temperature (mT), mean SpO2(mSpO2), urine output].

The outcomes of this study were ICU and in-hospital mortality.

Statistical Analyses

In this study, Pearson's correlation coefficient was adopted to determine the correlation between characteristic variables and to remove strongly correlated variables (Zhang et al., 2022). The following variables had coefficients higher than 0.6, so were removed since they were considered strongly correlated: RBC, hematocrit, MCV, chloride, BE, calculated total CO_2, pH, BUN, PT, AST, and LD (**Supplementary Figure S1**). For other variables with less than 20% of missing values, the "mice" package of R software was used to perform multiple imputation.

The data for continuous variables were represented as mean and standard-deviation or median and interquartile (IQR) values, while those for categorical data were represented as frequencies. The Mann-Whitney U test was used for continuous variables, and the χ^2 test or Fisher's exact test was used for categorical variables.

The Kaplan-Meier (KM) method draws a cumulative incidence curve, indicating the occurrence of ICU and in-hospital deaths in different groups of patients, and the differences in risk between the groups were compared using log-rank tests. Furthermore, after adjusting different covariates, two Cox proportional-hazards models were constructed to analyze the influence of the relationship between bronchoscopy and outcomes. There were no adjustments for covariates in model I. In model II, all variables, including age, sex, race, BMI, first care unit, APSIII, vasopressors, CRRT, duration of IMV, sepsis, MI, CHF, hypertension, CD, CPD, LD, RD, diabetes, malignancy, WBC, neutrophils, lymphocytes, basophils, eosinophils, monocytes, hemoglobin, MCH, MCHC, RDW, platelet, AG, bicarbonate, calcium total, calcium free, magnesium phosphate, sodium, potassium, PaCO2, PaO2, lactate, creatinine, glucose, INR, PTT, ALT, AP, bilirubin total, albumin, mHR, mMAP, mRR, mT, mSpO2, urine output were

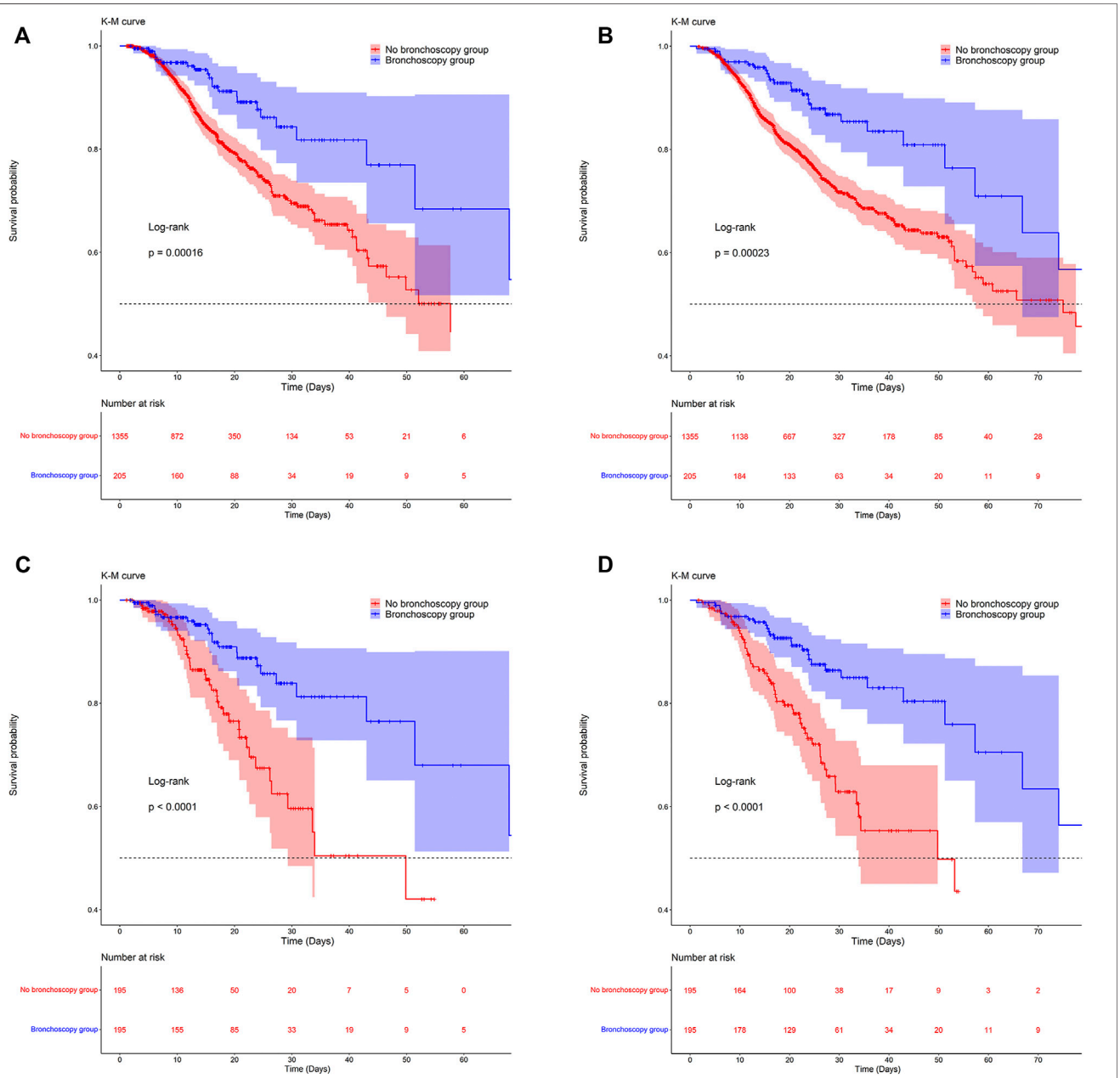

FIGURE 2 | (Continued).

adjusted for confounding factors. In the multivariate COX regression, we also evaluated the multicollinearity between the variables using variance inflation factors (VIF). **Supplementary Table S1** revealed that the VIF of each variable was less than 4, indicating that there was no multicollinearity between them.

To ensure that the results were stable and reliable, we further adjusted for covariates using propensity score matching (PSM) and propensity score-based inverse probability of treatment (IPTW) after analyzing the original population. Multivariate logistic regression model was used to estimate patient propensity scores by using one-to-one nearest neighbor matching with a caliper width of 0.05. The IPTW model was created using estimated propensity scores as weights. Differences in baseline levels between the two groups were evaluated using *p* values. Then, COX regression was performed on the matched population and the weighted population, respectively.

Causal mediation analysis (CMA) can distinguish the total effect of treatment into direct effects and indirect effects, If the independent variable X has a certain influence on the dependent variable Y through a certain variable M, then M is called the mediating variable of X and Y (Imai et al., 2010). In the present study, we hypothesized that changes in a particular indicator were mediating variables, that is, we assumed that bronchoscopy might lead to changes in that indicator and that such changes were

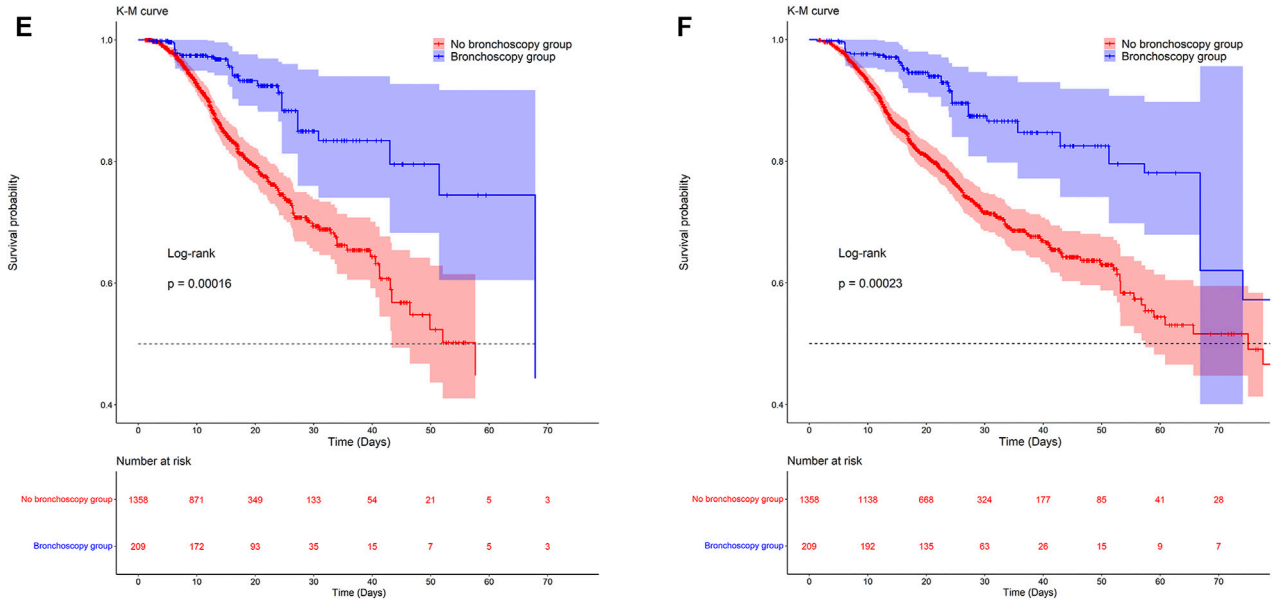

FIGURE 2 | Kaplan-Meier survival curves between groups. **(A,C,E)** are the ICU mortality risk for the original population, the PSM population and the IPTW population; **(B,D,F)** are the in-hospital mortality risk for the original population, the PSM population and the IPTW population.

TABLE 2 | Results of Cox proportional hazard models.

Outcomes	Model I		Model II	
	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Original population				
ICU Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.44 (0.29,0.68)	<0.001	0.33 (0.20,0.55)	<0.001
In-hospital Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.49 (0.33,0.72)	<0.001	0.40 (0.26,0.60)	<0.001
After PSM				
ICU Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.36 (0.21,0.61)	<0.001	0.33 (0.17,0.64)	0.001
In-hospital Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.38 (0.24,0.59)	<0.001	0.33 (0.20,0.58)	<0.001
After IPTW				
ICU Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.37 (0.23,0.60)	<0.001	0.26 (0.14,0.50)	<0.001
In-hospital Mortality				
Bonchoscopy				
no	Reference		Reference	
yes	0.42 (0.27,0.65)	<0.001	0.35 (0.21,0.57)	<0.001

associated with the prognosis of VAP patients. The average causal mediating effect (ACME), average direct effect (ADE) and total effect obtained through CMA can help us verify the above conjecture. Finally, WBC, platelets and RDW, three indicators that were further investigated for the presence of mediating effects because of their low absence rate after the cessation of IMV. Their change level was expressed as the difference between the corresponding first examination results and recorded as delta-WBC, delta-RDW, delta-platelet.

We also analyzed the impact of bronchoscopy on the prognoses of patients from different subgroups. The subgroups included age (dichotomized at 65 years), sex, type of first care unit, APSIII (dichotomized at the median of 69), and all comorbidities. The interactions between subgroups were further analyzed.

A two-tailed probability value of $p < 0.05$ was considered statistically significant. All statistical analyses in this study were performed using R software (version 4.1.0).

RESULTS

Baseline Characteristics

This study enrolled 1,560 patients with VAP: 1,355 in the no-bronchoscopy group and 205 in the bronchoscopy group (**Figure 1**). The median (IQR) ages of the patients in these two groups were 64.00 years (53.00–75.00 years) and 62.00 years (50.00–74.00 years), respectively; there were more male patients in the two groups (62.7 and 65.9%, respectively); the median (IQR) APSIII were 69.00 (51.00–90.00) and 70.00 (56.00–91.00), respectively; the median (IQR) durations of IMV were 136.00 h (60.50–253.50 h) and 145.00 h (59.00–286.00 h), respectively; and sepsis was the most common complication, present in 96.5 and 97.6% of patients in the two groups, respectively. The remaining baseline characteristics of the patients are listed in detail in **Table 1**.

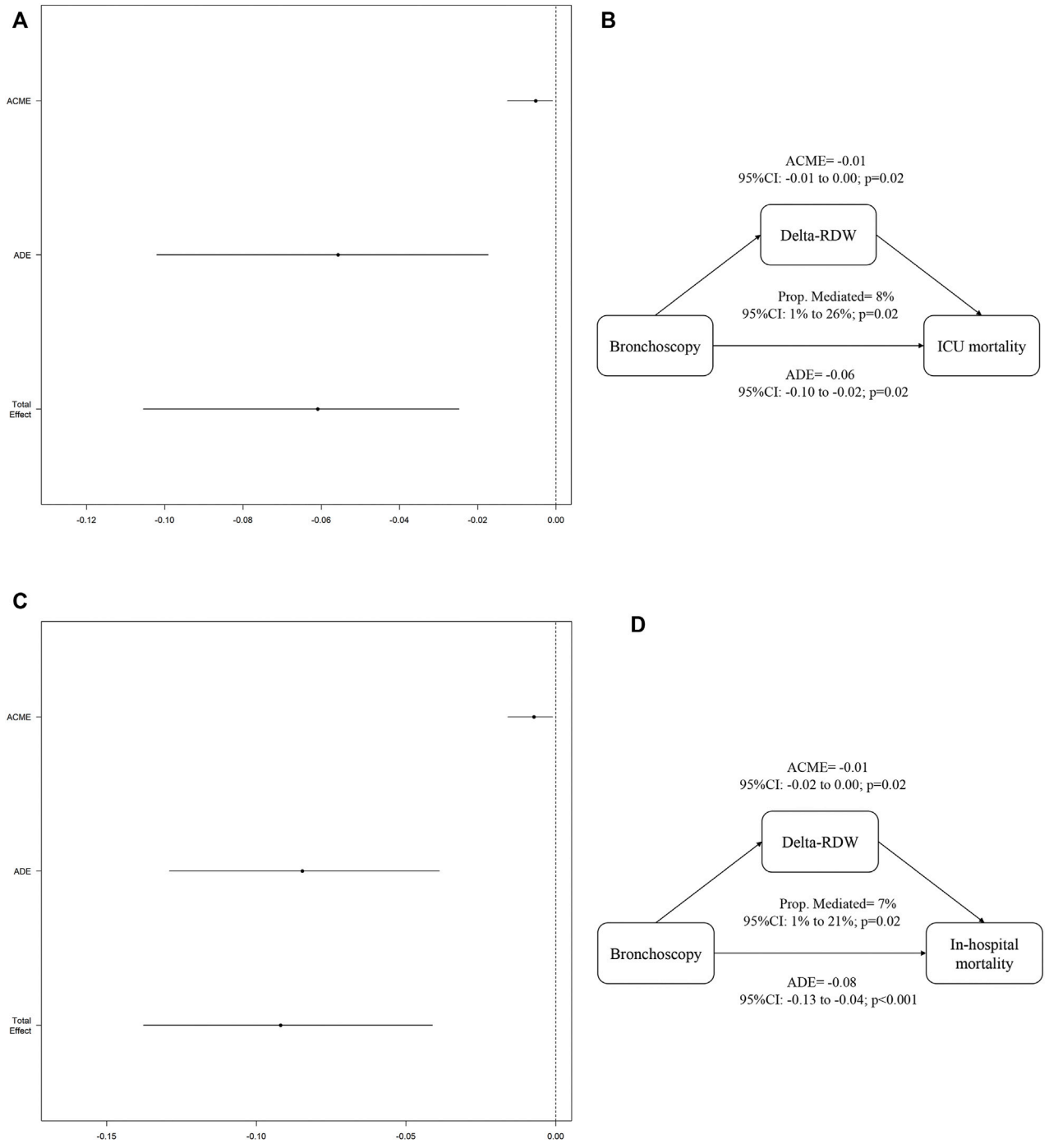

FIGURE 3 | Causal mediation analysis for RDW reduction.

Survival Analysis and Cox Proportional-Hazards Regression Model

The KM survival curve in **Figure 2** indicated a significant difference in survival probability between the two groups. The survival probability in the ICU and in hospital was significantly higher in the bronchoscopy group than in the no-bronchoscopy group (**Figure 2**). The results of the log-rank test indicated that the mortality risks in the ICU and in hospital differed between the two groups.

Table 2 lists the results for the Cox proportional-hazards model. In two models with no adjustment for confounders, or adjustment for all confounders, the hazard ratio (HR) was significantly lower than one for the bronchoscopy group compared with the no-bronchoscopy

TABLE 3 | Subgroup analysis of relationship between groups and mortality.

	ICU Mortality			Hospital Mortality		
	HR (95%CI)	*p*-value	p-interaction	HR (95%CI)	*p*-value	p-interaction
Age			0.773			0.442
<65 (*n* = 791)	0.61 (0.26,1.44)	0.261		0.59 (0.28,1.25)	0.167	
≥65 (*n* = 769)	0.28 (0.15,0.53)	<0.001		0.34 (0.20,0.57)	<0.001	
Gender			0.055			0.150
Male (*n* = 985)	0.23 (0.11,0.46)	<0.001		0.32 (0.18,0.55)	<0.001	
Female (*n* = 575)	0.36 (0.17,0.82)	0.014		0.47 (0.23,0.92)	0.029	
First care unit			0.941			0.576
MICU/SICU (*n* = 1,181)	0.32 (0.18,0.56)	<0.001		0.35 (0.22,0.57)	<0.001	
Others (*n* = 379)	0.30 (0.09,1.03)	0.056		0.44 (0.16,1.16)	0.097	
APSIII			0.381			0.390
<69 (*n* = 763)	0.49 (0.18,1.36)	0.170		0.40 (0.17,0.93)	0.032	
≥69 (*n* = 797)	0.30 (0.17,0.54)	<0.001		0.36 (0.22,0.59)	<0.001	
Microbiology			0.162			0.597
Gram positive (*n* = 290)	0.46 (0.08,2.49)	0.369		0.36 (0.11,1.20)	0.095	
Gram negative (n = 627)	0.27 (0.11,0.64)	0.003		0.35 (0.18,0.68)	0.002	
Sepsis						
No (*n* = 52)	NA			NA		
Yes (*n* = 1,508)	0.33 (0.20,0.55)	<0.001		0.39 (0.25,0.59)	<0.001	
Myocardial infarct			0.007			0.118
No (*n* = 1,279)	0.44 (0.26,0.75)	0.002		0.44 (0.28,0.70)	<0.001	
Yes (*n* = 281)	0.21 (0.05,0.86)	0.031		0.24 (0.07,0.82)	0.022	
Congestive heart failure			0.207			0.647
No (*n* = 1,048)	0.45 (0.24,0.84)	0.012		0.39 (0.23,0.67)	<0.001	
Yes (*n* = 512)	0.17 (0.07,0.42)	<0.001		0.31 (0.15,0.61)	<0.001	
Hypertension			0.317			0.725
No (*n* = 834)	0.22 (0.10,0.48)	<0.001		0.35 (0.20,0.63)	<0.001	
Yes (*n* = 726)	0.33 (0.16,0.68)	0.003		0.28 (0.14,0.56)	<0.001	
Cerebrovascular disease			0.240			0.223
No (*n* = 1,167)	0.28 (0.15,0.51)	<0.001		0.40 (0.25,0.64)	<0.001	
Yes (*n* = 393)	0.31 (0.09,1.02)	0.054		0.34 (0.13,0.89)	0.028	
Chronic pulmonary disease			0.004			0.151
No (*n* = 1,102)	0.55 (0.30,1.01)	0.053		0.48 (0.28,0.82)	<0.001	
Yes (n = 458)	0.13 (0.05,0.34)	<0.001		0.16 (0.07,0.36)	<0.001	
Renal disease			0.563			0.556
No (*n* = 1,215)	0.29 (0.16,0.54)	<0.001		0.36 (0.21,0.59)	<0.001	
Yes (*n* = 345)	0.36 (0.12,1.08)	0.069		0.50 (0.22,1.13)	0.096	
Liver disease			0.015			0.379
No (*n* = 1,272)	0.24 (0.13,0.45)	<0.001		0.36 (0.22,0.57)	<0.001	
Yes (*n* = 288)	0.64 (0.18,2.27)	0.491		0.56 (0.21,1.50)	0.246	
Diabetes			0.563			0.549
No (*n* = 1,082)	0.38 (0.21,0.68)	0.001		0.43 (0.27,0.69)	<0.001	
Yes (*n* = 478)	0.36 (0.11,0.94)	0.039		0.16 (0.07,0.36)	<0.001	
Malignant cancer						
No (n = 1,410)	0.25 (0.14,0.44)	<0.001		0.33 (0.21,0.53)	<0.001	
Yes (n = 150)	NA			NA		

group. In other words, compared with patients in the no-bronchoscopy group, those in the bronchoscopy group had a lower risk of ICU and in-hospital mortality. In model II, after adjusting for all covariates as confounding factors, the HR (95% CI) values for ICU and in-hospital mortality in the bronchoscopy group were 0.33 (0.20–0.55) and 0.40 (0.26–0.60), respectively, indicating that the risks of ICU and in-hospital mortality were 0.67 and 0.60 lower than in the no-bronchoscopy group. (**Table 2**).

Propensity Score Matching and Inverse Probability of Treatment Weighing

After PSM and IPTW, there was no significant difference in baseline levels between the two groups (**Supplementary Table S2**). Kaplan-Meier survival curves of matched and weighted populations are consistent with the original population (**Figure 2**). Univariate and multivariate COX regressions were then performed on the matched and weighted populations, respectively, yielding results consistent with the original population (**Table 2**). After multivariate COX regression, HR (95% CI) values for ICU mortality in the bronchoscopy group were 0.33 (0.17,0.64) and 0.26 (0.14,0.50), and in-hospital mortality the HR (95% CI) values of 0.33 (0.20,0.58) and 0.35 (0.21,0.57), respectively.

Causal Mediation Analysis

In the CMA analysis, after analyzing the three indicators, only the changes of RDW were significant. **Figures 3A, B** showed that in terms of in-hospital mortality of VAP patients, delta-RDW mediated 8% (95% CI:1–26%; $p = 0.02$) of the beneficial effect

of bronchoscopy (ACME:$p = 0.02$). At the same time, **Figures 3C, D** implied that in terms of ICU mortality, delta-RDW mediated 7% (95% CI: 1–21%; $p = 0.02$) of the beneficial effects of bronchoscopy (ACME:$p = 0.02$).

Subgroup Analysis

There was no significant difference between the subgroups of in-hospital mortality, meaning that no interaction was present. However, bronchoscopy was associated with a reduced risk of ICU mortality in MI and CPD populations, with HRs (95% CI) of 0.21 (0.05–0.86) and 0.13 (0.05–0.34), respectively. Meanwhile, patients without LD were more likely to benefit from bronchoscopy than those with LD (HR = 0.29, 95% CI = 0.16–0.54) (**Table 3**).

DISCUSSION

Few previous studies have investigated the prognostic effects of bronchoscopy among patients with VAP, with many studying the value of bronchoscopy for diagnosing or preventing VAP (Timsit et al., 2001), or limited to specific populations such as trauma or pediatric patients (Bush, 2003; Nannapaneni et al., 2021). The present study included patients with VAP from the large public MIMIC-IV database as the study population. In this present study, we took the VAP patients in the large public database MIMIC-IV as the study population, whether it was univariate analysis, or adjusted for a number of confounding factors such as demography, intervention, disease severity score, complications, vital signs, laboratory examination indicators, etc., and reached a consistent conclusion, that is, in patients with IMV in ICU, the risk of ICU mortality and in-hospital mortality in VPA patients who underwent bronchoscopy were significantly lower than those who did not receive bronchoscopy. In addition, after PSM and IPTW, the trend was consistent with that of the original population, which proved that our results were robust and reliable.

We believe that most VAP patients in the ICU are critically ill patients with severe diseases such as sepsis (Markwart et al., 2020), with unclear self-consciousness (Hübscher and Isenmann, 2016) and poor sputum expectoration ability (Tang et al., 2011), causing large amounts of sputum or respiratory secretions cannot be discharged and block the trachea, aggravating the condition. At the same time, there are a large number of inflammatory factor secretions in the patient's airway, forming a cascade amplification effect, which can spread to cause systemic inflammation (Zaragoza et al., 2020), which seriously affects the patient's prognosis. While bronchoscopy can go deep into the lower respiratory tract and directly reach the lungs with the most severe disease (Estella, 2012). When patients are undergoing IMV, bronchoscopy can not only help suck out the obstructions such as sputum and foreign bodies in the trachea, remove secretions, but also repeatedly suction and wash the lungs, which is of great significance for reducing inflammation and improving lung ventilation.

RDW is a parameter that reflects the heterogeneity of red blood cells in the blood, and is also a new marker of inflammation (Hou et al., 2018). At present, there are a lot of published evidences indicating that there is an inseparable connection between RDW and inflammatory response (Fava et al., 2019; Jandaghian et al., 2021). The inflammatory response causes red blood cell maturation disorder through iron metabolism disorder and erythropoietin destruction, resulting in immature red blood cells into the bloodstream, but also reduce the survival rate of red blood cells, resulting in the mixing of red blood cell volume in circulation and other ways to change RDW (Salvagno et al., 2015). And some other studies have also shown that RDW is related to some conventional inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate, tumor necrosis factor-α and interleukin-6 (He et al., 2018; Fava et al., 2019). In the CMA analysis, we have demonstrated that the beneficial effects of bronchoscopy on VAP patients are partially mediated by changes in RDW levels. Bronchoscopy is also essential for clinical targeted antimicrobial therapy to control inflammation, as it helps to obtain more accurate etiology, resulting in better diagnostic information. The findings of Christopher et al. showed that diagnostic bronchial therapy can help reduce the use of antibiotics and shorten the length of hospital stay (Guidry et al., 2014). Another retrospective study also indicated that the correct use of bronchoscopy can help intensive care clinicians formulate specific antibacterial treatments (Allen et al., 1994). Therefore, we can think that the reason why bronchoscopy can improve the prognosis of VAP patients is that whether it is aspiration of sputum or getting more accurate pathogens to help the adjustment of antibiotics, they all help to improve the inflammatory response of patients to some extent. Some scholars' study has shown that early bronchoscopy is associated with a lower 90-days mortality rate in mechanically ventilated patients, which also supports our findings (Lee et al., 2015).

The results of the present subgroup analysis indicated no significant interaction between the subgroups regarding in-hospital mortality, indicating that whether the ICU patient population with different characteristics underwent bronchial examination was consistent with the in-hospital mortality risk. Among the outcomes for ICU mortality, after bronchoscopy, patients with MI or CPD had a lower mortality risk than those who did not undergo bronchoscopy. When bronchoscopy is performed on patients in the ICU without LD, the mortality risk was lower than for those with LD. We will explain it step by step, chronic obstructive pulmonary disease and bronchiectasis are common chronic lung diseases chronic obstructive pulmonary disease and bronchiectasis are common chronic lung diseases (Raherison and Girodet, 2009; Hill et al., 2019), such patients are prone to infection and inflammation due to dysfunctional airway clearance and defense, and long-term chronic inflammation leads to bronchial epithelial cell degeneration, necrosis, bronchial scarring, distortion, impaired lung function, and sputum accumulation, which are more likely to occur in the presence of acute inflammation (Tantucci and Modina, 2012; Hill et al., 2019). There is research evidence that the hemodynamic changes in patients with MI will lead to varying degrees of pulmonary function changes (Hales and Kazemi, 1977), and there is a pathophysiological basis for the interaction between some chronic lung diseases and MI (Goedemans et al., 2020). Therefore, we can consider that when VAP is present, bronchoscopy is more likely to benefit in patients with chronic inflammatory disease of the lungs and varying degrees of changes in lung function. The liver can synthesize and remove most coagulation factors, plasminogen,

etc., and so LD can lead to coagulation and anticoagulation balance disorders (Amitrano et al., 2002; Monroe and Hoffman, 2009). One of the main complications of bronchoscopy is tracheal mucosal bleeding, so patients without LD are more likely to benefit from bronchoscopy than those with LD.

STRENGTHS AND LIMITATIONS

This research study was the first to explore the entire patients with VAP in the ICU from MIMIC-IV database, the large data sample provides a solid basis for the results. Furthermore, PSM and IPTW further confirmed the results. We also found that alterations in RDW mediated part of the positive effect of bronchoscopy on the prognosis of VAP patients by CMA methods. The technology for bronchoscopy is currently quite mature, and the results of this study provide evidence to support the guidance of clinicians for using this technique. Of course, our research still had certain limitations. First of all, because the specific diagnosis time of patients is not recorded in the analyzed database, this study cannot determine the duration between bronchoscopy and VAP, only how bronchoscopy impacts the prognoses of patients with VAP who received mechanical ventilation. Because of this we were also unable to analyze the specific antibiotics for VAP treatment, and so we did not consider the impact of antibiotic use on the outcome. Second, although we adopted multivariate analysis to control for confounding factors that may affect the results as much as possible, there are still potential confounding factors that cannot be corrected in a retrospective study. Finally, the interaction between subgroups needs to be analyzed prospective in a larger sample of people for further confirmation.

CONCLUSION

Bronchoscopy during MV was associated with reducing the risk of ICU and in-hospital mortality in patients with VAP in ICUs, and this beneficial effect was partially mediated by changes in RDW levels.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: The data were available on the MIMIC-IV website at https://mimic.physionet.org/, https://doi.org/10.13026/a3wn-hq05.

AUTHOR CONTRIBUTIONS

LZ created the study protocol, performed the statistical analyses and wrote the first manuscript draft. SL conceived the study and wrote the first manuscript draft. SY assisted with manuscript revision and data confirmation. XL assisted with the study design. JiL assisted with data collection. YL and TH guided the literature review. JuL and HY conceptualized the research aims. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (No. 82072232; 81871585), the Natural Science Foundation of Guangdong Province (No. 2018A030313058), Technology and Innovation Commission of Guangzhou Science, China (No. 201804010308), Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.868920/full#supplementary-material

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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.

GLOSSARY

AST aspartate aminotransferase
ALT alanine aminotransferase
AP alkaline phosphatase
APSIII Acute Physiology Score III
AG anion gap
BE base excess
BUN blood urea nitrogen
ACME average causal mediating effect
ADE average direct effect
BMI body mass index
CRRT continuous renal replacement therapy
CHF congestive heart failure
CD cerebrovascular disease
CPD chronic pulmonary disease
LD liver disease
CMA causal mediation analysis
LDH lactate dehydrogenase
IMV invasive mechanical ventilation
ICU intensive care unit
INR international normalized ratio
KM Kaplan-Meier
VIF variance inflation factor
IPTW propensity score-based inverse probability of treatment
MCV mean corpuscular volume
MCH mean corpuscular hemoglobin
MCHC mean corpuscular hemoglobin concentration
MICU Medical Intensive Care Unit
MIMIC Medical Information Mart for Intensive Care
MI myocardial infarction
mHR mean heart rate
mMAP mean value of mean arterial pressure
mRR mean respiratory rate
mT mean temperature
mSpO2 mean SpO2()
PT prothrombin time
PTT partial thromboplastin time
PSM propensity score matching
RD renal disease
RBC red blood cells
RDW red blood cell distribution width
SICU Surgical Intensive Care Unit
VAP: ventilator-associated pneumonia
WBC white blood cells

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ORIGINAL RESEARCH
published: 13 June 2022
doi: 10.3389/fphar.2022.869499

The Use of Antibiotics for Ventilator-Associated Pneumonia in the MIMIC-IV Database

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Edited by:
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 04 February 2022
Accepted: 30 May 2022
Published: 13 June 2022

Citation:
Yang R, Huang T, Shen L, Feng A, Li L, Li S, Huang L, He N, Huang W, Liu H and Lyu J (2022) The Use of Antibiotics for Ventilator-Associated Pneumonia in the MIMIC-IV Database. Front. Pharmacol. 13:869499. doi: 10.3389/fphar.2022.869499

Purpose: By analyzing the clinical characteristics, etiological characteristics and commonly used antibiotics of patients with ventilator-associated pneumonia (VAP) in intensive care units (ICUs) in the intensive care database. This study aims to provide guidance information for the clinical rational use of drugs for patients with VAP.

Method: Patients with VAP information were collected from the Medical Information Mart for Intensive Care IV (MIMIC-IV) database, including their sociodemographic characteristics, vital signs, laboratory measurements, complications, microbiology, and antibiotic use. After data processing, the characteristics of the medications used by patients with VAP in ICUs were described using statistical graphs and tables, and experiences were summarized and the reasons were analyzed.

Results: This study included 2,068 patients with VAP. Forty-eight patient characteristics, including demographic indicators, vital signs, biochemical indicators, scores, and comorbidities, were compared between the survival and death groups of VAP patients. Cephalosporins and vancomycin were the most commonly used. Among them, fourth-generation cephalosporin (ForGC) combined with vancomycin was used the most, by 540 patients. First-generati49n cephalosporin (FirGC) combined with vancomycin was associated with the highest survival rate (86.7%). More than 55% of patients were infected with Gram-negative bacteria. However, patients with VAP had fewer resistant strains (<25%). FirGC or ForGC combined with vancomycin had many inflammation-related features that differed significantly from those in patients who did not receive medication.

