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Changes in immune indicators and bacteriologic profile were associated with patients with ventilator-associated pneumonia

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Abstract

The aim of this study is to explore and identify ventilator-associated pneumonia (VAP)-related prognostic immune factors and further detect the drug-resistant pathogens to establish the theoretical guidance for clinical prevention and treatment strategies of VAP. A total of 478 patients using ventilator who were hospitalized in July 2014 to November 2016 in our hospital were enrolled in this study. About 103 patients with VAP (21.5%, 103/478) among 478 cases of patients using ventilator. Among the 103 patients with VAP, the distribution of pathogenic bacteria and drug resistance in patients with VAP were detected and analyzed. In the VAP group, 35 patients died and 43 patients had simultaneous sepsis. Compared with those of non-VAP group, the proportion of $CD3^+$ ($P = .012$), $CD3^+CD4^+$ ($P = .024$) and $CD8^+CD28^+$ ($P = .017$) T cells in VAP group increased significantly, which indicated more severe immune response. Multivariate regression model analysis revealed that tracheotomy of mechanical ventilation ($P = .013$), mechanical ventilation time ≥ 7 days ($P = .02$) and aspiration and reflux ($P = .011$) were independent risk factors associated with VAP. According to the results of bacterial culture and drug sensitivity test, rational selection of antibiotics and monitoring of patients within intensive care unit can effectively control the incidence of VAP and improve the prognosis of patients.

Abbreviations: CI = confidence interval, FITC = fluorescein isothiocyanate, ICU = intensive care unit, OR = odds ratio, PE = phycoerythrin, SIRS = systemic inflammatory response syndrome, VAP = ventilator-associated pneumonia.

Keywords: immune factors, pathogen analysis, ventilator-associated pneumonia

1. Introduction

Ventilator-associated pneumonia (VAP) is one of the most serious complications during mechanical ventilation and frequently occurred in the intensive care unit (ICU). VAP was defined as pneumonia occurring 48 to 72 hours after tracheal intubation.[1] VAP is characterized by the presence of new or progressive infiltrates in the lungs, signs of systemic infection (fever, changes in white blood cell count), changes in sputum characteristics, and detection of pathogens. The incidence of VAP was between 1.2 and 8.5 per thousand, and the occurrence of VAP depends on the

definition of VAP diagnosis.[2] VAP accounted for about half of all cases of hospital-acquired pneumonia.[3–5] The risk of VAP was highest in the first 5 days (3%) of mechanical ventilation, and the average duration of intubation was 3.3 days.[6,7]

There is currently no consensus on the diagnosis and definition of VAP.[8] Early onset VAP is defined as pneumonia that occurs within 4 days of intubation, which is usually attributed to antibiotic-sensitive pathogens, and late-onset VAP is more likely to be caused by multidrug resistance bacteria and occurred 4 days later after mechanical ventilation. Once the VAP occurs, it is easy to cause offline difficulties, prolonged hospital staying, hospitalization costs, serious life-threatening, or even death.[1,9] Therefore, an in-depth analysis of the independent risk factors related to the development of VAP is of great significance in preventing the occurrence of VAP and actively treating VAP.[10,11] *Pseudomonas aeruginosa* is one of the most common pathogens causing VAP and is independently associated with increased mortality; in China, it has been staying in the top 3 pathogens.[12,13] Antibiotic treatment is the primary method for managing *P aeruginosa* VAP; however, it constitutes a risk factor for the development of multidrug-resistant *P aeruginosa*. Increasing drug resistance, especially in ICUs, could result in *P aeruginosa* VAP becoming uncontrollable,[14–17] and the prognosis of VAP is closely related with the drug resistance of variance pathogens. One possible mechanism contributing to the risk of VAP in critically ill patients is "immunoparalysis," also known as the compensatory anti-inflammatory response syndrome. In this procedure, impaired lymphocyte and phagocyte function, and a shift from a Th1 to a Th2 immune phenotype played important roles.

