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Original Article

Risk factors and peripheral blood lymphocyte subset analysis of patients with ventilator-associated pneumonia: a Chinese population-based study

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Abstract: The aim of this study is to analyze and identify ventilator-associated pneumonia (VAP) risk factors related to pathogens and drug resistance, and explore the theoretical guidance for clinical prevention and treatment strategies of VAP. 478 cases using a ventilator who were hospitalized in July 2014 to November 2016 in our hospital were analyzed in this study. Among them there were 103 patients with VAP. The distribution of pathogenic bacteria and drug resistance in VAP patients was detected and analyzed. 103 patients had VAP (21.5%, 103/478) among 478 cases of patients using a ventilator. Among the 103 patients with VAP, 35 patients died and 43 had simultaneous sepsis. Compared with those of the non-VAP group, the proportion of $CD3^+$ ($p = 0.012$), $CD3^+$ $CD4^+$ ($P = 0.024$) and $CD8^+$ $CD28^+$ ($P = 0.017$) T cells in VAP group increased significantly, which showed a more severe immune response. Multivariate regression model analysis revealed that tracheotomy for mechanical ventilation ($P = 0.013$), mechanical ventilation time ≥ 7 days ($P = 0.02$) and aspiration and reflux ($P = 0.011$) were independent risk factors associated with VAP. Multi-drugs resistance was observed in this study. Modality of mechanical ventilation, mechanical ventilation ≥ 7 days, and aspiration and reflux 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 in the ICU can effectively control the incidence of VAP and improve prognosis.

Keywords: Ventilator-associated pneumonia, risk factors, pathogen analysis

Introduction

Ventilator-associated pneumonia (VAP) is one of the most serious complications during mechanical ventilation. VAP is 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 is between 1.2 and 8.5 per thousand, and the occurrence of VAP depends on the definition of VAP diagnosis. VAP accounts for about half of all cases of hospital-acquired pneumonia [2-4]. 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 [5, 6].

There is currently no consensus on the diagnosis and definition of VAP [7]. 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 multi-drugs resistance (MDR) bacteria and occurred > 4 days after mechanical ventilation. Once VAP occurs, it is easy to incur offline difficulties, prolonged hospital stay and hospitalization costs, life-threatening infection, or death [1, 8]. 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 [9, 10]. *Pseudomonas aeruginosa* is one of the most common pathogens causing VAP and is independently associated with increased mortality; in China, it remains among the top three pathogens. Antibiotic treatment is the primary method for managing *P. aeruginosa* VAP; however, it constitutes a risk factor for the development of multi-drug resistant *P. aeruginosa*. Increasing drug resistance, especially in intensive care

units (ICUs), could result in *P. aeruginosa* VAP becoming uncontrollable [11-14].

According to the third 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 [15]. Sepsis that is associated with VAP is the primary cause of death from infection; its recognition mandates urgent attention. Pathogen factors and host factors shape this syndrome: sex, age, comorbidities, and environment.

This study analyzes the risk factors associated with VAP, and further analyzes the pathogenic bacteria and their resistance in VAP. Moreover, this study discusses the prevention and treatment strategies of VAP, and provides theoretical guidance for clinical prevention and control of VAP.

Materials and methods

Patients and tissue samples

We selected 478 patients who used ventilators from November 2014 to July 2016 in our intensive care unit, 103 of whom had VAP, and the incidence was 21.5%. All patients underwent chest X-ray examination without pulmonary infection before admission. The study was approved by the Research Ethics Committee of Heze City Hospital. Informed consent was obtained from all patients. The study was conducted in accordance with the recognized ethical guideline of Declaration of Helsinki.