Conclusion: Understanding antibiotic use, pathogenic bacteria compositions, and the drug resistance rates of patients with VAP can help prevent the occurrence of diseases, contain infections as soon as possible, and promote the recovery of patients.

Keywords: ventilator-associated pneumonia, antibiotics, pathogenic bacteria, MIMIC-IV database, resistance rate

Abbreviations: VAP, ventilator-associated pneumonia; ICUs, Intensive Care Units; MIMIC, Medical Information Mart for Intensive Care; ForGC, forth-generation of cephalosporin; FirGC, first-generation of cephalosporin; ForGC + Van, ForGC combined with vancomycin; FirGC + Van, FirGC combined with vancomycin.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is a common hospital-acquired infection and is the most common complication of mechanical ventilation for patients in intensive care units (ICUs) (Legras et al., 1998; Urli et al., 2002). Once VAP presents, it further aggravates the condition and seriously harms the prognosis of the patient. The VAP incidence in ICUs has been reported to range from 5.0% to as high as 67%, and the mortality rate of patients with VAP exceeds 14% (Kalil et al., 2016; Pozuelo-Carrascosa et al., 2018). The general hospitalization time of patients with VAP is also longer, as is the time required for intensive care, which also increases the economic burden on both patients and the medical system (Kollef et al., 2012; Vandana Kalwaje and Rello, 2018). In order to avoid the increased incidence of diseases and the consumption of more medical resources caused by subjective deviation in clinical diagnosis or treatment, it is very important to formulate more accurate and more detailed clinical diagnosis and treatment specifications. The particular focus of recent clinical research has been on how to apply more appropriate drug interventions to patients with VAP in a timely and accurate manner.

The most commonly used drugs for clinically treating patients diagnosed with VAP are antibiotics, especially cephalosporins and vancomycin (Tseng et al., 2012; Yue et al., 2015; Torres et al., 2017). However, medical staff often prioritize antibacterial treatment for patients based on their personal experience. Improving the rationality, safety, and effectiveness of clinical medications, and converting empirical treatment into targeted treatments as soon as possible, will help medical staff to determine more objective medical treatments for patients with VAP.

To provide a reference for guiding rational clinical drug use in patients with VAP, this study analyzed patients with VAP in ICUs from an intensive care medical database using factors such as clinical characteristics, pathogenic bacteria distribution in respiratory secretions and their drug resistance characteristics, and changes in inflammatory response indicators.

MATERIALS AND METHODS

Data Source

The Medical Information Mart for Intensive Care (MIMIC) database was funded in 2003 by Beth Israel Deaconess Medical Center (BIDMC), Massachusetts Institute of Health, and the National Institutes of Health Technology, Massachusetts General Hospital, emergency room physicians, critical care physicians, computer science experts, and other professional critical care medicine database. MIMIC is the largest open source and free clinical database in the critical care and emergency department, based on BIDMC's intensive care inpatient system. MIMIC-IV (version 1.0) is the latest version, which contains data from 2008 to 2019 (Guo et al., 2021; Lu et al., 2021; Song et al., 2021). We completed the courses required to use the database and obtained the corresponding certificate. The requirement for individual patient consent was waived because the project did not impact clinical care and all protected health information was anonymized.

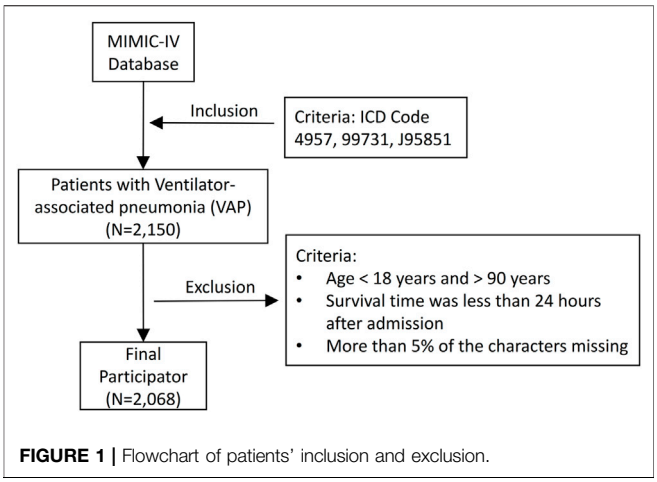

FIGURE 1 | Flowchart of patients' inclusion and exclusion.

Study Population and Data Extraction

We used the official MIMIC-IV tutorial to construct the study database using PostgreSQL (version 13.0, PostgreSQL Global Development Group). Structured Query Language was used to extract the data of the patients, which included sociodemographic characteristics, vital signs, laboratory measurements, complications, and microbiology and antibiotic use information (Yang et al., 2020; Wu et al., 2021). ICD-9 codes (4957 and 99,731) and ICD-10 code (J95851) were used to identify patients with VAP in the MIMIC-IV database. Data from the first hospital admission (if a patient had been admitted multiple times) was included in the study. If the data of a patient were measured multiple times, we used the first measurement. The survival rates in this article refer to in-hospital survival rates. The patient enrollment process of this study is illustrated in **Figure 1**.

Statistical Analysis

Univariate analyses were applied to all of the study variables. Shapiro-Wilk tests were used to assess the distribution of variables. Non-normally distributed continuous variables were reported as medians with interquartile ranges (IQRs), and the Kruskal–Wallis rank-sum test or the Mann-Whitney U test were used to compare these data. All classified variables were expressed as numbers and percentages, and they were compared using chi-square or Fisher's exact tests. Values in continuous variables that exceed 1.5 times the upper and lower quartiles are judged as outliers and the outliers are deleted. The Numpy (version 1.19.5) and Pandas (version 1.1.5) packages were used for data computation. **Supplementary Figure S1** shows which variables were absent. The "miceforest" (version 2.0.5) package of Python was used for the multiple interpolation of missing values (Zhao et al., 2021).

TABLE 1 | Basic characteristics of patients with VAP.

Characteristics	Overall (N = 2068)	Alive (N = 1608)	Death (N = 460)	*p* value
Age, years	64.0 (53.0, 74.0)	63.0 (51.0, 73.0)	69.0 (59.0, 79.0)	<0.001
Sex, *n*				0.490
Female	783 (37.9)	602 (37.4)	181 (39.3)	
Male	1285 (62.1)	1006 (62.6)	279 (60.7)	
Race, *n*				<0.001
Asian	62 (3.0)	38 (2.4)	24 (5.2)	
White	1216 (58.8)	963 (59.9)	253 (55.0)	
Hispanic	65 (3.1)	53 (3.3)	12 (2.6)	
Black	247 (11.9)	207 (12.9)	40 (8.7)	
Other	478 (23.1)	347 (21.6)	131 (28.5)	
Frist Service, *n*				0.561
Medical	906 (43.8)	704 (43.8)	202 (43.9)	
Surgical	139 (6.7)	107 (6.7)	32 (7.0)	
Thoracic Surgical	50 (2.4)	43 (2.7)	7 (1.5)	
Other	973 (47.1)	754 (46.9)	219 (47.6)	
First Care Unit, *n*				<0.001
CCU/CVICU	416 (20.1)	301 (18.7)	115 (25.0)	
MICU/SICU	1139 (55.1)	880 (54.7)	259 (56.3)	
NSICU	103 (5.0)	77 (4.8)	26 (5.7)	
TSICU	410 (19.8)	350 (21.8)	60 (13.0)	
Admission Type, *n*				0.335
Elective	44 (2.1)	31 (1.9)	13 (2.8)	
Urgent	505 (24.4)	389 (24.2)	116 (25.2)	
Emergency	1214 (58.7)	941 (58.5)	273 (59.3)	
Other	305 (14.7)	247 (15.4)	58 (12.6)	
BMI, kg/m^2	29.0 (25.1, 34.3)	29.1 (25.4, 34.6)	28.5 (24.1, 33.3)	0.004
Heart Rate, bpm	90.0 (76.0, 106.0)	90.0 (76.0, 106.0)	91.5 (77.0, 108.0)	0.174
Systolic Blood Pressure, mmHg	121.0 (103.0, 139.0)	121.0 (104.0, 139.0)	119.0 (101.8, 138.0)	0.086
Respiratory Rate, insp/min	20.0 (16.0, 24.0)	20.0 (16.0, 24.0)	20.0 (16.0, 24.0)	0.250
Temperature Fahrenheit, °F	98.4 (97.8, 99.0)	98.4 (97.8, 99.1)	98.3 (97.7, 98.8)	<0.001
Arterial O2 pressure, mmHg	114.0 (82.0, 185.0)	113.0 (82.0, 187.0)	115.0 (83.8, 182.2)	0.983
Arterial CO2 Pressure, mmHg	41.0 (35.0, 48.0)	41.0 (35.0, 48.0)	39.0 (33.0, 46.0)	<0.001
Creatinine, mg/dl	1.0 (0.7, 1.5)	1.0 (0.7, 1.4)	1.2 (0.8, 1.8)	<0.001
Characteristics	**Overall (N = 2068)**	**Alive (N = 1608)**	**Death (N = 460)**	***p* value**
Urea Nitrogen, mg/dl	21.0 (14.0, 33.0)	20.0 (13.0, 31.0)	26.0 (17.0, 40.0)	<0.001
Glucose, mg/dl	134.0 (109.0, 170.0)	134.0 (108.0, 169.0)	136.0 (111.0, 172.0)	0.415
RDW, %	14.9 (13.7, 16.6)	14.7 (13.6, 16.4)	15.3 (13.9, 17.2)	<0.001
RBC, m/μl	3.4 (2.9, 4.0)	3.4 (2.9, 4.1)	3.3 (2.8, 3.9)	0.003
WBC, K/μl	11.7 (8.4, 15.7)	11.6 (8.4, 15.4)	12.1 (8.5, 16.9)	0.102
Lymphocytes, %	9.4 (5.9, 14.3)	9.8 (6.1, 14.9)	8.5 (5.0, 12.0)	<0.001
Monocytes, %	5.5 (3.7, 7.8)	5.5 (3.7, 7.8)	5.3 (3.2, 7.7)	0.048
Neutrophils, %	81.0 (74.1, 86.2)	80.3 (73.9, 86.0)	82.8 (76.0, 87.1)	0.001
Basophils, %	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	0.2 (0.2, 0.4)	0.001
Eosinophils, %	1.0 (0.4, 2.0)	1.0 (0.4, 2.2)	1.0 (0.4, 2.0)	0.041
pH, units	7.4 (7.3, 7.4)	7.4 (7.3, 7.4)	7.4 (7.3, 7.4)	0.609
Lactate, mmol/L	1.6 (1.1, 2.4)	1.5 (1.1, 2.4)	1.7 (1.3, 2.5)	<0.001
Anion Gap, mEq/L	14.0 (12.0, 17.0)	14.0 (12.0, 17.0)	15.0 (13.0, 18.0)	0.001
ALT, IU/L	27.0 (16.0, 45.0)	27.0 (17.0, 44.0)	28.0 (16.0, 46.0)	0.842
ALP, IU/L	82.0 (59.0, 111.0)	80.5 (58.8, 110.0)	85.0 (61.0, 116.0)	0.023
AST, IU/L	39.0 (25.0, 66.0)	38.0 (25.0, 65.0)	43.0 (27.0, 76.0)	0.007
LDH, IU/L	287.5 (219.0, 406.0)	283.0 (215.0, 400.0)	314.0 (231.0, 453.2)	<0.001
Albumin, g/dl	2.9 (2.5, 3.3)	2.9 (2.6, 3.4)	2.8 (2.4, 3.3)	<0.001
Hemoglobin, g/dl	10.2 (8.6, 12.0)	10.3 (8.7, 12.1)	10.0 (8.4, 11.7)	0.017
Platelet Count, K/μl	196.0 (136.8, 261.0)	198.5 (140.0, 264.0)	182.5 (121.8, 250.0)	0.003
INR	1.2 (1.1, 1.4)	1.2 (1.1, 1.4)	1.3 (1.2, 1.5)	<0.001
PT, sec	13.7 (12.4, 15.7)	13.6 (12.4, 15.5)	14.3 (12.8, 16.3)	<0.001
PTT, sec	30.5 (27.1, 36.1)	30.2 (26.9, 35.7)	31.5 (27.5, 37.4)	<0.001
SOFA Score	1.0 (0.0, 4.0)	1.0 (0.0, 3.0)	2.0 (1.0, 5.0)	<0.001

(Continued on following page)

TABLE 1 | (*Continued*) Basic characteristics of patients with VAP.

Characteristics	Overall (N = 2068)	Alive (N = 1608)	Death (N = 460)	*p* value
Respiratory Secretions, *n*				0.522
Positive	987 (47.7)	774 (48.1)	213 (46.3)	
Negative	1081 (52.3)	834 (51.9)	247 (53.7)	

Nonnormal continuous variables were presented as Median (IQR). Categorical variables were presented as number (precentage %). CCU, coronary care unit; CVICU, cardiac vascular intensive care unit; MICU, medical intensive care unit; SICU, surgical intensive care unit; NSICU, neuro surgical intensive care unit; TSICU, traume surgical intensive care unit; BMI, body mass index; RDW, red blood cell volume istribution width; RBC, red blood count; WBC, write blood count; pH, hydrogen ion concentration; ATL, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate transaminase; LDH, lactate dehydrogenase; PT, prothrombin time; PTT, partial thromboplastin time; INR, international normalized ratio; SOFA, score, Sequential Organ Failure Assessment.

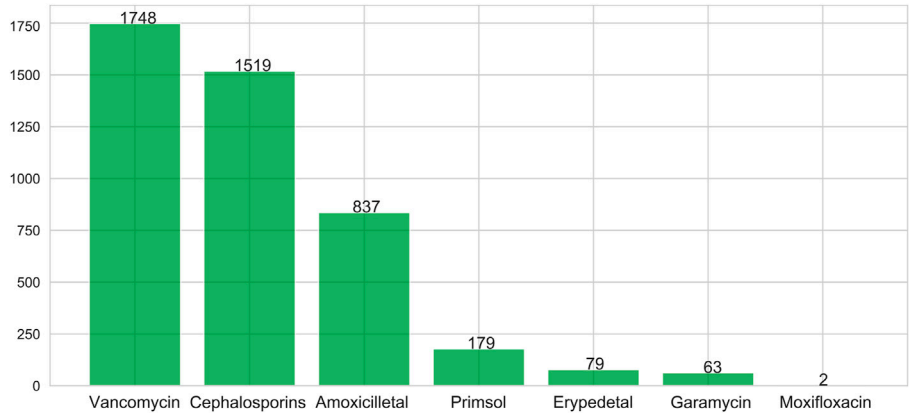

FIGURE 2 | Antibiotic used in patients with VAP.

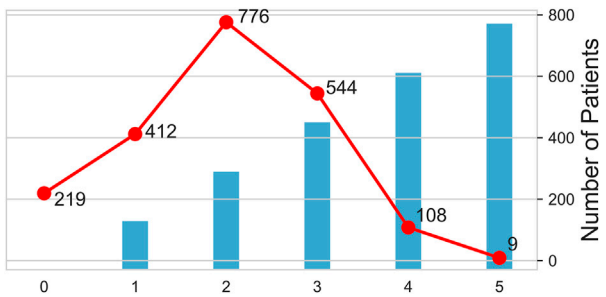

FIGURE 3 | Pattern and number of antibiotics used. The columns show the number of antibiotics used, and the broken lines shows the number of people taking drugs. The horizontal axis shows the number of antibiotics used. 0 indicates that no antibacterial drugs have been used, one indicates that only one antibacterial drug has been used, and two indicates the combination of two antibacterial drugs, and so on for 3, 4, and 5.

Statistical plots (include column charts, line charts, Venn diagrams, correlation charts, etc.) were used to present data distributions, and statistical tables are used to describe differences between patients. Seaborn (version 0.11.2) and Matplotlib (version 3.3.4) packages were used to graphically display data. Python software (version 3.7.0) was used for all statistical analyses. A probability value of $p < 0.05$ in two-sided tests was considered significant.

RESULTS

This study included 2,068 patients with VAP (**Table 1**), among whom 1,608 (77.8%) survived and 460 (22.2%) died. The patients were aged 64.0 years (53.0–74.0 years) [median (IQR)], and the survival group was younger than the death group [63.0 years (51.0–73.0 years) vs. 69.0 years (59.0–79.0 years)]. The patients had a BMI of 29.0 kg/m^2 (25.1–34.3 kg/m^2), which was lower in the death group than in the survival group [28.5 kg/m^2 (24.1–33.3 kg/m^2) vs. 29.1 kg/m^2 (25.4–34.6 kg/m^2)]. The first admission reason of thoracic surgery was the least common, accounting for only 2.4% of cases. VAP presented in different ICUs, most commonly in medical/surgical ICUs (55.1%). Patients admitted through emergency departments accounted for 58.7% of cases. Except for diabetes, the other eight complications showed statistically significant differences between survival group and death group (**Supplementary Table S1**).

Statistics of the application of antibiotics in all patients with VAP are shown in **Figure 2**. The most common used was vancomycin, followed by cephalosporins. Eight patients had used the fifth generation of cephalosporin, which was too few to be included in the analyses. In the subsequent analysis, antibiotic use therefore referred to using the first through to

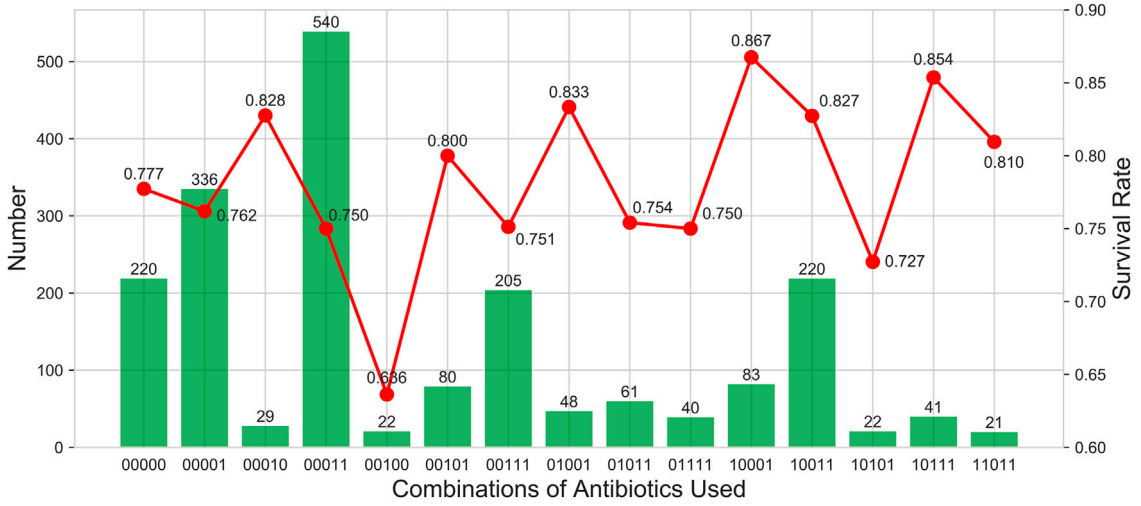

FIGURE 4 | Describe the use of antibiotics, the number of people and survival rates. The columns show the number of people who took drugs. The broken line shows the survival rate. The horizontal coordinate is a 5-digit sequence from left to right representing the first to fourth generations of cephalosporins and vancomycin. 0 indicates that it is not used, and one indicates that it is used.

TABLE 2 | Comparison of differences in inflammatory related characteristics. (a) FirGC + Vancomycin vs. Unused antibiotics; (b) ForGC + Vancomycin vs. Unused antibiotics.

(a) FirGC + Vancocin vs. Unused antibiotics

Inflammation Characteristics	Unused (N = 220)	FirGC + Vancomycin (N = 83)	*p* value
Temperature Fahrenheit, °F	98.3 (97.8,98.8)	98.3 (97.8,98.8)	0.468
Basophils, %	0.3 (0.2,0.5)	0.3 (0.2,0.4)	0.064
Eosinophils, %	1.6 (0.8,2.7)	1.0 (0.3,2.0)	0.002
Lymphocytes, %	10.4 (7.0,16.3)	10.8 (7.4,14.2)	0.578
Neutrophils, %	79.5 (71.9,84.8)	81.6 (75.8,85.8)	0.019
RBC, m/ul	3.3 (2.8,3.7)	3.3 (2.7,3.9)	0.646
WBC, K/ul	10.1 (7.3,13.8)	12.3 (9.1,15.5)	0.018
RDW, %	15.4 (14.0,17.0)	14.4 (13.6,15.4)	<0.001

(b) ForGC + Vancocin vs. Unused antibiotics

Inflammation Characteristics	Unused (N = 220)	ForGC + Vancomycin (N = 540)	*p* Value
Temperature Fahrenheit, °F	98.3 (97.8,98.8)	98.4 (97.7,99.1)	0.018
Basophils, %	0.3 (0.2,0.5)	0.3 (0.2,0.4)	0.003
Eosinophils, %	1.6 (0.8,2.7)	1.0 (0.4,2.0)	<0.001
Lymphocytes, %	10.4 (7.0,16.3)	8.9 (5.0,13.3)	<0.001
Neutrophils, %	79.5 (71.9,84.8)	81.3 (75.4,87.0)	<0.001
RBC, m/ul	3.3 (2.8,3.7)	3.4 (2.9,4.1)	0.002
WBC, K/ul	10.1 (7.3,13.8)	12.3 (8.5,16.3)	<0.001
RDW, %	15.4 (14.0,17.0)	15.0 (13.7,16.6)	0.026

the fourth generation of cephalosporins and vancomycin in patients with VAP.

Figure 3 shows how antibiotics were used and the corresponding number of people. The largest number of patients used two antibiotics in combination (n = 776), while the smallest number of patients used all five antibiotics (n = 9).

Supplementary Figure S2 shows the use of different antibiotics and their combinations in patients with VAP. The most common combination was using vancomycin and fourth-generation cephalosporin (ForGC) (n = 540). Vancomycin alone was used by 336 patients, while relatively few patients used cephalosporin antibiotics alone.

Supplementary Figure S3 shows the correlations between different antibiotics. The medical staff in the data source center most commonly used ForGC and vancomycin in combination, followed by first-generation cephalosporin (FirGC) and vancomycin.

TABLE 3 | Types and composition ratio of main pathogenic bacteria in respiratory tract specimens of patients with VAP treated with first-generation of cephalosporin combined with vancomycin.

Pathogenic bacteria	Number of strains (*n*)	Constituent ratio (%)
Fungus	19	4.44
Yeast	18	4.21
Mold	1	0.23
Gram Positive Bacteria	155	36.21
Staph Aureus Coag +	147	34.35
Streptococcus pneumoniae	6	1.40
Other	2	0.47
Gram Negative Bacteria	254	59.35
Enterobacteriaceae	138	32.24
Pseudomonas aeruginosa	49	11.45
Serratia marcescens	42	9.81
Klebsiella pneumoniae	12	2.80
Other	13	3.05
Total	428	100.00

We further counted the use of antibiotics, the number of people, and survival rates (**Figure 4**). The results indicated that ForGC combined with vancomycin (ForGC + Van) was the most common. Patients who used FirGC combined with vancomycin (FirGC + Van) had the highest survival rate (86.7%).

To explore the reasons behind medical staff using antibiotics, we compared the differences between FirGC + Van or ForGC + Van and no antibiotics (**Table 2**). This revealed that only four inflammation indicators of patients who used FirGC + Van differed from those in the no-antibiotics group, and that all inflammatory indicators of patients who used ForGC + Van differed from those in the no-antibiotics group.

Table 3 lists the distributions of pathogenic bacteria in respiratory tract specimens of patients with VAP treated using FirGC + Van. For FirGC + Van treatment, 428 pathogens were detected among 83 patients with VAP. These comprised 254 strains (59.35%) of Gram-negative bacteria, 155 strains (36.21%) of Gram-positive bacteria, and 19 strains (4.44%) of fungi. **Table 4** lists the resistance characteristics of these pathogens. The resistance rate to cephalosporin or vancomycin in respiratory tract specimens of patients with VAP treated using FirGC + Van was 0%.

Supplementary Table S2 presents the distribution of pathogenic bacteria in the respiratory tract specimens of patients with VAP treated using ForGC + Van. For ForGC + Van treatment, 2,320 pathogens were detected in 540 patients with VAP, comprising 1,505 strains (64.87%) of Gram-negative bacteria, 700 strains (30.17%) of Gram-positive bacteria, and 115 strains (4.96%) of fungi. **Supplementary Table S3** presents the drug resistance characteristics of Gram-positive bacteria in the respiratory tract specimens of patients with VAP treated using ForGC + Van. The results indicated that among 700 strains of Gram-positive bacteria, 163 (23.28%) were resistant, comprising 155 resistant strains of *Staphylococcus aureus* coagulase+, and eight resistant strains of *Streptococcus pneumoniae*. **Supplementary Table S4** presents the drug resistance characteristics of Gram-negative bacteria in the respiratory tract specimens of patients with VAP treated using. ForGC + Van. The results indicated that among 1,505 strains of Gram-negative bacteria, 147 were resistant, which included 46 strains resistant to cephalosporin or vancomycin, although the resistance was low (<2.4%).

DISCUSSION

This is the first study based on the clinical characteristics and medication characteristics of patients with VAP in the latest and largest open database in the field of severe and emergency medicine, aiming to have a deeper understanding of antibiotic use and bacterial infection in patients with VAP, and provide more medical evidence for drug intervention. This was achieved by understanding the distributions and drug resistance rates of pathogenic bacteria in patients with VAP, and further analyzing factors related to inflammatory characteristics. Formulating a more-scientific treatment plan would be helpful in reducing the occurrence of drug-resistant pathogenic bacteria. This has important clinical significance for diagnostic and prognostic evaluations of VAP.

TABLE 4 | Resistance of main Gram bacteria to different antibacterial drugs in respiratory tract specimens of patients with VAP treated with first-generation cephalosporin combined with vancomycin.

Antimicrobials	Gram positive bacteria				Gram Negative bacteria	
	Staph aureus coag + (N = 147)		*Streptococcus pneumoniae* (N = 6)		*Pseudomonas aeruginosa* (N = 49)	
	Number of resistant strains (*n*)	Resistant ratio (%)	Number of resistant strains (*n*)	Resistant ratio (%)	Number of resistant strains (*n*)	Resistant ratio (%)
Clidamycin	4	2.72	—		—	
Erythromycin	7	4.76	1	16.67	—	
Levofloxacin	4	2.72	—		—	
Oxacillin	5	3.40	—		—	
Penicillin	3	2.04	—		—	
Trimethoprim/Sulfa	—		1	16.67	—	
Meropenem	—		—		3	6.12

According to the significant difference between the survival group and the death group, such as sex, heart rate, systolic blood pressure, respiratory rate, arterial O_2 pressure, glucose, pH, ALT, positive/negative microorganisms in respiratory secretions showed no difference between the groups ($p > 0.05$). Sharpe et al. (2014) reported higher mortality in women with VAP, but our results and other studies suggest that gender is an irrelevant factor (de Miguel-Díez et al., 2017) for VAP. Other features with no difference between groups, such as heart rate, systolic blood pressure and respiratory rate, were the patients' vital signs; arterial O_2 pressure, glucose, pH, ALT, positive/negative microorganisms in respiratory secretions were the general blood gas, biochemical and microbiological status of patients. These characteristics may differ significantly before and after patients progress to VAP. This suggests that we need to further analyze the changes of these indicators when studying whether they are directly related to in-hospital death of VAP patients, and conclusion drawn from only one test may not be reliable. However, the characteristics that were significantly different between groups ($p < 0.05$), such as RDW, lymphocytes, monocytes, neutrophils, basophils, eosinophils and other inflammatory indicators; complications such as hypertension, liver disease, renal disease and cancer; biochemical indicators such as albumin and platelet count; and scores such as SOFA score reflected the types and severity of diseases in patients at admission, and their values showed that the diseases in the death group were more complex and severe than those in the survival group, which was consistent with clinical experience. BMI is a special indicator. According to our results, the BMI of the death group was lower than that of the survival group, which is consistent with the "obesity paradox" (Park et al., 2020), indicating that patients with higher BMI at admission may be more able to support the body's consumption of nutrients during the disease, and thus have a higher probability of survival.

The analysis results indicated that the most commonly used medications for patients with VAP were vancomycin and cephalosporins, which was consistent with the findings of [Agrafiotis et al., 2011; Bassetti et al., 2012; Zhang et al. (2019)]. This indicates that the clinical drug intervention measures (including diagnosis and treatment plans) for patients with VAP are currently relatively consistent between China and other countries. The results of the present study indicated that the composition of the primary pathogens in patients with VAP was consistent with that reported by [Giard et al., 2008; Shorr et al., 2009; Djordjevic et al. (2017); Kradin and Digumarthy, 2017]. This indicated that several pathogens (e.g., yeast, *Staphylococcus aureus* coagulase+, and *Pseudomonas aeruginosa*) invading the respiratory tract of patients were the main cause of lung damage, and so should receive attention from clinical medical staff. Patients in ICUs also often use ventilators more frequently than patients in conventional wards due to the severity of their medical conditions, which may also increase the occurrence of pathogenic bacteria, thereby aggravating lung damage, leading to VAP.

Vancomycin is a glycopeptide antibiotic widely used to treat infections caused by pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. It is more effective than cephalosporin antibiotics, exhibits no cross-resistance with other antibiotics, and very few strain are resistant to it (van der Veen et al., 2021). The present study also found that no strain was resistant to vancomycin. In addition, according to the results of this paper, most patients were co-infected with gram positive bacteria and gram negative bacteria, so the combined use of vancomycin and cephalosporin was consistent with the medication habits of clinicians.

The pathogens targeted by each generation of cephalosporins are different (Pomorska-Mól et al., 2015; Belley et al., 2021; Pilmis et al., 2021; Roach et al., 2021). FirGC was developed during 1962–1970. The antibacterial spectrum of FirGC has good effects on anti-Gram-positive, poor antistreptococcal effects, and a weak ability for blood–brain barrier penetration, meaning it is not suitable for central infections. Second-generation cephalosporins were mostly developed during 1970–1976, and their antibacterial effect on Gram-negative bacteria was superior to that of FirGC. The antibacterial spectrum of third-generation cephalosporins expanded on that of the second generation, but their effect on Gram-positive bacteria is weaker than that of FirGC. The recently developed ForGC has a broader antibacterial spectrum, which not only has a good antibacterial effect on Gram-negative bacteria, but also can resist *Staphylococcus aureus*.

Considering the research results of this study and the characteristics of different cephalosporins, we speculated that the patients treated with FirGC + Van would have the highest survival rate, which was due to the mild condition of these patients and the clearer bacterial flora of the infection, so it would have the highest cure rate. Patients were most commonly treated using ForGC + Van. This might be due to these two drugs having the broadest antibacterial spectrum, therefore making them suitable for a wider range of patients. It is the first choice for clinical antibiotic treatment for patients with VAP infected with different bacteria or with more complex flora. However, the survival rate of patients treated using ForGC + Van was low. This reminds us that in order to maximize the survival of patients with VAP, we need to be aware of the occurrence of drug abuse and accurately administer drugs at the appropriate time.

We therefore suggest that early treatment of patients with VAP should employ more adaptable broad-spectrum antibiotic therapies based on the clinical symptoms of the patient. Later, as the understanding of pathogen drug resistance increases, more sensitive antibiotics should be used. In clinical practice, doctors should designate more personalized and precise drug intervention programs for patients, and make timely adjustments to the dosage, treatment course, and antibiotic administration so as to improve the recovery of patients with VAP.

Inflammation-related indicators of patients can assist in diagnosing VAP occurrence and the rational use of drugs (Stoeppel et al., 2014; Nobahar et al., 2016; Moradi Moghaddam et al., 2019). However, the results of this study did not show that the median and IQR values of inflammatory indicators in the medication group were significantly higher

than the median and IQR values of inflammatory indicators in the untreated group. This may be attributed to the high sensitivity but poor specificity of the inflammatory response index. No significant increase was observed in the median and IQR values. This may be because the data used were from the first admission of patients with VAP, when the inflammatory responses of the patients were still in their initial stages. Although there were significant differences between groups, the value of inflammatory indicators did not change significantly.

The results of this study on the drug resistance of pathogens indicated that the included patients with VAP had poor pathogen resistance, meaning that antibiotic treatment was suitable and would have a better curative effect, which affects the mortality of patients with VAP. This further indicates that the medication experience of the medical institution or center of the study data can be used as a reference by other medical institutions.

This study inevitably had some limitations. First, the MIMIC-IV database includes mostly white patients. Therefore, due to genetic differences, the results described in this study may need further confirmation in different populations. Second, we did not attempt to analyze the factors that may lead to VAP occurrence and the factors that may affect the prognosis of patients with VAP. These work will be performed as one of the main focuses of our next study.

