Original Article

Distribution and antibiotic resistance of pathogens isolated from ventilator-associated pneumonia patients in pediatric intensive care unit

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BACKGROUND: With mechanical ventilation widely used in intensive care unit, the ventilator associated pneumonia (VAP) has become a common and serious complication in critically ill patients. Compared with adults, the incidence of VAP and the mortality are higher in children in pediatric intensive care unit (PICU) because of immune deficiency, severe basic diseases, and increased use of artificial airway or mechanical ventilation. Hence it is of significance to study the epidemiology and changes of antibacterial susceptibility in order to reduce the incidence and mortality of VAP in children.

METHODS: From January 2008 to June 2010, 2758 children were treated in PICU of Wuhan Children's Hospital. Among them, 171 received mechanical ventilation over 48 hours in PICU, and 46 developed VAP. The distribution and drug-resistance pattern of the pathogenic bacteria isolated from lower respiratory tract aspirations were analyzed.

RESULTS: A total of 119 pathogenic microbial strains were isolated. Gram-negative bacilli (G^-) were the most (65.55%), followed by fungi (21.01%) and gram-positive cocci (G^+, 13.45%). Among them, the most common pathogens were *Acinetobacter baummannii*, *Escherichia coli, Klebsiella pneumoniae, candida albicans and coagulase-negative staphylococci*. Antibiotic susceptibility tests indicated that the multiple drug-resistances of G^- and G^+ to antibiotics were serious. Most of G^- was sensitive to ciprofloxacin, amikacin, imipenem, meropenem, cefoperazone-sulbactam and piperacillin-tazobactam. The susceptibility of G^+ to vancomycin, teicoplanin and linezolid were 100%. Fungi were almost sensitive to all the antifungal agents. The primary pathogens of VAP were G^-, and their multiple drug-resistances were serious.

CONCLUSION: In clinical practice we should choose the most sensitive drug for VAP according to pathogenic test.

KEY WORDS: Pediatric; Intensive care unit; Ventilator-associated pneumonia; Pathogen; Drug-resistance; Retrospective clinical study

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INTRODUCTION

Patients in the intensive care unit (ICU) are at risk for dying not only from their critical illness but also from secondary processes such as nosocomial infection. With mechanical ventilation widely used in ICU, ventilator associated pneumonia (VAP) has become a common and serious complication in critically ill patients. VAP is defined as pneumonia occurring more than 48 hours after patients have been intubated and received mechanical ventilation. In adult ICU, the incidence of VAP can reach 9%-27%, and the mortality of VAP has been reported to range from 20% to 50%.[1-4] The incidence and mortality of VAP are higher in children in pediatric intensive care unit (PICU) than in adults because of immune deficiency, severe basic diseases, and increased use of artificial airway or mechanical ventilation. Hence

it would be of significance to study the epidemiology and changes of antibacterial susceptibility in order to reduce the incidence and mortality of VAP in children. We retrospectively studied the pathogenic bacteria distribution and drug resistance in VAP children in the PICU of Wuhan Children's Hospital between January 2008 and June 2010.

METHODS

General data

From January 2008 to June 2010, 2758 children were treated in the PICU of Wuhan Children's Hospital. Among them, 171 received mechanical ventilation over 48 hours in the PICU, and 46 developed VAP.

The diagnostic criteria of VAP were as follows: 1) pneumonia occurring more than 48 hours after intubation and mechanical ventilation; 2) two or more of the four criteria (i) fever of >38.3 °C, (ii) leukocytosis of 10 000 cells/mL or<5 000 cells/mL, (iii) purulent tracheobronchial secretion, (iv) and/or new pathogenic bacteria isolated from bronchial secretions; 3) a new and persistent (>48 h) infiltrate on chest radiograph.[5-7] The basic information of 46 children with VAP was listed in Table 1.

