Original Article

Risk factors and antibiotic resistance of pneumonia caused by multidrug resistant *Acinetobacter baumannii* in pediatric intensive care unit

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BACKGROUND: With beta-lactam drugs and immunosuppressants widely used, the infection caused by *Acinetobacter baumannii* (Ab) has become more and more serious with multidrug resistant *Acinetobacter baumannii* (MDRab) emerging and worsening rapidly. Compared with other patients, the incidence and multidrug resistance of MDRab are higher in children in pediatric intensive care unit (PICU) because of immune deficiency, severe basic diseases, prolonged hospitalization and invasive operations. Hence it is significant to study the epidemiology and changes of antibacterial susceptibility in order to reduce the incidence of MDRab in children.

METHODS: A total 115 patients with MDRab pneumonia and 45 patients with negative MDRab (NMDRab) pneumonia who had been treated from January 2009 to August 2011 were studied retrospectively at the PICU of Wuhan Children's Hospital. Clinical data were analyzed with univariate and multivariate Logistic regression.

RESULTS: In 176 clinical strains of *Acinetobacter baumannii* isolated, there were 128 strains of MDRab, accounting for 72.73%. Drug susceptibility tests showed that the resistance rates of β -lactam antibiotics were more than 70% except for cefoperazone sulbactam. The rates to carbapenems were higher than 90%. They were significantly higher than those of NMDRab. Amikacin, levofloxacin, ciprofloxacin and minocycline had the lowest drug-resistance rates (<20%). Multivariate Logistic regression revealed that ICU stay, the time of mechanical ventilation, anemia, hypoproteinemia and the use of carbapenems were independent risk factors for MDRab pneumonia.

CONCLUSIONS: MDRab is an important opportunistic pathogen to pneumonia in PICU, and its drug-resistance is severe. It increases significantly the mortality of patients. It is important to take the effective prevention measures for controlling it.

KEY WORDS: Pediatric; Intensive Care Unit; Multidrug resistance; *Acinetobacter baumannii*; Pneumonia; Risk factor; Retrospective study

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INTRODUCTION

Acinetobacter baumannii (Ab) is an important opportunistic pathogen that causes nosocomial infection, especially infection of the lower respiratory

tract.^[1] In recent years, with beta-lactam drugs and immunosuppressants widely used, the Ab infection has become more and more serious with multidrug resistant *Acinetobacter baumannii* (MDRab) emerging

and worsening rapidly.^[2] The incidence and multidrug resistance of MDRAb are higher in children in pediatric intensive care unit (PICU) than in other patients because of immune deficiency, severe basic diseases, prolonged hospitalization and invasive operations. Hence it would be of significance to study the epidemiology and changes of antibacterial susceptibility in order to reduce the incidence of MDRAb in children. We retrospectively studied the risk factors and antibiotic resistance of patients with pneumonia caused by MDRAb who had been treated at the PICU of Wuhan Children's Hospital between January 2009 and August 2011.

METHODS

Subjects

One hundred and sixty children with pneumonia caused by Ab, who had been treated from January 2009 to August 2011 at the PICU of Wuhan Children's Hospital, were enrolled in this retrospective study. The inclusion criteria of children were as follows: 1) meeting the diagnostic criteria of hospital-acquired pneumonia formulated by the Society of Respiratory Diseases, Chinese Medical Association^[3]; and 2) two consecutive Ab strains isolated from sputum or bacteria quantitative culture of Ab $\geq 10^6$ CFU/mL.

The 160 children were divided into two groups: infected by MDRAb (MDRAb group, $n=115$), and infected by non-MDRAb (NMDRAb group, $n=45$). In the MDRAb group, 91 were male and 24 female, with an average age of (6.08 ± 3.7) months. In the NMDRAb group, 36 were male and 9 female, with an average age of (5.59 ± 3.17) months.

Outcome measures

Sex, age, length of stay, ICU stay, infection time, basic diseases, critical illness score, WBC, Hb, ALB, invasive operation before infection, mechanical ventilation, immunosuppressive agents, antacids, tranquilizers, anti-bacterial drugs, mixed infection and prognosis were recorded by checking the discharges retrospectively and compared between the study groups.

Sputum test

Under the condition of sterility, a disposable sterile sputum collector was used to collect sputum samples from the nasal cavity deeply (10–15 cm) after cleaning the oropharyngeal areas by using sterile saline. For the children with an artificial airway tract, the secretion samples were collected 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, and antimicrobial susceptibility test was carried out.

Instruments and reagents

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

Susceptibility test

Routine drug susceptibility test was performed using the disk diffusion method and the VITEK-32 Auto Microbic System. Susceptibilities to all antimicrobial agents were determined and interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) set in 2008.^[4] The quality control strain was *Pseudomonas aeruginosa* ATCC 27853. If more than two consecutive results were the same within one week in one patient, we recorded the results of sputum culture one time.