CONCLUSION

This study has described the basic clinical characteristics, pathogen compositions, drug resistances, and antibiotic use of patients with VAP in ICUs from the MIMIC-IV database. The findings provide a reference path and theoretical basis for formulating more reasonable drug intervention measures for patients with VAP.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: https://mimic.mit.edu/.

AUTHOR CONTRIBUTIONS

RY, TH, and LS conceived of and designed the work. TH, AF, and LL acquired and check the data. RY and TH performed statistical and computational analyses. LS, WH, SL, and LL provided professional clinical analyses. RY, LS, and TH drafted the work. LH and NH assisted with manuscript revision and data confirmation. All authors participated in interpretation of the data and substantively revised the work.

FUNDING

This work was supported by the Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007) and the Scientific research project of Guangdong Bureau of Traditional Chinese Medicine (No.20221106).

ACKNOWLEDGMENTS

We appreciate the researchers at the MIT Laboratory for Computational Physiology for publicly sharing of the MIMIC-IV clinical database.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.869499/full#supplementary-material

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frontiers | Frontiers in Pharmacology

ORIGINAL RESEARCH
published: 20 June 2022
doi: 10.3389/fphar.2022.921422

Effect of Stress Ulcers Prophylaxis, Sedative and Statin on Ventilator-Associated Pneumonia: A Retrospective Analysis Based on MIMIC Database

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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: *15 April 2022*
Accepted: *30 May 2022*
Published: *20 June 2022*

Citation:
Kong X, Wu Y, Wen B, Meng D, Wei L and Yu P (2022) Effect of Stress Ulcers Prophylaxis, Sedative and Statin on Ventilator-Associated Pneumonia: A Retrospective Analysis Based on MIMIC Database. Front. Pharmacol. 13:921422. doi: 10.3389/fphar.2022.921422

Background: The use of MV can easily lead to VAP especially in ICU patients. SUP, sedatives, statin and insulin have been proved to prevent VAP and improve the prognosis of patients. Our aim was to analyze the effects of SUP, sedative, statin, and insulin on patients with MV.

Methods: The occurrence of VAP and death in MV patients and VAP patients were explored by multivariate logistic regression and Cox regression to analyze analyses.

Results: Totally, 5277 cases who received MV in ICU from MIMIC IV database were included. There were 826 (15.7%) cases in VAP-group and 4451 (84.3%) cases in non-VAP group and there were 1914 (36.3%) cases in hospital mortalities altogether. No protective effect of drugs on VAP was found in MV patients. The risk of death was 1.43 times higher in MV patients taking midazolam than in propofol (aHR = 1.43 95% CI: 1.04,1.97). No protective effect of drugs on death was found in VAP patients.

Conclusion: Compared with midazolam, propofol is more recommended as sedation regimen in ICU patients with MV. Further high-quality studies are needed to confirm this finding.

Keywords: mechanical ventilation, ventilator-associated pneumonia, MIMIC IV database, stress ulcers prophylaxis, sedative, statin

Abbreviations: MV, mechanical ventilation; VAP, ventilator-associated pneumonia; SAPS II, simplified acute physiology score II; CCI, Charlson comorbidity Index; PPI, proton pump inhibitors; H2RA, Histamine2-receptor antagonist; MRSA, methicillin resistant Staphylococcus aureus; aOR, adjusted odds ration; aHR, Adjusted Hazard Ratio.

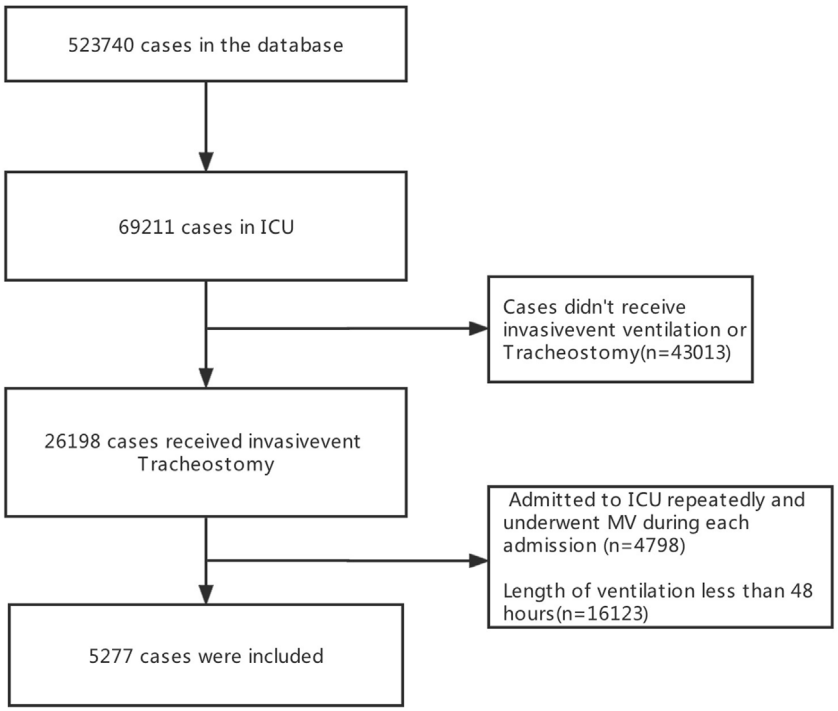

FIGURE 1 | Flowchart of screening phases.

BACKGROUND

Ventilator-associated pneumonia (VAP) is the second most common nosocomial infection in ICU, with an incidence rate ranging from 8%–28% and a mortality rate ranging from 8%–76% (Vincent et al., 1995; Rello et al., 2002), and patients in intensive care unit (ICU) with VAP are 1–9 times more likely to die. If the patients admitted to the intensive care unit (ICU) are complicated with VAP, the mortality rate will increase by 1–9 times (Celik et al., 2012; Yamada et al., 2012). The presence of a tracheal tube is the most important risk factor for the development of VAP. The existence of the catheter can interfere with the normal protective upper airway reflexes and inhibits effective coughing, leading to the inhalation of contaminated oropharyngeal secretions and gradually developing into pulmonary infection lesions (Adair et al., 1999; Zolfaghari and Wyncoll, 2011; Hunter, 2012). Therefore, the chance of pneumonia increases when the duration of MV is prolonged. The methods of treating VAP in ICU mainly include antibiotic therapy, sputum suction and nutritional support, but most patients have a poor prognosis (Luo et al., 2021). Since no effective drugs have been found to treat VAP, it has become the focus of attention to find drugs that can prevent the occurrence of VAP or improve the prognosis of patients with VAP.

Oversedation can cause serious adverse reactions to patients, such as cardiorespiratory depression, decreased gastrointestinal motility, immunosuppression and unnecessary prolongation of MV, thus, indirectly increasing the risk of infection. Undersedation may result in hypertension, tachycardia and severe discomfort (Garrett, 2016). Therefore, choosing an appropriate sedation regimen is crucial. Propofol, dexmedetomidine and midazolam are common sedative drugs used in clinical practice. A preliminary study showed that dexmedetomidine reduced the incidence of coma and insanity in patients and shortened the duration of MV compared to propofol or midazolam (Pandharipande et al., 2007). Another meta-analysis found that propofol improved clinical outcomes in the ICU and reduced time to extubation in critically ill patients compared with midazolam (Garcia et al., 2021). The results of the above studies have been inconsistently described and no definitive conclusions have been made regarding the effect of the three sedation regimens on patient sedation and prognosis.

ICU patients are at risk for upper gastrointestinal stress ulcer bleeding, and patients requiring long-term MV are at higher risk (Peura, 1987). Therefore, stress ulcer prevention (SUP) is generally considered to be the standard of care in the ICU (Krag et al., 2013). SUP drugs usually include proton pump inhibitors (PPI), Histamine2-receptor antagonist (H2RA) and sucralfate. A previous study compared the efficacy of ranitidine and sucralfate in patients receiving MV over 48 h. The investigators found a significant decrease in the rate of clinically significant bleeding for patients receiving ranitidine, accompanied by a nonsignificant increase in VAP (Cook et al., 1998). A randomized, open-label, crossover clinical trial reported a higher mortality rate in the PPI group (18.3%) than in the H2RA group, although the difference was not statistically significant (Young et al., 2020). The potential benefits and

TABLE 1 | Baseline characteristics of mechanical ventilation cases. (n = 5277).

	VAP group (*N* = 826)	Non-VAP group (*N* = 4451)	Overall (*N* = 5277)	*p*-value
Age				
Median [min, max]	65.1 [19.6, 96.9]	65.5 [18.0, 98.7]	65.4 [18.0, 98.7]	0.468
Gender				
Male	512 (62.0%)	2506 (56.3%)	3018 (57.2%)	**0.003**
Female	314 (38.0%)	1945 (43.7%)	2259 (42.8%)	
Ethnicity				
American Indian/Alaska native	2 (0.2%)	12 (0.3%)	14 (0.3%)	0.999
Asian	24 (2.9%)	132 (3.0%)	156 (3.0%)	
Black/African American	89 (10.8%)	491 (11.0%)	580 (11.0%)	
Hispanic/Latino	27 (3.3%)	156 (3.5%)	183 (3.5%)	
White	481 (58.2%)	2698 (60.6%)	3179 (60.2%)	
Missing	203 (24.6%)	962 (21.6%)	1165 (22.1%)	
SAPS II				
Median [min, max]	42.0 [6.00, 107]	43.0 [6.00, 107]	43.0 [6.00, 107]	**0.014**
SOFA				
Median [min, max]	9.00 [0, 21.0]	9.00 [0, 23.0]	9.00 [0, 23.0]	0.246
CCI				
Median [min, max]	6.00 [0, 15.0]	6.00 [0, 19.0]	6.00 [0, 19.0]	0.084
Diagnoses				
Respiratory	126 (15.3%)	668 (15.0%)	794 (15.0%)	0.762
CNS	20 (2.4%)	108 (2.4%)	128 (2.4%)	
Liver	10 (1.2%)	71 (1.6%)	81 (1.5%)	
Renal	7 (0.8%)	46 (1.0%)	53 (1.0%)	
Diabetes	4 (0.5%)	22 (0.5%)	26 (0.5%)	
Trauma	57 (6.9%)	254 (5.7%)	311 (5.9%)	
Other	468 (56.7%)	2654 (59.6%)	3122 (59.2%)	
Missing	134 (16.2%)	628 (14.1%)	762 (14.4%)	
Acinetobacter baumannii				
N	799 (96.7%)	4412 (99.1%)	5211 (98.7%)	<**0.001**
Y	27 (3.3%)	39 (0.9%)	66 (1.3%)	
Pseudomonas aeruginosa				
N	701 (84.9%)	4168 (93.6%)	4869 (92.3%)	<**0.001**
Y	125 (15.1%)	283 (6.4%)	408 (7.7%)	
Klebsiella pneumoniae				
N	741 (89.7%)	4234 (95.1%)	4975 (94.3%)	<**0.001**
Y	85 (10.3%)	217 (4.9%)	302 (5.7%)	
Escherichia coli				
N	739 (89.5%)	4098 (92.1%)	4837 (91.7%)	**0.016**
Y	87 (10.5%)	353 (7.9%)	440 (8.3%)	
MRSA				
N	769 (93.1%)	4187 (94.1%)	4956 (93.9%)	0.322
Y	57 (6.9%)	264 (5.9%)	321 (6.1%)	
Stenotrophomonas maltophilia				
N	777 (94.1%)	4348 (97.7%)	5125 (97.1%)	<0.001
Y	49 (5.9%)	103 (2.3%)	152 (2.9%)	
WBC				
Median [min, max]	9.85 [0.100, 54.5]	9.80 [0.100, 208]	9.80 [0.100, 208]	0.522
Missing	0 (0%)	6 (0.1%)	6 (0.1%)	
INR				
Median [min, max]	1.30 [0.900, 27.4]	1.30 [0.800, 15.2]	1.30 [0.800, 27.4]	0.439
Missing	4 (0.5%)	60 (1.3%)	64 (1.2%)	
Lactate				
Median [min, max]	1.50 [0.300, 21.6]	1.60 [0, 28.2]	1.60 [0, 28.2]	<**0.001**
Missing	33 (4.0%)	210 (4.7%)	243 (4.6%)	
SUP				
PPI	130 (15.7%)	714 (16.0%)	844 (16.0%)	**0.015**
H2RA	135 (16.3%)	646 (14.5%)	781 (14.8%)	
PPI or Sucralfate	5 (0.6%)	20 (0.4%)	25 (0.5%)	
H2 or Sucralfate	0 (0%)	2 (0.0%)	2 (0.0%)	
PPI or H2RA	68 (8.2%)	203 (4.6%)	271 (5.1%)	
PPI, H2RA or Sucralfate	0 (0%)	1 (0.0%)	1 (0.0%)	
Missing	488 (59.1%)	2865 (64.4%)	3353 (63.5%)	

(Continued on following page)

TABLE 1 | (*Continued*) Baseline characteristics of mechanical ventilation cases. (n = 5277).

	VAP group (*N* = 826)	Non-VAP group (*N* = 4451)	Overall (*N* = 5277)	*p*-value
Sedative				
Propofol	106 (12.8%)	649 (14.6%)	755 (14.3%)	<**0.001**
N	507 (61.4%)	2977 (66.9%)	3484 (66.0%)	
Dexmedetomidine	7 (0.8%)	26 (0.6%)	33 (0.6%)	
Midazolam	19 (2.3%)	67 (1.5%)	86 (1.6%)	
Dexmedetomidine or Propofol	86 (10.4%)	335 (7.5%)	421 (8.0%)	
Midazolam or Propofol	46 (5.6%)	247 (5.5%)	293 (5.6%)	
Dexmedetomidine, Midazolam or Propofol	55 (6.7%)	150 (3.4%)	205 (3.9%)	
Statin				
N	701 (84.9%)	3930 (88.3%)	4631 (87.8%)	**0.007**
Y	125 (15.1%)	521 (11.7%)	646 (12.2%)	
Insulin				
N	599 (72.5%)	3446 (77.4%)	4045 (76.7%)	**0.003**
Y	227 (27.5%)	1005 (22.6%)	1232 (23.3%)	
Antibiotic				
N	18 (2.2%)	263 (5.9%)	281 (5.3%)	<**0.001**
Single antibiotic	35 (4.2%)	516 (11.6%)	551 (10.4%)	
Combined antibiotics	773 (93.6%)	3672 (82.5%)	4445 (84.2%)	
Vasopressor				
N	596 (72.2%)	3097 (69.6%)	3693 (70.0%)	0.149
Y	230 (27.8%)	1354 (30.4%)	1584 (30.0%)	
Length of Ventilation (day)				
Median [min, max]	5.68 [2.00, 52.5]	3.70 [2.00, 85.3]	3.92 [2.00, 85.3]	<**0.001**
Length of ICU stays (day)				
Median [min, max]	13.5 [2.33, 79.0]	8.11 [2.10, 99.6]	8.83 [2.10, 99.6]	<**0.001**
In-hospital mortalities				
N	574 (69.5%)	2789 (62.7%)	3363 (63.7%)	<**0.001**
Y	252 (30.5%)	1662 (37.3%)	1914 (36.3%)	

SAPS II, Simplified acute physiology score; SOFA, Sequential Organ Failure Assessment; CCI, Charlson comorbidity Index; CNS, Central nervous system; MRSA, Methicillin-resistant Staphylococcus; WBC, White blood cell; INR, International Normalized Ratio; PPI, Proton pump inhibitors; H2RA, Histamine2-receptor antagonist; SUP, stress ulcer prevention; VAP, Ventilator-associated pneumonia; N, No; Y, Yes.

harms of PPI, H2RA and sucralfate for the prevention of stress ulcers in critically ill patients still require further analysis.

In addition to their proven ability to regulate blood lipids, statins have been shown to have various other pharmacological effects on independent cholesterol pathways, such as antithrombotic and anti-inflammatory effects (Mortensen et al., 2005; van de Garde et al., 2006; Chalmers et al., 2008). Based on this principle, many studies have linked them to VAP, aiming to explore the benefits of statins in ICU patients. A previously published meta-analysis of cohort studies showed that preadmission statin use is associated with beneficial outcomes in critically ill patients, including lower short-term mortality and less use of MV (Yu et al., 2021a). In contrast, another study concluded that in adults with suspected VAP, adjunctive simvastatin therapy compared with placebo did not improve day-28 survival, and VAP patients cannot benefit from it (Papazian et al., 2013a). There are conflicting results regarding the benefit of pleiotropic effects of statins in ICU patients.

Patients admitted to the ICU often have hyperglycemia and insulin resistance in stressful situations even in the absence of a history of diabetes, and significant blood glucose elevations are associated with a worsening prognosis in critically ill patients, mainly including stroke, myocardial infarction, head trauma and postoperative wound infection (Krinsley, 2004). Two previous reports by Van den Berghe stated that intensive insulin therapy (IIT) reduced patient mortality and morbidity in surgical ICU patients, and decreased the risk of death in 767 patients with hospital stay ≥3 days (van den Berghe et al., 2001; Van den Berghe et al., 2006). In contrast, another recently published study showed that intensive insulin therapy was not associated with improved survival in patients in medical and surgical ICUs and was associated with an increased incidence of hypoglycemia (Arabi et al., 2008). Despite the above disputes and concerns, people still tend to make strict blood glucose control in critically ill patients a major treatment goal.

Based on previous studies, we found that the use of sedative drugs, SUP, statins and insulin may be candidates for the prevention and treatment of VAP patients, but the application of drugs has been controversial due to inconsistent results of the research. Therefore, we conducted this retrospective study to verify the effects of the above drugs on patients in ICU.

MATERIALS AND METHODS

Study Design and Participants

Cross-sectional research was conducted in this study. The data was sourced from the MIMIC IV database from 2008 to 2019 which was downloaded from (https://mimic.physionet.org/) (Johnson et al., 2021). The database contains more than 40000 ICU patient medical data from Beth Israel Deaconess Medical Center. We gained access to the MIMIC IV database files upon

TABLE 2 | Logistic regression analysis of VAP in MV patients. (n=5277)

Covariate	aOR (95%CI)	*p*-value
Gender		**0.023**
Male	reference	
Female	0.73 (0.56,0.96)	
SAPS II	1.00 (0.99,1.01)	0.535
CCI	0.98 (0.94,1.03)	0.528
Acinetobacter baumannii		0.070
N	reference	
Y	2.48 (0.93,6.61)	
Pseudomonas aeruginosa		**0.008**
N	reference	
Y	1.79 (1.17,2.76)	
Klebsiella pneumoniae		**0.002**
N	reference	
Y	2.03 (1.29,3.18)	
Escherichia coli		0.377
N	reference	
Y	1.23 (0.78,1.92)	
Stenotrophomonas maltophilia		0.719
N	reference	
Y	1.14 (0.56,2.31)	
WBC	1.00 (0.98,1.01)	0.800
Lactate	0.93 (0.88,0.99)	**0.017**
SUP		0.880
PPI	reference	
H2RA	1.11 (0.83,1.47)	0.487
PPI or Sucralfate	1.54 (0.54,4.37)	0.414
H2 or Sucralfate	NA	NA
PPI or H2RA	1.23 (0.85,1.8)	0.275
PPI, H2RA or Sucralfate	NA	NA
Sedative		0.210
Propofol	reference	
N	0.94 (0.54,1.65)	0.835
Dexmedetomidine	1.61 (0.65,4.02)	0.304
Midazolam	1.54 (0.81,2.9)	0.190
Dexmedetomidine or Propofol	1.26 (0.9,1.76)	0.187
Midazolam or Propofol	0.84 (0.56,1.27)	0.419
Dexmedetomidine, Midazolam or Propofol	1.42 (0.93,2.16)	0.108
Statin		0.161
N	reference	
Y	1.22 (0.92,1.62)	
Insulin		0.684
N	reference	
Y	0.94 (0.71,1.25)	
Antibiotic		**0.005**
N	reference	
Single	1.14 (0.38,3.4)	0.817
Combined	2.59 (1.01,6.62)	**0.047**
Length of Ventilation (day)	1.01 (0.97,1.04)	0.720
Length of ICU stays (day)	1.07 (1.04,1.09)	<**0.001**
In-hospital mortalities		0.314
N	reference	
Y	0.86 (0.64,1.16)	

aOR (95%CI), adjusted Odds ratio (95% confidence interval).

completion of the required training (COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE). (Record ID: 47402444).

Eligible participants were age ≥18 years and the duration of MV ≥ 48 h. Patients with multiple mechanical ventilation at different ICU admissions were excluded. To analyze the risk factors of VAP, all patients will be divided into VAP group and non-VAP group and drugs were taken as the independent variable. Because all patient data was de-identified, informed consent was waived.

Data Collection

Baseline clinical data were extracted from the first admission including age (year), sex, ethnicity (American Indian/Alaska National, Asian, black/African American, Hispanic/Latino, white), simplified acute physiology score II (SAPSII), Charlson comorbidity Index (CCI),

TABLE 3 | Survival analysis of patients with mechanical ventilation.

Covariate	aHR(95%CI)	*p*-value
Age	1.01 (1.00,1.01)	**0.024**
Gender		0.147
Male	reference	
Female	1.12 (0.96,1.30)	
SAPS II	0.99 (0.99,1.00)	**0.0281**
SOFA	1.01 (0.99,1.04)	0.210
CCI	1.07 (1.04,1.10)	<**0.001**
Diagnoses		0.580
Respiratory	reference	
CNS	0.71 (0.42,1.22)	0.218
Liver	0.80 (0.47,1.36)	0.412
Renal	0.90 (0.51,1.57)	0.706
Diabetes	0.58 (0.18,1.86)	0.357
Trauma	0.94 (0.70,1.27)	0.705
Other	0.82 (0.65,1.04)	0.099
Pseudomonas aeruginosa		0.202
N	reference	
Y	0.81 (0.59,1.12)	
Stenotrophomonas maltophilia		0.181
N	reference	
Y	0.75 (0.50,1.14)	
WBC	1.02 (1.01,1.02)	<**0.001**
INR	1.10 (1.03,1.17)	**0.006**
Lactate	1.08 (1.06,1.10)	<**0.001**
SUP		0.256
PPI	reference	
H2RA	1.17 (0.99,1.38)	0.0712
PPI or Sucralfate	0.93 (0.51,1.70)	0.811
H2 or Sucralfate	2.25 (0.55,9.15)	0.256
PPI or H2RA	1.25 (1.01,1.55)	**0.043**
PPI, H2RA or Sucralfate	NA	NA
Sedative		<**0.001**
Propofol	reference	
N	1.99 (1.53,2.58)	<**0.001**
Dexmedetomidine	0.75 (0.41,1.40)	0.370
Midazolam	1.43 (1.04,1.97)	**0.026**
Dexmedetomidine or Propofol	0.66 (0.53,0.81)	<**0.001**
Midazolam or Propofol	1.13 (0.91,1.39)	0.272
Dexmedetomidine, Midazolam or Propofol	0.56 (0.42,0.76)	<**0.001**
Statin		0.305
N	reference	
Y	1.09 (0.92,1.29)	
Insulin		0.098
N	reference	
Y	0.87 (0.74,1.03)	
Antibiotic		**0.044**
N	reference	
Single	0.53 (0.30,0.93)	**0.027**
Combined	0.8 0 (0.50,1.26)	0.332
Vasopressor		<**0.001**
N	reference	
Y	1.91 (1.61,2.26)	
Length of Ventilation (day)	1.15 (1.12,1.18)	<**0.001**
VAP		0.927
N	reference	
Y	0.99 (0.80,1.22)	
Length of ICU stays (day)	0.85 (0.83,0.86)	<**0.001**

aHR (95%CI), adjusted Hazard Ratio (95% confidence interval); p-value < 0.05 is highlighted with bold values.

clinical disease classification (respiratory, circulation, CNS, liver, renal, diabetes, trauma, other). Leukocyte count, INR, and lactate were extracted from the first day of MV. We extracted bacterial infection [*Acinetobacter* baumannii, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, methicillin resistant Staphylococcus aureus (MRSA), Stenotrophomonas *maltophilia*]. And drugs (antiplatelet, insulin, sedative, statin, PPI, H2, sucralfate) during MV. We also extracted the length of hospital stay (day), ICU duration (day), and ventilation duration (day). The clinical classification of diseases is based on clinical classifications software suggested by the agency for healthcare research and quality (AHRQ) (Cowen et al., 1998).

Outcomes

The primary outcomes were incidence of VAP and VAP patients' mortalities and the secondary outcome was MV patients' mortalities. The definition of VAP is based on the International Classification of Diseases 9th edition and International Classification of Diseases 10th edition (ICD-9: 4957 and 99731; ICD-10: J95851) (World, 2004). The observation period began on the date MV of started and it end at the date of discharge or in-hospital death of the patient.

Statistical Analysis

Wilcoxon rank sum test was for non-normally distributed data. We used Chi-square and Fisher's exact test (when the expected value of data was lower than 5) for categorical variables. Several descriptive statistics, including medians and interquartile ranges, are presented. We select confounder variables for multivariable analysis if they had a $p \leq 0.1$ on univariate analysis. Multivariable logistic regression was used to analyze the VAP independent risk factor and odds ratio (OR), 95% confidence intervals (CI), and p-value were presented. The Cox regression model was used to analyze the factor affecting MV patient survival and VAP patient survival. Statistical analyses were performed in R version 4.1.2.

RESULTS

Baseline Characteristics

23566 patients were screened in the database and 5277 patients were finally included in the present study (**Figure 1**). There were 826 (15.7%) patients with VAP and 4451 (84.3%) patients were non-VAP. Among all patients, the median age was 65.4years, 3018 (57.2%) were male, 3179 (60.2%) were White, 794 (15.0%) were diagnosed with respiratory diseases, the median stays in ICU were 8.83 days, and 1914 (36.3%) died in hospital. (**Table 1**).

Comparison of Characteristics Between VAP Group and Non-VAP Group

The gender of the VAP group and the non-VAP group was different, the SAPS II score of the VAP group was lower than that of the non-VAP group (42.0 vs. 43.0, $p < 0.014$). VAP Patients detected *Acinetobacter* baumannii, *Pseudomonas aeruginosa*, *Klebsiella* pneumonia, *Escherichia coli*, and Stenotrophomonas *maltophilia* more than non-VAP patients (3.3 *vs.* 0.9%, $p < 0.001$; 15.1 *vs.* 6.4%, $p < 0.001$; 10.3 *vs.* 4.9%, $p < 0.001$; 10.5 *vs.* 7.9%, $p = 0.016$; 5.9 *vs.* 2.3%, $p < 0.001$). The lactate of the VAP group was lower than that of the non-VAP group (1.50 *vs.* 1.60, $p < 0.001$). The use of sedatives and stress ulcer prophylaxis was different

between the VAP group and the non-VAP group. More statins users in VAP group (15.1 *vs*. 11.7%, $p = 0.007$). More insulin users in VAP group (27.5 *vs*. 22.6%, $p = 0.007$). The use of antibiotics was different between the VAP group and the non-VAP group. The length of ventilation of VAP patients was longer than that of non-VAP patients (5.68 *vs*. 3.70, $p < 0.001$). The length of ICU stays of VAP patients was longer than that of non-VAP patients (13.5 *vs*. 8.11, $p < 0.001$). And the in-hospital mortalities in VAP group was lower (30.5 *vs* 37.3%, $p < 0.001$). (**Table 1**).

Influence of Drugs on VAP in MV Patients

After adjustment for confounders, there was no statistically significant difference in VAP risk between PPI and H2RA (aOR = 1.11 95% CI: 0.83, 1.47). There was also no statistically significant difference in the risk of VAP between propofol, dexmedetomidine and midazolam (aOR = 1.61, 95% CI: 0.65,4.02; aOR = 1.54, 95% CI: 0.81,2.9). Taking statins did not significantly increase risk factor for VAP (aOR = 1.22 95% CI: 0.92, 1.62). Insulin also did not significantly reduce the risk of VAP (aOR = 0.94 95% CI: 0.71,1.25). (**Table 2**).

TABLE 4 | Survival analysis of VAP patients. (n = 826).

Covariate	aHR (95%CI)	*p*-value
Age	1.01 (0.99,1.03)	0.259
SAPS II	1.00 (0.98,1.02)	0.718
SOFA	1.01 (0.94,1.08)	0.759
Diagnoses		0.077
Respiratory	reference	
CNS	0.96 (0.21,4.45)	0.963
Liver	2.21 (0.57,8.51)	0.251
Renal	1.50 (0.28,8.14)	0.637
Diabetes	NA	NA
Trauma	2.32 (1.12,4.79)	**0.023**
Other	0.98 (0.53,1.81)	0.951
WBC	1.06 (1.04,1.09)	**<0.001**
INR	1.10 (0.82,1.47)	0.543
Lactate	1.04 (0.95,1.14)	0.420
CCI	1.03 (0.95,1.11)	0.598
SUP		0.320
PPI	reference	
H2RA	1.35 (0.81,2.25)	0.242
PPI or Sucralfate	3.45 (0.75,15.78)	0.111
PPI or H2RA	1.30 (0.76,2.24)	0.339
Sedative		**0.027**
Propofol	reference	
N	2.44 (1.13,5.28)	**0.023**
Dexmedetomidine	0.88 (0.19,4.07)	0.874
Midazolam	1.69 (0.70,4.06)	0.244
Dexmedetomidine or Propofol	0.61 (0.35,1.08)	0.093
Midazolam or Propofol	0.76 (0.41,1.39)	0.369
Dexmedetomidine, Midazolam or Propofol	0.72 (0.37,1.38)	0.324
Statin		0.987
N	reference	
Y	1.00 (0.64,1.56)	
Insulin		0.506
N	reference	
Y	1.20 (0.71,2.02)	
Antibiotic		0.410
N	reference	
Single	1.59 (0.13,18.74)	0.713
Combined	3.01 (0.39,23.45)	0.292
Vasopressor		0.116
N	reference	
Y	1.45 (0.91,2.31)	
Length of Ventilation (day)	0.96 (0.93,0.99)	**0.012**

p-*value < 0.05 is highlighted with bold values.*

Influence of Drugs on the Risk of Death in MV Patients

There were 1914 (36.3%) in-hospital mortalities among mechanical ventilation patients. After adjusting for confounding factors (**Supplementary Table S1**), the results showed no statistically significant difference in VAP risk between PPI and H2RA (aOR = 1.11 95% CI: 0.83, 1.47). There was also no statistically significant difference in the risk of VAP between propofol, dexmedetomidine and midazolam (aOR = 1.61, 95% CI: 0.65,4.02; aOR = 1.54, 95% CI: 0.81,2.9). Taking statins did not significantly increase risk factor for VAP (aOR = 1.22 95% CI: 0.92, 1.62). Insulin also did not significantly reduce the risk of VAP (aOR = 0.94 95% CI: 0.71,1.25). (**Table 3**).

Effect of Drugs on Death in VAP Patients

There were 252 (30.5%) in-hospital mortalities among ventilator-associated pneumonia patients. After adjusting for confounding factors (**Supplementary Table S2**), compared with VAP patients taking PPI, patients taking H2RA do not have a greater risk of death (aHR = 1.35 95% CI: 0.81,2.25). There was also no significant reduction in the risk of death after taking dexmedetomidine (aHR = 0.88 95% CI: 0.19,4.07). Taking midazolam also did not increase the risk of death in VAP patients (aHR = 1.69 95% CI: 0.70,4.06). Taking statin and insulin did not increase the risk of death in VAP patients (aHR = 1.00 95% CI: 0.64, 1.56; aHR = 1.20 95% CI: 0.71, 2.02) (**Table 4**).

DISCUSSION

The present study retrospectively analyzed the underlying therapeutic effects of sedative drugs, SUP, statins, and insulin in patients with MV by using the MIMIC database. Our results found that patients with MV benefited more with propofol than with midazolam.

This study analyzed the effects of different SUP regimens in patients with MV. The results showed that patients with MV using sucralfate or H2RA had a similar risk of death to those with MV using PPI, and there were no differences in the occurrence of VAP and the prognosis of VAP patients. A previous meta-analysis including randomized clinical trials showed that PPI may be more effective for preventing upper gastrointestinal bleeding than H2RA, but had no differences between drugs in the risk of pneumonia, death, or ICU length of stay. This result has some limitations, such as insufficient data, differences between lower and higher quality trials, methodological limitations, and possible publication bias (Alhazzani et al., 2013). Another study concluded that the incidence of nosocomial pneumonia were not different between patients

using PPIs and those using H2RA. However, for prevention of stress-related mucosal diseases, the rate of clinically important bleeding decreased significantly in patients using PPI (Pongprasobchai et al., 2009). All of the above reports are consistent with our findings. For MV patients who need to receive SUP, since there is no clear conclusion to prove the difference between H2RA and PPI, when using acid suppressants, we should give priority to the characteristics of the patient and disease, and choose a more appropriate SUP regimen. In addition, previous studies have shown that sucralfate did not affect the gastric pH of patients, and did not increase the risk of bacteria infection (Kappstein et al., 1991; Sun et al., 2019). Thus, many studies have illuminated that sucralfate should be the preferred option for SUP compared to PPI and H2RA. However, in our study, due to few patients used sucralfate, the corresponding p-values could not be calculated. We hope that a prospective cohort study with a larger sample size will confirm this conclusion in the future.