VAP diagnostic criteria

According to the diagnostic criteria of VAP as follows [16, 17]: (1) After 48 hours of mechanical ventilation, the chest X-rays showed infiltrated lungs or new infiltrated shadows. The physical examination of the lungs revealed wet rales; one of the following conditions was also met: White blood cell count > 10×10^{11}/L or < 4.0×10^{9}/L; body temperature > 37.5°C; purulent respiratory secretions; isolation from the bronchial secretions of pathogenic bacteria. (2) Clinical Pulmonary Infection Scores: CPISS scores were calculated to be > 6 for confirmed or suspected cases. (3) 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: (1) Mechanical ventilation time is less than 48 h. (2) Pulmonary infection has been diagnosed before entering the ICU. (3) Incomplete data. (4) Pulmonary embolism, ARDS, tuberculosis and other diseases.

Sepsis and systemic inflammatory response syndrome (SIRS)

In 1991 sepsis was first defined as a "SIRS to the presence of infection", requiring the presence of 2 or more of: alterations in heart and respiratory rate, body temperature and white blood cell count as criteria. In addition, when sepsis was associated with an organ dysfunction it was called severe sepsis and when it was associated with refractory hypotension, septic shock [15, 18]. 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 by the host with consequent organ failure.

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 used the K-B method or VITEK system. Bacterial resistance was defined according to bacterial species: resistance of Staphylococcus aureus to methicillin; resistance of tiamethicillin, ceftazidime or imipenem to Pseudomonas aeruginosa; and broad-spectrum β-lactamase producing and cephalosporin resistance to Enterobacteriaceae.

Analysis of the circulating immune response

PBMC were incubated with combinations of fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-cyanine 5.5 (PE-cy5.5), and peridinin chlorophyll protein (PerCP) 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, USA). About

Table 1. Clinical and pathologic features of patients with ventilator-assisted pneumonia (N = 478)

Variable	VAP (n = 103)	Non-VAP (n = 375)	*P* values
Age (years)	59.2 ± 13.4	58.8 ± 14.5	0.327
Gender			0.002
Male	71	192	
Female	32	183	
Hospital stay (days)			0.001
≥ 15 days	82	182	
< 15 days	21	193	
APACHE II scores			0.088
≥ 18	66	205	
< 18	37	170	
Mechanical ventilation			0.015
Endotracheal intubation	55	249	
Tracheotomy	48	126	
Mechanical ventilation time			0.001
≥ 7 days	70	172	
< 7 days	33	203	
Basic diseases			0.602
Yes	47	182	
No	56	193	
Aspiration and reflux			0.008
Yes	48	122	
No	55	253	
Use of sedatives and antacids			0.391
Yes	52	168	
No	51	207	
Use of glucocorticoids			0.271
Yes	46	145	
No	57	230	
Retained stomach tube			0.123
Yes	64	201	
No	39	174	

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.

Statistical methods

Continuous variables were expressed as mean ± SD (standard deviation) and compared using a two-tailed unpaired Student t test; categorical variables were compared using χ^2 or Fisher analysis. The Greenwood formula was used for the standard deviation. A logistic regression approach [19] was chosen for the evaluation of the risk factors of VAP. Potential predicting variables were analyzed both univariately with one factor taken at a time, and then in a multivariate model combining all factors. Results were showed as odd hazard ratios (OR) and their 95% confidence intervals (CI) An OR > 1 indicated an elevated risk with respect to the reference category. A confidence interval 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 $P < 0.05$ was considered significant in all analyses.

Results

Patient characteristics

Among 478 patients with ventilator usage, 103 cases suffered ventilator-associated pneumonia (21.5%, 103/478). Among the 103 patients with VAP, 35 patients died and 43 patients had simultaneous sepsis. Among 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 ($P < 0.05$). These variables were confirmed as associated risk factors for VAP. There were no significant differences in age and gender between the two groups ($P > 0.05$). The baseline characteristics of patients are summarized in **Table 1**.