According to the 3rd international consensus definitions for sepsis and septic shock (sepsis-3), sepsis should be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[18] Sepsis is commonly associated with VAP

the primary cause of death from infection; its recognition mandates urgent attention. Pathogen factors and host factors shape this syndrome: sex, age, comorbidities, environment, etc.

The aim of this study is to analyze and identify VAP-related prognostic immune factors and pathogens and detect the drug resistance, and explore the theoretical guidance for clinical prevention and treatment strategies. Moreover, this study discusses the prevention and treatment strategies of VAP and provides theoretical guidance for clinical prevention and control of VAP.

2. Materials and methods

2.1. Patients and tissue samples

We selected 478 patients who used ventilators from July 2014 to November 2016 in our ICU, 103 of whom had VAP, and the incidence was 21.5%. All patients underwent chest X-ray examination without pulmonary infection before admission. VAP was diagnosed according to CDC (2015) and HELICS criteria, namely: the presence of newly developed or progressive infiltrates on chest radiographs plus at least 2 other signs of respiratory tract infection: temperature >38°C, purulent sputum, leukocytosis (WBC > $10 \times 10^3/mm^3$) or leukopenia (WBC < $4 \times 10^3/mm^3$), signs of inflammation during auscultation, cough, and/or respiratory insufficiency with a PaO_2/FiO_2 ratio of ≤300 mm Hg. The guideline was implemented after a multifaceted dissemination and implementation teaching period. The study was approved by the Research Ethics Committee of Anhui Medical University. Informed consent was obtained from all patients. The study was conducted in accordance with the recognized ethical guideline of Declaration of Helsinki.

2.2. VAP diagnostic criteria

According to the diagnostic criteria of VAP as follows[19,20]: After 48 hours of mechanical ventilation, the chest X-rays showed infiltrated lungs or new infiltrated shadows. The physical examination of the lungs could smell wet rales; one of the following conditions was also met: White blood cell count >$10 \times 10^{11}/L$ or <$4.0 \times 10^9/L$; body temperature >37.5°C; purulent respiratory secretions; isolated from the bronchial secretions of pathogenic bacteria. Clinical pulmonary infection scores: CPISS scores were calculated to be >6 for confirmed or suspected cases. Johanson criteria: New infiltrating shadows or infiltrates in the chest X-ray progression plus at least 2 of the following: body temperature >38°C; white blood cell count increased or decreased; purulent secretions.

Exclusion criteria: mechanical ventilation time is <48 hours; pulmonary infection has been diagnosed before entering the ICU; incomplete data; and pulmonary embolism, ARDS, tuberculosis, and other diseases.

2.3. Sepsis and systemic inflammatory response syndrome

In 1991, sepsis was 1st defined as a "systemic inflammatory response syndrome to the presence of infection," setting the presence of 2 or more alterations in heart and respiratory rate, body temperature, and white blood cell count as criteria; in addition, when sepsis was associated with an organic dysfunction, it was called severe sepsis and when it was associated with refractory hypotension, septic shock.[18,21] The definition of sepsis was updated by the European Society of Medical Care

Intensive Care Society and the Critical Care Medicine Society as an infection associated with an excessive immune response of the host with a consequent organ failure.

2.4. Specimen detection method

The acquisition of specimens for etiological results was performed by using a disposable sterile suction tube or a branch fiberscope to take a deep suction tube, and the sterile container was directly sent for examination. Bacterial identification strains were identified by ATB and VITEK identification systems. Drug susceptibility test adopts K-B method or VITEK system. Bacterial resistance defined according to bacterial species: resistance of *Staphylococcus aureus* to methicillin; resistance of tiamethicillin, ceftazidime, or imipenem to *P aeruginosa*; and broad-spectrum β-lactamase produce and cephalosporin resistance to Enterobacteriaceae.

