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Original Article

Analysis of distribution and drug resistance of pathogens in ICU newborns with ventilator-associated pneumonia

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Abstract: Objective: The goal of this study was to analyze the distribution and drug resistance of main pathogens in ventilator-associated pneumonia in ICU newborns. Methods: A total of 55 newborns with ventilator-associated pneumonia (VAP) and another 60 newborns without VAP were selected. The secretions at the end of endotracheal tube were acquired for pathogen culture and drug sensitivity test. Moreover, serum levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were also measured. Results: 1) The average weight and gestational age in the VAP group were smaller than those in the non-VAP group, and times of tracheal intubation, duration of mechanical ventilation and duration of hospital stays in the VAP group were significantly longer than those in the non-VAP group ($P < 0.05$). 2) In the VAP group, Gram-positive cocci was 36.36%, with Staphylococcus aureus, Staphylococcus hemolyticus and Enterococcus fecalis being prevalent. Gram-negative bacilli occupied 54.55%, with Escherichia coli and Stenotrophomonas maltophilia as the main pathogens. In the non-VAP group, Gram-positive cocci accounted for 48.33%, with Staphylococcus aureus and Staphylococcus epidermidis predominant. Gram-negative bacilli occupied 41.67%, with Escherichia coli as the common one. The constituent ratios of fungi in both VAP and non-VAP groups were relatively low. 3) The Gram-positive cocci showed resistance to penicillin sodium and erythromycin with low resistance rate to vancomycin, linezolid and clindamycin. 4) Gram-negative bacilli were completely resistant to piperacillin without resistance to cefoperazone/tazobactam and meropenem. 5) The differences in serum levels of TNF-α and IL-6 levels between the VAP and non-VAP groups before mechanical ventilation were not significant, while they were significantly higher than those in the non-VAP group at 1, 3, 5, and 7 days after mechanical ventilation ($P < 0.05$). Conclusion: Gram-negative bacilli are dominated in the infection in newborns with VAP in ICU.

Keywords: Newborns, ventilator-associated pneumonia, drug resistance, inflammatory factors

Introduction

Ventilator-associated pneumonia (VAP) is the associated pneumonia which occurs after mechanical ventilation. There is generally no pulmonary infection with relatively no latency before mechanical ventilation, so VAP refers in particular to pneumonia which is acquired after mechanical ventilation [1]. In recent years, VAP is becoming increasingly common in neonatal patients. Among a series of relevant factors causing neonatal infection, lower respiratory tract infection accounts for the highest proportion. There are many contributing factors of lower respiratory tract infection, among which the invasive manipulation represented by tracheal intubation is predominant [2]. Accurately speaking, VAP is a pulmonary infection that occurs within 48 hours after mechanical ventilation in patients without a history of pulmonary infection, or new pulmonary infection that occurs at 48 hours after mechanical ventilation in patients with pulmonary infection. It is the determining factor for the failure of the mechanical ventilation treatment, and increases the mortality rate, duration of hospital stays and hospitalization costs of newborns in the meantime. Currently, once newborns develop various critical diseases, they can be generally transferred to the Intensive Care Unit (ICU) for monitoring and treatment in clinic through different systems. Treatment with tracheal intubation is also more and more common, but the incidence of VAP becomes increasingly higher [4]. In critical care medicine, how to treat the VAP in the most effective way has been received more and more attention. Detecting the pathogen distribution and drug resistance of VAP is more help-

Table 1. Comparisons of general data between the VAP and non-VAP groups

General data	VAP group (n = 55)	Non-VAP group (n = 60)	*P*
Days of age (d)	6.35 ± 2.8	6.30 ± 2.7	0.067
Gender (male/female)	30/25	36/24	0.059
Average weight (g)	1836.29 ± 525.73	2316.18 ± 611.29	0.016
Number of tracheal intubation (time)	3.01 ± 2.08	1.79 ± 1.41	0.025
Time of mechanical ventilation (h)	170.26 ± 101.83	117.83 ± 205.40	0.021
Hospitalization time (d)	21.44 ± 10.58	12.93 ± 7.97	0.005
Gestational age (week)	33.85 ± 6.53	36.83 ± 3.74	0.047

ful in guiding the clinical application of antibiotics and improving prognosis effectively. However, it still lacks a reliable detection index with guiding significance in the diagnosis of VAP. This study aims to analyze the distribution and drug resistance of main pathogens in ventilator-associated pneumonia in ICU newborns, in order to provide guidance on the diagnosis and treatment of VAP.