Multiple logistic regression analysis of risk factors associated with VAP

The risk factors associated with the occurrence of ICU ventilator-associated pneumonia, including gender, duration of hospitalization, mechanical ventilation, mechanical ventilation time, and aspiration and reflux, were included in the

Table 2. Multivariate logistic regression analysis of risk factors related to VAP

Variable	Multivariate logistic regression analysis		
	OR	95% CI	*P* value
Gender: male	1.046	0.968-1.249	0.417
Hospital stay (days) ≥ 15 days	1.083	0.963-1.125	0.632
Mechanical ventilation: tracheotomy	1.446	1.168-3.482	0.013
Mechanical ventilation time ≥ 7 days	1.355	1.271-3.347	0.021
Aspiration and reflux: Yes	1.667	1.461-2.971	0.011

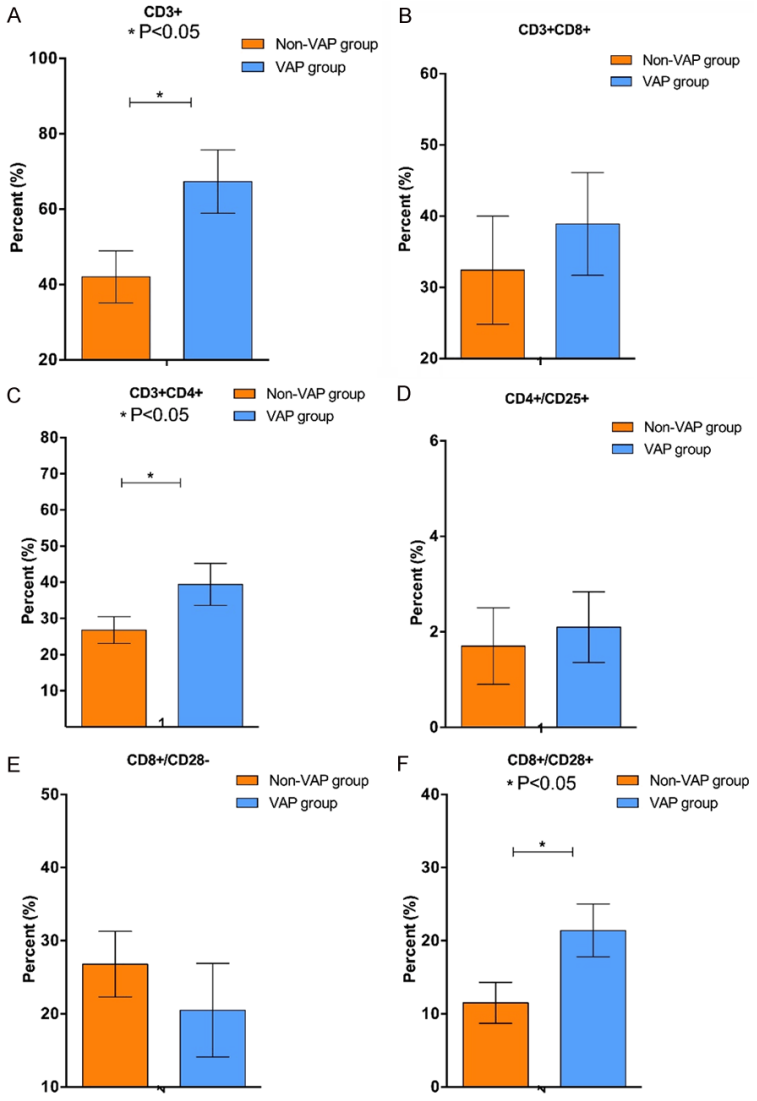

Figure 1. Peripheral lymphocyte subset analysis of patients between VAP group and non-VAP group.

multivariate logistic regression model after univariate analysis. We found that mechanical ventilation: tracheotomy (P = 0.013, OR = 1.446, 95% CI: 1.168-3.482), mechanical ventilation time ≥ 7 days (P = 0.021, OR = 1.355, 95% CI: 1.271-3.347) and aspiration and reflux (P = 0.011, OR: 1.667, 95% CI: 1.461-2.971) were independent risk factors associated with VAP (**Table 2**).