2.5. Analysis of the circulating immune response

The PBMC were incubated with combinations of fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-cyanine 5.5 (PE-cy5.5), and peridinin chlorophyll protein monoclonal antibodies. The monoclonal antibodies were CD3-FITC, CD3-Percp/Cy5.5, CD4-PE, CD4-FITC, CD8-FITC, CD8-PE, CD16-FITC, CD56-PE, CD19-PE, CD25-FITC, CD127-Percp/Cy5.5, and CD28-PE (Beckman Coulter). About 10,000 lymphocytes were assessed with FC500 software to determine the percentage of $CD3^+$, $CD4^+$, $CD8^+$, $CD3^-CD16^+CD56^+$, $CD19^+$, $CD4^+CD25^+CD127^+$, $CD8^+CD28^-$, and $CD8^+CD28^+$ lymphocytes.

2.6. Statistical methods

Continuous variables were expressed as mean ± standard deviation and compared using a 2-tailed unpaired Student t test; categorical variables were compared using Chi-squared or Fisher analysis. The Greenwood formula was used for the standard deviation. A logistic regression approach[22] was chosen for the evaluation of the risk factors of VAP. Potential predicting variables were analyzed both univariately with 1 factor taken at a time, and then in a multivariate model combining all factors. Results were showed as odds ratios (ORs) and their 95% confidence interval (CI). A OR >1 indicated an elevated risk with respect to the reference category. A CI which did not include the value 1 indicated statistical significance at the 5% level. All statistical evaluations were carried out using SPSS software (Statistical Package for the Social Science, version 15.0; SPSS Inc, Chicago, IL). A value of $P < .05$ was considered to be statistically significant in all the analyses.

3. Results

3.1. Patients' characteristics

Among 478 patients with ventilator usage, 103 cases suffered VAP (21.5%, 103/478). Among the 103 patients with VAP, 35 patients died and 43 patients had simultaneous sepsis. Of the 103 patients with VAP, the APACHE II score (30.74 ± 3.13), application of sedative antacids (32.50%), the rate of aspiration and reflux (27.50%), and ventilation time (13.84 ± 2.76 days) were significantly higher in observation group (patients with VAP) compared with that in the control group (patients without VAP) ($P < .05$). These variables were confirmed as associated risk

Table 1

Clinical and pathological features of patients with ventilator-assisted ventilation (N = 478).

Variables	VAP (n = 103)	Non-VAP (n = 375)	P-value
Age, yr	59.2 ± 13.4	58.8 ± 14.5	.327
Gender			.002
Male	71	192	
Female	32	183	
Hospital stay, d			.001
≥15	82	182	
<15	21	193	
APACHE II scores			.088
≥18	66	205	
<18	37	170	
Mechanical ventilation			.015
Endotracheal intubation	55	249	
Tracheotomy	48	126	
Mechanical ventilation time, d			.001
≥7	70	172	
<7	33	203	
Basic diseases			.602
Yes	47	182	
No	56	193	
Aspiration and reflux			.008
Yes	48	122	
No	55	253	
The use of sedatives and antacids			.391
Yes	52	168	
No	51	207	
Use of glucocorticoids			.271
Yes	46	145	
No	57	230	
Retain stomach tube			.123
Yes	64	201	
No	39	174	

VAP = ventilator-associated pneumonia.

Table 2

Multivariate logistic regression analysis of risk factors related to ventilator-associated pneumonia.

	Multivariate logistic regression analysis		
Variables	OR	95% CI	P-value
Gender: male	1.046	0.968–1.249	.417
Hospital stay, d, ≥15 d	1.083	0.963–1.125	.632
Mechanical ventilation: tracheotomy	1.446	1.168–3.482	.013
Mechanical ventilation time ≥7 d	1.355	1.271–3.347	.021
Aspiration and reflux: yes	1.667	1.461–2.971	.011

CI = confidence interval, OR = odds ratio.

$CD3^+CD4^+$ and $CD8^+CD28^+$ T cells in VAP group are significantly higher than those in non-VAP group ($P < .05$), we performed multiple factors analysis and found that $CD8^+CD28^+$ T cells was independent risk factor relation to VAP ($P < .05$, Fig. 2). We further divided the patients into a death group and a survivor group with respect to VAP group, then, analyzing the differences between the 2 groups. Firstly, we divided the VAP group into the death group and the survivor group. And then, we divided the VAP group into the sepsis group and without sepsis group, respectively. Finally, we compared the characteristics of lymphocyte subsets between the subgroups, respectively.