Table 1. General data of children with VAP

Variables	Number of cases	Rate (%)
Sex		
Male	33	71.74
Female	13	28.26
Age		
1 month to 1 year	25	54.35
1 to 3 years	12	26.09
Over 3 years	9	19.56
Central venous catheter	12	26.09
Indwelling catheter	11	23.91
Indwelling nasogastric tube	46	100.00
TPN	22	47.83
Use of glucocorticoids	13	28.26
≥2 broad-spectrum antibiotics	33	71.74
VAP occurrence of mechanical ventilation		
<5 d	11	23.91
≥5 d	35	76.09
Basic diseases		
Severe pneumonia (combined ARDS)	23 (7)	50 (15.22)
Congenital heart disease with pneumonia	7	15.22
Central nervous system infection	4	8.70
Drowning syndrome (combined ARDS)	4 (1)	8.70 (2.17)
Guillain-Barre syndrome	3	6.52
Sepsis and ARDS	2	4.35
Intracranial hemorrhage	2	4.35
Drug poisoning	1	2.17

Sputum test

Under the condition of sterility, a disposable sterile sputum collector was used to collect secretion samples from the trachea at bifurcation through an endotracheal intubation catheter. Routine microscopic examination was performed before sputum culture. Squamous epithelial cells<10/low-power field and multi-nucleated cells>25/low-power field were regarded as qualified specimens. Then the qualified specimens were inoculated into the culture medium, pathogens with a concentration of≥10^5 cfu/mLwere isolated, and then antimicrobial susceptibility test was carried out.

Instruments and reagents

Bacteria were identified using a VITEK-32 Auto Mcrobic System produced by Marcel Mérieux. Susceptibility paper and susceptibility medium (MH medium) were purchased from the UK Oxiod and Marcel Mérieux, respectively.

Susceptibility test

Routine drug susceptibility test was performed using the disk diffusion method and the VITEK-32 Auto Mcrobic System. The extended-spectrum β-lactamases (ESBLs) strains were detected by double disk test. Quality control strains included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, and *Escherichia coli* ATCC 35218. The criteria of drug resistance followed the CLSI set in 2008. If more than two consecutive results were the same in one patient, we recorded the results of sputum culture one time; if the results were different, we recorded the each result of sputum culture.

Statistical analysis

All test data were analyzed by the WHONET 5.4 software.

RESULTS

Distribution of pathogenic bacteria

Among the 46 VAP children, 24 (52.17%) had mixed infection. A total of 119 pathogenic microbial strains were isolated, including 78 strains of gram-negative bacilli (G^-, 65.55%), 25 strains of fungi (21.01%), and 16 strains of gram-positive cocci (G^+, 13.45%). Among pathogens, the most common pathogens were *Acinetobacter baummannii*, *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae* (*K.pneumoniae*), *Candida*

albicans and coagulase-negative staphylococci (Table 2).

Prevalence of methicillin-resistant staphylococcus and ESBLs

The detection rate of ESBL-producing *E.coli* strain, ESBL-producing *K.pneumoniae* strain, methicillin-resistant *Staphylococcus aureus* (MRSA), and methicillin-resistant coagulase-negative staphylococci (MRCNS) was 57.89 % (11/19), 58.82 % (10/17), 0.75% (6/8), respectively.

Table 2. Distribution of pathogens isolated from patients with VAP

Pathogens	Number of strains	Proportion (%)
G^- bacilli	78	65.55
Acinetobacter baummannii	25	21.01
E.coli	19	15.97
K.pneumoniae	17	14.29
Pseudomonas aeruginosa	7	5.88
Stenotrophomonas maltophilia	5	4.20
Enterobacter cloacae	2	1.68
Enterobacter amnigenus	1	0.84
Kata Braham coli	1	0.84
Chryseobacterium meningosepticum	1	0.84
Fungi	25	21.01
Candida albicans	11	9.24
Candida	7	5.88
Candida glabrata	3	2.52
Candida tropicalis	2	1.68
Mucor spp	2	1.68
G^+ cocci	16	13.45
Coagulase-negative staphylococci	8	6.72
Staphylococcus aureus	6	5.04
Streptococcus pneumoniae	2	1.68
Total	119	100