Peripheral lymphocyte subset analysis of patients between VAP group and non-VAP group

Compared with those of the non-VAP group, the proportion of $CD3^+$ (P = 0.012), $CD3^+CD4^+$ (P = 0.024) and $CD8^+CD28^+$ (P = 0.017) T cells in the VAP group were increased significantly, which showed a more severe immune response (**Figure 1**). Since the proportion of $CD3^+CD4^+$ and $CD8^+CD28^+$T cells in VAP group are significantly higher than those in the non-VAP group (P < 0.05), we performed multifactor analysis and found that $CD8^+CD28^+$T cells had an independent risk factor relation to VAP (P < 0.05, **Figure 2**). We further divided the patients into a death group and a survivor group with respect to the VAP group, then, analyzed the differences between the two groups. First, we divided the VAP group into the death group and the survivor group. 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 < 0.05) (**Figure 3A**, **3B**). The $CD3^+CD4^+$T cells and $CD8^+CD28^+$T cells in the without-sepsis

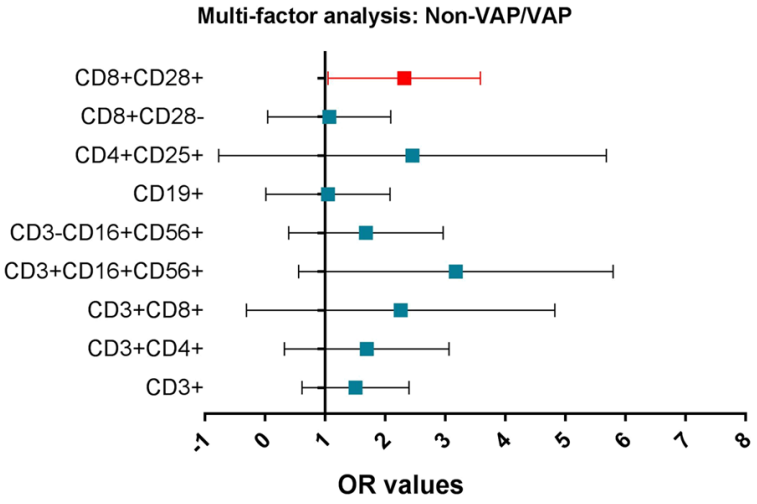

Figure 2. Multiple analysis of peripheral lymphocyte subsets for VAP.

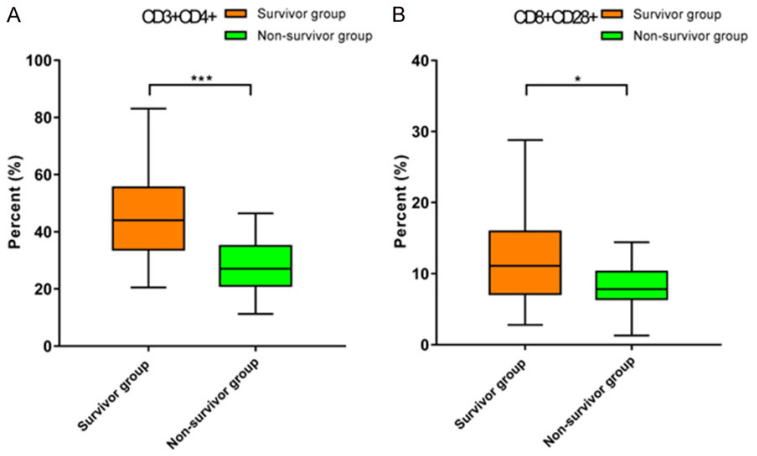

Figure 3. Peripheral lymphocyte subset analysis of patients stratified by survival in patients with VAP.

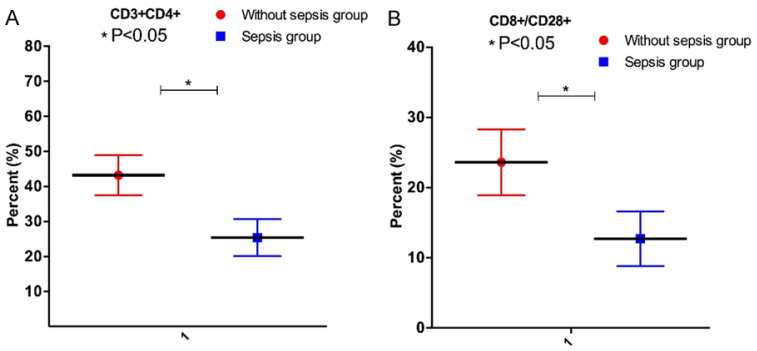

Figure 4. Peripheral lymphocyte subset analysis of patients stratified by sepsis in patients with VAP.

subgroup were significantly higher than those in the sepsis subgroup, respectively ($P < 0.05$) (**Figure 4A**, **4B**).