The subgroup analysis results showed that $CD3^+CD4^+$ T cells and $CD8^+CD28^+$ T cells in the survivor subgroup were significantly higher than those in the dead subgroup ($P < .05$) (Fig. 3A, B). The $CD3^+CD4^+$ T cells and $CD8^+CD28^+$ T cells in the without sepsis subgroup were significantly higher than those in the sepsis subgroup, respectively ($P < .05$) (Fig. 4A, B).

3.4. Distribution of pathogens in infected patients

The pathogenic microorganisms of 103 infected patients were cultured and a total of 137 pathogenic bacteria were isolated; gram-negative bacteria were the major bacteria, and a total of 179 strains accounted for 72.3%. The details are shown in Table 3.

3.5. Antimicrobial drug resistance of major gram-negative bacteria

The main gram-negative bacteria include *Escherichia coli*, *Klebsiella pneumoniae*, *P aeruginosa*, and *Acinetobacter baumannii*. The highest resistance rate of *E coli* to ampicillin was 57.1%. The highest resistant rate to Aztreonam was 53.8% and 68.8% for *K pneumoniae* and *P aeruginosa*. Pipercisin resistance to *A baumannii* was the highest. The highest rate is 59.5%. The details are shown in Table 4.

3.6. Antimicrobial drug resistance of major gram-positive bacteria

The gram-negative bacteria required include *S aureus* and *Streptococcus pneumoniae*. Both *S aureus* and *S pneumoniae* have the highest resistance rate to penicillin of 100%, and the resistance rates to vancomycin are the lowest at 5.6% and 0, as shown in Table 5.

4. Discussion

As a common iatrogenic infectious disease in ICU, VAP is one of the most common complications in mechanical ventilation

factors for VAP. There were no significant differences in age and gender between the 2 groups ($P > .05$). The baseline characteristics of patients are summarized in Table 1.

3.2. Multiple logistic regression analysis of risk factors associated with VAP

The risk factors associated with the occurrence of ICU VAP, including gender, duration of hospitalization, mechanical ventilation, mechanical ventilation time, and aspiration and reflux, were included in the multivariate logistic regression model after univariate analysis. We found that mechanical ventilation: tracheotomy ($P = .013$, OR = 1.446, 95% CI: 1.168–3.482), mechanical ventilation time ≥7 days ($P = .021$, OR = 1.355, 95% CI: 1.271–3.347), and aspiration and reflux ($P = .011$, OR: 1.667, 95% CI: 1.461–2.971) were independent risk factors associated with VAP (Table 2).