Our study also compared the effects of different sedation regimens. The results showed that patients in the midazolam group had a higher risk of death than those in the propofol group, but we did not find differences between several sedation regimens in terms of the occurrence of VAP and the prognosis of patients with VAP. The 2013 Pain, Agitation, and Delirium (PAD) guidelines stated that non-benzodiazepine is better choice than benzodiazepines for mechanically ventilated adult patients sinceof the former improved length of stay, duration of MV, 90-day mortality and psychological dysfunction in ICU patients (Barr et al., 2013). Another study showed that the 28-day mortality rate of patients treated with midazolam was 30.8%, and the propofol group was 25.5%, the adjusted odds ratio (OR) value was 1.421 [95% confidence interval (CI): 1.118–1.806, $p < 0.001$], with a significantly lower mortality rate in the propofol group (Sun et al., 2022). Many previous studies do not support the choice of midazolam as a sedation regimen in patients with MV, mainly considering that midazolam is easy to accumulate in patients' tissues, resulting in prolonged metabolism and elimination time (Spina and Ensom, 2007). On the other hand, propofol has the advantages of quick effect, rapid distribution and metabolism, and is less likely to produce sequelae, which make it an ideal drug for rapid recovery of consciousness after discontinuation (Mirenda and Broyles, 1995). In addition, accumulating evidence has shown that the occurrence of ICU delirium is a strong predictor of increased mortality and prolonged hospitalization (Ely et al., 2004). One potential mechanism for inducing delirium is the activation of the γ-aminobutyric acid receptor (Maldonado, 2008). Propofol and midazolam are γ-aminobutyric agonists, and both drugs have the potential to cause delirium in patients (Levine, 1994). However, due to the rapid metabolic distribution of propofol, the delirium induced by propofol may be not long-lasting and harmful as midazolam. In addition, previous studies have reported that dexmedetomidine and propofol have similar sedative effects, and there is no difference in the prognostic effects of dexmedetomidine and propofol on patients with MV. However, given that dexmedetomidine appears to be associated with a higher risk of adverse effects, such as hypotension, bradycardia and other cardiopulmonary complications (Anger et al., 2010; Jakob et al., 2012), propofol is more recommended in this study when choosing a sedation regimen.

This study reviewed the effects of statins on patients receiving MV therapy in ICU. Our results showed that there was no difference in mortality and the incidence of VAP between patients who took statins during MV and patients who did not receive statins, and the use of statins did not reduce mortality in patients with VAP. A meta-analysis reported by Yu SS mentioned that the use of statins before admission will benefit critically ill patients and can significantly reduce the duration of mechanical ventilation and short-term mortality (Yu et al., 2021b). However, Laurent's study was against the view that statins improve the prognosis of VAP patients. The results showed that the 28-day mortality rate of VAP patients with simvastatin was 22.6% (95% CI, 15.7–31.5%), while Placebo was 14.3% (95% CI, 8.9–22.2%) ($p = 0.06$) (Papazian et al., 2013b). Previous studies had suggested that there was a significant difference in the prognosis of patients with a history of statin prescriptions compared with patients with those who urgently use statins. This anti-inflammatory effect of statins may need sufficient dose and treatment time (Brealey et al., 2011; Bruyere et al., 2014). Because our study did not have access to a patient's prescription history of statins, inability to distinguish whether a patient is on temporary or long-term medication. we hope that this point can be fully taken into account in follow-up studies.

By comparing the prognosis of MV patients treated with and without insulin, we found that the use of insulin did not affect mortality of MV patients, the incidence of VAP or the mortality of VAP patients. However, in previous studies, MV patients who were intensively treated with insulin appeared to have a better prognosis, with possible mechanisms including the elimination of glucose-induced osmotic diuresis, enhancement of erythropoiesis, prevention of acute renal failure, maintenance of macrophage and neutrophil function, reduction of cholestasis, promotion of direct anabolic effects of respiratory muscles, and protection of patients the central and peripheral nervous systems. A study by Krinsley et al. showed that the mortality rate of critically ill patients who use insulin to control blood glucose has decreased by 29.3% ($p = 0.02$), and the length of stay in ICU has been reduced by 10.8% ($p = 0.01$) (Krinsley, 2004). Another prospective observational study performed a multivariate Logistic regression analysis of data on patients undergoing MV and found that the degree of blood glucose control was associated with risk of death and organ system dysfunction, and independent of insulin dose (Van den Berghe et al., 2003). Using insulin to control the blood glucose concentration to less than 100–110 mg/dl appears to provide the best benefit to the patient (Lewis et al., 2004). In this study, wedistinguished the specific conditions of insulin use in patients, but did not dynamically monitor the blood glucose concentration of the patients. Therefore, there is

uncertainty whether it is the actual insulin dose received per se or the degree of euglycemia achieved that is responsible for the beneficial effects of intensive glycemic management. We hope that more literature in the future to assess the clinical and economic effects of insulin on ICU patients.

Our research has the following limitations. First, due to database limitations, we could not know the exact time of VAP onset, so we could not build a competing risk model. In this regard, we include VAP onset in cox regression and found that VAP occurrence did not elevate mortality risk. In addition, we also supplemented the subgroup analysis of mortality risk in VAP patients, the results of which did not differ significantly from those of MV patients, we think the results are stable. Secondly, our data mainly comes from the MIMIC database, so the risk of losing confusing data is real, such as the dynamic changes of gastric pH in patients using SUP and the history of statins in MV patients. Thirdly, our study also analyzes the combination of drugs, but the specific situation of the combination of drugs is not clear, and it is not possible to determine whether patients were on combination or alternate use, so we have not interpreted the results of this piece of the study in detail. Finally, this study only investigated the effect of drugs on the mortality of patients with MV, the incidence of VAP and the prognosis of patients with VAP, but did not explain the adverse effects of the drugs. Therefore, further high-quality original research or more scientific research is needed to draw a clear conclusion.

CONCLUSION

We conducted this retrospective analysis to explore the effects of different drugs in patients with MV. Results showed that propofol was superior to midazolam in reducing in-hospital mortality in patients requiring mechanical ventilation. Further high-quality original research or a more scientific approach is required to draw definitive conclusions.

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.

AUTHOR CONTRIBUTIONS

Conception and design: PY Administrative support: PY and LW. Provision of study materials: PY, Collection and assembly of data: XK, PY, Data analysis and interpretation: XK and YW, Manuscript writing: All authors revising it critically for intellectual content: All authors final approval of manuscript.

FUNDING

This work was supported by the Guangzhou Municipal Science and Technology Bureau under Grant No. 202102010313 and 202102010186.

ACKNOWLEDGMENTS

The authors would like to express their sincere appreciation and gratitude to the staff members of Beth Israel Deaconess Medical Center for their great assistance in field surveying in the current study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.921422/full#supplementary-material

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ORIGINAL RESEARCH
published: 23 June 2022
doi: 10.3389/fphar.2022.898566

Thiamine May Be Beneficial for Patients With Ventilator-Associated Pneumonia in the Intensive Care Unit: A Retrospective Study Based on the MIMIC-IV Database

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OPEN ACCESS

Edited by:
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Wuhan University, China

Reviewed by:
Chong Lei,
Fourth Military Medical University, China
Sergio Ramirez-Estrada,
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 17 March 2022
Accepted: 06 June 2022
Published: 23 June 2022

Citation:
Zhang L, Li S, Lu X, Liu Y, Ren Y, Huang T, Lyu J and Yin H (2022) Thiamine May Be Beneficial for Patients With Ventilator-Associated Pneumonia in the Intensive Care Unit: A Retrospective Study Based on the MIMIC-IV Database. Front. Pharmacol. 13:898566. doi: 10.3389/fphar.2022.898566

Background: Ventilator-associated pneumonia (VAP) is a common infection complication in intensive care units (ICU). It not only prolongs mechanical ventilation and ICU and hospital stays, but also increases medical costs and increases the mortality risk of patients. Although many studies have found that thiamine supplementation in critically ill patients may improve prognoses, there is still no research or evidence that thiamine supplementation is beneficial for patients with VAP. The purpose of this study was to determine the association between thiamine and the prognoses of patients with VAP.

Methods: This study retrospectively collected all patients with VAP in the ICU from the Medical Information Mart for Intensive Care-IV database. The outcomes were ICU and in-hospital mortality. Patients were divided into the no-thiamine and thiamine groups depending upon whether or not they had received supplementation. Associations between thiamine and the outcomes were tested using Kaplan-Meier (KM) survival curves and Cox proportional-hazards regression models. The statistical methods of propensity-score matching (PSM) and inverse probability weighting (IPW) based on the XGBoost model were also applied to ensure the robustness of our findings.

Results: The study finally included 1,654 patients with VAP, comprising 1,151 and 503 in the no-thiamine and thiamine groups, respectively. The KM survival curves indicated that the survival probability differed significantly between the two groups. After multivariate COX regression adjusted for confounding factors, the hazard ratio (95% confidence interval)

Abbreviations: VAP: ventilator-associated pneumonia; ICU: intensive care unit; KM: Kaplan-Meier; PSM: propensity-score matching; IPW: inverse probability weighting; IMV: invasive mechanical ventilation; HAT therapy: hydrocortisone, ascorbic acid and thiamine; MIMIC: Medical Information Mart for Intensive Care; ICD: International Statistical Classification of Disease; BMI: Body Mass Index; APSIII: Acute Physiology Score III; CRRT: continuous renal replacement therapy; WBC: white blood cells; RDW: red blood cell distribution width; AG: anion gap; AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR: international normalized ratio; PTT: partial thromboplastin time; mHR: min heart rate; mMAP: min value of mean arterial pressure; mRR: min respiratory rate; mT: max temperature; mSpO2: min SpO2; IQR: interquartile; VIF: variance inflation factors; XGBoost: Extreme Gradient Boosting; HR: hazard ratios.

values for ICU and in-hospital mortality in the thiamine group were 0.57 (0.37, 0.88) and 0.64 (0.45, 0.92), respectively. Moreover, the results of the PSM and IPW analyses were consistent with the original population.

Conclusion: Thiamine supplementation may reduce ICU and in-hospital mortality in patients with VAP in the ICU. Thiamine is an inexpensive and safe drug, and so further clinical trials should be conducted to provide more-solid evidence on whether it improves the prognosis of patients with VAP.

Keywords: ICU, ventilator-associated pneumonia, thiamine, IPW, mortality

INTRODUCTION

Ventilator-associated pneumonia (VAP) refers to a lung parenchyma infection that occurs in patients with artificial airways (tracheal intubation or tracheotomy) who receive invasive mechanical ventilation (IMV) for at least 48 h (Kalil et al., 2016). With the maturity of modern rescue technology, ventilators and invasive diagnosis and treatment techniques have been widely used. IMV is one of the main cornerstones of life support in intensive care units (ICUs). This increases the VAP incidence, which studies have found to range from 5 to 40% in patients receiving IMV(Čiginskienė et al., 2019; Papazian et al., 2020). Once VAP occurs, it often causes weaning difficulties, thereby prolonging ICU stays and hospitalization times, increasing the costs for the patient, or even endangering their life and causing death (American Thoracic Society; Infectious Diseases Society of America, 2005; Zimlichman et al., 2013). Epidemiology suggests that the VAP-related all-cause mortality rate in critically ill patients can be as high as 50% (Papazian et al., 2020). The timely diagnosis and treatment of VAP is therefore of great significance. The main treatment for VAP is currently antibiotics (Metersky and Kalil, 2018), but primary disease treatment, prevention and treatment of risk factors leading to VAP, nutritional support, immunotherapy, and enhanced nursing can improve VAP prognoses (Modi and Kovacs, 2020; Ścisło et al., 2022).

Thiamine (also called vitamin B1) is a water-soluble vitamin, and its biologically active form is thiamine pyrophosphate in cells, which is an essential coenzyme in the tricarboxylic acid cycle during glucose metabolism and participates in human energy production (Polegato et al., 2019). Thiamine can also maintain the redox state of cells and participate in the antioxidant pathway by producing reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (Mallat et al., 2016). In addition to these traditional functions, recent studies have found that thiamine derivatives also have some nonenzymatic functions, such as involvement in gene expression, stress response, and neural signal transduction regulation (Tylicki and Siemieniuk, 2011; Aleshin et al., 2019). These important roles of thiamine form the basis of thiamine supplementation in critically ill patients, and it has been explored in many trials in intensive care environments. For example, thiamine is often studied in the form of a drug combination with hydrocortisone and ascorbic acid (so-called HAT therapy) (Iglesias et al., 2020). Several studies have found that HAT therapy is associated with organ dysfunction improvement, decreased Sequential Organ Failure Assessment scores, increased lactate clearance, and reduced mortality in patients with sepsis (Marik, 2018; Woolum et al., 2018). Other than in patients with sepsis, a retrospective cohort found that HAT therapy is also associated with a reduced in-hospital mortality risk in patients with severe pneumonia (Kim et al., 2018). Thomas

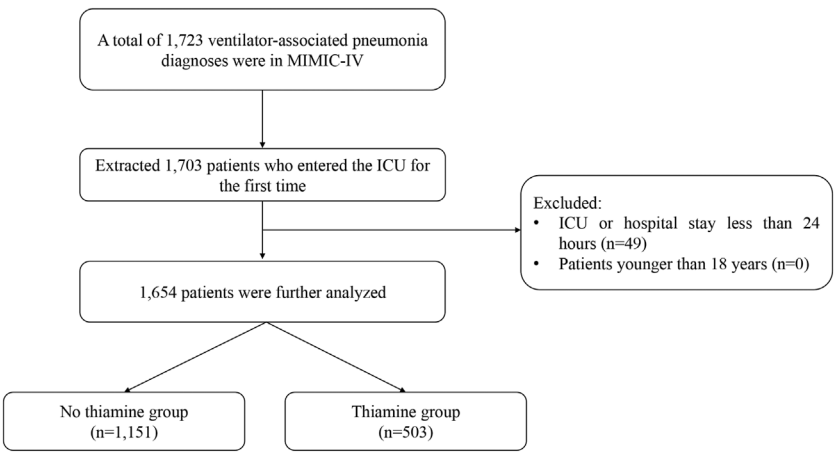

FIGURE 1 | Inclusion and exclusion flowchart of the study.

TABLE 1 | Baseline characteristics of the original population.

	No Thiamine Group	Thiamine Group	*p*-Value	Missing data (%)
	1,151	503		
Age	66.00 (55.00, 77.00)	61.00 (49.00, 70.00)	<0.001	0
Gender (%)			0.143	0
male	710 (61.7)	330 (65.6)		
female	441 (38.3)	173 (34.4)		
BMI	28.31 (24.47, 34.17)	27.99 (23.68, 33.67)	0.365	0
APSIII	66.00 (50.00, 86.00)	71.00 (51.00, 92.00)	0.003	0
Ethnicity (%)			0.049	0
White	690 (59.9)	275 (54.7)		
Black	128 (11.1)	52 (10.3)		
others	333 (28.9)	176 (35.0)		
Admission type (%)			0.008	0
emergency	702 (61.0)	271 (53.9)		
others	449 (39.0)	232 (46.1)		
First careunit (%)			0.298	0
MICU/SICU	861 (74.8)	389 (77.3)		
others	290 (25.2)	114 (22.7)		
Vasopressor (%)			0.079	0
no	756 (65.7)	307 (61.0)		
yes	395 (34.3)	196 (39.0)		
CRRT (%)			0.002	0
no	1,128 (98.0)	478 (95.0)		
yes	23 (2.0)	25 (5.0)		
Duration of IMV(hour)	124.00 (56.00, 241.00)	169.00 (74.00, 310.00)	<0.001	0
Comorbidities				
Sepsis (%)			0.109	0
no	67 (5.8)	19 (3.8)		
yes	1,084 (94.2)	484 (96.2)		
Myocardial infarct (%)			0.283	0
no	934 (81.1)	420 (83.5)		
yes	217 (18.9)	83 (16.5)		
Congestive heart failure (%)			0.020	0
no	750 (65.2)	358 (71.2)		
yes	401 (34.8)	145 (28.8)		
Hypertension (%)			0.013	0
no	636 (55.3)	244 (48.5)		
yes	515 (44.7)	259 (51.5)		
Cerebrovascular disease (%)			0.004	0
no	835 (72.5)	399 (79.3)		
yes	316 (27.5)	104 (20.7)		
Chronic pulmonary disease (%)			0.184	0
no	808 (70.2)	370 (73.6)		
yes	343 (29.8)	133 (26.4)		
Liver disease (%)			<0.001	0
no	1,014 (88.1)	341 (67.8)		
yes	137 (11.9)	162 (32.2)		
Renal disease (%)			0.015	0
no	874 (75.9)	410 (81.5)		
yes	277 (24.1)	93 (18.5)		
Diabetes (%)			0.047	0
no	777 (67.5)	365 (72.6)		
yes	374 (32.5)	138 (27.4)		
Malignant cancer (%)			0.287	0
no	1,029 (89.4)	459 (91.3)		
yes	122 (10.6)	44 (8.7)		
Vital signs				
mMAP (mmhg)	58.00 (51.00, 65.00)	59.00 (52.00, 66.00)	0.111	0.06
mHR (-min)	69.00 (60.00, 82.00)	74.00 (61.00, 86.00)	<0.001	0.06
mRR (-min)	13.00 (10.00, 15.38)	13.00 (10.00, 16.00)	0.966	0.12
mSpO2 (%)	93.00 (90.00, 96.00)	93.00 (90.00, 95.00)	0.218	0.06
mT (°C)	37.61 (37.17, 38.22)	37.64 (37.11, 38.22)	0.735	5.08
Laboratory tests				
WBC(k/uL)	12.00 (8.80, 16.25)	11.60 (8.20, 16.40)	0.174	0.36
Lymphocytes (%)	9.00 (5.30, 14.40)	9.20 (5.97, 14.62)	0.504	0.36

(Continued on following page)

TABLE 1 | (*Continued*) Baseline characteristics of the original population.

	No Thiamine Group	Thiamine Group	*p*-Value	Missing data (%)
Neutrophils (%)	81.90 (74.88, 87.00)	79.95 (72.07, 85.40)	0.001	0.36
Hemoglobin (g/dl)	10.50 (8.90, 12.30)	10.70 (8.88, 12.50)	0.525	0.36
Platelet (k/uL)	198.00 (144.00, 265.50)	181.00 (112.00, 245.00)	<0.001	0.36
RDW (%)	14.60 (13.60, 16.10)	14.70 (13.60, 16.80)	0.151	0.48
AG(mEq/L)	14.00 (12.00, 17.00)	15.00 (13.00, 19.00)	<0.001	0
Lactate (mmol/L)	1.60 (1.10, 2.60)	1.80 (1.20, 3.20)	0.004	4.53
PaCO2(mmhg)	42.00 (36.00, 50.00)	41.00 (34.00, 49.00)	0.018	1.69
PaO2(mmhg)	109.00 (71.00, 200.50)	101.00 (59.50, 182.00)	0.006	1.69
INR	1.20 (1.10, 1.50)	1.30 (1.10, 1.60)	0.212	2.06
PTT(s)	30.60 (26.90, 37.88)	31.10 (27.30, 38.10)	0.532	2.06
AST (IU/L)	39.00 (25.00, 78.00)	53.00 (29.00, 125.25)	<0.001	8.65
ALT (IU/L)	29.00 (17.00, 56.75)	31.00 (18.00, 66.50)	0.024	9.25
Bilirubin, total (mg/dL)	0.60 (0.40, 1.00)	0.80 (0.40, 1.80)	<0.001	9.61
Albumin (g/dl)	2.90 (2.50, 3.40)	2.90 (2.50, 3.30)	0.235	16.51
Glucose (mg/dl)	138.00 (111.00, 179.00)	136.00 (108.00, 178.50)	0.163	0
Creatinine (mg/dL)	1.00 (0.70, 1.50)	1.00 (0.70, 1.90)	0.199	0
Urine output (ml)	1,590.00 (962.50, 2350.00)	1,371.50 (757.50, 2223.75)	<0.001	2.72

et al. found beneficial effects from thiamine, vitamin C, and vitamin D in patients with COVID-19, acute respiratory distress syndrome, and sepsis (Jovic et al., 2020). Thiamine is a particularly safe and inexpensive treatment, but warrants larger clinical trials to provide more evidence for its utility. At present, there is no research or evidence to show whether thiamine supplementation can improve the prognosis of patients with VAP. This study therefore aimed to determine the effect of thiamine on the prognosis of patients with VAP based on the Medical Information Mart for Intensive Care (MIMIC)-IV database, which will provide more evidence for clinical thiamine application and thus help to improve prognoses.

METHODS

Data Source and Population

MIMIC is a large, single-center, freely available database developed by the Massachusetts Institute of Technology (Yang et al., 2020; Wu et al., 2021). Several versions have been released, and the latest version, MIMIC-IV (version 1.0), was released on 16 March 2021. This version contains comprehensive information on more than 200,000 patients hospitalized between 2008 and 2019, and it uses a modular approach to organize its data structure and highlight data sources to better utilize different data sources (Goldberger et al., 2000). The database was approved by the Massachusetts Institute of Technology (Cambridge, Mass.) and the Beth Israel Deaconess Medical Center (Boston, Mass.), and consent was obtained for collection of the original data (Johnson et al., 2021). Patients in the database were anonymized, so informed consent was not required. The data in this database can be accessed and extracted after researchers have completed the appropriate coursework and obtained the associated certificate.

All patients with a diagnosis of VAP in the ICU were included in this study on the basis of International Statistical Classification of Diseases, 9th and 10th Revisions (ICD-9 and ICD-10). If patients were admitted to ICU more than once, only data on their first admission was selected. Patients who stayed in the ICU or hospital for less than 24 h and who were younger than 18 years were excluded. Patients with VAP were divided into the no-thiamine and thiamine groups depending on whether or not they had received supplementation (including via intravenous and oral routes).

Data Extraction

Patient information was extracted from the database using Structured Query Language. Demographic information included age, sex, Body Mass Index [BMI = weight (kg)/height^2(m)], and ethnicity. Patient clinical information included admission type, first care unit, Acute Physiology Score III (APSIII), and interventional therapy [vasopressor use, continuous renal replacement therapy (CRRT) use]. The main comorbidities of patients included sepsis, myocardial infarction, congestive heart failure, hypertension, cerebrovascular disease, chronic pulmonary disease, liver disease, renal disease, diabetes, and malignant cancer. Laboratory test indicators were collected from the first record after admission to the ICU, vital signs were the worst values on the first day of the ICU, and urine output was the total on the first day of the ICU.The outcomes of this study were ICU and in-hospital mortality.

Statistical Analyses

Testing revealed that the continuous variables in this study did not conform to a normal distribution, and so they are expressed as median and interquartile (IQR) values, and differences between the two groups were determined using Mann-Whitney U tests. Categorical variables are presented as numbers and percentages, and differences between groups were determined using chi-square and Fisher's exact tests.

Kaplan-Meier (KM) curves and log-rank tests were used to assess whether thiamine supplementation influenced patient survival. We then constructed two Cox proportional-hazards models to analyze how thiamine affected the outcomes. No covariates were adjusted for in model I. While in model II, patients' general characteristics, disease severity scores,

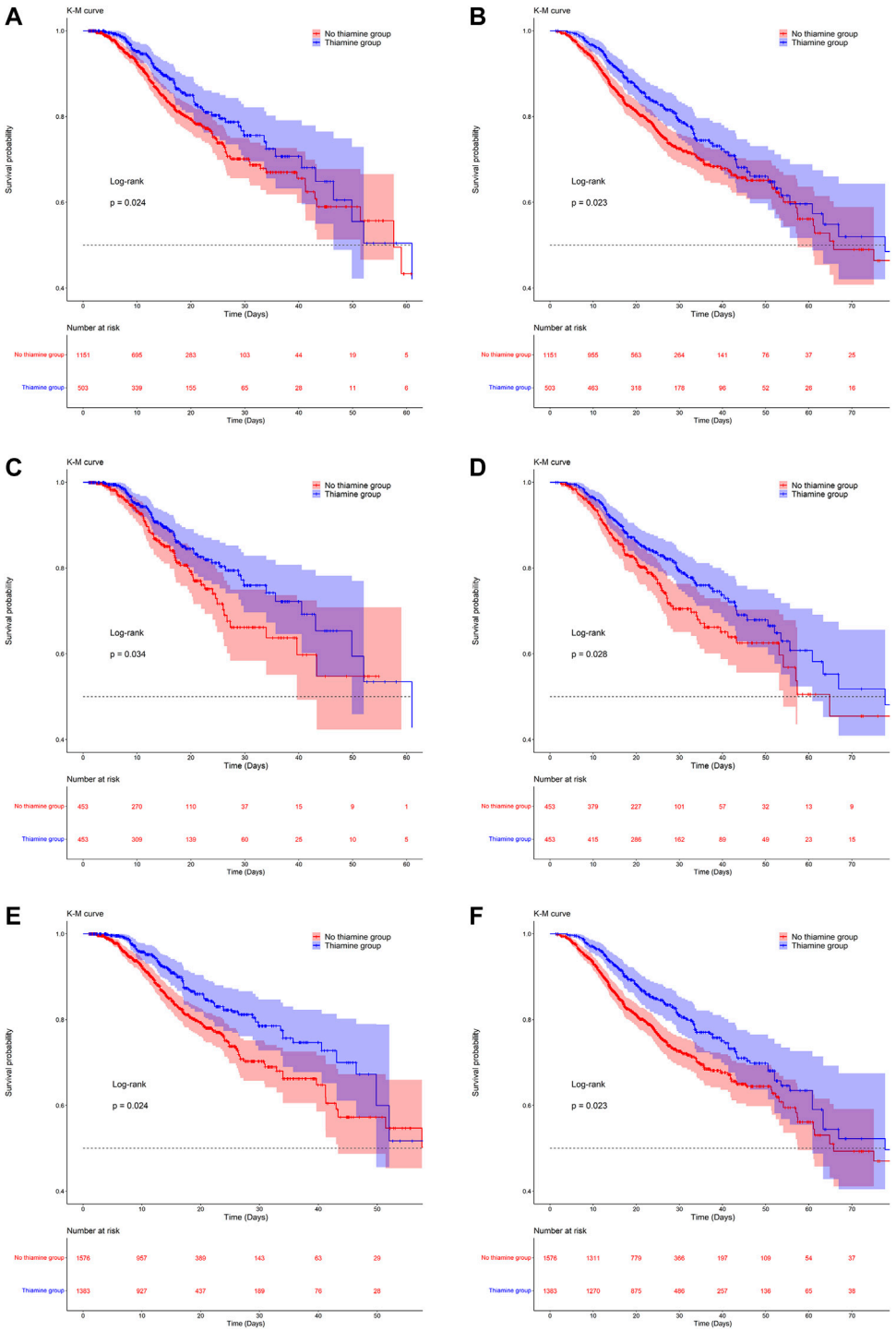

FIGURE 2 | Kaplan-Meier survival curves between groups. a, c and e are the ICU mortality risk for the original population, the PSM population and the IPW population; b, d, and f are the in-hospital mortality risk for the original population, the PSM population and the IPW population.

interventions, comorbidities, laboratory findings and vital signs were adjusted in order to balance the impact of these factors on patient outcomes. Variance inflation factors (VIFs) were used to test for multicollinearity among the independent variables before performing multivariate COX regression.

To guarantee the robustness of the findings, we used propensity-score matching (PSM) and inverse probability weighting (IPW) to reduce the baseline differences between the two groups. When performing PSM and IPW analyses, the presence of missing values was not allowed. Therefore, the

multiple imputation function in the "mice" package of our R software was applied to fill in the missing covariate values beforehand. The propensity scores for patients in the thiamine group were estimated using a multivariate logistic regression model followed by one-to-one nearest-neighbor matching with a 0.05 caliper width. XGBoost (Extreme Gradient Boosting) is an efficient gradient boosting decision tree algorithm, which can be used in the R software package "twang" to estimate relevant propensity scores (Yuan et al., 2020). We incorporated 43 covariates into the XGBoost model, obtained estimated propensity scores as weights, and finally used the IPW model to generate weighted cohorts (McCaffrey et al., 2013). This process introduced two new groups: the PSM and weighted populations. Then, similar to for the original population, univariate and multivariate Cox regression analyses were applied to these two populations to obtain a doubly robust estimation.

We also analyzed the effect of thiamine on the prognoses of different patient subgroups. Subgroups included age (<65 and ≥65 years), sex (male and female), and all of the comorbidities listed above. The interactions between the subgroups were further analyzed.

A two-tailed probability value of $p < 0.05$ was considered statistically significant. All statistical analyses in this study were performed using R software (version 4.1.0).

TABLE 2 | Results of Cox proportional hazard models.

	Model I		Model II	
Outcomes	**HR (95%CI)**	***p*-Value**	**HR (95%CI)**	***p*-Value**
Original population				
ICU Mortality				
Thiamine				
no	Reference		Reference	
yes	0.73 (0.55,0.96)	0.025	0.57 (0.37,0.88)	0.011
In-hospital Mortality				
Thiamine				
no	Reference		Reference	
yes	0.77 (0.61,0.96)	0.023	0.64 (0.45,0.92)	0.015
After PSM				
ICU Mortality				
Thiamine				
no	Reference		Reference	
yes	0.68 (0.49,0.97)	0.035	0.62 (0.42,0.91)	0.015
In-hospital Mortality				
Thiamine				
no	Reference		Reference	
yes	0.73 (0.56,0.96)	0.028	0.72 (0.53,0.98)	0.036
After IPW				
ICU Mortality				
Thiamine				
no	Reference		Reference	
yes	0.64 (0.48,0.85)	0.002	0.65 (0.48,0.89)	0.007
In-hospital Mortality				
Thiamine				
no	Reference		Reference	
yes	0.69 (0.54,0.87)	0.002	0.75 (0.57,0.97)	0.029

Abbreviations: HR, hazard ratio; CI, confidence interval.
Models were derived from Cox proportional hazards regression models.
Model I was not adjusted for covariates.
Model II covariates were adjusted for age, sex, BMI, race, admission type, first care unit, APSIII, vasopressor use, CRRT, sepsis, myocardial infarction, congestive heart failure, hypertension, cerebrovascular disease, chronic pulmonary disease, liver disease, renal disease, diabetes, malignant cancer, WBC, neutrophils, lymphocytes, hemoglobin, RDW, platelets, AG, PaCO2, PaO2, lactate, creatinine, AST, ALT, total bilirubin, albumin, INR, PTT, glucose, mHR, mMAP, mRR, mT, mSpO2, and urine output.

RESULTS

Baseline Characteristics

The study ultimately finally included 1,654 patients with VAP, comprising 1,151 in the no-thiamine group and 503 in the thiamine group (**Figure 1**). Patients in the no-thiamine group were older than those in the thiamine group (median [IQR] = 66.00 [55.00, 77.00] years old vs. 61.00 [49.00, 70.00] years old); larger proportions of patients in both groups were male (61.7 and 65.6%, respectively); patients in the thiamine group had higher APSIII scores than those in the no-thiamine group (median [IQR] = 66.00 [50.00, 86.00] vs. 71.00 [51.00, 92.00]); patients hospitalized due to an emergency in the two groups accounted for 61.0 and 53.9% of those in the no-thiamine and thiamine groups, respectively; and sepsis accounted for the highest proportion of comorbidities in both groups (94.2 and 96.2%, respectively). More baseline characteristic information is provided in **Table 1**.

Survival Analysis and Cox Proportional-Hazards Regression Model

ICU mortality rates were 15.6 and 13.9% in the no-thiamine and thiamine groups, respectively, and the corresponding in-hospital mortality rates were 22.6 and 21.7%. The KM survival curves indicated that the survival probability differed significantly between the two groups. Patients with VAP who received thiamine had significantly higher survival odds in both the ICU and in-hospital (**Figure 2**).

Supplementary Table S1 listed the VIFs of each covariate, they were all less than 4, indicating that there was no multicollinearity between the variables. The Cox proportional-hazards model results are listed in **Table 2**. The hazard ratios (HRs) were less than 1 in the thiamine group when compared with the no-thiamine group in both the unadjusted model and the model adjusted for all confounders; that is, patients in the thiamine group had lower ICU and in-hospital mortality risks than did those in the no-thiamine group. After adjusting for the covariates mentioned above as confounding factors, the HR (95% confidence interval [CI]) values for ICU and in-hospital mortality in the thiamine group were 0.57 (0.37, 0.88) and 0.64 (0.45, 0.92), respectively, indicating that the ICU and in-hospital mortality risks were 0.57 and 0.64 times higher than those in the no-thiamine group, respectively (**Table 2**).