Multidrug resistance was defined as resistance to more than three kinds of antibiotics. Intermediate susceptibility was considered as resistance. The same other pathogens being checked for two consecutive times in one period were identified as mixed infection strains.

Statistical analysis

All statistical analyses were performed using SPSS version 16.0. Tests performed in univariate analysis were the Chi-square test for categorical variables and Student's *t* test for continuous variables as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. All variables with a *P* value < 0.05 in univariate analysis were included in a logistic regression model for multivariate analysis. All tests were two-tailed, and a *P* value < 0.05 was considered statistically significant.

RESULTS

Prevalence of MDRAb

In 176 Ab strains detected from the 160 children, there were 128 MDRAb and 48 NMDRAb strains. The detection rate of MDRAb was 72.73 % (128/176).

Drug resistance results of MDRAb

MDRAb was resistant to carbapenems, most penicillins and cephalosporins and sulfa drugs. Their resistance rates were more than 70%. Only cefoperazone-sulbactam, amikacin, ciprofloxacin, levofloxacin and minocycline had a high sensitivity to MDRAb (>70%). Compared with the NMDRab group, the resistance rates of beta-lactam drugs (including carbapenems) were higher in the MDRAb group ($P<0.05$). Only aminoglycosides and fluoroquinolones had a similar low drug resistance between the two groups (Table 1).

Risk factors for appearance of MDRAb pneumonia

There were 23 risk factors analyzed for appearance of MDRAb pneumonia in this study. Comparing to the NMDRab group, more patients in the MDRAb group received endotracheal intubation (48.7% vs. 22.22%, $P<0.01$), and the time of mechanical ventilation was longer (mean±standard deviation, 11.76±5.98 vs. 8.29±3.1 days, $P<0.001$). Meantime, patients in the MDRAb group had a longer time for hospitalization (19.21±8.56 vs. 14.48±3.97 days, $P=0.001$), greater

length of ICU stay (17.39±7.05 vs. 14.43±3.92 days, $P=0.009$), later happiness of hospital-acquired infection (7.47±3.96 vs. 5.62±2.42 days, $P=0.004$), lower critical illness scores (76±7.23 vs. 80.29±8.56, $P=0.002$), more serious anemia (93.91% vs. 66.67%, $P<0.001$) and hypoproteinemia (39.13% vs. 8.89%, $P<0.001$).

The use of antimicrobial agents was reviewed, while precisely verifying their doses and days of the use. Patients in the MDRAb group received more types of antibiotics (7.92±2.8 vs. 5.91±2.09, $P<0.001$) and longer days of administration (19.21±8.56 vs. 14.48±3.97 days, $P=0.001$). They were exposed to more carbapenems (97.39% vs. 84.44%, $P=0.006$), aminoglycosides (33.91% vs. 13.33%, $P=0.009$), fluoroquinolones (57.39% vs. 33.33%, $P=0.013$), and macrolides (44.35% vs. 26.67%, $P=0.04$).

Among all the risk factors identified by univariate analysis, exposure to carbapenems, length of ICU stay, time of mechanical ventilation, anemia, and hypoproteinemia were the significant independent risk factors for the appearance of MDRAb pneumonia (Tables 2–4).

Table 1. Drug-resistance rates of MDRAb and NMDRab (%)

Antibiotics	NMDRab group (n=48)			MDRab group (n=128)			χ^2	P
	n	Resistant trains	Resistance rate	n	Resistant trains	Resistance rate		
Amikacin	48	1	2.08	128	12	9.38	3.06	0.118
Ampicillin-sulbactam	48	18	37.5	128	120	93.75	65.24	0.000
Cefepime	48	36	75	128	122	95.31	13.55	0.000
Cefoperazone-sulbactam	48	2	4.17	128	38	29.69	12.95	0.000
Cefazidime	48	40	83.33	128	122	95.31	5.30	0.014
Ceftriaxone	48	38	79.17	128	122	95.31	9.14	0.002
Ciprofloxacin	48	4	8.33	128	20	15.63	1.58	0.209
Imipenem	48	2	4.17	128	116	90.63	118.1	0.000
Levofloxacin	48	4	8.33	128	22	17.19	2.17	0.141
Meropenem	48	2	4.17	128	122	95.31	139.32	0.000
Piperazone-sulbactam	40	16	40	86	80	93.02	42.31	0.000
Sulfamethoxazole-trimethoprim	46	34	73.91	128	98	76.56	0.13	0.718
Minocycline	48	3	6.25	128	16	12.5	1.42	0.233

Table 2. Univariate analysis of risk factors for MDRAb pneumonia (continuous variables)