3.3. Peripheral lymphocyte subsets analysis of patients between VAP group and non-VAP group

Compared with those of non-VAP group, the proportion of $CD3^+$ ($P = .012$), $CD3^+CD4^+$ ($P = .024$), and $CD8^+CD28^+$ ($P = .017$) T cells in VAP group increased significantly, which showed more severe immune response (Fig. 1). Since he proportion of
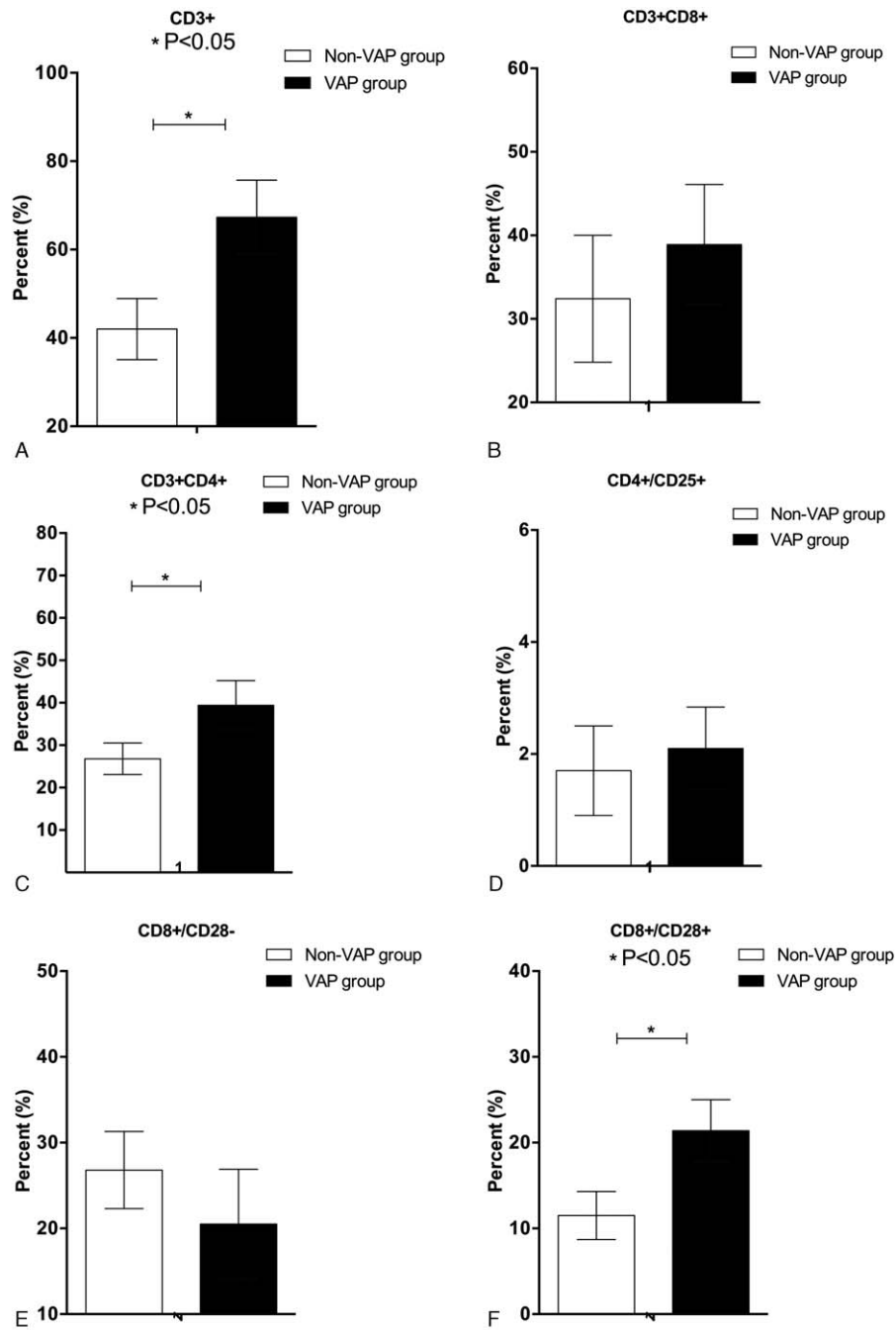
Figure 1. Peripheral lymphocyte subsets analysis of patients between ventilator-associated pneumonia (VAP) group and non-VAP group.

therapy. Patients with VAP have longer ICU staying, higher morbidity and mortality, and more infectious pathogens.[3,23] It has been reported that the incidence of VAP is about 20% to 71%, and the mortality rate of patients with VAP in ICU is relatively high, which is closely related to the various risk factors of VAP. Common VAP prevention measures, such as daily interruptions of sedative medications and assessments prior to preparation for extubating, do not work since related injuries such as severe chest trauma, intra-abdominal bleeding, and damage to other organs need to be considered. The prognosis of VAP is still very different. It is closely related to