Propensity-Score Matching and Inverse Probability of Treatment Weighting

After PSM and IPW, baseline differences between the two groups improved substantially, but there were still differences

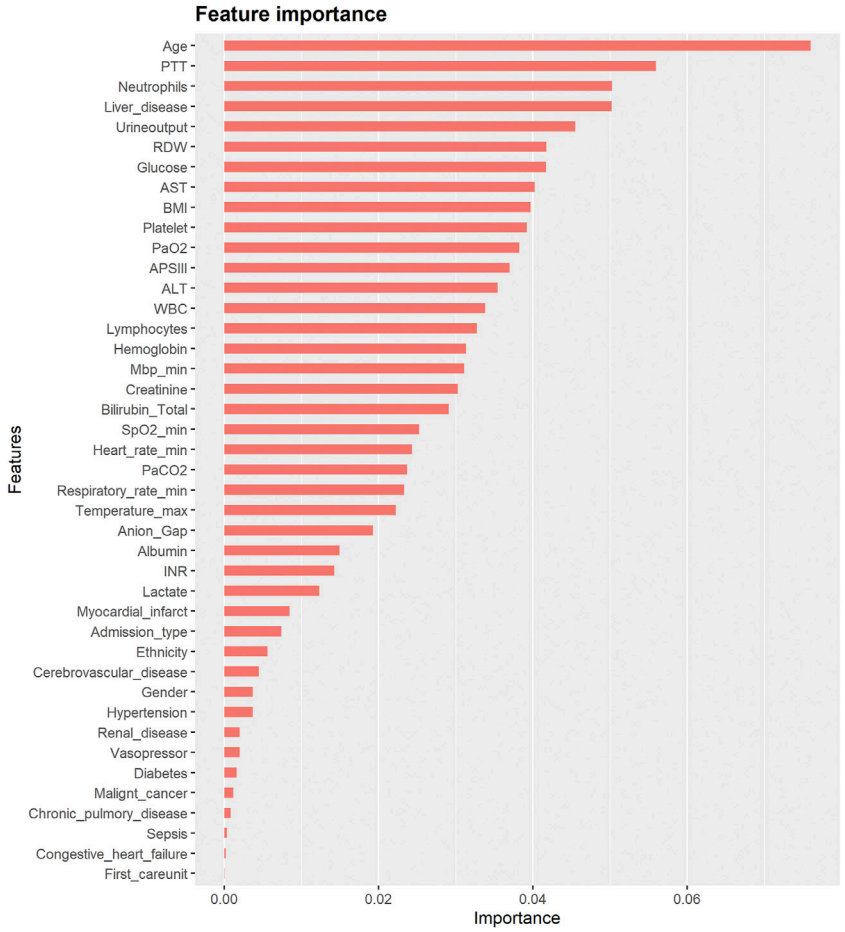

FIGURE 3 | The contribution of each covariate to the XGBoost model.

in a few variables (**Supplementary Figure S1**; **Supplementary Table S2**). The KM survival curves of the matched and weighted populations indicated a trend consistent with that for the original population (**Figure 2**). As with the original population, we also applied univariate and multivariate Cox regression analyses to the matched and weighted populations. After the multivariate Cox regression, the HRs (95% CI) for ICU and in-hospital mortality in the thiamine group were 0.62 (0.42, 0.91) and 0.72 (0.53, 0.98), respectively, in the PSM population, and 0.65 (0.48, 0.89) and 0.75 (0.57, 0.97) in the weighted population (**Table 2**).

Figure 3 also shows rankings of contributions to the propensity score of 43 covariates in the XGBoost model, which reflected the degree of influence of different covariates on the groups or the degree of imbalance between groups. The figure shows that the five highest-ranked variables in order were age, PTT, neutrophils, liver disease, and urine output.

Subgroup Analysis

The results of the subgroup analysis are listed in **Table 3**, and there was no significant interaction between the thiamine and no-thiamine groups in each stratified population.

DISCUSSION

Based on the MIMIC-IV database, this study was the first to determine the effects of thiamine supplementation on ICU and in-hospital mortality risks among patients with VAP. The results were very gratifying, confirming that thiamine—as an inexpensive, easily available, and relatively safe drug—is related to improving the prognosis of patients with VAP, which was also verified using PSM and IPW. The results were stable and reliable, providing a new basis for clinical VAP treatment research.

Many published studies have assessed the relationship between thiamine and patients with sepsis, or have conducted clinical studies involving combined HAT therapy. A subset of studies did not exhibit beneficial effects of thiamine, such as one multicenter randomized clinical study indicating that HAT therapy did not provide faster relief of septic shock compared with intravenous hydrocortisone alone, but this study did not evaluate the possible individual effects of vitamin C and thiamine separately (Fujii et al., 2020). There are also some studies with similar results to ours that thiamine was strongly associated with improved organ dysfunction and reduced mortality in patients

TABLE 3 | Subgroup analysis of relationship between groups and mortality.

	ICU Mortality			In-hospital Mortality		
	HR (95%CI)	*p*-value	p-interaction	HR (95%CI)	*p*-value	p-interaction
Age			0.887			0.865
<65 (*n* = 831)	0.84 (0.39,1.84)	0.667		0.79 (0.44,1.43)	0.440	
≥65 (*n* = 823)	0.50 (0.27,0.88)	0.021		0.64 (0.39,1.04)	0.073	
Gender			0.405			0.072
male (*n* = 1,040)	0.57 (0.31,1.03)	0.064		0.69 (0.44,1.09)	0.115	
female (*n* = 614)	0.500 (0.23,1.11)	0.088		0.47 (0.24,0.92)	0.029	
Sepsis						
no (*n* = 86)	(NA)			(NA)		
yes (*n* = 1,568)	0.59 (0.37,0.90)	0.015		0.67 (0.47,0.95)	0.024	
Myocardial infarct			0.649			0.476
no (*n* = 1,354)	0.56 (0.34,0.93)	0.023		0.68 (0.47,1.00)	0.051	
yes (*n* = 300)	0.45 (0.16,1.27)	0.131		0.53 (0.21,1.36)	0.186	
Congestive heart failure			0.654			0.894
no (*n* = 1,108)	0.53 (0.28,0.98)	0.044		0.66 (0.41,1.07)	0.089	
yes (*n* = 546)	0.59 (0.28,1.26)	0.171		0.58 (0.32,1.08)	0.171	
Hypertension			0.836			0.520
no (*n* = 880)	0.68 (0.36,1.26)	0.219		0.77 (0.47,1.27)	0.300	
yes (*n* = 774)	0.39 (0.19,0.80)	0.010		0.54 (0.31,0.94)	0.029	
Cerebrovascular disease			0.175			0.455
no (*n* = 1,234)	0.58 (0.35,0.95)	0.032		0.68 (0.45,1.01)	0.059	
yes (*n* = 420)	0.55 (0.13,2.25)	0.403		0.52 (0.18,1.47)	0.218	
Chronic pulmonary disease			0.277			0.074
no (*n* = 1,178)	0.62 (0.36,1.07)	0.086		0.74 (0.49,1.11)	0.152	
yes (*n* = 476)	0.32 (0.13,0.76)	0.010		0.35 (0.17,0.75)	0.006	
Liver disease			0.685			0.596
no (*n* = 1,355)	0.63 (0.38,1.05)	0.075		0.68 (0.44,1.03)	0.074	
yes (*n* = 299)	0.21 (0.05,0.82)	0.024		0.44 (0.10,0.95)	0.038	
Renal disease			0.797			0.854
no (*n* = 1,284)	0.57 (0.33,0.97)	0.039		0.66 (0.44,0.99)	0.047	
yes (*n* = 370)	0.44 (0.16,1.21)	0.112		0.39 (0.16,0.95)	0.037	
Diabetes			0.719			0.822
no (*n* = 1,142)	0.58 (0.34,1.00)	0.051		0.62 (0.41,0.96)	0.032	
yes (n = 512)	0.61 (0.26,1.45)	0.267		0.63 (0.31,1.26)	0.181	
Malignant cancer						
no (*n* = 1,488)	0.55 (0.34,0.90)	0.166		0.63 (0.42,0.94)	0.027	
yes (*n* = 166)	(NA)			(NA)		

Hazard ratio (95% CI): from Cox proportional hazards regression models. The covariate adjustment was consistent with Model II, in ***Table 2****.*

with sepsis (Donnino et al., 2010; Woolum et al., 2018). With the recent spread of COVID-19, the role of thiamine in this disease is gradually being explored (Jovic et al., 2020). A two-center, noninterventional, retrospective study found that the thiamine group had significantly lower 30-days mortality for critically ill patients admitted to the ICU with confirmed COVID-19 (Al Sulaiman et al., 2021).

Thiamine cannot be synthesized endogenously and so can only be obtained from food (Frank, 2015). However, patients with VAP in ICUs are often in a state of fasting or eating less due to their critical condition, resulting in insufficient thiamine intake (Attaluri et al., 2018). As a key coenzyme in glycolysis, thiamine plays a key regulatory role in the process of mitochondrial ATP synthesis to provide cells with energy, and so the lack of thiamine will inevitably affect mitochondrial function (Belsky et al., 2018). Impaired mitochondrial function can lead to cell dysfunction, leading to the dysfunction or even failure of various organs (Singer et al., 2004). Extensive published studies have found that thiamine deficiency is a common phenomenon in ICUs(Manzanares and Hardy, 2011; van Snippenburg et al., 2017), and can lead to serious complications in critically ill patients with heart failure, neuropathy, gastrointestinal dysfunction, and lactic acidosis (Katta et al., 2016; Attaluri et al., 2018; Woolum et al., 2018). Thiamine supplementation therefore helps to restore mitochondrial function and reduce the likelihood of organ dysfunction occurrence, thereby improving patient prognoses. In addition to playing an important role in energy metabolism, the production of the major components of redox reactions in the body are all inextricably linked to thiamine (Mallat et al., 2016). In patients with VAP, cell structure changes due to the inflammatory response and tissue hypoxia, and the balance of oxidation and antioxidant systems is dysfunctional, resulting in excessive levels of oxidative stress products such as reactive oxygen species (Galley, 2011). Experiments have confirmed that the thiamine level is positively correlated with glutathione peroxidase activity (Depeint et al., 2006), which is the main component of the cellular antioxidant system and has a strong scavenging effect on oxygen free radicals (Cominetti et al., 2011).

In summary, exogenous thiamine supplementation was observed to not only contribute to the energy recovery of patients with VAP, reducing the occurrence of some complications, but also relieve the state of oxidative stress and play an anti-inflammatory role, which is of great significance in improving patient prognosis and survival. Regarding drug safety, a clinical study of patients with Wernicke's encephalopathy found that there were no obvious side effects from thiamine treatment, even after high-dose oral administration at 500 mg three times a day, indicating that thiamine is relatively safe in clinical applications (Donnino et al., 2007).

Strengths and Limitations

To the best of our knowledge, this was the first study to investigate the relationship between thiamine and the prognosis of patients with VAP. Various statistical methods were used to ensure the stability of the results. The large number of population samples in the MIMIC-IV database also provided a solid foundation for our research. Of course, this study also had some limitations. Firstly, this study had a retrospective design. When identifying the study population, we determined the diagnosis of VAP by ICD codes in the MIMIC-IV database. However, we could not avoid this problem due to the possible interobserver variability in the diagnosis of VAP (Klompas, 2010). In future studies, focus on patients with ventilator-associated events may have a higher clinical applicability. Secondly, although we tried our best to balance confounding factors, there were still some potential confounding biases. Thirdly, our study only focused on whether patients with VAP had received thiamine supplementation, and so specific and optimal thiamine doses need to be explored in future prospective studies. Moreover, there is no routine test for measuring thiamine levels, and so no further studies were performed on the thiamine levels of patients in this study.

CONCLUSION

Thiamine supplementation may reduce ICU and in-hospital mortality in patients with VAP in the ICU. Thiamine is an inexpensive and safe drug, and so further clinical trials should be conducted to provide more-solid evidence on whether it improves the prognosis of patients with VAP.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: The data were available on the MIMIC-IV website at https://mimic.physionet.org/, https://doi.org/10.13026/a3wn-hq05.

AUTHOR CONTRIBUTIONS

LZ created the study protocol, performed the statistical analyses and wrote the first manuscript draft. SL conceived the study and critically revised the manuscript. XL assisted with the study design and performed data collection. YL assisted with data collection and manuscript editing. YR and TH assisted the analysis and explain of statistical methods. JL assisted with manuscript revision and data confirmation. HY contributed to data interpretation and manuscript revision. All authors read and approved the final manuscript.

FUNDING

This study received financial support from the National Natural Science Foundation of China (Nos. 82072232 and 81871585), the Natural Science Foundation of Guangdong Province (No. 2018A030313058), Technology and Innovation Commission of Guangzhou Science, China (No. 201804010308), Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization (2021B1212040007).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.898566/full#supplementary-material

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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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CLINICAL TRIAL
published: 01 July 2022
doi: 10.3389/fphar.2022.948236

Efficacy, Safety and Mechanism of Jinzhen Oral Liquid in the Treatment of Acute Bronchitis in Children: A Randomized, Double-Blind, Multicenter Clinical Trial Protocol

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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 19 May 2022
Accepted: 31 May 2022
Published: 01 July 2022

Citation:
Cui X, Liang L, Geng H, Liu Y, Xi J, Wang J, Ching TB, Bee EG, Chai Y, Wu S, Jin D and Xie Y (2022) Efficacy, Safety and Mechanism of Jinzhen Oral Liquid in the Treatment of Acute Bronchitis in Children: A Randomized, Double-Blind, Multicenter Clinical Trial Protocol. Front. Pharmacol. 13:948236. doi: 10.3389/fphar.2022.948236

Background: Acute bronchitis (AB) is a common disease in pediatrics. Prolonged AB may develop into chronic bronchitis. Bronchitis caused by the influenza virus can lead to severe hypoxia or insufficient ventilation, causing great harm to patients and increasing the burden on children and society. Presently, there is no specific treatment for AB except symptomatic supportive treatment. It is urgent to find an effective treatment for AB. Jinzhen Oral Liquid (JZOL) has been found to have a broad spectrum of anti-inflammatory and antiviral effects in previous clinical and basic studies and has a good effect on AB in children. However, the large-sample, randomized, double-blind, head-to-head, evidence-based studies are lacking. The purpose of this protocol is to evaluate the efficacy, safety, and mechanism of JZOL in the treatment of AB in children.

Methods: This is a randomized, double-blind, parallel-controlled multi-center clinical trial. The sample size is 500 participants in the intervention group and the control group respectively, with a total of 1000 participants. They will be recruited by 10 hospitals in China. The Intervention group takes JZOL and Ambroxol Hydrochloride and Clenbuterol Hydrochloride Oral Solution (AHCHOS) placebo, while the control group receives AHCHOS and JZOL placebo. The dosage of the two drugs varies according to age and weight. The medication lasts for 7 days. The disappearance time of cough is adopted as the primary outcome. Quality control will be carried out at every stage of data management and processing to ensure that all data are reliable and processed correctly. SAS is used for statistical analysis. Intention-to-treat analysis will be carried

Abbreviations: AB, acute bronchitis; AEs, adverse events; AHCHOS, ambroxol hydrochloride and clenbuterol hydrochloride oral solution; AUC, area under curve; CRF, case report form; DMP, data management plan; ECG, electrocardiogram; EDC, electronic data capture; IL-1β, interleukin-1β; JZOL, Jinzhen Oral Liquid; LPS, lipopolysaccharide; RMPP, refractory mycoplasma pneumoniae pneumonia; SAE, serious adverse event; SOPs, standard operating procedures; TCM, traditional Chinese medicine; TNF-α, tumor necrosis factor -α.

out in this trial. All statistical tests are conducted using a two-sided test, and p <0.05 would be considered statistically significant.

Discussion: We hypothesized that children with AB could get good health benefits from JZOL. This study not only evaluates the clinical efficacy and safety of JZOL but also conducts metagenomics analysis and metabolomics analysis of feces and saliva of participants to study the mechanism of JZOL against AB. Therefore, this protocol evaluates the efficacy, safety, and mechanism of JZOL from a comprehensive perspective, so as to obtain a more solid evidence chain, which will enhance the credibility of the evidence. If successful, this study will provide a high-level evidence-based reference for the treatment of AB in children and future relevant studies.

Keywords: Jinzhen Oral Liquid (JZOL), acute bronchitis (AB), Ambroxol Hydrochloride and Clenbuterol Hydrochloride Oral Solution (AHCHOS), traditional Chinese medicine (TCM), randomized controlled trial, protocol

INTRODUCTION

Acute bronchitis (AB), also known as lobular pneumonia, is a common disease in pediatrics (Koehler, et al., 2019; Wopker, et al., 2020). The main clinical manifestations of the disease are cough, asthma, dyspnea, fever, headache, and general malaise (Kantar, et al., 2017). This disease has complex pathogenic factors and high incidence, accounting for 40% of all healthcare-associated infections in pediatric long-term care facilities (Murray, et al., 2016). Children have lower immunity than adults, so their conditions tend to change quickly. If not treated in time, it may lead to various complications, such as chronic bronchitis, emphysema, chronic obstructive pulmonary disease, *etc*., seriously affecting the quality of life of the children and their guardians (Newcombe, et al., 2010; Rolfsjord, et al., 2016). Moreover, the risk of AB is increased due to complex conditions, frequent device use, immaturity of immunity, and behavioral factors. AB spends 10 times as much in the respiratory virus season as in the non-respiratory virus season (Murray, et al., 2016). AB causes the under-five mortality rate to reach 15 percent, resulting in an estimated 808,694 child deaths (Murray, et al., 2016). Therefore, AB not only has high morbidity and mortality but also imposes a huge economic burden on society and families (Kinkade, et al., 2016; Murray, et al., 2016; Unger, et al., 2017). Currently, there is no specific treatment for AB except symptomatic supportive treatment. Antibiotics are the first choice for pure western medicine (Aabenhus, et al., 2017; Ouldali, et al., 2017). However, in recent years, the abuse of antibiotics has increased the number of drug-resistant bacteria, leading to a prolonged illness, more likely to develop into a severe illness, and then endanger life (Zanasi, et al., 2016; Bauer, et al., 2019; Walters, et al., 2022). Therefore, it is urgent to find an effective treatment for AB.

Traditional Chinese Medicine (TCM) for AB has a long history and has been widely recognized and used in Asian countries (Gottschling, et al., 2013). Jinzhen Oral Liquid (JZOL) is a TCM compound preparation, which originated from the pediatric formula "Antelope Qingfei Prescription," with a history of more than 400 years (Tao, et al., 2020). JZOL is mainly used for acute bronchitis, mycoplasma pneumonia, viral pneumonia, and other diseases in children (Hu, et al., 2014; Yu, et al., 2019). JZOL consists of eight traditional Chinese herbs including Antelope Horn (*Saigae Tataricae Cornu*), Pingbeimu (*Fritillaria usuriensis Maxim. (Liliaceae))*, Dahuang (*Rheum officinale Baill. (Polygonaceae))*, Huangqin (*Scutellaria baicalensis Georgi (Lamiaceae))*, Niuhuang (*Bos Taurus domesticus Gmelin's dry gallstones*), Qingmengshi (*Metamorphic biotite schist or chloritic mica carbonate schist*), Shi-gao (*Sulfate minerals gypsum family gypsum*) and Gancao (*Glycyrrhiza glabra L. (Fabaceae))* (Tao, et al., 2020). JZOL has the effect of clearing heat, detoxifying, resolving phlegm, and relieving cough (Tao, et al., 2020). Some researchers used UPLC-Q/TOF-MS technology to analyze the prototype components and metabolites in plasma, urine, bile, and feces of JZOL after intragastric administration in rats. That study described the metabolic profile of JZOL *in vivo* and preliminarily revealed its material basis *in vivo*. And the results showed that the main components absorbed into the blood by JZOL were flavonoids, saponins, and anthraquinones. The main metabolic reactions in rats were hydrolysis, hydroxylation, glycolaldehyde acidification, and sulfation (Zhang, et al., 2021). In addition, a number of clinical studies have also proved the clinical benefits of JZOL in children with refractory mycoplasma pneumoniae pneumonia (RMPP), bronchopneumonia, community-acquired pneumonia, and viral pneumonia (Hu, et al., 2019; Sun, 2020; Yang, et al., 2021; Liu, et al., 2022). In the clinical study of RMPP in children, compared with the control group, JZOL combined with methylprednisolone can effectively improve the clinical symptoms and the level of lung function, and reduce the inflammatory response, with good safety (Liu, et al., 2022). In the clinical study on the treatment of mycoplasma pneumonia in children, JZOL combined with azithromycin can reduce the levels of IL-6, IL-8, and IL-10, regulate the inflammatory response and improve the clinical symptoms with good safety (Sun, 2020).

However, the previous clinical studies lacked large sample, multi-center randomized double-blind controlled studies in terms of trial design. At the same time, in terms of intervention methods, most clinical trials adopted western medicine combined with JZOL compared with western medicine alone, which failed to fully highlight the advantages

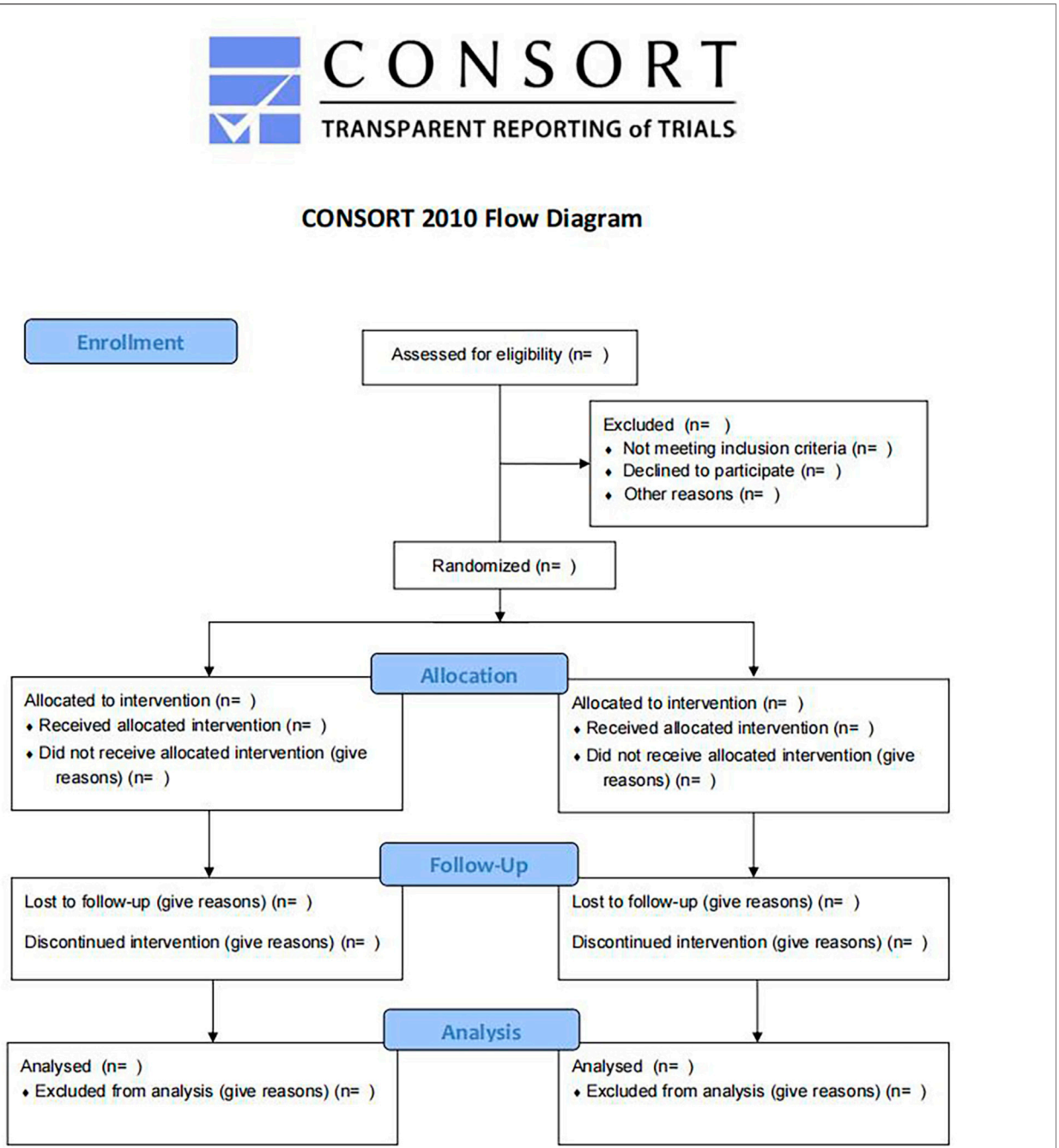

FIGURE 1 | Flow diagram of the trial.

of JZOL in the treatment of AB. In terms of mechanism research, there are few laboratory studies on the mechanism of TCM treatment of AB. Therefore, a randomized, double-blind, parallel-controlled, multi-center clinical trial is designed to accurately evaluate the clinical efficacy and safety of JZOL in the treatment of AB in children. Meanwhile, this protocol will also explore the *in vivo* mechanism of JZOL in the treatment of AB through metagenomic analysis of stool or blood and metabolomics analysis of stool or blood. If completed, this study will provide high-level evidence-based support and reference for clinical treatment and drug decision-making.

MATERIALS AND METHODS

Study Design

This clinical trial is designed as a stratified, randomized, double-blind, parallel-controlled, multi-center clinical study. The sample

size is 500 participants in the intervention group and the control group respectively, with a total of 1000 participants. The trial will be carried out in 10 hospitals in China: *1*) Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine, *2*) Beijing Children's Hospital, Capital Medical University, *3*) Children's Hospital of Soochow University, *4*) Children's Hospital Affiliated to Shandong University, *5*) The Second Affiliated Hospital of Heilongjiang University of TCM, *6*) Beijing Hepingli Hospital, *7*) Guangzhou Women and Children's Medical Centar, *8*) Children's Hospital of Shanghai, *9*) The First Affliated Hospital of Henan University of TCM, *10*) Xuzhou Children's Hospital. All of these hospitals are qualified and experienced to conduct clinical trials. All enrolled participants will sign an informed consent before randomization. The flow chart of the trial is shown in **Figure 1**. There are three main objectives of the study. The first aim is to evaluate the effect of JZOL on shortening the course of AB and improving the condition in children. The disappearance time of cough is adopted as the primary outcome. The second aim is to assess the safety of JZOL in the clinical setting. The incidence of adverse events (AEs), routine blood investigations, blood biochemistry investigations, urinalysis, electrocardiogram (ECG), and stool routine tests are the main indicators to evaluate safety. The third aim is to explore the mechanism of action of JZOL in the treatment of AB by feces/saliva metagenomic analysis and feces/blood metabolomics analysis. The protocol will be reported following the Standard Protocol Items for Clinical Trials with Traditional Chinese Medicine 2018: Recommendations, Explanation and Elaboration (SPIRIT-TCM Extension 2018) (Additional file 1) (Dai, et al., 2019).

Participants

Diagnostic Criteria

(1) Diagnostic criteria of Western medicine

AB is diagnosed if the child has respiratory symptoms such as cough, combined with lung crackles and/or chest radiographic changes (Hu, et al., 2014).

AB is diagnosed when there are primary symptoms, plus three secondary symptoms, concerning tongue and pulse condition (China Association of Chinese Medicine, 2012).

Inclusion Criteria

(1) Participants with AB under the diagnostic criteria of Western medicine.
(2) Those who meet the TCM diagnostic criteria of phlegm-heat stasis in lungs.
(3) Participants aged 2–14 years.
(4) Cough (day + night) score ≥4 points.
(5) Duration of disease ≤48 h, no antibiotics, antitussives, phlegm-reducing drugs, and other Chinese or Western medicine that influence cough has been used before treatment.
(6) The informed consent process complies with the regulations, and the legal representative and/or the child (≥8 years old) signs the informed consent.

Exclusion Criteria

(1) Participants with severe bronchitis, which is difficult to distinguish from pneumonia in the early stage.
(2) Participants with acute infectious diseases such as measles, whooping cough, and influenza.
(3) Participants with single acute upper respiratory tract infection, suppurative tonsillitis, asthmatic bronchitis, bronchial asthma, bronchiolitis pneumonia, tuberculosis, or tumor.
(4) White blood cell count >12.0 × 10^9/L, or a lot of purulent sputum in the lungs.
(5) Participants with severe malnutrition or immunodeficiency.
(6) Participants with severe diseases involving the heart, liver, kidney, digestive and hematopoietic system.
(7) Allergic to JZOL or Ambroxol Hydrochloride and Clenbuterol Hydrochloride Oral Solution (AHCHOS).
(8) Participants who are taking epinephrine, isoproterenol, or other catecholamines.
(9) Participants who are taking monoamine oxidase inhibitors or tricyclic antidepressants.
(10) Participants who are taking propranolol or other non-selective β-blockers.
(11) Participants who are taking large amounts of other sympathetic stimulants.
(12) Participants who are deemed unsuitable for inclusion in this study by the researcher.

Drop-Out Criteria

(1) Participants who develop an allergic reaction or serious adverse event (SAE) would be withdrawn from the trial according to the doctor's judgment.
(2) Participants with other concomitant diseases occurring during the trial, which affected the assessment of efficacy and safety of JZOL.
(3) Participants whom have poor compliance, changed their medication midway, or added drugs prohibited under this study.
(4) Any reason breaking the double-blind setting of this clinical trial.
(5) After medication, if the total score of cough and expectoration increased by four points or the armpit temperature >38.5°C continued for more than 48 h, or the child developed bronchial pneumonia, the tested medication should be stopped immediately, effective treatment should be given. The child should complete all laboratory tests, be withdrawn from the study, and be considered an invalid case.
(6) Participants who are found to have serious violations of inclusion or exclusion criteria after being enrolled in this study.

Voluntary Withdrawal of Participants

(1) The participants and their guardians are unwilling or unable to continue the trial for any reason and request the physician in charge to withdraw the candidate from the trial.
(2) Participants who no longer receive medication and testing, and were lost to follow-up visits.

Conditions for Suspension

(1) If an SAE occurs during the study, the trial should stop immediately.
(2) If there is a major error in the trial, or a serious deviation of protocol during implementation, which makes it difficult to evaluate the drug efficacy and safety, the trial should be discontinued.
(3) If the drug has a poor therapeutic effect or no clinical value during the trial, the trial should be stopped.
(4) The sponsor requests to stop the trial.
(5) The administrative authority cancels the trial.

Sample Size Calculation

In this study, the disappearance time of cough is used as the primary outcome. According to previous studies, the disappearance time of cough in the experimental group and the control group is about 5 ± 2 days. The non-inferiority threshold of this study is set as 0.5 days, unilateral $\alpha = 0.025$, power = 0.95, and the PASS (V14.0) is selected to estimate the sample size. Based on the calculation, 417 cases are needed for each group. After considering the proportion of withdrawals, the sample size of this study is 500 cases for each group, with a total of 1000 cases.

Randomization and Blindness

In this protocol, the stratified block randomization method is adopted. Participants will be stratified according to age. The age stratification is divided into five categories, which are 2–3 years old, 4–5 years old, 6–7 years old, 8–12 years old, and more than 12 years old. SAS 9.4 is used to generate random numbers and corresponding treatment groups. Random numbers are assigned by a central random platform (DAS for IWRS), and competitively selected by each clinical subcenter. There are two copies of the random scheme, which were sealed and stored respectively in the sponsor and the designer of the study scheme.

The double-blind method is used in this trial. Participants, clinicians, and outcome evaluators will be blinded. A two-stage blind design also will be used in the study. In this design, the statistical analyst will not know the grouping of medications in the study. Thus, the statistical analyst also will be blinded. To ensure the quality of blinding, all drugs and placebo are required to be uniformly packaged, while guaranteeing that there is no difference between real drugs and placebo in shape, color, sizer, taste, and smell. Entrust a third party to randomly number the corresponding medicines according to the random coding table and paste them in a conspicuous position in the external packaging of the medicines. The researchers logged on to the designed central random platform to obtain the random numbers of participants, and the pharmacy administrators of each clinical unit distributed the corresponding medicines to the patients according to the random number, to ensure that both researchers and patients were blinded. All the outcome measures and statistical analysts were not involved in the implementation of the trial, nor did they know the study design and hypotheses. If the participants experience SAEs during this trial, the principal investigator at each center has the authority to log in to the central randomization platform to unblind the patients, the reason for urgent unblinding should be noted, dated, and recorded on case report form (CRF).

Intervention Method

The Intervention group takes JZOL and AHCHOS placebo, while the control group receives AHCHOS and JZOL placebo. Both drugs and their placebos are taken orally. According to different ages and weights, the dosages of the two drugs are shown in **Table 1**. The conditions treated by the two drugs in this study are the same as publicly available information. Ibuprofen Suspension will be taken when two groups of participants appear in an emergency. The medication lasts for 7 days. The treatment medicine and their placebos are provided by Jiangsu Kanion Pharmaceutical Co., Ltd according to the double-blind principle. The schedule of enrollment, interventions, and assessments is shown in **Table 2**.

