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

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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%.\textsuperscript{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

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 baumannii, 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

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.

\section*{METHODS}

\subsection*{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. The basic information of 46 children with VAP was listed in Table 1.

\begin{table}[h!]
\centering
\caption{General data of children with VAP}
\begin{tabular}{llr}
\hline
Variables & Number of cases & Rate (\%)
\hline
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 \\
\hline
\end{tabular}
\end{table}

\section*{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≥10^3 cfu/mL were isolated, and then antimicrobial susceptibility test was carried out.

\section*{Instruments and reagents}

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

\section*{Susceptibility test}

Routine drug susceptibility test was performed using the disk diffusion method and the VITEK-32 Auto Microbic 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.

\section*{Statistical analysis}

All test data were analyzed by the WHONET 5.4 software.

\section*{RESULTS}

\subsection*{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 baumannii*, *Escherichia coli* (E.coli), *Klebsiella pneumoniae* (K.pneumoniae), *Candida*
albicans and coagulate-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 (MRS), and methicillin-resistant coagulate-negative staphylococci (MRCNS) was 57.89 % (11/19), 58.82 % (10/17), 0.75% (6/8), respectively.

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 baumannii 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, linezolid and teicoplanin (100%), and highly resistant

| Table 2. Distribution of pathogens isolated from patients with VAP |
| Pathogens | Number of strains | Proportion (%) |
| G bacilli | 78 | 65.55 |
| Acinetobacter baumannii | 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 | 1 | 0.84 |
| G’ cocci | 16 | 13.45 |
| Coagulate-negative staphylococci | 8 | 6.72 |
| Staphylococcus aureus | 6 | 5.04 |
| Streptococcus pneumoniae | 2 | 1.68 |
| Total | 119 | 100 |

| Table 3. Drug-resistance of the top five G’ bacilli (%) |
| Antibiotics | Acinetobacter baumannii (n=25) | E.coli (n=19) | K.Pneumoniae (n=17) | Pseudomonas aeruginosa (n=7) | Stenotrophomonas maltophilia (n=5) |
| Amoxicillin | — | 0 | 0 | 0 | 0 |
| Tazobactam | — | 0 | 0 | 0 | 0 |
| Cefoxitin | — | 0 | 0 | 0 | 0 |
| Ceftriaxone | — | 0 | 0 | 0 | 0 |
| Cefoperazone-sulbactam | — | 0 | 0 | 0 | 0 |
| Cefepime | — | 0 | 0 | 0 | 0 |
| Ciprofloxacin | — | 0 | 0 | 0 | 0 |
| Levofloxacin | — | 0 | 0 | 0 | 0 |
| Minocycline | — | 0 | 0 | 0 | 0 |
| Sulfamethoxazole-trimethoprim | — | 0 | 0 | 0 | 0 |
to penicillin, erythromycin, clindamycin, tetracycline and sulfamethoxazole-trimethoprim (>50%). The resistance rate of oxacillin against coagulase-negative staphylococci was up to 75%. Additionally, G⁺ cocci had lower resistance to cefoxitin, 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 baumannii*, *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 baumannii* ranked the first. *Acinetobacter baumannii* 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 baumannii* 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 baumannii* was very serious. In our study *Acinetobacter baumannii* 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 baumannii*. 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 baumannii*, 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 mononycic 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. Teicoplanin and linezolid 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. 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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