Risk factors	NMDRab group (n=45)	MDRab group (n=115)	t	P
Age (years)	5.59±3.17	6.08±3.7	0.78	0.437
Critical illness score	80.29±8.65	76±7.23	3.19	0.002
During of hospitalization (d)	14.48±3.97	19.21±8.56	3.56	0.001
ICU length of stay (d)	14.43±3.92	17.39±7.05	2.66	0.009
Time of hospital-acquired infection (d)	5.62±2.42	7.47±3.96	2.92	0.004
Time of mechanical ventilation (d)	8.29±3.1	11.76±5.98	3.70	0.000
Time of deep venous catheterization (d)	21±5.1	19.14±8.05	1.44	0.152
Type of antibiotics used	5.91±2.09	7.92±2.8	4.36	0.000
Time of antibiotics used (d)	14.48±3.97	19.21±8.56	3.56	0.001

Table 3. Univariate analysis of risk factors for MDRAb pneumonia (categorical variables) (n, %)

Risk factors	NMDRAb group (n=45)	MDRAb group (n=115)	t	P	OR (95%CI)
Sex (male)	36 (80)	91 (79.13)	0.01	0.920	0.948 (0.402-2.235)
Use of tranquilizers	8 (17.78)	36 (31.3)	2.97	0.085	2.108 (0.892-4.980)
Immunosuppressive agents	6 (13.33)	24 (20.87)	1.21	0.271	1.714 (0.650-4.523)
Use of antacids	4 (8.89)	6 (5.22)	0.25	0.469	0.564 (0.152-2.102)
Tracheal intubation	10 (22.22)	56 (48.7)	9.35	0.002	3.322 (1.504-7.336)
Urinary catheter	1 (2.22)	3 (2.61)	0.18	0.685	1.179 (0.119-11.637)
Venous catheterization	3 (6.67)	21 (18.26)	3.41	0.084	3.128 (0.884-11.062)
WBC (<4.0×10 ⁹ or >10×10 ⁹)	32 (71.11)	96 (83.48)	0.77	0.122	2.053 (0.912-4.619)
Hb (<100 g/L)	30 (66.67)	108 (93.91)	20.25	0.000	7.714 (2.883-20.640)
ALB (<30 g/L)	4 (8.89)	45 (39.13)	13.92	0.000	6.589 (2.209-19.655)
Fungal infection	20 (44.44)	54 (46.96)	0.08	0.888	0.951 (0.479-1.888)
Other bacterial infections	26 (57.78)	69 (60)	0.07	0.791	1.096 (0.545-2.206)
Use of antibiotics					
Second-generation cephalosporins	12 (26.67)	48 (41.74)	3.14	0.076	1.970 (0.924-4.203)
Third and fourth generation cephalosporins	38 (84.44)	97 (84.35)	0.00	1.000	0.993 (0.384-2.567)
Carbapenems	38 (84.44)	112 (97.39)	7.18	0.006	6.878 (1.693-27.936)
Aminoglycoside	6 (13.33)	39 (33.91)	6.78	0.009	3.336 (1.3-8.558)
Fluoroquinolones	16 (33.33)	66 (57.39)	6.17	0.013	2.441 (1.196-4.983)
Macrolide	12 (26.67)	51 (44.35)	4.24	0.040	2.191 (1.029-4.668)
Basic diseases					
Severe pneumonia	34 (75.56)	88 (76.52)	0.02	0.888	1.055 (0.471-2.359)
Congenital heart disease	12 (26.67)	36 (31.3)	0.33	0.566	1.253 (0.581-2.705)
Sepsis	10 (22.22)	24 (20.87)	0.04	0.841	0.923 (0.401-2.123)
Central nervous system infection	4 (8.89)	18 (15.65)	1.25	0.264	1.902 (0.606-5.967)
Severe diarrheal disease	3 (6.67)	9 (7.83)	0.01	0.920	1.189 (0.307-4.607)
Malnutrition	1 (2.22)	6 (5.22)	0.16	0.689	2.422 (0.283-20.705)
Poisoning	1 (2.22)	4 (3.48)	0.01	0.920	1.586 (0.172-14.584)
Bronchial foreign body	1 (2.22)	3 (2.61)	0.18	0.671	1.179 (0.119-11.637)
Hemophagocytic syndrome	1 (2.22)	3 (2.61)	0.18	0.671	1.179 (0.119-11.637)
Drowning syndrome	1 (2.22)	3 (2.61)	0.18	0.671	1.179 (0.119-11.637)
Guillain-Barre syndrome	1 (2.22)	2 (1.74)	0.2	0.655	0.779 (0.069-8.807)
Spinal muscular atrophy	1 (2.22)	1 (0.87)	0.01	0.920	0.386 (0.024-6.307)