Guidelines for Drug Combination

(1) During the trial, expectorants, bronchodilators, and other Chinese and Western medicines with antitussive and expectorant effects are not allowed to be used. For example, salbutamol, ipratropium bromide, budesonide, acetylcysteine, terbutaline, ambroxol hydrochloride, which are western medicines commonly used for expectoration and bronchiectasis; and compound fresh bamboo juice, Feilike Mixture, cough syrup, which are Chinese patent medicine used to remove phlegm and relieve cough.
(2) Antibiotics should not be given routinely. Whenever needed, antibiotics can be used under the doctor's discretion, but the duration, dosage, and frequency should be recorded in detail.
(3) When the axillary temperature exceeds 38.5°C during the study, physical cooling or antipyretic analgesics (e.g. ibuprofen suspension) can be used together, and usage information should also be recorded in detail.
(4) The physician should ask the participants to bring all medication taken by themselves during the follow-up to check for combination medication. For the drugs or other treatments that must be taken continuously due to the combination of diseases, the name, duration, dosage, and frequency of the drugs must be recorded in the medical record, so as to facilitate the summary, analysis and reporting later.

Outcome Evaluation

Primary Outcome

The disappearance time of cough should be recorded daily, then evaluated at the study endpoint.

Secondary Outcome

(1) Area Under Curve (AUC) of cough and expectoration symptom score-time, cough symptom score (**Table 3**), antitussive onset time, clinical recovery time, and TCM syndrome score (Li, 2015) (**Table 4**) will be daily recorded and evaluated at the study endpoint.
    i. Cough disappeared: cough symptom score (daytime + night) ≤1 point, and maintained for 24 h or more.

TABLE 1 | Usage instructions for both drugs.

Jinzhen oral liquid and its placebo	
Age	**Dosage**
2–3 years old	10 ml each time, twice a day
4–7 years old	10 ml each time, three times a day
8–14 years old	15 ml each time, three times a day

Ambroxol Hydrochloride and Clenbuterol Hydrochloride Oral Solution and its placebo		
Age	**Weight**	**Dosage**
2–3 years old	12–16 kg	7.5 ml each time, twice a day
4–5 years old	16–22 kg	10 ml each time, twice a day
6–12 years old	22–35 kg	15 ml each time, twice a day
More than 12 years old	—	20 ml each time, twice a day

Note: In case of inconsistency between age and weight, the dosage can be adjusted according to their weight.

TABLE 2 | Flow chart for clinical trial.

Stage procedure	**Screening stage /Baseline (1st Visit)**	**During treatment (2nd Visit)<**	**After treatment (3rd Visit)**
	−1 ~ 0 days	**5 ± 1 day**	**7 ± 1 day**
Patient screening	×	—	—
Sign informed consent form	×	—	—
Fill in demographic information	×	—	—
Past medical history	×	—	—
Medical comorbidities and current medication	×	—	—
Chest radiograph	×	—	—
General examination	×	×	×
Physical examination	×	×	×
TCM syndrome scoring and grading	×	×	×
Routine blood investigations, urinalysis and stool examination	×	—	×
Liver function tests (ALT, ALP, AST, TBil, γ-GT) and kidney function tests (BUN, Cr)	×	—	×
ECG	×	—	×
Stool/salivary metagenomic analysis	×	×	×
Stool/blood metabolomics analysis	×	×	×
Distribution and withdrawal of the remaining intervention medication	×	—	×
Distribution and withdrawal of patients' medication diary record cards	×	×	×
Adverse events record	—	×	×
Combination therapy medication record	—	×	×
Conclusion (Trial Summary)	—	—	×

TABLE 3 | Cough symptom scoring.

Score	**Daytime symptom**	**Night symptom**
0	No cough	No cough
1	Short occasional cough	Short occasional cough during sleep
2	Frequent cough, daily activities mildly affected	Slight disturbance of sleep due to cough
3	Frequent cough, daily activities severely affected	Severe disturbance of sleep due to cough

ii. Antitussive onset time: the number of days required for cough symptom score to decrease by one point after taking the medicine.
iii. Clinical recovery: cough, expectoration and pulmonary symptoms are all mild after treatment, without affecting study, living and sleep.
iv. Evaluation criteria for the efficacy of TCM syndromes
   A. Clinical recovery: the total score decreased by ≥90%.
   B. Significant effect: the total score decreased by ≥70% and <90%.
   C. Effective: the total score decreased by ≥30% and <70%.
   D. Ineffective: those who fail to meet the above standards.

(2) The disappearance rate of cough, sputum and pulmonary rales will be evaluated at the study endpoint.

TABLE 4 | Quantitative Grading evaluation for TCM syndrome.

Grading symptoms		**(–)**	**(+)**	**(++)**	**(+++)**
Primary symptoms		**0**	**1**	**2**	**3**
Cough	Daytime	No cough	Short occasional cough	Frequent cough, daily activities mildly affected	Frequent cough, daily activities severely affected
	Night	No cough	Short occasional cough during sleep	Slight disturbance of sleep due to cough	Severe disturbance of sleep due to cough
Expectoration	Daytime	No expectoration	Phlegm is whitish and slightly sticky	Phlegm is whitish/yellowish and sticky	Phlegm is yellowish and sticky
	Night	No expectoration	Phlegm is easy to cough out	Phlegm is slightly difficult to cough out	Phlegm is difficult to cough out
Secondary Symptoms		0	1	2	3
Fever (24 h maximum axillary temperature)		≤37.2°C	37.3–37.9°C	38.0–38.5°C	>38.5°C
Thirsty		No	Yes	Yes	Yes
Facial Erythema		No	Yes	Yes	Yes
Dysphoria		No	Yes	Yes	Yes
Scanty dark-colored urine		No	Yes	Yes	Yes
Dry stool		No	Yes	Yes	Yes
Tongue and pulse condition		Normal	Abnormal	Others	
Tongue		Pinkish	Reddish	—	
Tongue coating		Thin whitish coating	Yellowish coating	—	
Pulse condition		Normal	Slippery and rapid	—	

Note : Children who cannot expectorate is not included.

(3) The usage of antibiotics, antipyretics or analgesics will be evaluated at the study endpoint.

Safety Evaluation

General physical examination, such as body temperature, pulse rate, respiration and blood pressure, are measured at baseline, during the treatment period and at the end of the treatment. Routine blood investigations, urinalysis, stool routine tests, liver function tests, renal function tests and ECG are examined at baseline and at the end of the trial. Participants who are normal before treatment but abnormal after treatment should be re-examined regularly until the end of follow-up. Possible AEs and allergic reactions will be observed at any time after medication. If an AE occurs during the trial, the sponsor shall be responsible for the cost of treatment until the AE disappears or the condition becomes stable.

Mechanism Investigation

In order to understand the mechanism of the action for efficacy of JZOL in the treatment of AB, stool, saliva and blood are collected three times, i.e., before treatment, 5 days after treatment and at the end of treatment, followed by stool/saliva metagenomics analysis and stool/blood metabolomics analysis. In order to exclude the effect of antibiotics on the intestinal flora and its metabolites, participants who received antibiotics during treatment should be excluded.

Quality Control

In order to ensure the quality of the trial and the implementation of quality assurance system, investigators are required to carry out clinical trials according to standard operating procedures (SOPs). All the results and findings in the trial should be verified to ensure the reliability of the data and to ensure that the conclusions of clinical trials are derived from the original data. Researchers must carry out quality control at every stage of data processing to ensure that all data are reliable and processed correctly.

This trial will carry out quality control in the following aspects: progress of the trial; qualification of clinical sub-center; qualification of researchers; mastery of procedures; scientificity, authenticity, accuracy, and completeness of CRF; preservation of files; program implementation; AEs; preservation and storage of drugs; collection and management of biological samples; written informed consent; patients compliance; and laboratory examination data. In particular, the authenticity and accuracy of the CRF, the implementation of the program, and the determination of AEs will be strictly inspected. A diary record card for this trial will be provided to record medication status, symptom and syndrome score. In this way, recall bias can be avoided and study quality and compliance can be improved.

Before the drafting of the protocol, the study will invite experts in the pediatric and respiratory fields to hold a design meeting to determine the test objectives, ideas, and methods. After the protocol is drafted, all study sub-centers will be convened to discuss specific operation details and enhance the operability of the protocol. After the protocol is drafted, all study sub-centers will be convened to discuss specific operation details and enhance the operability of the protocol. During the implementation of the trial, research training sessions will be held in each clinical sub-center to strengthen the researchers' and quality control staff's grasp and understanding of the trial scheme and its key procedures. At the same time, interim coordination meetings

TABLE 5 | Secondary outcome.

Secondary outcome	Test method	Data set
Time-lapse analysis of measured value and changed value of cough symptom score	T test is used to compare the differences between groups	FAS[a]
AUC of cough and expectoration symptom score - time		
Onset of cough relief		
Clinical recovery time		
Disappearance rate of cough, expectoration and lung rales	$\chi 2$ test or Fisher's exact probability method is used to compare the differences between groups	
Use of antibiotics, antipyretics and analgesics		
Analysis on the curative effect of TCM syndrome	CMH chi-square is used to compare the differences between groups	

[a]*FAS, Full Analysis Set; AUC, Area Under Curve.*

will be convened as appropriate to discuss and solve technical problems in the operation process. After the completion of the trial, all clinical sub-centers will be invited to participate in the trial summary meeting to discuss the Multi-center Summary Report.

Data Management

Electronic data management and direct-attached storage for Electronic Data Capture (EDC) system are used in this study. The Data Management Plan (DMP) is written by the data manager as the guidance for data management and is approved by the sponsor. The data management will be carried out in accordance with the time, content, and method defined by the DMP. The integrity of the data will be checked by reviewing whether all paper and electronic materials are filled in and archived following SOPs. The authenticity of participants will be checked by telephone follow-up. Paper materials of the data will be collected after passing the review by composition of data monitoring committee. Researchers responsible for data input will log in to the established EDC and enter data into eCRF in the principle of double-person and double-enter, and conduct consistency tests to ensure the accuracy of the data.

Statistical Analysis

SAS will be used for statistical analysis. Intention-to-treat analysis will be carried out in this trial, which refers to the analysis of all cases that have been randomized and used drugs at least once. All statistical tests are conducted using a two-sided test, and $p < 0.05$ would be considered statistically significant. The missing data will be filled up by carrying forward the last measured value in this trial. Covariance analysis is used for the evaluation of the cough disappearance time. The group is taken as a fixed effect, and the baseline value of the cough symptom score is taken as covariance into the model for analysis. The difference between the different values of the two groups and their bilateral 95% confidence intervals are calculated. Then, the lower limit of bilateral 95% confidence intervals and the preset non-inferiority standard are compared to determine whether the test group is inferior to the control group. In addition, the subgroup analysis is performed according to age (2–3 years, 4–7 years, and 8–14 years). Measurement methods and data sets of secondary outcomes are shown in **Table 5**. In terms of safety, the number of AEs and SAEs during the study will be listed, and their incidence will be calculated.

DISCUSSION

Prolonged AB may develop into chronic bronchitis (Kinkade, et al., 2016). Bronchitis caused by the influenza virus can lead to severe hypoxia or insufficient ventilation, causing great harm to patients and increasing the burden on children and society (Unger, et al., 2017). Ciliated epithelial cells will be damaged when airway epithelial tissues are affected by air pollutants, viruses, bacteria, endotoxins, and other pathogenic factors. At the same time, this process will be accompanied by the activation of the corresponding cell membrane G protein receptor, which will activate the intracellular inflammatory stress response and start the downstream inflammatory response pathway, resulting in acute respiratory epithelial injury (Mokra, et al., 2015; Deshpande, et al., 2020). When an acute respiratory epithelial injury occurs, neutrophil infiltration may occur in the respiratory tract, releasing a large number of inflammatory factors, oxygen free radicals, proteolytic enzymes and so on (Narasaraju, et al., 2011). Under this action, the epithelium of the respiratory tract can undergo the reaction of cell self-melting, cilia necrosis and abscission (Narasaraju, et al., 2011). In the treatment of AB, antibiotics are the first choice for Western medicine intervention. However, the abuse of antibiotics has led to an increasing number of clinical drug resistance events. Some studies have shown that the resistance of Spn to β -lactam antibiotics in hospitalized children has decreased, but the resistance to erythromycin and other commonly used antibacterial drugs is still severe, and there are a large number of multiple drug resistance (Lin, et al., 2022). Therefore, there is an urgent need for a safe and effective strategy to treat AB.

JZOL is a compound prescription of TCM (Tao, et al., 2020). It can effectively improve lipopolysaccharide (LPS)-induced interstitial edema of lung tissue. JZOL can also reduce the levels of inflammatory cytokines, tumor necrosis factor -α (TNF-α), and interleukin (IL)-1β (Zong, et al., 2018). Besides, it can inhibit or kill the influenza virus, Coxsackie virus, respiratory syncytial virus, and mycoplasma pneumonia (Xiao, et al., 2008; Hou, et al., 2009; Xiao, et al., 2009). Experimental studies *in vitro* and *in vivo* have shown that Dahuang can inhibit a variety of viruses by inhibiting the viral synthesis and reducing the number of virus replication (Lin, et al., 2016). Dahuang also exerts strong anti-inflammatory effects by

inhibiting the production of pro-inflammatory factors such as IL-6, IL-1β, and TNF-α and inhibiting the NF-κB inflammatory signaling pathway (Ge, et al., 2017). An experiment showed that Gancao could inhibit the proliferation of HIV-1, SARS, and other viruses, which indicated that Gancao had good antiviral activity (Yi, et al., 2021). Gancao can also regulate the number and function of lymphocytes, and inhibit the levels of inflammatory mediators and proinflammatory cytokines, indicating that glycyrrhiza has a good anti-inflammatory effect (Li et al., 2021). Animal experiments showed that peimisine had a good therapeutic effect on LPS-induced airway mucus hypersecretion model and acute lung injury mice (Cui, et al., 2015; Wang, et al., 2016). Peiminine can treat bleomycin-induced lung injury by reducing TGF-β/MAPK ERK and MEK1/2 cell signal transduction (Guo, et al., 2016a; Guo, et al., 2016b). These *in vitro* and *in vivo* studies have proved that JZOL has broad spectrum antiviral and anti-inflammatory effects, and has a potential therapeutic effect on AB.

Some clinical studies have come to the same conclusion. In a clinical study of AB in children, JZOL combined with atomized inhalation of budesonide in the treatment of AB achieved a higher degree of clinical efficacy satisfaction, which can effectively shorten the rehabilitation time of children with a low incidence of AEs (Weng, et al., 2021). Meanwhile, another clinical study has shown that, compared with the control group, JZOL combined with ambroxol and clenbuterol tablets can significantly relieve the clinical symptoms of cough, wheezing and other symptoms in children with AB, and improve the level of inflammation with fast onset of effect (Li, 2021). These studies have demonstrated clinical efficacy and safety of JZOL. However, there is still a lack of large-sample, multi-center randomized double-blind controlled studies comparing JZOL head-to-head with Western medicine and the mechanism of its effect *in vivo*.

Even if the use of Chinese compound prescription is on the rise in China (Jin, 2021), the number of high-level evidence-based clinical trials is limited. To date, this study is the first prospective, multi-center, randomized controlled trial of JZOL in the treatment of AB in children strictly according to CONSORT Extension for CHM Formulas (Consort-CHM Formulas 2017) (Cheng, et al., 2017). Simultaneously, we selected ten hospitals from different regions of China as sub-centers of this clinical study, which can reduce regional bias and obtain more accurate results. In addition, standardized methods such as stratified block randomization, double blindness, and double simulation were adopted in this study, which played a positive role in further promoting and improving the level of the evidence-based methodology of TCM worldwide. Furthermore, this study not only evaluates the clinical efficacy of JZOL but also conducts metagenomics analysis and metabolomics analysis of feces and saliva of participants to study the mechanism of JZOL against AB. Therefore, this protocol evaluates the efficacy, safety, and mechanism of JZOL from a comprehensive perspective, so as to obtain a more solid evidence chain, which will enhance the credibility of evidence.

Although strict quality control will be carried out in this study, there might also be potential limitations for this study. Firstly, different hospitals use different testing machines and testing standards, which may lead to test data bias. Secondly, the efficacy criteria of TCM syndromes are not included in the international guidelines. However, the evaluation criteria of TCM efficacy in this study were determined after several rounds of discussion by high-level TCM experts and have been highly recognized in the TCM guidelines (Li, 2015).

We hypothesized that children with AB could get good health benefits from JZOL. If successful, this study will provide a high-level evidence-based reference for the treatment of AB in children and future relevant studies.

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 Ethics Committee of Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine (No. DZMEC-KY-2019-183-01). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XC, YMX, and SXW conceived and designed this study. XC and LL wrote the manuscript with contributions from all authors. YMX, DJ, and SXW critically revised the manuscript. HJG, YL, JYX, JHW, TBC, EGB, and YC refined the protocol. All authors contributed to the article and approved the submitted version.

FUNDING

The study is supported by the National Key Research and Development Program of The Ministry of Science and Technology of China in 2018, "Research on Modernization of TCM" project, "Demonstration Study on Evidence-based Evaluation and Effect mechanism of Ten Chinese patent Medicines and Classic famous prescriptions in the Treatment of Major Diseases after marketed" (2018YFC1707400).

ACKNOWLEDGMENTS

We would like to thank to all the investigators who are participating in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.948236/full#supplementary-material

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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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ORIGINAL RESEARCH
published: 10 August 2022
doi: 10.3389/fphar.2022.944965

Clinical Efficacy and *In Vitro* Drug Sensitivity Test Results of Azithromycin Combined With Other Antimicrobial Therapies in the Treatment of MDR *P. aeruginosa* Ventilator-Associated Pneumonia

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Objective: The aim of the research was to study the effect of azithromycin (AZM) in the treatment of MDR *P. aeruginosa* VAP combined with other antimicrobial therapies.

Methods: The clinical outcomes were retrospectively collected and analyzed to elucidate the efficacy of different combinations involving azithromycin in the treatment of MDR-PA VAP. The minimal inhibitory concentration (MIC) of five drugs was measured by the agar dilution method against 27 isolates of MDR-PA, alone or in combination.

Results: The incidence of VAP has increased approximately to 10.4% (961/9245) in 5 years and 18.4% (177/961) caused by *P. aeruginosa* ranking fourth. A total of 151 cases of MDR *P. aeruginosa* were included in the clinical retrospective study. Clinical efficacy results are as follows: meropenem + azithromycin (MEM + AZM) was 69.2% (9/13), cefoperazone/sulbactam + azithromycin (SCF + AZM) was 60% (6/10), and the combination of three drugs containing AZM was 69.2% (9/13). The curative effect of meropenem + amikacin (MEM + AMK) was better than that of the meropenem + levofloxacin (MEM + LEV) group, $p = 0.029$ ($p < 0.05$). The curative effect of cefoperazone/sulbactam + amikacin (SCF + AMK) was better than that of the cefoperazone/sulbactam + levofloxacin (SCF + LEV) group, $p = 0.025$ ($p < 0.05$). There was no significant difference between combinations of two or three drugs containing AZM, $p > 0.05$ ($p = 0.806$). From the MIC results, the AMK single drug was already very sensitive to the selected strains. When MEM or SCF was combined with AZM, the sensitivity of them to strains can be significantly increased. When combined with MEM and AZM, the MIC_{50} and MIC_{90} of MEM decreased to 1 and 2 ug/mL from 8 to 32 ug/mL. When combined with SCF + AZM, the MIC_{50} of SCF decreased to 16 ug/mL, and the curve shifted obviously. However, for the combination of SCF + LEV + AZM, MIC_{50} and MIC_{90}

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Edited by:
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Specialty section:
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

Received: 16 May 2022
Accepted: 07 June 2022
Published: 10 August 2022

Citation:
Huang Y, Wang W, Huang Q, Wang Z, Xu Z, Tu C, Wan D, He M, Yang X, Xu H, Wang H, Zhao Y, Tu M and Zhou Q (2022) Clinical Efficacy and In Vitro Drug Sensitivity Test Results of Azithromycin Combined With Other Antimicrobial Therapies in the Treatment of MDR P. aeruginosa Ventilator-Associated Pneumonia. Front. Pharmacol. 13:944965. doi: 10.3389/fphar.2022.944965

could not achieve substantive changes. From the FIC index results, the main actions of MEM + AZM were additive effects, accounting for 72%; for the combination of SCF + AZM, the additive effect was 40%. The combination of AMK or LEV with AZM mainly showed unrelated effects, and the combination of three drugs could not improve the positive correlation between LEV and AZM.

Conclusion: AZM may increase the effect of MEM or SCF against MDR *P. aeruginosa* VAP. Based on MEM or SCF combined with AMK or AZM, we can achieve a good effect in the treatment of MDR *P. aeruginosa* VAP.

Keywords: ventilator-associated pneumonia, *Pseudomonas aeruginosa*, azithromycin, *in vitro* drug sensitivity test, multidrug-resistant

INTRODUCTION

Ventilator-associated pneumonia (VAP) refers to the pneumonia that occurs after endotracheal intubation or tracheotomy patients receiving mechanical ventilation (MV) for 48 h. The pneumonia that occurs within 48 h after MV withdrawal and extubation also belongs to the category of VAP (Kalil et al., 2016; Torres et al., 2017; Shi et al., 2019). VAP is a common nosocomial infection in critically ill patients. With the extensive application of invasive MV in the rescue of intensive care unit (ICU) patients, VAP has become one of the most common complications, presenting high incidence rate and mortality (Shi et al., 2019). Statistical results showed that the incidence rate of VAP was 7.9%–48.4%, and the mortality was 21.2%–43.2% (Metersky and Kalil, 2018; Papazian et al., 2020). Once patients are combined with VAP, the time of MV, the length of hospital stay, and the cost of hospitalization will increase, and some cases are even life-threatening. All of these directly affect the short-term and long-term prognosis of patients (Kalanuria et al., 2014). Gram-negative flora is the majority in the VAP pathogen spectrum, including *Acinetobacter baumannii*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and so on (Kalil et al., 2016). *P. aeruginosa* is widely recognized as a common conditional pathogen of hospital-acquired infection (Faure et al., 2018). *P. aeruginosa* has the characteristics of easy colonization, variation, and multi-drug resistance (MDR) (Miyoshi-Akiyama et al., 2017; Liao et al., 2019; Al-Orphaly et al., 2021). Among Gram-negative strains, the most common MDR pathogens are *Acinetobacter baumannii*, *P. aeruginosa*, and Enterobacteriaceae (Otsuka, 2020; Mills and Marchaim, 2021). In China, carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) has been included in one of the five MDR bacteria targeted for prevention and control, in accordance with the requirements of the National Health Commission. The US Centers for Disease Control and Prevention antibiotic resistance threat report has stated that three groups of antimicrobial resistant Gram-negative bacteria pose particular therapeutic challenges: 1) extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), 2) carbapenem-resistant Enterobacterales (CRE), and 3) *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR *P. aeruginosa*). These pathogens have been designated urgent or serious threats by the CDC in the United States.

MDR and pan-drug resistant (PDR) strains of *P. aeruginosa* are particularly frequent in ICU-acquired pneumonia (Ribeiro et al., 2019; Souza et al., 2021). The isolation of a MDR pathogen has been identified as an independent predictor of increased mortality in VAP. In recent years, with the abuse of broad-spectrum antibiotics, MDR and PDR *P. aeruginosa* have been increasing, which bring great difficulties to clinical treatment. The mechanism of *P. aeruginosa* resistance is complex, especially the formation of biofilm (Maurice et al., 2018), which leads to strong bacterial resistance at the lesion. Biofilm formation is the main reason for the recurrence and difficulty to control disease after *P. aeruginosa* infection.

In the past few years, the combination of the two drugs has been frequently used to treat MDR *P. aeruginosa* VAP in the ICU (Shi et al., 2019). The combined antibacterial scheme was based on carbapenems or cephalosporins β-lactamase inhibitor combinations, combined with fluoroquinolones or aminoglycosides, in accordance with the recommendations of the guidelines (Kalil et al., 2016; Torres et al., 2017; Shi et al., 2019). Among these types of drugs, the most commonly used drugs are: meropenem, cefoperazone sulbactam, levofloxacin, and amikacin. In the past, there was no polymyxin in our hospital. For PDR *P. aeruginosa* VAP, our treatment was very difficult. However, we found that a regimen of azithromycin combined with the aforementioned drugs may improve the symptoms of patients and achieve good therapeutic results. However, azithromycin is not the drug recommended in the guidelines for the treatment of *P. aeruginosa* pneumonia. Even *P. aeruginosa* is naturally resistant to azithromycin. So, when it is used in combination, how does it play an antibacterial role? Therefore, this study aimed to research the efficacy of azithromycin combined with other treatment regimens in the treatment of MDR-PA VAP through retrospective analysis of clinical data and *in vitro* drug sensitivity tests.

In this study, 5 years of clinical data from January 2017 to December 2021 were studied retrospectively to describe the characteristics of PA-VAP, and determine the clinical efficacy of antimicrobial regimens. A total of 27 strains of MDR-PA were isolated from our ICU from June 2021 to February 2022. According to the principle of clinical medication, five

antibiotics, namely, meropenem (MEM), cefoperazone sulbactam (SCF), amikacin (AMK), levofloxacin (LEV), and azithromycin (AZM), as single drug or combination, were used for the *in vitro* drug sensitivity test to provide the evidence for the clinical treatment. It aimed to study the efficacy and mechanism of azithromycin combined with other regimens in the treatment of MDR *P. aeruginosa* VAP by combining the results of clinical analysis and *in vitro* drug sensitivity test.

MATERIALS AND METHODS

Setting and Study Design

A retrospective study was conducted in the general ICU of Suizhou Central Hospital Affiliated to Hubei University of Medicine from 1 January 2017 to 31 December 2021. Suizhou Central Hospital is a 2380-bed tertiary care comprehensive hospital, which receives about 73,300 admissions per year. The ICU has 52 beds and covers all medical and surgical cases. This study was approved by the Ethics Committee of Suizhou Central Hospital.

The study included all adult patients who were mechanically ventilated for >48 h and developed VAP caused by *P. aeruginosa*. The first episode of *P. aeruginosa* VAP or polymicrobial VAP was recorded for each patient. The patients with other previous or concurrent infections were excluded from the study. Eligible patients were recognized by the microbial culture results to identify MDR isolates. The patients with COVID-19 were not included in this study. (Special management requirements based on the hospital, since the occurrence of novel coronavirus pneumonia, we have always had a special isolation ward to treat patients with severe COVID-19 pneumonia).

Definitions

VAP was defined according to the guidelines of Chinese Thoracic Society (CTS) and the ATS-IDSA (Kalil et al., 2016; Shi et al., 2019). Diagnosis of VAP required radiographic appearance of a new or persistent pulmonary infiltrate and two or more of the following criteria:

① Temperature of >38°C or <36°C.
② Leukocytosis (peripheral blood leukocyte count, $>10\times10^9$/L) or leukopenia (peripheral blood leukocyte count, $<4\times10^9$/L).
③ The presence of purulent bronchial secretions.

Pneumonia was considered to be ventilator-associated when onset occurred 48 h after the initiation of MV, and was judged not to have been incubating before the initiation of MV. The patients with no clinical symptoms or radiological evidence of an infiltrate were excluded from the study. The onset of VAP was defined as the date of collection of the first clinical positive microbial cultures of aspirate:

① Specimen cultures obtained by endotracheal aspiration cultures (ETA) $>10^5$ CFU/ml; or ② bronchoalveolar lavage cultures (BAL) $>10^4$ CFU/ml.

MDR pathogens were commonly resistant to at least three classes of the following five antibiotics: cephalosporins, carbapenems, compound preparation containing β-lactamase inhibitor, fluoroquinolone, and aminoglycoside antibiotics. Clinical pulmonary infection scores (CPIS) were a retrospective calculation for the studied cases given the nature of this study.

Empirical Antimicrobial Agents' Plan and Curative Effect Judgment

The usage and dosage of each antibacterial drug are as follows:

Meropenem, 1g pump in for 3 h, every 8 h.
Cefoperazone/sulbactam, 3 g intravenous drip, every 12 h.
Amikacin, 15 mg/kg, intravenous drip, once a day.
Levofloxacin, 0.4 g intravenous drip, once a day.
Azithromycin, 0.5 g intravenous drip, once a day.

All patients involved in the study received appropriate antibiotic therapy. The course of all drug combination treatments was for at least 7–10 days. Experiential treatment schemes are as follows:

① Meropenem + amikacin.
② Meropenem + levofloxacin.
③ Cefoperazone/sulbactam + amikacin.
④ Cefoperazone/sulbactam + levofloxacin.
⑤ Meropenem or cefoperazone/sulbactam + levofloxacin or amikacin + azithromycin.

The clinical outcome of PA-VAP was a comprehensive judgment based on the clinical symptoms and CPIS of the patients.

Cured: the clinical symptoms were eliminated, and the results of sputum culture turned negative.

Improved: the clinical symptoms were obviously improved, and the CPIS was declined before combination therapy.

Aggravated: the clinical symptoms were worse, and the CPIS was increased before combination therapy.

Dead: VAP-related death was defined as death that occurred during the treatment period when the signs of pneumonia remained, or due to septic shock.

Effective treatment cases = cured + improved cases.
Ineffective treatment cases = aggravated + dead cases.

Clinical Data Collection

Clinical, biological, and treatment data were obtained retrospectively from patient medical records and department of nosocomial infection management databases. Clinical data included age, sex, Acute Physiology and Chronic Health Evaluation (APACHE) II scores, ICU admission diagnosis, comorbidities, days of MV to VAP, as well as possible risk factors for MDR.

Drug sensitivity data of *P. aeruginosa* to 14 antibiotics from 2017 to 2021 were collected for analysis of drug resistance rate and trend. Data on antimicrobial therapy for the group of *P. aeruginosa*

VAP were recorded for assessment of the effectiveness. The cases were grouped according to the different treatment schemes mentioned earlier. Clinical outcomes were analyzed to elucidate the effect of these empiric antibiotic regimens.

Combined Drug Sensitivity Test *In Vitro*

A total of 27 strains of MDR *P. aeruginosa* were isolated from different patients in the ICU of our hospital in June 2021–February 2022. Quality control strains: *Pseudomonas aeruginosa* ATCC 27853. The minimum inhibitory concentration (MIC) of a single drug was determined in accordance with the method recommended by CLSI (M100ED32-2022) (Clinical and Laboratory Standards Institute, 2022). The MIC value of meropenem, cefoperazone/sulbactam, amikacin, levofloxacin, and azithromycin against 27 strains of *P. aeruginosa* was determined using the agar dilution method. Mueller–Hinton (MH) broth was diluted to a series of concentrations by a double ratio, and all of the five antibiotics were diluted to 11 concentration gradients. The concentrations in the combined drug sensitivity test are 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5 (ug/mL). The specific experimental steps are as follows: ①preparation of the culture medium: MH agar was used to prepare the culture medium according to requirements; ② preparation of agar plate containing drugs: add the diluted antibacterial drugs of different concentrations into the quantitative MH agar melted and cooled to about 50°C, and the plate containing antibacterial drugs of different decreasing concentrations was made. Put it in a sealed plastic bag and store it in a refrigerator at 2–8°C for 5 days; ③ inoculation: inoculate the bacterial solution on the surface of the agar plate, incubate at 35°C for 16–20 h after inoculation; ④ result judgment: place the plate on the surface of dark and non-reflective objects to judge the test end point, and the minimum drug concentration contained in the agar plate that inhibits bacterial growth was regarded as MIC. The single drug MIC ($MIC_{A\ alone}$ and $MIC_{B\ alone}$) and the MIC value of the optimal combination effect ($MIC_{A\ combined}$ and $MIC_{B\ combined}$) were selected to record. The combined drug sensitivity test usually uses the fractional inhibitory concentration (FIC) value to evaluate the effect of the combined drug use. The calculation method and criterion of interpretation of the FIC index are: FIC index = $MIC_{A\ combined}/MIC_{A\ alone}$ + $MIC_{B\ combined}/MIC_{B\ alone}$, synergistic: FIC ≤ 0.5, addictive: 0.5 < FIC ≤ 1, indifference: 1 < FIC ≤ 2, and antagonistic: FIC > 2.

Statistical Analysis

SPSS 24.0 and Excel software were used for statistical analysis, $p < 0.05$ was found to be statistically significant. Qualitative variables were expressed as percentages, whereas quantitative variables are expressed as means ± standard deviations (SD) or medians.

RESULT

Between January 2017 and December 2021, 10,272 adult patients were admitted to our ICU and 9,245 cases were mechanically ventilated patients. The diagnostic criteria for VAP were fulfilled in 961 patients (10.4%, 961/9245), 177 episodes of VAP were due to *P. aeruginosa*, and the incidence of PA-VAP has approximately 18.4% (177/961) of all VAP patients, ranked fourth (*Acinetobacter baumannii*, *Escherichia* coli, and *Klebsiella pneumoniae* ranked in the top three).