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**.

Antimicrobial drug resistance of major gram-negative bacteria

The main gram-negative bacteria included E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii. The highest resistance rate of Escherichia coli to ampicillin was 57.1%. The highest resistant rates to aztreonam were 53.8% and 68.8% for Klebsiella pneumoniae and Pseudomonas aeruginosa. Pipercisin resistance to Acinetobacter baumannii was the highest. The highest rate is 59.5%. Details are shown in **Table 4**.

Antimicrobial drug resistance of major Gram-positive bacteria

The Gram-negative bacteria encountered include Staphylococcus aureus and Streptococcus pneumoniae. Both Staphylococcus aureus and Streptococcus pneumoniae had the highest resistance rate to penicillin of 100%, and the resistance rates to vancomycin were the lowest, at 5.6% and 0, as shown in **Table 5**.

Discussion

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

Table 3. Distribution of pathogenic bacteria in VAP patients (%)

Pathogen	N	Percent (%)
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

Patients with VAP have longer ICU stays, higher morbidity and mortality, and more infectious pathogens [2, 20]. It has been reported that the incidence of VAP is about 20%-71%, and the mortality rate of VAP patients in the ICU is relatively high, which is closely related to the various risk factors for VAP. Common VAP prevention measures, such as daily interruptions of sedative medications and assessments prior to preparation for extubation, may 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 variable. It is closely related to the patient's primary disease, pathogenic characteristics, and use of antibiotics [21, 22].

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 [23, 24]. 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 lead to VAP. Other risks depended on factors that increased the likelihood of colonization of the alimentary canal by pathogenic bacteria (previous antibiotic exposure, age older than 60 years, chronic obstructive pulmonary disease) and induced contaminated secretions causing aspiration (supine position, coma, and head injury) [25]. In this study, we analyzed by multivariate logistic regression models and found that mechanical ventilation: tracheotomy ($P = 0.013$), mechanical ventilation time ≥ 7 days ($P = 0.02$) and aspiration and reflux ($P = 0.011$) were independent risk factors associated with VAP.

Early-onset VAP is caused by antibiotic-sensitive pathogens, but late-onset VAP is caused by multidrug-resistant bacteria, which is more difficult to treat. The microbiological environment has a significant effect on VAP strains, particularly in late-onset VAP, but also affects early-onset VAP [26]. Choice of the right antibiotic depended on the duration of mechanical ventilation. Late-stage VAP (> 4 days) requires broad-spectrum antibiotics but early-stage disease (≤ 4 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 an optimal dose [27, 28]. 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 [29]. In critically ill patients, assisted mechanical ventilation and antibiotic treatment are necessary measures to prevent and treat ventilator-associated pneumonia. The study found that pathogenic microorganisms in respiratory secretions of patients with severe ventilator-associated pneumonia were mainly gram-negative bacilli. Our study also confirmed that the distribution of VAP pathogens chiefly include Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli with a rate of high drug resistance, which were mainly to ampicillin, gentamicin, cefazolin, cefotaxime sodium, and others, while serious multidrug resistance was 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.

In summary, the occurrence and development of VAP in patients under 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 need intense 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 the ICU. Comprehensive prevention and control of risk factors in all aspects can

Table 4. Results of antimicrobial resistance analysis of major gram-negative bacteria

	E. coli (n = 14)		Klebsiella pneumoniae (n = 26)		Pseudomonas 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

effectively control the incidence of VAP and improve prognosis.

Disclosure of conflict of interest

None.

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