Drug resistance results of the top five G^- bacilli

The antimicrobial resistance of G^- bacilli showed multiple drug resistance. The most sensitive antibiotics against *Acinetobacter baummannii* were amikacin, ciprofloxacin, levofloxacin and minocycline (100%), followed by cefoperazone-sulbactam (68%). The resistance rates of penicillins and cephalosporins were more than 70%, and the resistance rates of meropenem and imipenem were up to 72%. *E.coli* and *K.pneumoniae* were resistant to most penicillins and cephalosporins, and their antibiotic resistance was lower for imipenem, meropenem, amikacin, ciprofloxacin, levofloxacin, piperacillin-tazobactam and cefoperazone-sulbactam. The antibiotic resistance of *Pseudomonas aeruginosa* was 0 for amikacin, ciprofloxacin and levofloxacin; 14.29% for imipenem and meropenem; 28.57% for piperacillin-tazobactam and cefoperazone-sulbactam; >70% for other antibiotics. For *Stenotrophomonas maltophilia*, the antibiotic resistance was 0 for sulfamethoxazole-trimethoprim, minocycline, and levofloxacin; 20% for ciprofloxacin; and > 60% for other antibiotics (Table 3).

Susceptibility results of G^+ cocci

G^+ cocci were highly sensitive to vancomycin, linazolid and teicoplanin (100%), and highly resistant

Table 3. Drug-resistance of the top five G^- bacilli (%)

Antibiotics	*Acinetobacter baummannii* (*n*=25)	*E.coli* (*n*=19)	*K.Pneumoniae* (*n*=17)	*Pseudomonas aeruginosa* (*n*=7)	*Stenotrophomonas maltophilia* (*n*=5)
Ampicillin	100	94.74	100	100	100
Carbenicillin	100	94.74	100	100	100
Mezlocillin	100	89.47	94.12	85.71	100
Ampicillin-sulbactam	92	63.16	82.35	85.71	80
Amoxicillin-clavulanic acid	92	63.16	82.35	85.71	100
Piperacillin-tazobactam	88	10.53	29.41	28.57	80
Cefoperazone-sulbactam	32	31.58	35.29	28.57	80
Cefoxitin	—	52.63	70.59	—	—
Cefuroxime	96	94.74	82.35	100	100
Ceftazidime	88	47.37	70.59	85.71	100
Cefazolin	100	94.74	88.24	100	100
Cefotaxime	100	78.95	88.24	100	100
Cefepime	84	42.11	64.71	85.71	80
Imipenem	72	0	0	14.29	100
Meropenem	72	0	11.76	14.29	100
Amikacin	0	0	0	0	—
Gentamicin	48	52.63	47.06	100	60
Ciprofloxacin	0	0	0	0	20
Levofloxacin	0	0	0	0	0
minocycline	0	—	—	—	0
Sulfamethoxazole-trimethoprim	100	—	—	100	0

"-" indicates that the drug has not been selected to do antimicrobial susceptibility testing.

to penicillin, erythromycin, clindamycin, tetracycline and sulfamethoxazole-trimethoprim (>50%). The resistance rate of oxcillin against coagulase-negative staphylococci was up to 75%. Additionally, G^+ cocci had lower resistance to cefuxine, cefazolin and levofloxacin (<30%).

Susceptibility results of fungi

The fungi remained sensitive to antifungal agents. One strain of *Candida glabrata* was resistant to fluconazole and itraconazole, and one strain of Mucor was resistant to fluconazole.

DISCUSSION

PICU is an area within a hospital specializing in the care of critically ill infants, children, and teenagers. Complex technology and equipment is often in use, particularly mechanical ventilators and patient monitoring systems. Children in PICU are characterized by severe pathophysiological disorders, immune dysfunction and other critical conditions. They are often treated with tracheal intubation, tracheotomy, mechanical ventilation and other rescue measures. At the same time, the impaired respiratory tract barriers allow bacteria to enter the respiratory system, and thus increase bacterial colonization and infection. Most children in PICU are given high-dose broad-spectrum antibiotics in combination, which change the normal bacterial colonization, and thus lead to VAP. In PICU of Wuhan Children's Hospital from January 2008 to June 2010, 171 children received mechanical ventilation (≥48 hours), and 46 children (26.9%) were diagnosed with VAP. In the isolated pathogens and pathogenic bacteria, the detection rate of G^- bacilli was 65.55%, followed by 21.01% for fungi and 13.45% for G^+ cocci. The most common pathogens were *E.coli, K.pneumoniae, Candida albicans* and coagulase-negative staphylococci.