TABLE 1 | Clinical characteristics of patients treated for PA-VAP (n = 151).

Characteristic	
No.	151
Age, mean ± SD (years)	50.4 ± 11.3
Female sex [n (%)]	45 (29.8)
Male sex [n (%)]	106 (70.2)
APACHE II score, mean ± SD	23 ± 5
ICU admission diagnosis [n (%)]	
Multiple trauma	52 (34.4)
Severe craniocerebral trauma	49 (32.5)
Respiratory failure	3 (23.8)
Severe nervous system disease	30 (19.9)
Hemorrhagic shock	16 (10.6)
Various kinds of poisoning	13 (8.6)
Acute exacerbation of COPD	10 (6.6)
Other reasons	12 (7.9)
Comorbidities [n (%)]	
Hypertension	28 (18.5)
COPD	16 (10.6)
Other respiratory diseases	14 (9.3)
Diabetes	11 (7.3)
Coronary heart disease	9 (6.0)
Digestive system disease	11 (7.3)
None	68 (45)
Days of hospital admission to VAP, mean ± SD	8.4 ± 5.3
Days of ICU admission to VAP, mean ± SD	7.5 ± 4.6
Days of MV to VAP, mean ± SD	6.8 ± 3.4
Early-onset VAP [n (%)]	24 (15.9)
Late-onset VAP [n (%)]	127 (84.1)
Duration of MV, days, mean ± SD	13.4 ± 11.4
Length of ICU stay, days, mean ± SD	20.3 ± 7.8
Length of hospital stay, days, mean ± SD	31.2 ± 11.5

Abbreviations: APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; MV, mechanical ventilation.

A total of 151 patients were included in our study and 26 patients were excluded from this analysis because VAP treatment time was not enough or took other plans for treatment. They were divided into seven groups based on the treatment regimen (**Table.3** for details). We analyzed the drug sensitivity of five different combination regimens of antibiotics based on the 25 strains of MDR-PA from different patients in the ICU of our hospital in June 2021–February 2022. Note: 27 strains and one quality control strain were used for the *in vitro* drug sensitivity test. However, during the test, two strains were not successful and were excluded from the analysis of the results.

Clinical Characteristics of 151 Patients Treated for PA-VAP

The mean age of the patients was 50.4 ± 11.3 years old (ranging from 25 to 86 years). The male to female ratio was 2.4 (males 106: females 45). The APACHE II score was 23 ± 5. In the hospital, 52 patients (34.4%) were admitted because of multiple trauma, 49 (32.5%) were admitted because of severe craniocerebral trauma, and 30 (19.9%) were admitted for severe nervous system disease. Also, 28 patients (18.5%) had a previous history of hypertension, 16 patients (10.6%) had a previous history of chronic obstructive

TABLE 2 | Antimicrobial resistance of the 151 isolates of *P. aeruginosa* to 14 antibiotics from 2017 to 2021 (%).

Antibiotic	S	R	I	Sensitivity rate (%)	Resistance rate(%)
Amikacin	133	18	0	88.1	11.9
Ceftazidime	56	54	41	37.1	35.8
Ciprofloxacin	68	70	13	45.0	46.4
Gentamicin	105	44	2	69.5	29.1
Furantoin	10	141	0	6.6	93.4
Cefepime	77	39	35	51.0	25.8
Imipenem	49	76	26	32.5	50.3
Levofloxacin	71	71	9	47.0	47.0
Tobramycin	93	54	4	61.6	35.8
Piperacillin/tazobactam	57	32	62	37.7	21.2
Aztreonam	52	95	4	34.4	62.9
Meropenem	71	68	12	47.0	45.0
Cefoperazone/sulbactam	80	54	17	53.0	35.8
Polymyxin	148	3	0	98.0	2.0

Abbreviations: S, susceptible; R, resistant; I, intermediate.

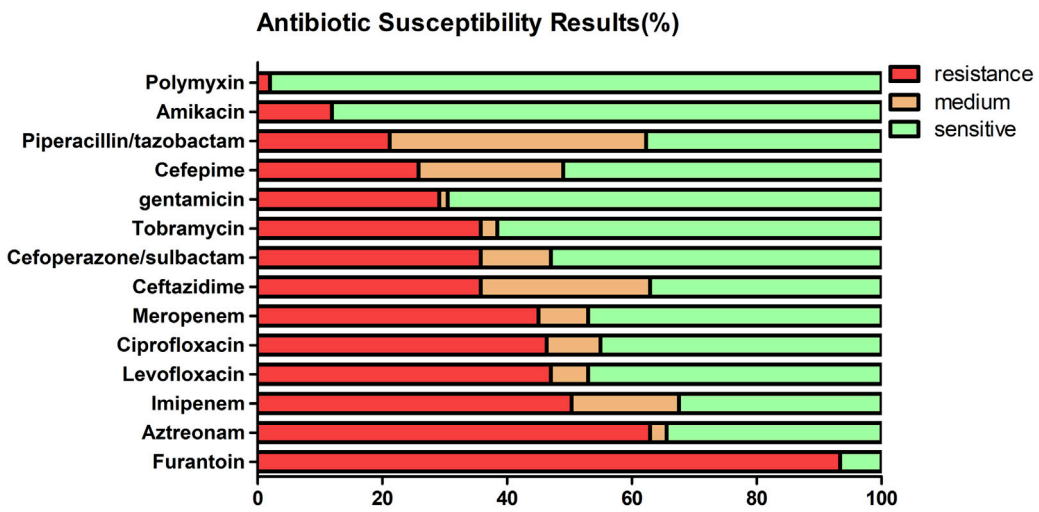

FIGURE 1 | Antimicrobial resistance of the 151 isolates of *P. aeruginosa* to 14 antibiotics from 2017 to 2021 (%).

pulmonary disease (COPD), 14 patients (9.3%) had other respiratory diseases, diabetes mellitus was known for 11 patients (7.3%), coronary heart disease was known for 9 patients (6.0%), and digestive system disease was known for 11 patients (7.3%). The mean length of hospital admission to VAP was 8.4 ± 5.3 days, the mean length of ICU admission to VAP was 7.5 ± 4.6 days, and the mean time from MV to VAP was 6.8 ± 3.4 days. The duration of MV was 13.4 ± 11.4 days, the length of ICU stay was 20.3 ± 7.8 days, and the length of hospital stay was 31.2 ± 11.5 days. The clinical characteristics and outcomes of all patients are summarized in **Table 1**.

Antimicrobial Resistance of *P. aeruginosa* of VAP From 2017 to 2021

In this retrospective study, 151 strains of *P. aeruginosa* were all MDR bacteria. In 5 years, the highest incidence is in 2019, up to 57 of these 151 cases, accounting for 37.75%.

Table 2 and **Figure 1** show the sensitivity and resistance of *P. aeruginosa* to 14 antibiotics in 5 years. Among the 14 antibiotics, the three most sensitive are polymyxin (98%), amikacin (88.1%), and gentamicin (69.5%). Accordingly, the three most resistant are furantoin (93.4%), aztreonam (62.9%), and imipenem (50.3%), the resistance rates were over 50%. The resistance rates of *P. aeruginosa* to meropenem, cefoperazone/sulbactam, amikacin, and levofloxacin are as follows: 45.0%, 35.8%, 11.9%, and 47.0%, respectively. The details are shown in **Table 2**. It was worth mentioning that *P. aeruginosa* showed high resistance to carbapenem antibiotics from the clinical drug sensitivity results. The resistance rates of *P. aeruginosa* to imipenem and meropenem are 50.3% and 45.0%, respectively.

Therapeutic Effect of the Combination of Five Antibiotics

Table 3 shows the clinical grouping of different empirical schemes based on meropenem (MEM) or cefoperazone/

TABLE 3 | Clinical grouping of 151 cases.

		Antibiotic therapy (n)	n
Combination of two drugs (138)	Based on MEM (75)	MEM + AMK	34
		MEM + LEV	28
		MEM + AZM	13
	Based on SCF (63)	SCF + AMK	30
		SCF + LEV	23
		SCF + AZM	10
Combination of three drugs containing AZM (13)		MEM or SCF + AMK or LEV + AZM[a]	13

Abbreviations: MEM, meropenem; SCF, cefoperazone/sulbactam; AMK, amikacin; LEV, levofloxacin; AZM, azithromycin.
[a]*There is no further subdivision of subgroups because of the small number of clinical cases.*

TABLE 4 | Therapeutic effect of different empirical schemes.

Antibiotic therapy (n)		Effective rate (%)	*P* value
Based on MEM (75)	MEM + AMK (34)	73.5 (25/34)	0.029[a] * 0.173[b] 0.768[c]
	MEM + LEV (28)	46.4 (13/28)	
	MEM + AZM (13)	69.2 (9/13)	
Based on SCF (63)	SCF + AMK (30)	70.0 (21/30)	0.025[d] * 0.269[e] 0.559[f]
	SCF + LEV (23)	39.1 (9/23)	
	SCF + AZM (10)	60.0 (6/10)	
Combined with AMK (64)	MEM + AMK (34)	73.5 (25/34)	0.754
	SCF + AMK (30)	70.0 (21/30)	
Combined with LEV (51)	MEM + LEV (28)	46.4 (13/28)	0.601
	SCF + LEV (23)	39.1 (9/23)	
Combined with AZM (23)	MEM + AZM (13)	69.2 (9/13)	0.645
	SCF + AZM (10)	60.0 (6/10)	
Combination of two or three drugs containing AZM (36)	MEM or SCF + AMK or LEV + AZM (13)	69.2 (9/13)	0.806
	MEM or SCF + AZM (23)	65.2 (15/23)	

Abbreviations: MEM, meropenem; SCF, cefoperazone/sulbactam; AMK, amikacin; LEV, levofloxacin; AZM: azithromycin.
[a]*MEM + AMK vs. MEM + LEV.*
[b]*MEM + LEV vs. MEM + AZM.*
[c]*MEM + AKM vs. MEM + AZM.*
[d]*SCF + AMK vs. SCF + LEV.*
[e]*SCF + LEV vs. SCF + AZM.*
[f]*SCF + AKM vs. SCF + AZM.*
* $p < 0.05$.

sulbactam (SCF). In 151 cases, the combination of two drugs was selected in 138 cases, 13 cases were treated with a combination of three drugs containing AZM. Of the 138 cases, 75 cases were based on MEM and 63 were based on SCF. In this retrospective study, the most commonly used antibiotic therapy for PA-VAP was MEM or SCF combined with AMK, AZM is rarely used in clinical treatment of *P. aeruginosa*. **Table 4** shows the statistical analysis results of the clinical efficacy of different empirical treatment schemes.

Among the 75 cases of two drug combination schemes based on MEM, 34 cases were MEM combined with AMK, and the effective rate was 73.5%; there were 28 cases of MEM combined with LEV, and the effective rate was 46.4%; 13 cases combined with AZM, and the effective rate was 69.2%. Intra group comparison found that the curative effect of MEM combined with AMK was significantly better than that of the LEV group, $p = 0.029$ ($p < 0.05$). There was no significant difference in the efficacy of MEM combined with LEV compared with MEM combined with AZM, at the same time, the efficacy of MEM combined with AMK was similar to that of MEM combined with AZM, with both $p > 0.05$.

Among the 63 cases of two drug combination schemes based on SCF, 30 cases were SCF combined with AMK, and the effective rate was 70%; there were 23 cases of SCF combined with LEV, and the effective rate was only 39.1%; 10 cases combined with AZM, and the effective rate was 60%. The efficacy of the combined LEV group was worse than that of the other two combinations. Further intra group comparison found that the curative effect of SCF combined with AMK was significantly better than that of the LEV group, $p = 0.025$ ($p < 0.05$). There was no significant difference in the efficacy of SCF combined with LEV compared with SCF combined with AZM, at the same time, the efficacy of SCF combined with AMK was similar to that of SCF combined with AZM, with both $p > 0.05$.

Among the two drug combination cases, 64 cases were combined with amikacin, 51 cases with levofloxacin, and 23 cases with azithromycin. In terms of effective rate alone, the effective rate of MEM combined with the aforementioned three drugs was higher than that of SCF. Further intra group statistical analysis found that there was no significant difference in the efficacy of MEM combined with AMK and SCF combined with AMK. The efficacy of LEV combined with MEM or SCF was

TABLE 5 | MIC values of MEM, SCF, AMK, and LEV single drugs or after being combined with AZM against the 25 isolates of MDR *P. aeruginosa* (ug/mL).

Antibiotics	Alone			Combined with AZM			Combination of three drugs included AZM			Reference standard (CLSI-M100)		
	MIC_{50}	MIC_{90}	MIC_G	MIC_{50}	MIC_{90}	MIC_G	MIC_{50}	MIC_{90}	MIC_G	S	I	R
MEM	8	32	0.5–32	1	2	0.5–16	—	—	—	≤2	4	≥8
SCF	64	128	1–128	16	64	2–64	8	64	2–64	≤16	32	≥64
AMK	4	16	0.5–16	4	16	0.5–16	—	—	—	≤16	32	≥64
LEV	8	64	1–128	8	32	0.5–64	4	16	0.5–16	≤2	4	≥8

Abbreviations: MIC, minimum inhibitory concentration; S, susceptible; I, intermediate; R, resistant.

TABLE 6 | MIC values of the AZM single drug or after combination against the 25 isolates of MDR *P. aeruginosa* (ug/mL).

Antibiotic	MIC_{50}	MIC_{90}	MIC_G	Reference standard
AZM alone	256	256	32–512	None
AZM + MEM	128	256	0.5–256	
AZM + SCF	128	256	4–256	
AZM + AMK	256	256	0.5–256	
AZM + LEV	128	256	0.5–256	
AZM (combination of three drugs)	128	256	0.5–256	

Abbreviations: MIC, minimum inhibitory concentration. S, susceptible; I, intermediate; R, resistant.

similar, and there was no significant difference between MEM combined with AZM and SCF combined with AZM. All three *P* values were greater than 0.05.

Among 151 cases, 36 cases were combined with AZM, of which 23 cases were combined with two drugs and 13 cases were combined with three drugs. Because of the small number of cases, there was no further subgroup. It was found that there was no significant difference between combinations of two or three drugs containing AZM, $p > 0.05$ ($p = 0.806$). The details are shown in **Table 4**.

Minimal Inhibitory Concentration Results of the *In Vitro* Drug Sensitivity Test

A total of 27 strains and one quality control strain (ATCC 27853) were used for the *in vitro* drug sensitivity test. However, two strains were excluded from the analysis of the results due to failure in the experiment. **Tables 5**, **6**, **Figures 2**, **3** show the MIC results of the five antibiotics against the 25 isolates of MDR-PA. **Table 5** shows the MIC values of MEM, SCF, AMK, and LEV single drugs or after combined with AZM. **Table 6** shows the MIC values of a AZM single drug or after combination. **Figures 2**, **3** show these percentage curves of concentration cumulative inhibition rate, respectively.

The MIC_{50} and MIC_{90} of a MEM single drug are 8 and 32 ug/mL, respectively. After being combined with AZM, MIC_{50} and MIC_{90} decreased to 1 and 2 ug/mL, respectively (which is reduced to the sensitivity critical point of MEM, i.e., ≤2 ug/mL). The MIC_{50} and MIC_{90} of the MEM decreased significantly and the concentration cumulative bacteriostatic percentage curve shifted significantly to the left (the inhibition effect was better) after being combined with AZM, as shown in **Figure 2A1**. The MIC_{50} and MIC_{90} of a SCF single drug are 64 and 128 ug/mL, respectively. After being combined with AZM, MIC_{50} decreased to 16 ug/mL, which is reduced to the sensitivity critical point of SCF, that is, ≤16 ug/mL, however, MIC_{90} decreased to 64, it was still in its resistant critical point, that is, ≥64 ug/mL. The concentration cumulative bacteriostatic percentage curve of SCF shifted obviously to the left after combined with AZM, as shown in **Figure 2B1**. The MIC_{50} and MIC_{90} of AMK single drug are 4 and 16 ug/mL, respectively (which were in sensitivity critical point of AMK, i.e., ≤16 ug/mL). After combined with AZM, MIC_{50} and MIC_{90} of AMK were same as single drug. The two curves of SCF were almost overlapping as shown in **Figure 2C1**. The MIC_{50} and MIC_{90} of LEV single drug were 8 and 64 ug/mL respectively. After being combined with AZM, MIC_{50} and MIC_{90} of LEV were 8 and 32 ug/mL, respectively. They were still at its resistance point, that is, ≥8. After being combined with AZM, the curve of LEV shifted implicitly, as shown in **Figure 2D1**. The MIC_{50} and MIC_{90} of AZM single drug were 256 ug/mL. After being combined with the other four drugs respectively, all MIC_{50} and MIC_{90} of AZM did not vary significantly. Four curves of AZM moved left in different degrees, as shown in **Figures 3A2–D2**.

To summarize, the AMK single drug was already very sensitive to the selected strains. When MEM or SCF was combined with AZM, the sensitivity of them to strains can be significantly increased; the sensitivity of LEV was improved after being combined with AZM, but it was not obvious.

Based on the aforementioned experimental results, we selected the scheme of SCF + LEV + AZM on the strains for further study. The analysis found that after the triple combination, the MIC_{50} and MIC_{90} of SCF and LEV were lower than those of the double combination, but they still could not achieve substantive changes. The MIC_{90} of SCF was reduced to 64, which was still in the range of the resistance level, that is, ≥64, and the MIC_{50} of LEV was reduced to 4,

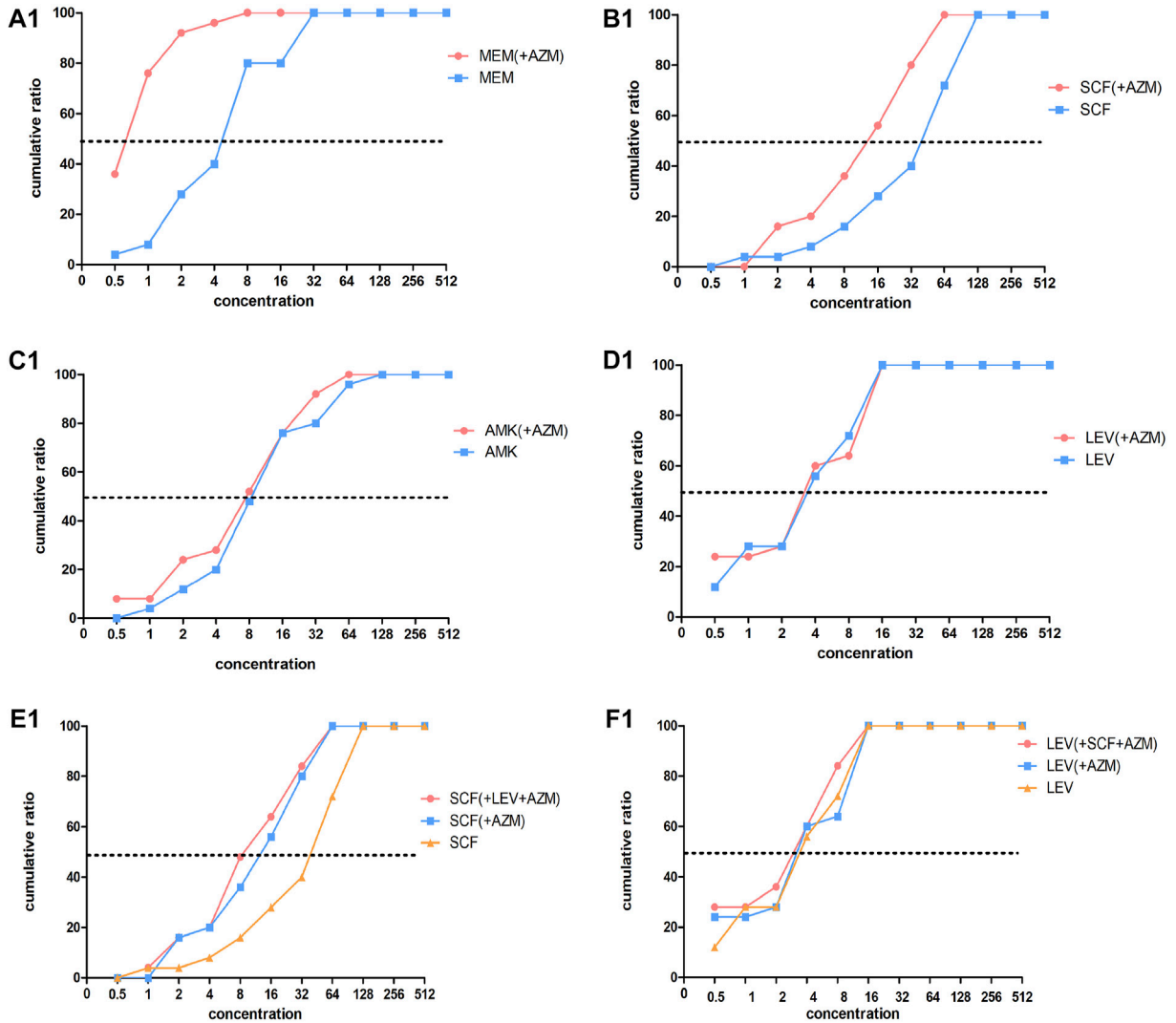

FIGURE 2 | Concentration cumulative bacteriostatic percentage curves of four antibiotics. Abbreviations: MEM, meropenem; SCF, cefoperazone/sulbactam; AMK, amikacin; LEV, levofloxacin; AZM: azithromycin.

which still could not reach the sensitivity critical point, that is, ≤ 2. The left shift of SCF and LEV curves was not significant compared with the combination of three drugs and two drugs, as shown in **Figures 2E1,F1**. At the same time, after the combination of three drugs, the MIC_{50} and MIC_{90} of AZM were the same as those of the combination of two drugs, and the curve shift was slight, as shown in **Figures 3E2,F2**. The details are shown in **Tables 5**, **6**.

Fractional Inhibitory Concentration Index Results of the *In Vitro* Drug Sensitivity Test

The FIC index results of **Table 7** suggested that, the main actions of MEM combined with AZM were the additive effect, accounting for 72%, the proportion of synergistic and additive effect added up to 80%, and 20% was the indifference effect; when SCF was combined with AZM, the addictive effect was 40%, and the unrelated effect was 60%; when LEV was combined with AZM, the additive effect accounted for 16%, and the unrelated effect accounted for 84%. After the combination of SCF + LEV + AZM, the additive effect accounted for 64%, and the unrelated effect accounted for 36% calculated based on SCF and AZM. In addition, the additive effect accounted for 32%, and the unrelated effect accounted for 68% analysis from the MIC of Lev and AZM. The details are shown in **Table 7**.

In conclusion, the combination of MEM and AZM showed the obvious additive effect. After the combination of SCF and AZM, the additive effect was 40%, and after the combination of three drugs, the additive effect was slightly increased to 64%. AMK or LEV combined with AZM mainly showed the unrelated effect, and the combination of three drugs could not improve the positive correlation between LEV and AZM.

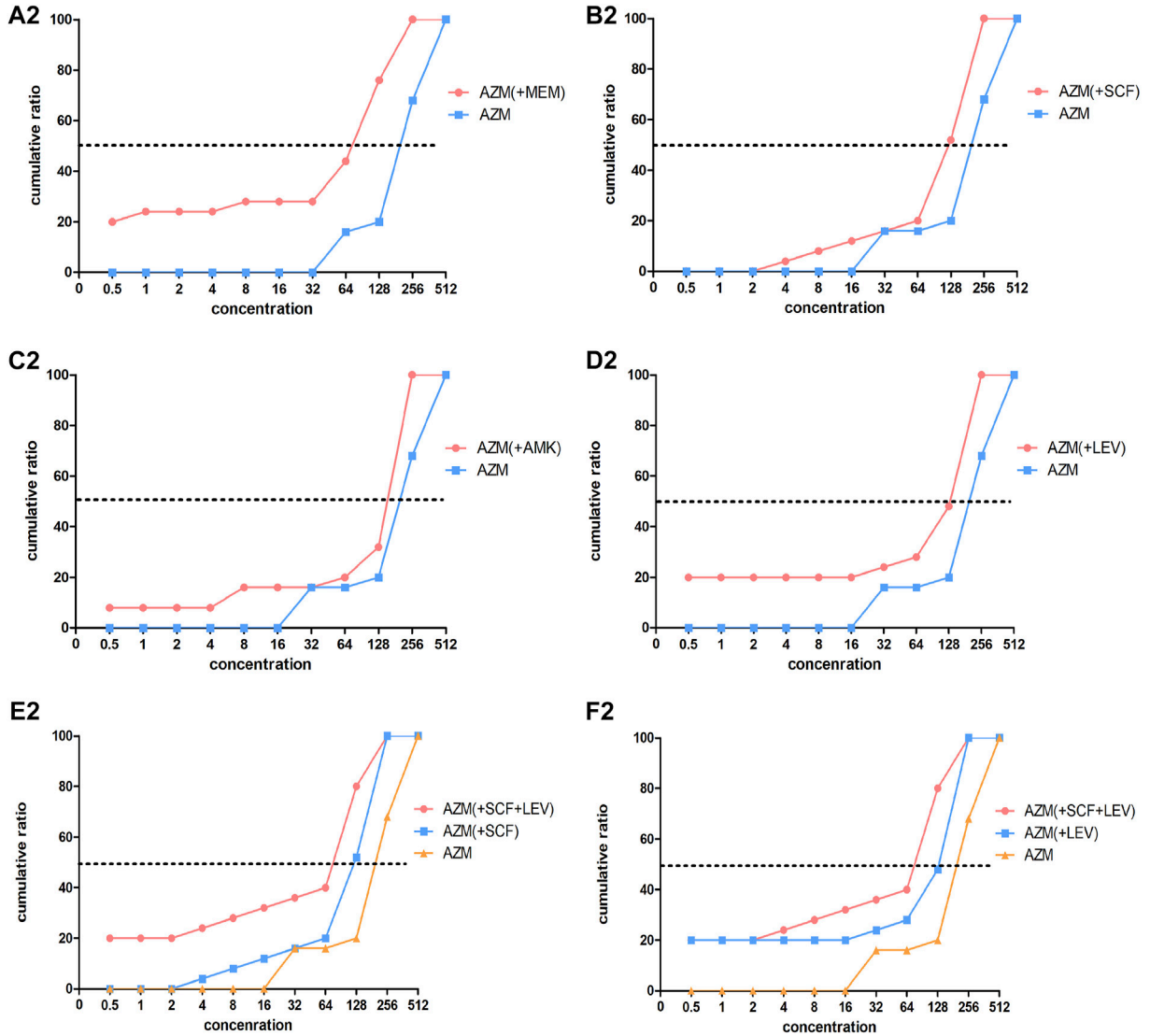

FIGURE 3 | Concentration cumulative bacteriostatic percentage curves of azithromycin. Abbreviations: MEM, meropenem; SCF, cefoperazone/sulbactam; AMK, amikacin; LEV, levofloxacin; AZM: azithromycin.

TABLE 7 | Distribution (%) of the FIC index to the MDR *P. aeruginosa* (*n* = 25).

Antibiotic (n)		FIC ≤ 0.5	0.5 < FIC ≤ 1	1 < FIC ≤ 2	FIC>2
Combined with AZM	MEM	8 (2)	72 (18)	20 (5)	—
	SCF	—	40 (10)	60 (15)	—
	AMK	—	28 (7)	72 (18)	—
	LEV	—	16 (4)	84 (21)	—
SCF + LEV + AZM	SCF	—	64 (16)	36 (9)	—
	LEV	—	32 (8)	68 (17)	—

Abbreviations: FIC, fractional inhibitory concentration.

DISCUSSION

The updated IDSA/ATS HAP/VAP guideline in 2016 specifically emphasizes that Hospital-acquired pneumonia (HAP) only refers to the pneumonia occurring after hospital admission in the patients without endotracheal intubation and is not associated with MV, while VAP represents the pneumonia occurring after endotracheal intubation and MV (Kalil et al., 2016). In China,

people still assume that VAP is a special type of HAP (Shi et al., 2019). VAP is one of the most frequent ICU-acquired infections. Large scale studies worldwide have shown that the incidence of VAP is 2.5–40.0% (or 1.3 to 20.2 cases per 1,000 mechanical ventilation days) in ICU patients, associated with mortality of 13.0–25.2% (Kollef et al., 2012; Melsen et al., 2013). In our study, the incidence of VAP is approximately 10.4% (961/9245) of all mechanically ventilated patients in 5 years, consistent with the results of relevant studies (Melsen et al., 2013). VAP is associated with prolonged duration of MV and prolonged ICU stay (Papazian et al., 2020) and increased health-care costs (Zimlichman et al., 2013). Usual Gram-negative microorganisms involved in VAP are *P. aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter* species; *Staphylococcus aureus* is the major Gram-positive microorganism (Bailey and Kalil, 2015; Huang et al., 2018; Luyt et al., 2018). A large proportion of VAP is caused by MDR pathogens and VAP in patients with risk factors for MDR pathogens is more likely to be due to MDR pathogens (Kalil et al., 2016). The non-standard use of antibiotics is one of the main factors for the occurrence of MDR pathogens.

MDR isolates of *P. aeruginosa* are increasingly prevalent (Denis et al., 2019). *P. aeruginosa* strains have recently become issues of public health concern (Oliver et al., 2015). One of three groups of antimicrobial-resistant Gram-negative bacteria posing particular therapeutic challenges is *P. aeruginosa* with difficult-to-treat resistance (DTR *P. aeruginosa*) according to the report of the US Centers for Disease Control and Prevention (CDC) antibiotic resistance threat (Thomas et al., 2007; Oliver et al., 2015). Recently, new tools using polymerase chain reaction (PCR) directly applied to fresh (bronchoscopic) samples have been developed to identify pathogens, which can shorten the time of organism identification and increased susceptibilities (Thomas et al., 2007). However, this technique is not available to determine *P. aeruginosa*. The prevalence of MDR *P. aeruginosa* is probably increasing worldwide, although with major geographical differences. The prevalence of MDR *P. aeruginosa* has increased over the last few decades and is now within the 15–30% range in multiple areas (Walkty et al., 2017; Sader et al., 2018).

Research showed *P. aeruginosa* had a high resistance to ciprofloxacin, levofloxacin, ceftazidime, piperacillin, imipenem, piperacillin and tazobactam, tobramycin, gentamicin, and meropenem, according to the data of a single center in Germany for 10 years (Yayan et al., 2015). Our statistical results of the resistance of *P. aeruginosa* to 14 antibiotics in 5 years showed that the resistants are furantoin (93.4%), aztreonam (62.9%), imipenem (50.3%), levofloxacin (47%), ciprofloxacin (46.4%), and meropenem (45%). It was worth mentioning that *P. aeruginosa* showed high resistance to carbapenem antibiotics from the clinical drug sensitivity results. The resistance rates of *P. aeruginosa* to imipenem and meropenem are 50.3% and 45.0%. In China, carbapenem-resistant *P. aeruginosa* (CR-PA) has been included in one of the five MDR bacteria targeted for prevention and control, in accordance with the requirements of the National Health Commission. During the last decade, there has been a global increase in the incidence and prevalence of carbapenem-resistant Gram-negative bacteria (Barbier and Luyt, 2016). In Europe, the population-weighted mean percentage of invasive isolates resistant to carbapenems in 2015 was 17.8% for *P. aeruginosa*. In the United States, 19.2% of *P. aeruginosa* submitted to the National Healthcare Safety Network was resistant to carbapenems in 2014 (Centers for Disease Control and Prevention, 2016; Tomczyk et al., 2019).

One of the main consequences of MDR is the difficulty of selecting an appropriate empirical antibiotic treatment. VAP caused by MDR bacteria puzzles every doctor in the ICU. Physicians face a dilemma, between avoiding ineffective treatment, inappropriate initial antimicrobial treatment being associated with increased mortality; and on the other hand, reducing the consumption of broad-spectrum antibiotics, the latter being associated with increased bacterial resistance (Yayan et al., 2015).

For patients with VAP due to *P. aeruginosa*, there are many choices according to the drug sensitivity results for sensitive *P. aeruginosa*, such as anti-PA cephalosporins and their combination with β-lactamase inhibitor complex preparations (such as ceftazidime and cefoperazone sulbactam), anti-PA carbapenems (including meropenem and biapenem), fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (amikacin and isopamicin), polymyxin, and fosfomycin based on clinical guidelines (Kalil et al., 2016; Torres et al., 2017; Shi et al., 2019). For patients with MDR-PA, the domestic and foreign guidelines for the treatment of PA-VAP recommend combination medication (ADSA-ATS in 2016 and Chinese guideline of 2018 Edition). For example, β-lactamase inhibitor compound preparation combined with fluoroquinolones or aminoglycosides, carbapenems combined with fluoroquinolones or aminoglycosides. For CR-PA, especially extremely drug-resistant (XDR) pulmonary infection, polymyxin (Kalil et al., 2016; Shi et al., 2019) and ceftazidime–avibactam are recommended (32–33).