General data and methods

General data

A total of 55 newborns with VAP who were treated with mechanical ventilation in our hospital from August 2016 to September 2017 were selected, and another 60 newborns without VAP who were treated with mechanical ventilation were also selected. There were 66 males and 49 females aged 1-28 days old with an average age of (6.35 ± 2.8) days old. All cases selected were treated with mechanical ventilation. The diagnostic criteria for VAP group included new invasive shadows on the chest film at 48 hours after mechanical ventilation, and meeting any 2 of the following 3 criteria: 1) body temperature > 38.3°C, 2) increased white blood cell count, and 3) purulent secretions in the respiratory tract, positive bacterial culture of secretions or new infectious pathogenic bacteria. Cases receiving mechanical ventilation for less than 48 hours, or complicated with congenital disease or neonatal death were excluded. The study was approved by the Ethics Committee of Yantai Yuhuangding Hospital and informed consents were signed by the patients' guardians.

Methods

General data of all newborns were collected, including age, gender, weight, number of tracheal intubation, time of mechanical ventilation and hospitalization time. Detection methods of pathogenic bacteria in the respiratory tract: After mechanical ventilation for more than 48 hours, secretions in the lower respiratory tract were collected from all newborns using the disposable suction catheter under aseptic conditions, or the end of cannula was cut for bacterial culture in the case of ventilator withdrawal and change of tracheal cannula. The drug sensitivity test of pathogenic bacteria was performed using the K-B agar diffusion method. Detection of inflammatory factors: 5 mL fasting peripheral blood was drawn from all newborns before mechanical ventilation, and at 1, 3, 5, and 7 days after mechanical ventilation followed by isolation of serum which was used for measuring and levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) via immunoturbidimetry.

Statistical methods

Statistical Product and Service Solutions (SPSS) 19.0 software was used for data processing. Measurement data are presented as mean ± standard deviation (SD) and compared by student t-test. Chi-square test was used for the comparison of enumeration data. $P < 0.05$ suggested that the difference was statistically significant.

Results

Comparisons of general data

In the VAP group, the average weight and gestational age were significantly smaller than those in the non-VAP group, but the number of tracheal intubation, time of mechanical ventilation and hospitalization time were significantly larger and longer than those in the non-VAP group ($P < 0.05$). However, there were no statistically significant differences in days of age and gender (**Table 1**).

Table 2. Comparisons of distribution ratios of pathogenic bacteria between the VAP and non-VAP groups

Pathogenic bacteria	VAP group (n = 55)		Non-VAP group (n = 60)	
	Strain	Constituent ratio (%)	Strain	Constituent ratio (%)
G+ cocci	20	36.36	29	48.33
Staphylococcus aureus	5	9.09	12	20
Staphylococcus haemolyticus	5	9.09	3	5
Enterococcus faecalis	6	10.91	2	3.37
Staphylococcus epidermidis	4	7.27	12	20
G- bacilli	30	54.55	25	41.67
Escherichia coli	10	18.18	11	18.33
Stenotrophomonas maltophilia	7	12.73	4	6.67
Klebsiella pneumoniae	5	9.09	4	6.67
Pseudomonas aeruginosa	4	7.27	3	5
Acinetobacter baumannii	4	7.27	3	5
Fungi	5	9.09	6	10

Comparisons of distribution ratios of pathogenic bacteria

In the VAP group, Gram-positive cocci accounted for 36.36%, dominated by Staphylococcus aureus, Staphylococcus hemolyticus and Enterococcus fecalis (10.91%), while Gram-negative bacilli accounted for 54.55%, dominated by Escherichia coli (18.18%) and Stenotrophomonas maltophilia (12.73%). In the non-VAP group, Gram-positive cocci accounted for 48.33%, dominated by Staphylococcus aureus (20%) and Staphylococcus epidermidis (20%), while Gram-negative bacilli accounted for 41.67%, dominated by Escherichia coli (18.33%). The constituent ratio of fungi was low in both the VAP and non-VAP groups (**Table 2**).

Analysis of drug resistance rate of Gram-positive cocci

Gram-positive cocci were basically resistant to penicillin sodium and erythromycin, with a drug resistance rate of nearly 0, and their resistance rates to vancomycin, linezolid and clindamycin were low. Drug resistance rates of Staphylococcus epidermidis, Staphylococcus aureus and Enterococcus faecalis were 0 to vancomycin, 0, 5.88% and 12.5% to linezolid, and 31.25%, 17.65% and 25% to clindamycin (**Table 3**).

Analysis of drug resistance rate of Gram-negative bacilli

Gram-negative bacilli were almost resistant to piperacillin completely. Drug resistance rates of Escherichia coli, Stenotrophomonas maltophilia and Klebsiella pneumoniae were 95.24%, 100% and 88.89% to piperacillin, almost 0 to cefoperazone/tazobactam and meropenem, 4.76%, 0 and 11.11% to cefoperazone/tazobactam, and 0, 18.18% and 11.11% to meropenem (**Table 4**).