the patient's primary disease, pathogenic characteristics, and use of antibiotics.[24,25] Studies showed that there was an increased proinflammatory cytokine response in the lungs of humans in the setting of VAP, consistent with previous studies, the percentage of T cells are lower in patients with VAP compared to those without.[26–29] Levels of CD4+ IFN-γ producing cells were no different between the 2 groups suggesting that activated CD4+ cells are not preferentially lost, but that the decrease is specific to the Th17 compartment. These data suggested that T-cell subsets might be protective against VAPs in humans.
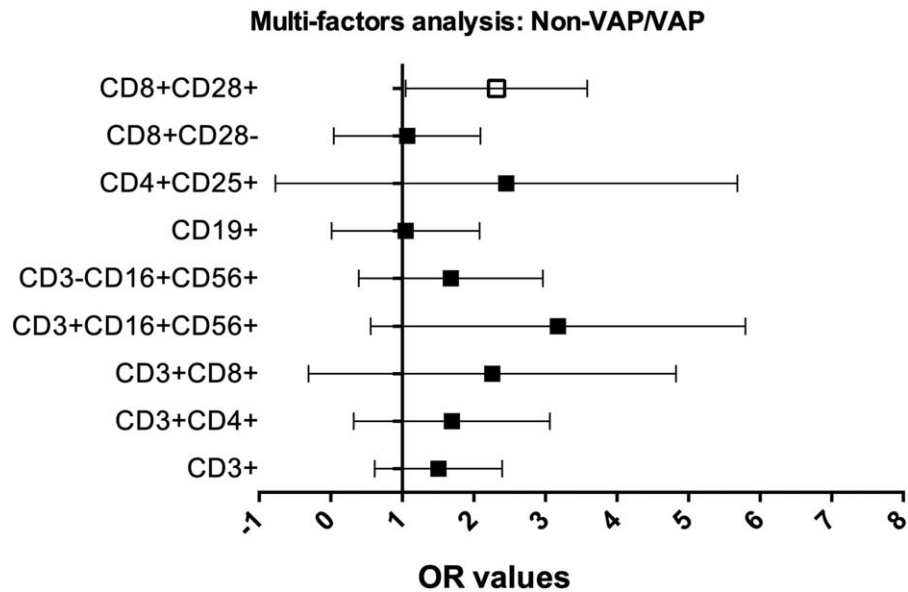
Multi-factors analysis: Non-VAP/VAP

Figure 2. Multiple analysis of peripheral lymphocyte subsets for ventilator-associated pneumonia (VAP). OR = odds ratio.

Although the occurrence and development of VAP are basically the same as those associated with other nosocomial infections, VAP still has certain predisposing factors for pulmonary infection, mainly tracheal intubation and mechanical ventilation.[30,31] The risk factors for VAP induction depended in part on the time of exposure to the ICU environment, the host factors, and factors associated with the development of treatment that leads to VAP. The other part depended on factors that increasing the likelihood of colonization of the alimentary canal of pathogenic bacteria (e.g., previous antibiotic exposure, older than 60 years old, chronic obstructive pulmonary disease) and

induced contaminated secretions = aspiration (e.g., supine position, coma, head injury, etc).[32] In this study, we analyzed by multivariate logistic regression models and found that mechanical ventilation: tracheotomy ($P = .013$), mechanical ventilation time ≥ 7 days ($P = .02$) and aspiration and reflux ($P = .011$) were independent risk factors associated with VAP.