Table 4. Multivariate logistic regression of risk factors for MDRAb pneumonia

Independent risk factors	Wald	P	OR	95%CI
ICU length of stay (d)	5.589	0.018	0.351	0.147-0.836
Time of mechanical ventilation (d)	5.023	0.025	1.271	1.031-1.567
Hb (<100 g/L)	8.46	0.004	6.92	1.879-25.48
ALB (<30 g/L)	5.439	0.02	5.119	1.298-20.191
Use of carbapenems	4.455	0.035	9.239	1.172-72.819

Clinical outcomes

Cefoperazone / sulbactam, moxifloxacin, levofloxacin, amikacin and minocycline were the recommended antimicrobial agents for MDRAb eradication, based on the *in vitro* susceptibility test. The patients received one or two of the above antibiotics after appearance of MDRAb. In the MDRAb group which had a higher in-hospital mortality than the NMDRAb group (18.26% vs. 4.44%, $P < 0.05$), 53 patients (46.09%) were cured, 41 patients (35.65%) improved and 21 patients (18.26%) dead.

DISCUSSION

Ab, a non-fermentative bacterium, is widely distributed in nature, in hospitals and human skin. With the decrease of immunity in patients, Ab can cause various infections of the lung, urinary tract, blood, wound, biliary tract, etc.^[5] Children in PICU are characterized by severe pathophysiological disorders, immune dysfunction and other critical conditions. Thus the rate of Ab infection and its drug resistance become more serious while MDRAb strains appear rapidly.^[6] In the PICU of Wuhan Children's Hospital 128 MDRAb strains were isolated

from January 2009 to August 2011, with a rate (72.73%) higher than that reported elsewhere.^[7] The finding indicated that the drug resistance of Ab was very serious. MDRAb infection leads to complex refractory diseases, difficult treatment and high mortality. In this study, the mortality rate in the MDRAb group was 18.26%, which was significantly higher than that in the NMDRab group (4.44%) because of lack of effective treatment.

MDRab is resistant to most pediatric antimicrobial agents, including penicillins, cephalosporins, single-ring antibiotics and carbapenems. MDRab produces 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.^[8-10] Additionally, MDRab could induce drug resistance through plasmid integration, while causing multiple drug resistance plasmids.^[11] In our study, the susceptibility results showed that the drug resistance rates of MDRab to beta-lactam antibiotics were more than 70% except for cefoperazone-sulbactam (<30%). Compared with the NMDRab group, the resistance rates of beta-lactam drugs (including carbapenems) were higher in the MDRab group. Cefoperazone-sulbactam has a lower resistance rate (<30%) because sulbactam may be combined with the important PBPs or change the outer membrane permeability of G-bacteria, making the beta-lactamase leakage while increasing the opportunity of other antibacterial drugs into biomass.^[12] So cefoperazone-sulbactam can be used as the choice of treatment of Ab infection. Carbapenems are no longer the first choice to treat Ab infection because of the high resistance rate (>90%). Aminoglycosides and quinolones which rarely used in the pediatric field remained lower resistance rates (<20%) because of probable damage to children's hearing and cartilage, and they have become the last helplessly choice to treat the children with MDRab infection. So close attention should be paid to the results of bacteria at any time in order to adjust or control the use of carbapenems.

We conclude that the independent risk factors for appearance of MDRAB pneumonia include the use of carbapenems, length of ICU stay, time of mechanical ventilation, anemia and hypoproteinemia. (1) Prolonged ICU stay often prompts that patients' basic diseases are more serious, for example, host defense and immune dysfunction, leading to opportunistic pathogen infections. In addition, ICU environment often results in a large number of different resistant opportunistic pathogens

colonized in it and in the skin and mucous membranes of the patients and staff, waiting to invade the human body. Hence, ICU is the place which provides the chance for opportunistic pathogen infection, also contributes to MDRab infection in it. (2) Tracheal intubation and mechanical ventilation make the upper respiratory tract lose the ability of filtering pathogens and non-specific immune protective effect, leading to exogenous and endogenous lung infection.^[13] The longer duration of mechanical ventilation, the more opportunities acquired ventilator-associated pneumonia. (3) Anemia and hypoproteinemia would decrease patients' immunity, eradicate bacteria difficultly, increase in the amount and type of antimicrobial agents, prolong the use of antibiotics and lead to MDRab generation and spread. (4) Carbapenems can induce non-fermentative bacteria efflux pumps expressed highly, which can make bacteria low sensitive to a variety of antimicrobial agents. Carbapenems have been used extensively before the outbreak of hospital infection.^[14] Possibly, the resistant bacteria are dominant while the sensitive strains are eradicated because of wide use of highly effective broad-spectrum antimicrobial drugs. Judicious use of carbapenem with antibiotics stewardship programs would be the most effective measure to avoid the emergence of MDRab and the associated unfavorable outcome.

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Conflicts of interest: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Contributors: Cai XF proposed the study, and wrote the first draft. All authors contributed to the design and interpretation of the study and to further drafts.

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