Comparisons of inflammatory factors in peripheral serum

No statistically significant differences were found in serum levels of TNF-α and IL-6 between the VAP group and the non-VAP group before mechanical ventilation. However, at 1, 3, 5, and 7 days after mechanical ventilation, serum levels of TNF-α and IL-6 in the VAP group were significantly higher than those in the non-VAP group ($P < 0.05$) (**Figure 1A** and **1B**). With prolongation of time of mechanical ventilation, serum levels of TNF-α and IL-6 in the non-VAP group were gradually decreased. After mechanical ventilation, serum levels of TNF-α and IL-6 in VAP group were decreased and then increased reaching the peak on 5 days, followed by a decreasing trend again finally (**Figure 1A** and **1B**).

Discussion

With continuous improvement of medical and scientific research progress, the incidence rate of neonatal death in clinic has been lower, which is closely related to the construction of related transfer system in the neonatal intensive care unit (NICU) in hospitals. Moreover, correct and effective rescue approaches, such as mechanical ventilation, adopted by NICU physicians also plays an important role [3]. Increasingly more newborns in NICU are treated with tracheal intubation, raising the survival rate. However, the incidence of related complications, such as VAP, displaying an increasing trend year by year [5]. The occurrence of VAP increases the risk of neonatal nosocomial infection, prolongs the time of hospitalization and ventilator withdrawal and increases medical expenses. During mechanical ventilation, tracheal cannula can easily damage the soft

Table 3. Analysis of drug resistance rate of Gram-positive cocci

Antibacterial agent	Staphylococcus epidermidis (n = 16)		Staphylococcus aureus (n = 17)		Enterococcus faecalis (n = 8)	
	Strain	Drug resistance rate (%)	Strain	Drug resistance rate (%)	Strain	Drug resistance rate (%)
Penicillin sodium	16	100	17	100	-	-
Erythromycin	15	93.75	14	82.35	8	100
Gentamicin	14	87.5	14	82.35	1	12.5
Clindamycin	5	31.25	3	17.65	2	25
Vancomycin	0	0	0	0	0	0
Levofloxacin	8	50	4	23.53	1	12.5
Chloramphenicol	7	43.75	1	5.88	3	37.5
Tetracycline	4	25	8	47.06	3	37.5
Linezolid	0	0	1	5.88	1	12.5

Table 4. Analysis of drug resistance rate of Gram-negative bacilli

Antibacterial agent	Escherichia coli (n = 21)		Stenotrophomonas maltophilia (n = 11)		Klebsiella pneumoniae (n = 9)	
	Strain	Drug resistance rate (%)	Strain	Drug resistance rate (%)	Strain	Drug resistance rate (%)
Ceftazidime	13	61.9	4	36.36	4	44.44
Cefotaxime	15	71.43	8	72.73	6	66.67
Cefepime	10	47.62	5	45.45	4	44.44
Cefoperazone	12	57.14	7	63.64	3	33.33
Cefoperazone/tazobactam	1	4.76	0	0	1	11.11
Meropenem	0	0	2	18.18	1	11.11
Piperacillin	20	95.24	11	100	8	88.89
Piperacillin/tazobactam	4	19.05	2	18.18	0	0
Levofloxacin	2	9.53	0	0	1	11.11

oropharynx and respiratory mucosa of newborns, and a new respiratory passage is formed by mechanical ventilation and passes through the throat [6]. The throat is an important immune barrier in the human body, but the protection, filtration and humidification effects of the original barrier are lost in newborns under mechanical ventilation [7]. Additionally, the immune system in newborns has not developed or matured yet. Therefore, the risk of bacterial infection *in vivo* or *in vitro* is increased, leading to a high incidence rate of VAP [8]. According to recent data, the incidence rate of VAP is about 30% currently, and its mortality rate is approximately 28% [9]. Therefore, it is of great significance regarding how to avoid the occurrence of VAP or realize early diagnosis and treatment, as well as reduce the mortality rate of VAP in newborns.