The microbiological environment has a significant effect on VAP strains, particularly in late-onset VAP, but also affects early onset VAP.[33] Choosing the right antibiotic depended on the duration of mechanical ventilation. Late-stage VAP (>4 days) requires broad-spectrum antibiotics and early stage disease (≤ 4
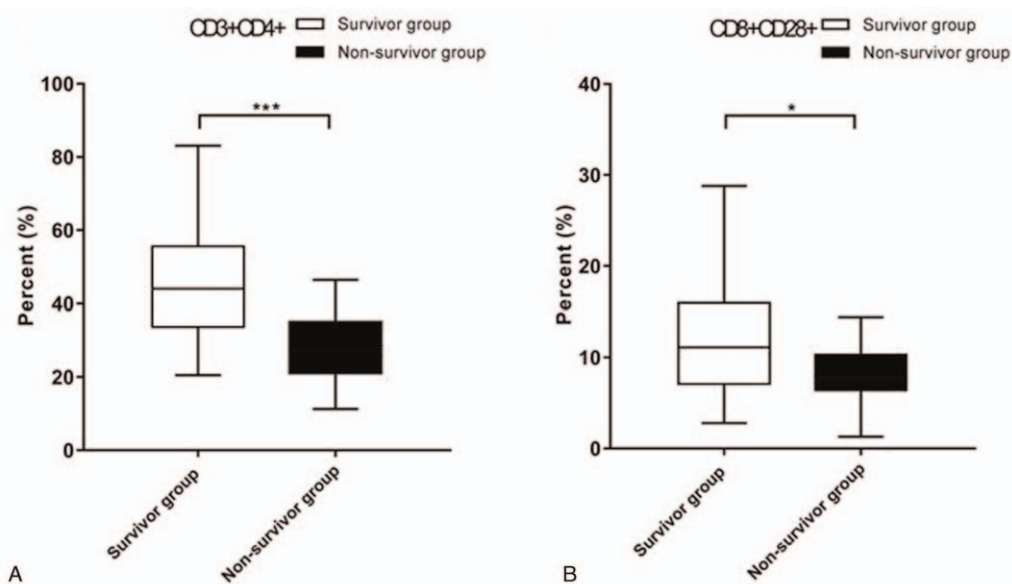

Figure 3. Peripheral lymphocyte subsets analysis of patients stratified by survival in the patients with ventilator-associated pneumonia.
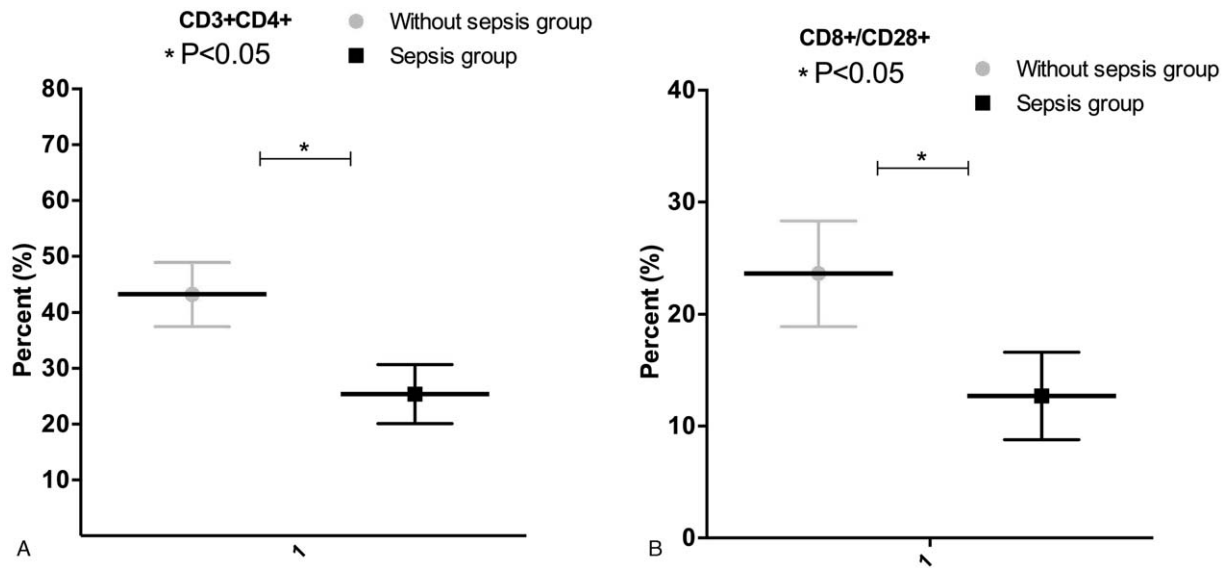
Figure 4. Peripheral lymphocyte subsets analysis of patients stratified by sepsis in the patients with ventilator-associated pneumonia.

Table 3

Distribution of pathogenic bacteria in patients with ventilator-associated pneumonia (%).