Our study showed that the effective rate of MEM + AMK was 73.5% (25/34) and the SCF + AMK was 70% (21/30). The curative effect of MEM + AMK was better than that of the MEM + LEV group, $p = 0.029$ ($p < 0.05$) and this of SCF + AMK was better than that of the SCF + LEV group, $p = 0.025$ ($p < 0.05$). It indicated that the efficacy of MEM or SCF combined with AMK was better than that combined with LEV in the treatment of MDR-PA VAP. This can be explained by results of clinical drug sensitivity and *in vitro* drug sensitivity test. For 151 clinical cases, the sensitivity rate of amikacin was 88.1%, amikacin showed good sensitivity to most strains of *P. aeruginosa* (Pericolini et al., 2018). From the results of the drug sensitivity test *in vitro*, the MIC_{50} and MIC_{90} of AMK single drug are 4 and 16 ug/mL, respectively, which were in the sensitivity critical point of AMK, that is, ≤16 ug/mL. The high sensitivity of amikacin was further explained for *P. aeruginosa*. In contrast, the MIC_{50} and MIC_{90} of MEM, SCF, and LEV single drugs were in the range of drug resistance. American IDSA guidelines also pointed out that if DTR *P. aeruginosa* was not sensitive to all preferred drugs, a sensitive aminoglycoside can be considered in combination with cefloza–tazobactam, ceftazidime–avibactam, or imipenem–cilastatin–relibatan. The MIC closest to its sensitivity critical point was the preferred β-lactams β-lactamase inhibitor

(Tamma et al., 2021). If DTR *P. aeruginosa* was also not sensitive to aminoglycosides, polymyxin B was considered in combination with preferred β-lactams β-lactamase inhibitor (Tamma et al., 2021).

However, amikacin alone is very rare because of its side reaction and the comprehensive situation of patients in ICU. In recent years, the application of polymyxin has gradually increased at home and abroad (Kalil et al., 2016; Vaara, 2019). However, compared with large provincial and teaching hospitals, the use in grass-roots hospitals is still very limited due to factors such as high costs or restrictions on prescription rights. Polymyxin was only available in our area last year. Also, a new β-lactams β-lactamase inhibitor (such as cefloza–tazobactam and ceftazidime–avibactam) is still in the clinical trial stage in China and has not been widely used in clinic (Papp-Wallace et al., 2019). Therefore, when the patient is with VAP caused by CR-PA, DTR-PA, or XDR *P. aeruginosa*, the treatment of VAP is more difficult for doctors in ICU. If the progress of the disease cannot be curbed in about a week, the function of other organs of the patient is bound to be affected, and serious secondary conditions such as septic shock and multiple organ failure (MOF) will develop.

In the ICU of our hospital, most patients with MDR *P. aeruginosa* VAP were treated with carbapenems (meropenem and biapenem) or commonly used β-lactamase inhibitor (cefoperazone sulbactam and piperacillin tazobactam) combined with aminoglycosides (amikacin and etimicin) or fluoroquinolones (moxifloxacin and levofloxacin). Based on the local drug supply at that time, the largest combination was meropenem or cefoperazone sulbactam combined with amikacin or levofloxacin. It is worth mentioning that a small number of patients were treated with the aforementioned drugs combined with azithromycin, possibly based on the medication situation at that time. Among the 75 cases of two drug combination scheme based on MEM, 13 cases combined with AZM, and the effective rate was 69.2% and the efficacy of MEM + AZM was similar to that of MEM + AMK, $p > 0.05$. Among the 63 cases of two-drug combination schemes based on SCF, 10 cases combined with AZM, and the effective rate was 60%. Also, the curative effect of SCF + AZM did not show significant difference in the efficacy of SCF + AMK, $p > 0.05$. We had surprisingly found that the combination of azithromycin has achieved a good therapeutic effect in the treatment of MDR *P. aeruginosa* VAP.

Azithromycin is the first broad-spectrum therapeutic drug, an azalide, a subclass of macrolide antibiotics. It prevents bacteria from growing by interfering with their protein synthesis. It binds to the 50S subunit of the bacterial ribosome and thus inhibits translation of mRNA (Bakheit et al., 2014). Azithromycin is used to treat or prevent certain bacterial infections, most often those causing middle ear infections, strep throat, typhoid, bronchitis, and sinusitis. Azithromycin (AZM) was used to treat chronic inflammatory airway diseases because it regulates the cell–cell contact between airway epithelial cells. AZM can inhibit the ability of TNF-α-to induce interleukin (IL)-8 production (Yang, 2020). In fact, azithromycin is not often selected by doctors in ICU. However, in the Strategic Plan for Biodefense Research by the US Department of Health and Human Services, azithromycin is one such broad-spectrum therapeutic that is both included in the University of Oxford's RECOVERY and excluded from the World Health Organization's SOLIDARITY trialsI. The Strategic Plan will demarcate the need for drugs which target multiple types of pathogens to prepare for infectious threat (Firth and Prathapan, 2020). The latest research has shown that azithromycin was used to treat COVID-19 (Damle et al., 2020; Oldenburg et al., 2021).

The treatment options for nosocomial Gram-negative infections are very limited. The poor activities of these antibiotics on bacterial biofilms and the increasing prevalence of MDR *P. aeruginosa* leave the physicians with very limited choices to effectively treat these patients (Bassetti et al., 2018; Ciofu and Tolker Nielsen, 2019; Kumar et al., 2019). Antibiotic-resistant biofilms exist widely in *P. aeruginosa* infection, which is one of the important reasons for the failure of antibacterial treatment. Biofilm is an architecture built mostly by autogenic extracellular polymeric substances which functions as a scaffold to encase the bacteria together on surfaces, and to protect them from environmental stresses, impeding phagocytosis and thereby conferring the capacity for colonization and long-term persistence (Mah et al., 2003; Sharma et al., 2014; Kang and Kirienko, 2018; Thi et al., 2020). Macrolide antibiotics have little activity against *P. aeruginosa*. However, it can inhibit the formation of biofilm, regulate immunity, enhance the phagocytosis of phagocytes, and inhibit some toxic factors of *P. aeruginosa*. Macrolide antibiotics may enhance the antibacterial activity of other drugs against *P. aeruginosa* by destroying the biofilm and improving the curative effect. The results of Ren et al. (2019) indicated that the trans-translation system played an essential role in *P. aeruginosa* tolerance to azithromycin and multiple aminoglycoside antibiotics which was a ribosome rescue system that plays an important role in bacterial tolerance to environmental stresses. The experimental result showed that the ciprofloxacin–azithromycin sinus stent (CASS) maintained a uniform coating and sustained delivery of ciprofloxacin and azithromycin, providing anti-biofilm activities against *P aeruginosa* (Lim et al., 2020). Raouf et al. (2021) indicated that combined free and ciprofloxacin–azithromycin nanoparticles on chitosan nanocarrier (Cipro-AZM-CS) showed promising results *in vitro* and *in vivo* overcoming high resistance of biofilm producing *P. aeruginosa*. The present study was conducted to evaluate the effect of azithromycin on *P. aeruginosa* biofilm. We showed that azithromycin exhibited a potent activity against *P. aeruginosa* biofilm, and microscopic observation revealed that azithromycin substantially inhibited the formation of solid surface biofilms. Interestingly, we observed that azithromycin restricted the *P. aeruginosa* biofilm formation by inhibiting the expression of pel genes. We concluded that azithromycin attenuates *P. aeruginosa* biofilm formation, impairs its ability to produce the extracellular biofilm matrix, and increases its sensitivity to the immune system (Kumar et al., 2021).

From the MIC results of our vitro drug sensitivity test, we found that after MEM + AZM, MIC_{50} and MIC_{90} of MEM reduced the sensitivity critical point of MEM, that is, ≤2 ug/mL from 8 to 32 ug/mL; after SCF + AZM, MIC_{50} of SCF decreased to 16 ug/mL, which is reduced to the sensitivity critical point of SCF, that is, ≤16 ug/mL. The concentration cumulative bacteriostatic percentage curve of MEM shifted significantly to the left. The FIC index results suggested that the main actions of MEM combined with AZM were the additive effect, accounting for 72%, and the proportion of

synergistic and additive effect is added up to 80%. The proportion of the additive effect of SCF + AZM was 40%. We speculated that azithromycin may increase the bioactivity of meropenem and cefoperazone sulbactam by destroying the biofilm of multidrug-resistant *Pseudomonas aeruginosa*. This effect is particularly obvious after combining meropenem from our experimental results. The underlying mechanism related to this will be further studied in our future research. At the same time, we also found that the combination of three drugs containing azithromycin is not necessary to treatment of MDR *P. aeruginosa* ventilator-associated pneumonia, whether from the clinical results or *in vitro* drug sensitivity test results.

CONCLUSION

Our study suggested that MDR *P. aeruginosa* was highly sensitive to amikacin in our region. Carbapenems or cephalosporins-β-lactamase compound combined with amikacin had a good effect in the treatment of VAP of MDR *P. aeruginosa*. At the same time, azithromycin was combined with carbapenems or cephalosporins-β-lactamase compound could to be selected as the recommended scheme. In primary hospitals, we recommend azithromycin to treat MDR *P. aeruginosa* VAP when amikacin is resistant and polymyxin or ceftazidime–avibactam is not available. Moreover, the second is enough, and the third is unnecessary, which cannot further increase the therapeutic effect.

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.

AUTHOR CONTRIBUTIONS

YH, WW, and QH conceived the study, performed statistical analyses, and generated graphs. YH, QH, ZX, CT, DW, MH, and XY performed clinical data collection and *in vitro* drug sensitivity test studies. ZW performed the statistical analysis and discussion on clinical data. HX, HW, YZ, MT, and QZ performed the project management. YH, WW, QH, and QZ wrote the first draft of the manuscript. All authors contributed to manuscript editing, discussed the results, and approved the final submitted version.

FUNDING

This study was supported by the Advantages Discipline Group (Medicine) Project in Higher Education of Hubei Province (2021–2025) (No. 2022XKQY4).

ACKNOWLEDGMENTS

We thank the department of Clinical laboratory, Suizhou Central Hospital, Hubei University of Medicine, for collecting, reserving, and providing MDR *P. aeruginosa*. We were also grateful to Qiuying Zhang and Yang Liu, for performing professional consultation and explanation related to the *in vitro* drug sensitivity test.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.944965/full#supplementary-material

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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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frontiers | Frontiers in Pharmacology

TYPE Mini Review
PUBLISHED 19 October 2022
DOI 10.3389/fphar.2022.945892

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Respiratory Pharmacology, a section of the journal Frontiers in Pharmacology

RECEIVED 17 May 2022
ACCEPTED 07 October 2022
PUBLISHED 19 October 2022

CITATION
Deng J, Li F, Zhang N and Zhong Y (2022), Prevention and treatment of ventilator-associated pneumonia in COVID-19.
Front. Pharmacol. 13:945892.
doi: 10.3389/fphar.2022.945892

Prevention and treatment of ventilator-associated pneumonia in COVID-19

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Ventilator-associated pneumonia (VAP) is the most common acquired infection in the intensive care unit. Recent studies showed that the critical COVID-19 patients with invasive mechanical ventilation have a high risk of developing VAP, which result in a worse outcome and an increasing economic burden. With the development of critical care medicine, the morbidity and mortality of VAP remains high. Especially since the outbreak of COVID-19, the healthcare system is facing unprecedented challenges. Therefore, many efforts have been made in effective prevention, early diagnosis, and early treatment of VAP. This review focuses on the treatment and prevention drugs of VAP in COVID-19 patients. In general, prevention is more important than treatment for VAP. Prevention of VAP is based on minimizing exposure to mechanical ventilation and encouraging early release. There is little difference in drug prophylaxis from non-COVID-19. In term of treatment of VAP, empirical antibiotics is the main treatment, special attention should be paid to the antimicrobial spectrum and duration of antibiotics because of the existence of drug-resistant bacteria. Further studies with well-designed and large sample size were needed to demonstrate the prevention and treatment of ventilator-associated pneumonia in COVID-19 based on the specificity of COVID-19.

KEYWORDS

ventilator-associated pneumonia, COVID-19, SARS-CoV-2, nosocomial infection, antibiotic, acute respiratory distress syndrome, ARDS

Introduction

Ventilator-associated pneumonia (VAP) is usually regarded as a pneumonia phenomenon that occurs within 48 h after mechanical ventilation to 48 h after extubation, and is the main type of hospital-acquired pneumonia. The risk of VAP in patients with invasive mechanical ventilation is approximately 5%–40%, and has been reported to be different depending on the country, the type of intensive care unit (ICU), and the criteria of VAP identification (American Thoracic and Infectious Diseases Society of, 2005; Seguin et al., 2014). Not only does VAP have a significant attributable mortality rate (4.6%), and VAP remains an integral part of the spectrum of adverse events such as acute respiratory distress syndrome (ARDS) (Spalding et al., 2017). VAP may place a

longer hospital course and a greater financial burden on patients (Kalil et al., 2016), with a VAP-related cost of $40,144 (95% CI $36,286–44,220) reported in a survey in the United States in 2013 (Zimlichman et al., 2013). Although our knowledge of VAP has increased, its incidence has not decreased (Wang et al., 2014). Since the outbreak of COVID-19, Earth shaking changes have taken place in global medical care. COVID-19 patients had a high incidence of severe ARDS, many of whom require invasive ventilation and are at a high risk of VAP (Razazi et al., 2020; Maes et al., 2021; Fumagalli et al., 2022; Gosangi et al., 2022), which can make the state of COVID-19 patients more complicated and more strenuous to manage. Besides, COVID-19-associated VAP shows novel characters compared to non-COVID-19 disease (Wicky et al., 2021). Thus, a literature search on the PubMed and Web of Science was done for this review, using the following keywords from 2005 to 2022: "ventilator associated pneumonia" or "ventilator-associated pneumonia" or "ventilator-acquired pneumonia" or "VAP", "COVID-19" or "SARS-CoV-2". We aim to review the characteristics, diagnosis, drug prevention and therapy for the management of VAP in COVID-19 patients.

Ventilator-associated pneumonia in patients with COVID-19

During the COVID-19 pandemic, severe COVID-19 patients were admitted to ICUs, received mechanical ventilation (Chang et al., 2021; COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators, 2021), and have a high incidence of VAP (Ippolito et al., 2021; Wicky et al., 2022).

Occurrence and outcome of ventilator-associated pneumonia in COVID-19 patients

VAP is thought to be the most common fatal nosocomial infections in ICU (Kalanuria et al., 2014; Haque et al., 2018; Papazian et al., 2020). The reported incidence of VAP varies widely in COVID-19 patients, but was different from studies. We summarized these studies in Table 1. Some studies have shown a higher VAP risk in COVID-19 ARDS patients than in non-COVID-19 ARDS patients (Maes et al., 2021; Nseir et al., 2021; Rouzé et al., 2021; Vacheron et al., 2022a). It has been reported that VAP may bring poor prognosis to patients, with prolonged durations of both mechanical ventilation and ICU stay, and even a high mortality (Papazian et al., 2020). Similarly, the association of VAP with some relevant poor outcomes was also reported in COVID-19 patients in recent studies. Compared with non-COVID-19 patients, VAP in COVID-19 cases was significantly related with 28-day mortality, shock, VAP recurrence, and polymicrobial culture, as well as a significantly longer mechanical ventilation time (Razazi et al., 2020; Maes et al., 2021; Nseir et al., 2021; Rouyer et al., 2021). Meanwhile, compared with the influenza or non-virus infection group, no significant difference in the relationship between VAP with mortality was found in the COVID-19 group (Nseir et al., 2021).

Risk factors for ventilator-associated pneumonia in COVID-19 patients

Potential causes for the increased incidence of VAP include prolonged mechanical ventilation and hospital stay, viral immunomodulation, steroids use, sedating and neuromuscular blocking agents use, vasopressor use, ARDS, prone positioning, applications of extracorporeal mechanical oxygenation, shortages of healthcare workers and inadequate protective equipment corresponding to the increased medical resource demands (Fumagalli et al., 2022). The number of patients requiring invasive ventilation has significantly increased during the COVID-19 pandemic, which has overwhelmed the medical resources (Nacoti et al., 2021).

The prolonged mechanical ventilation was thought to be the most relevant risk factor of VAP (Kalil and Cawcutt, 2022). The patients treated with invasive mechanical ventilation who were accompanied with COVID-19 tend to present a delayed mechanical ventilation and a higher incidence of ARDS than without COVID-19, which are both recognized as risk factors for VAP (Razazi et al., 2020; Blonz et al., 2021; Nseir et al., 2021; Rouzé et al., 2021). It has been suggested that in COVID-19 patients the use of immunomodulatory drugs may promote the development of VAP (Martínez-Martínez et al., 2021). What is more, there are also studies indicating that dexamethasone could accelerate the process of the VAP occurrence (Cour et al., 2021; Gragueb-Chatti et al., 2021). In addition, the prone position, widely applied in the treatment of COVID-19, is associated with an elevated risk of microaspiration (Ayzac et al., 2016), thereby leading to the occurrence of VAP.

Microorganisms responsible for ventilator-associated pneumonia

The kind of microorganisms responsible for VAP reported is various, which may be explained by the duration of invasive mechanical ventilation, length of hospital and ICU stays before VAP episode, the local ecology, and cumulative exposure and timing to antimicrobials (Papazian et al., 2020). Some studies suggested that gram-negative bacteria were the predominant microorganisms (primarily *Pseudomonas aerginosa*, *Enterobacter* spp., *Klebsiella* spp.) followed by gram-positives

TABLE 1 The characteristics of studies reported ventilator-associated pneumonia in COVID-19.

Reference no.	Sample size	VAP incidence in COVID-19 patients (VAP no./total patients no.)	VAP incidence in non-COVID-19 patients (VAP no./total patients no.)	VAP-associated outcomes
Maes et al. (2021)	225	28/1000 ventilator days (an incidence density)	13/1000 ventilator days	NA
Nseir et al. (2021)	1576	late-onset VAP: 169/200	Influenza: 79/102	1) VAP was associated with a higher risk for 28-day mortality
			No viral infection: 57/87	2) VAP was significantly associated with longer duration of mechanical ventilation
Rouyer et al. (2021)	288	42/100	188/188	1)VAP had a significantly higher rate of shock
				2) a higher rate of death in ICU
				3) a higher rate of VAP recurrence
				4) a higher risk of positive blood culture
				5) a higher rate of polymicrobial culture
				6) a higher rate of clinical worsening at day 3 and 7
Rouzé et al. (2021)	1576	205/568	Influenza:107/482	NA
			No viral infection: 87/526	
Razazi et al. (2020)	172	58/90	36/82	1) longer duration of mechanical ventilation
Vacheron et al. (2022a)	3758	550/1879	242/1879	NA
Vacheron et al. (2022b)	9129	623/1687	995/7442	1) VAP attributable mortality was higher for COVID-19 patients
				2) less likely to be extubated after a VAP

Abbreviations: VAP, ventilator-associated pneumonia; COVID-19, Coronavirus disease 2019; NA, not available.

(mainly *Staphylococcus aureus*) responsible for VAP in patients with COVID-19 (Grasselli et al., 2021; Rouzé et al., 2021). Furthermore, there is an increased occurrence of multidrug-resistant (MDR) bacterial strains in patients with COVID-19, one of the reasons may be that most cases of COVID-19-related VAP were diagnosed when invasive mechanical ventilation had been initiated for more than 7 days (late VAP) (Fumagalli et al., 2022). It is reported that multiple drug resistance of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated from COVID-19 patients (Gregorova et al., 2020; Baba et al., 2021; Ghanizadeh et al., 2021). In addition, a monocenter retrospective study involving 172 patients suggested that compared with non-COVID-19 patients, COVID-19 cases were more likely to develop MDR-related VAP (Razazi et al., 2020). Besides, COVID-19 could lead to severe organ dysfunction and prone to secondary bacterial infections, and then even facilitate the emergence of infectious diseases caused by rare microorganisms. There are also studies of VAP contributed by the rare bacteria Hafnia alvei (Cutuli et al., 2021; Méndez et al., 2021).

It is worth noting that bacteria are not the only cause of VAP. Secondary causes of fungal infection have been reported from first noticed in China, to become a clear manifestation as indicated in European in severe COVID-19 patients (Marr et al., 2021). A clinical study showed that all 134/134 (100%) patients with VAP caused by fungal were already receiving the treatment of corticosteroids and tocilizumab (Meawed et al., 2021). Current studies have shown a higher prevalence of Aspergillus pneumoniae in critical COVID-19 patients compared with the other patients in ICUs. In addition, patients affected by COVID-19 associated invasive pulmonary aspergillosis have a high mortality rate (Lahmer et al., 2021). Moreover, COVID-19 patients can also develop virus-related VAP. Herpesvirade (HSV) activation was also numerically more frequent among COVID-19 than non-COVID-19 cases (Maes et al., 2021). HSV-1 reactivation was related to the increased risks of VAP and mortality in critical COVID-19 patients (Meyer et al., 2021).

Diagnosis of ventilator-associated pneumonia in COVID-19 patients

Diagnosing VAP in patients with COVID-19 is an appreciable challenge, since substantial overlap exists between the basic clinical symptoms and signs of COVID-19 with secondary infection (Fumagalli et al., 2022). It is considered traditionally that the diagnosis of VAP should be based on three criteria followed: clinical suspicion, new or progressive radiographic infiltrates, and microbiological diagnosis meaning

positive microbiological cultures from the lower respiratory tract (Papazian et al., 2020).

Adjustment of diagnosis for ventilator-associated pneumonia in COVID-19 patients

We should realize that it is time to consider whether there exists VAP when new clinical signs of respiratory deterioration potentially attributed by infection appear (Papazian et al., 2020). The clinical presentation of COVID-19 includes high fever, severe hypoxemia, hyperleukocytosis, biological inflammatory syndrome, and extensive bilateral radiologic infiltrates in detail. These symptoms overlap highly with VAP, making traditionally clinic diagnosis criteria for VAP invalid in the critical population with COVID-19 (Francois et al., 2020). While discovering new infiltrations in patients based on baseline infiltrations is arduous, the conventional assessment of VAP, chest X-ray or computed tomography imaging, are not suitable for real-time measurement in critical patients. Hence, lung ultrasound has been widely used as a real-time monitoring tool to monitor VAP for critically ill patients in recent years (Kameda et al., 2001; Dargent et al., 2020; Dargent et al., 2021). It must be stated that confirmed diagnosis of VAP depends on the identification of the pathogen. Regardless of the technique used (endotracheal aspiration or bronchoscopy-guided bronchoalveolar lavage), a challenge in diagnosing VAP is reducing the time from sampling to pathogen identification (Papazian et al., 2020).

The widespread use of new molecular technology effectively alleviates this problem and contributes to the increasing incidence of VAP. Tools based on multiplex polymerase chain reaction make it possible to diagnose early VAP and identify VAP usually underdiagnosed by conventional culture-based methods (Cohen et al., 2021; Wicky et al., 2022). It has been reported in the literature, the combination of sequential PCR and electrospray ionization mass spectrometry was a potential rapid technique to diagnose VAP within 6 h (Hou et al., 2020). In addition, the next-generation sequencing (NGS) and even metagenomic NGS (mNGS) are in some distance more effective and rapid for pathogen detections (Toma et al., 2014; He et al., 2022). NGS-based methods and mNGS-based methods can help clinicians to make accurate and precise diagnosis, leading to targeted antimicrobial therapy and improve the prognosis of patients in time.

Other methods to help identify ventilator-associated pneumonia in COVID-19 patients

It is reported that procalcitonin (PCT) can help distinguish virus from bacterial pathogens in patients with VAP, and typical bacteria tend to present higher procalcitonin levels than atypical bacteria or viruses (Self et al., 2017; Modi and Kovacs, 2020). The data of a clinical study suggested that the patients whose PCT was over 0.975 ng/ml were more likely to have VAP, showing that PCT may be an applicable biomarker for VAP diagnosis (Côrtes et al., 2021). In addition, immature granulocytes (IGs) was considered that the threshold was 18% or 2 g/L, and the sensitivity and specificity to identify patients with ventilator-associated pneumonia were 100%, supporting IGs could be a biomarker to help identify pulmonary bacterial infections in this population (Daix et al., 2021).

Drug treatment of ventilator-associated pneumonia in COVID-19 patients

Antimicrobial therapy

Intravenous antimicrobial therapy is the cornerstone for drug treatment of VAP. It must be stressed that the emergence of antimicrobial resistance and determining the appropriate type, timing and duration of antibiotics is worthy of attention.

COVID-19 patients on mechanical ventilation have a very high risk of exposure to extensive antibiotic treatment. During their hospitalization, most patients were prescribed several different classes of antibiotics, of which broad-spectrum coverage was commonly used. Cefepime and vancomycin have been reported to be the most commonly used antibiotics, with an average duration of 1 week. (Risa et al., 2021). It is generally recommended that the empirical treatment plan should be based on the local distribution of VAP-related pathogens and their antimicrobial susceptibility, and resistance rates vary widely among countries, regions, and hospitals. Guideline-based empirical antibiotic management results in antibiotic overuse (Pickens et al., 2021). Inappropriate empiric antibiotic use may lead to the emergence of more resistant bacteria.

Antimicrobial resistance is not only a global crisis but also a global problem that attracts the attention of governments and society. Multiple studies showed that drug-resistant and even multidrug-resistant frequently occurred in COVID-19 patients with VAP. Since the outbreak of COVID-19, antibiotic resistance is increasing. The resistance to the ceftazidime and levofloxacin for *P. aeruginosa* strains was significantly increased, as well as A. baumannii strains (Bahçe et al., 2022). Therefore, drugs for multidrug resistant bacteria infection of VAP, such as polymyxins, ceftazidime avibatan et al. were used to improve the outcomes of COVID-19 patients. Moreover, recent researches suggested that PBT2 may act as a drug resistance inhibitor to rescue the efficacy of commonly used tetracycline antibiotics in the treatment of multidrug-resistant baumannii infection (De Oliveira and Walker, 2022).

The best way to determine a specific antibiotic depends on the evidence of pathogen culture from bronchoalveolar lavage or endotracheal aspiration of the lower airways (Fumagalli et al., 2022). New antimicrobials are undergoing rapid development, aiming to keep pace with the development of multidrug resistance (Cusack et al., 2022). We expect more target drugs to be developed earlier in the future, to implement more precise etiological treatment.

Other drugs treatments

An observational study found that it may be a reasonable therapeutic option to decrease the intubation rate in COVID-19 patients (Mushtaq et al., 2022). Compared with high-dose dexamethasone, tocilizumab seemed to be a much better and safer for controlling the cytokine storm in COVID-19 patients with moderate to severe ARDS (Naik et al., 2021). In contrast to this study, tocilizumab was reported to increase the incidence of VAP in critical COVID-19 patients (Ceccarelli et al., 2021). In addition, it is reported that combination therapy of tocilizumab and steroids is likely to be conducive to managing COVID-19-associated cytokine release syndrome (Dravid et al., 2021). While another research suggested that adding tocilizumab to methylprednisolone did not improve outcomes significantly (Hamed et al., 2021). Further, interferon gamma is proved to have a plausible efficacy in the treatment of recurrent VAP by recovering monocyte activation (Nguyen et al., 2021).

Drug prevention of ventilator-associated pneumonia in COVID-19 patients

VAP is difficult to manage and it will complicate existing diseases, so we should pay attention to its prevention which is thought to be more important than the treatment of VAP. It is generally believed that the main way to prevent VAP is to reduce the timing of invasive mechanical ventilation, and this part focuses on the pharmacological prevention of VAP.

Stress ulcer prophylaxis

Ventilated patients usually need to use proton pump inhibitors (PPI) and gastric mucosal protective agent prophylactically because of the risk of stress ulcers. These acid suppressive medications inhibit gastric acid secretion, increase the hydrogen of the gastric juice, and promote bacterial growth (Buendgens et al., 2016). It may be a reason of stress ulcer prophylaxis was reported to be associated with higher VAP rates (Alhazzani et al., 2018; Huang et al., 2018). There were a lot of researches focused on the association of PPI use with COVID-19 (Almario et al., 2020; Elmunzer et al., 2021; Fan et al., 2021; Lee et al., 2021; Ramachandran et al., 2022), and the results showed that PPI use was associated with the increased susceptibility of COVID-19 infection and poor outcome including disease severity and mortality (Fatima et al., 2022). However, the relationship between PPI use and VAP in COVID-19 patients still needs to be demonstrated. A meta-analysis suggested that sucralfate, a gastric mucosal protective agent, could significantly decrease the occurrence of VAP, but cannot affect the days on ventilator, duration of ICU stay, and ICU mortality (He et al., 2014). Hence, sucralfate would be a good choice to prevent stress ulcers in critical ill patients with invasive mechanical ventilation.

Selective digestive decontamination

Selective digestive decontamination (SDD) is demonstrated to be effective in reducing the occurrence of VAP in non-COVID-19 patients (Liberati et al., 2009; Minozzi et al., 2021; van der Meer et al., 2021). In COVID-19 patients, a retrospective observational study that included 178 subjects on invasive mechanical ventilation more than 2 days, showed that the use of SDD significantly reduced the incidence of VAP (Luque-Paz et al., 2022). But, the evidence level of this study limited the reliability for its retrospective and observational design. Therefore, SDD deserves more consideration to use in critical COVID-19 patients.

Chlorhexidine

Chlorhexidine, a drug used in oral hygiene care for over 2 decades, can reduce oral colonization, and prevent the occurrence of VAP (Zand et al., 2017). Besides, the combination of chlorhexidine with toothbrushing was more satisfactory in preventing VAP in patients on mechanical ventilation, compared with chlorhexidine alone (Silva et al., 2021). However, several studies were questioning the efficacy and safety of oral chlorhexidine. It has been reported in some studies that the use of chlorhexidine oral care may increase mortality, owing to the occurrence of acute lung injury resulting from aspirating the anticorrosive composition of chlorhexidine (Klompas et al., 2014a; Klompas, 2017; Deschepper et al., 2018; Harris et al., 2018). In COVID-19 patients, Chlorhexidine was reported to be effective in reducing SARS-CoV-2 load in the oral cavity (Costa et al., 2021). More research is needed in the future to clarify the safety and efficacy of chlorhexidine in preventing VAP for COVID-19 patients.

Prophylactic probiotics

The microbiota plays an important role in the risk of intestinal complications and the disease severity of COVID-19 patients

(Zanza et al., 2021). Probiotics may be an attractive intervention for preventing VAP in adult hospitalized patients by modulating the intestinal microbiome and reducing the colonization of pathogens (Papazian et al., 2020; Su et al., 2020; Kullar et al., 2021). Systematic reviews also support the protective role of probiotics in preventing VAP (Rozga et al., 2021). But the evidence levels of these included studies were low and existing significant heterogeneity, the use of probiotics for preventing VAP in COVID-19 patients remains controversial.

Early enteral nutrition

Early enteral nutrition is recommended to prevent VAP in critically ill non-COVID-19 patients (Klompas et al., 2014b) and critical COVID-19 patients (Haines et al., 2022). A study of real-world clinical practice showed that early enteral nutrition within 3 days after invasive mechanical ventilation can shorten the time of invasive mechanical ventilation and improve the outcome of COVID-19 patients (Haines et al., 2022). The mechanisms of early enteral nutrition in preventing VAP may involve reducing pathogen colonization and bacterial translocation through facilitating intestinal peristalsis and maintaining intestinal mucosal structure and barrier function. However, a large number of patients failed to initiate early enteral nutrition for hemodynamic instability, fear of aspiration, and significant gastrointestinal complications.

Intravenous selenium

A narrative literature review proved that selenium supplementation could reduce the incidence of VAP, shorten the length of hospital stay, and decrease mortality through decreasing the inflammatory cytokines (Mahmoodpoor et al., 2018; Oliveira et al., 2022). In COVID-19 patients, a higher prevalence of selenium deficiencies was found, especially in older cases (Voelkle et al., 2022). However, direct evidence of selenium supplementation in preventing VAP is needed in further research.

Conclusion

As mentioned above, during the COVID-19 era, VAP has drawn more attention than before due to its high incidence and high mortality. Patients who develop ARDS and require invasive mechanical ventilation after SARS-CoV-2 infection are at a higher risk to experience VAP episodes than non-COVID-19 ARDS patients. Despite extensive research, the diagnosis, prevention and treatment of VAP remain a challenge. At present, strict hospital management measures and standardized procedures to prevent VAP. As for clinical treatment, the application of antibiotics remains recommended, especially the resistance of antibacterial drugs is a serious problem, attracting people's attention. Further studies with well-designed and large sample size were needed to demonstrate the prevention and treatment of ventilator-associated pneumonia in COVID-19 based on the specificity of COVID-19.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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.

Publisher's note

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