Currently, it is believed in both China and foreign countries that the most common infectious pathogenic bacteria of VAP are Gram-negative bacilli, followed by Gram-positive cocci dominated by Staphylococcus aureus [10]. In China, it is recognized that Klebsiella pneumoniae is the most common Gram-negative bacilli, followed by Pseudomonas aeruginosa [11]. With the influence of various factors, especially the inappropriate application of antibiotics, however, the infection rate of Gram-positive cocci has been increased in recent years, but that of Gram-negative bacilli has shown a decreasing trend, in which Staphylococcus is the dominant strain in Gram-positive cocci [12]. The above phenomenon is possibly related to the preventive application of antibiotics in NICU, such as ceftazidime, resulting in excessive inhibition of Gram-negative bacilli [13]. Moreover, Staphylococcus aureus can be transmitted hand to hand among people, which is also one of the causes for the increased infection rate of Gram-positive cocci, suggesting that the hand hygiene needs further

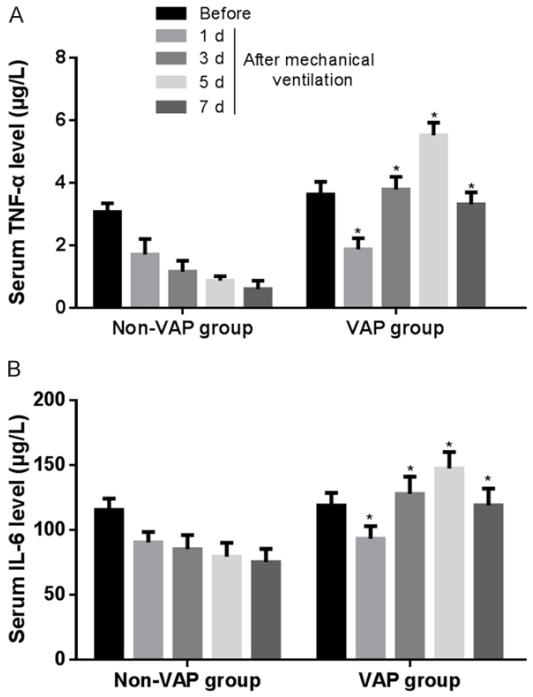

Figure 1. Serum levels of TNF-α and IL-6 before and after mechanical ventilation. Serum was isolated from patients before and after mechanical ventilation followed by measuring the levels of TNF-α (A) and IL-6 (B) by ELISA. Compared with corresponding value in the non-VAS group, *P < 0.05.

strengthening [14]. Here, pathogenic bacteria were found to be successfully cultured in 55 newborns in the VAP group, dominated by Gram-negative bacilli (54.55%). In Gram-negative bacilli, there were 10 strains of Escherichia coli, accounting for 18.18%. In Gram-positive cocci, there were 6 strains of Enterococcus fecalis, accounting for 10.91%. Chen SQ et al. reported that Escherichia coli occupied the largest proportion in Gram-negative bacilli in VAP group, while Staphylococcus aureus occupied the largest proportion in Gram-positive cocci, which might have a significant correlation with the small sample size in this study. The drug resistance rate of meropenem was the lowest in Gram-negative bacilli, while that of vancomycin was the lowest in Gram-positive cocci. With the development of antibiotics, the clinical effects of some synthetic antibiotics, such as cefoperazone/tazobactam and piperacillin/tazobactam, on Gram-positive bacilli are satisfactory. More serious adverse reactions of meropenem and vancomycin are renal toxicity and ototoxicity, but a large number of clinical studies have revealed that there are generally no adverse reactions under the usage of the conventional recommended dose. Drug-induced renal insufficiency may occur in case of overdose, but it can be corrected if it is detected in time [15].

The toxin will stimulate the immune system in case of serious infection in the body, and inflammatory cytokines will be secreted into the blood under the action of DNA transcription [16]. TNF-α and IL-6 play important and different roles in the regulation of immune system due to their own specificity [17]. TNF-α is a kind of polypeptide formed upon the stimulation of endotoxins, inflammatory factors, etc., which can directly attack lung endothelial cells in case of severe infection in the body, leading to coagulation disorders and hypercoagulable state and activating the complement system [18]. IL-6 is mainly produced from monocytes and macrophages, which mediates the local inflammatory response [19]. According to relevant studies in China, the high levels of TNF-α and IL-6 in the peripheral blood indicate the infection, whose levels are related to the degree of infection and can be used to assess the curative effect and evaluate the prognosis [20]. It was found in this study that with the prolongation of time of mechanical ventilation, levels of inflammatory factors in the peripheral serum were decreased gradually in the non-VAP group, while they were decreased first, increased then and decreased again finally in the VAP group, indicating that TNF-α and IL-6 display decreasing trends finally with improvement of disease and drug control.

Conclusion

Therefore, detecting levels of TNF-α and IL-6 in the peripheral serum of VAP newborns might be used to assess the severity of disease and evaluate the curative effect and prognosis. However, the sample size was small in this study, so the conclusion needs to be supported through large cohort clinical studies.

Disclosure of conflict of interest

None.

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