In recent years, the infection rate of non-fermentative bacteria such as *Acinetobacter baummannii, Stenotrophomonas maltophilia* has been increasing year by year. The bacteria have become the main pathogens in nosocomial infections, especially in VAP. In this study the infection rate of non-fermentative bacteria was high, accounting for 50% of G^- bacilli (39/78), and *Acinetobacter baummannii* ranked the first. *Acinetobacter baummannii* produce antimicrobial resistance by increasing the expression of Ampc enzyme, producing OXA-23 carbapenem enzyme, decreasing the expression of outer membrane pore channel protein, efflux pump system hyperactivity, and loss of PBPs.[6,7] Additionally, *Acinetobacter baummannii* could induce drug resistance through plasmid integration, while causing multiple drug resistance plasmids.[8] Gundogan et al[9] found that the resistance of cephalosporins and imipenem against *Acinetobacter baummannii* was very serious. In our study *Acinetobacter baummannii* was only sensitive to aminoglycoside antibiotics, quinolone antibiotics and cefoperazone-sulbactam, and the resistance rate of carbapenem reached 72%. Imipenem and meropenem are no longer the first choice to treat *Acinetobacter baummannii*. In the present study, the resistance rate of β-lactam antibiotics such as imipenem and meropenem increased year by year. The resistance rate of imipenem and meropenem to *Pseudomonas aeruginosa* was 14.39%, which indicated that carbapenems can temporarily serve as the first choice to treat *Pseudomonas aeruginosa*.[10] The resistance rate of *Stenotrophomonas maltophilia* to imipenem was 100%. This may be connected with the natural drug-resistance to imipenem.[11] Since compound sulfamethoxazole is highly sensitively to *Stenotrophomonas maltophilia*, this agent can be used as the first choice for critically ill patients. Because of declined susceptibility in *Acinetobacter baummannii*, increased resistance in *Pseudomonas aeruginosa*, and the natural drug resistance in *Stenotrophomonas maltophilia*, close attention should be paid to the results of bacteria at any time so as to adjust or control the use of carbapenems.

Because β-lactam antibiotics are widely used, strains such as *E.coli* and *K.pneumoniae*, can produce extended spectrumβ-lactamase (ESBLs). Because ESBLs can hydrolyze cephalosporins and monocyclic β-lactam antibiotics and spread through the formation of plasmids[12] at the same time, cephalosporin can induce the G^- bacilli to produce ESBLs. Thus the resistance mediated by ESBLs expands rapidly. In our study the detection rates of *E.coli* and *K.pneumoniae*, which produce ESBLs, were 57.89% and 58.82% respectively, which were similar to those reported by Wang et al.[13] Susceptibility test results showed that *E.coli* and *K.pneumoniae* were highly sensitive to carbapenems, aminoglycosides, quinolones and some compound formulations containing enzyme inhibitors, but resistant to penicillins and cephalosporins.

The results of sensitivity test also showed that G^+ cocci were highly resistant to penicillins. Vancomycin resistant strains were not seen, and vancomycin is still the most effective agent to treat severe *Staphylococcus aureus* infection. β-lactam antibiotics, aminoglycosides,

macrolides, clindamycin and tetracyclines were not recommended to treat critically ill patients.[14] Teicoplanin and linezolide against multi-resistant bacteria were very effective to multidrug resistant bacteria, just as vancomycin.

Our data showed that the proportion of fungal infection increased (21.01%), and took the second position before G^+ cocci. This was related to the long use of antibiotics (especially the use of third generation cephalosporins and carbapenem ≥7d or unreasonable use of corticosteroids), mechanical ventilation and other invasive operations.[15] Therefore corticosteroids and antimicrobial agents must be limited and rationally used to control fungal infection. *Candida albicans* were the main fungal strains in VAP, but the infection of non-*Candida albicans* strains increased, and natural drug-resistance fungi-*Candida glabrata* emerged. In our study, among the three *Candida glabrata*, one was resistant to fluconazole and itraconazole. In clinical practice, therefore, we should choose the most sensitive agents according to the results of pathogenic test.

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