Pathogens	N	%
Klebsiella pneumoniae	26	25.2
Acinetobacter baumannii	37	35.9
Escherichia coli	14	13.6
Pseudomonas aeruginosa	16	15.5
Proteobacteria	6	5.8
Staphylococcus aureus	18	5.8
Pneumococcus	13	12.7
Others	7	6.8

days) can be selected for narrow-spectrum antibiotic therapy. Various hospitals and ICUs need to constantly update the use of antibiotics based on local bacterial morphology and sensitivity, and accumulate initial experience of optimal dose.[34,35] In any empirical antibiotic regimen, step down is the key to reducing drug resistance. It is thought to provide the greatest benefit for

individual patients. Delayed antibiotic treatment may increase the risk of death from VAP.[36] In critically ill patients, assisted mechanical ventilation and antibiotic treatment are necessary measures to prevent and treat VAP. The study found that pathogenic microorganisms in respiratory secretions of patients with severe VAP were mainly gram-negative bacilli. Our study also confirmed that the distribution of VAP pathogens is mainly concentrated in *A baumannii*, *P aeruginosa*, *K pneumoniae*, and *E coli* with a high drug-resistance rate, which were mainly to ampicillin, gentamicin, cefazolin, cefotaxime sodium, and other drug resistance, while serious multidrug resistance were also observed. Therefore, the analysis of the characteristics of VAP pathogens and their drug resistance is of guiding significance for future clinical prevention and treatment of VAP.

Several limitations existed in this study. Firstly, this study is a retrospective cohort study and the sample size is relatively small; secondly, the exact mechanisms of characteristics of VAP pathogens involved in the immune phenotypes of T cells are still not elucidated. Thus, further studies are needed to solve these problems.

Table 4

Results of antimicrobial resistance analysis of major gram-negative bacteria.

	Escherichia coli (n=14)		Klebsiella pneumoniae (n=26)		Pseudomons aeruginosa (n=16)		Acinetobacter baumannii (n=37)	
	N	Resistance rate, %	N	Resistance rate, %	N	Resistance rate, %	N	Resistance rate, %
Ampicillin	8	57.1	14	53.8	10	62.5	16	43.2
Gentamicin	9	64.3	10	38.5	11	68.8	12	32.4
Aztreonam	10	71.4	14	53.8	8	50	21	56.8
Piperacillin	9	64.3	13	50	7	43.8	22	59.5
Cefazolin	6	42.8	9	34.6	5	31.3	10	27.1
Cefaclor	3	21.4	10	38.5	5	31.3	9	24.3
Ceftazidime	5	35.7	11	42.3	7	43.8	11	29.7
Ceftriaxone	8	57.1	9	34.6	7	43.8	12	32.4
Cefotaxime sodium	7	50	7	26.9	8	50	12	32.4
Levofloxacin	5	35.7	9	34.6	5	31.3	9	24.3
Ciprofloxacin	3	21.4	12	46.2	6	37.5	9	24.3
Imipenem	0	0	3	11.5	1	6.3	3	8.1

Table 5

Analysis of antimicrobial resistance of major gram-positive bacteria.

	Staphylococcus aureus (n = 18)		Pneumococcus (n = 13)	
	n	Resistance rate, %	n	Resistance rate, %
Penicillin	18	100	13	100
Oxacillin	12	66.7	6	46.2
Clindamycin	8	44.4	8	61.5
Levofloxacin	10	55.6	5	38.5
Vancomycin	1	5.6	0	0

In summary, the occurrence and development of VAP in patients with ICU control is of utmost importance and is also a key and difficult task in ICU work. Patients with advanced age, coma, and diabetes mellitus should strengthen monitoring to ensure curative effect, shorten mechanical ventilation time and length of hospital stay, reasonably select antimicrobial drugs according to bacterial culture and drug susceptibility test results, and strengthen patient care management in ICU. The comprehensive prevention and control of risk factors in all aspects can effectively control the incidence of VAP and improve the prognosis of patients.

Author contributions

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