

# Beta-Lactamase Gene Expression Level of Hospital-Acquired CRAB Isolated from Children in Picu

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**Purpose:** *Acinetobacter baumannii* is a major cause of hospital-acquired infections. Studies showed that carbapenem resistance was related to mortality. Carbapenem resistance depends on expression of  $\beta$ -lactamase in adults. The present study explores the relationship between  $\beta$ -lactamase gene expression and carbapenem resistance and outcomes in children with *A. baumannii* infections.

**Patients and Methods:** We gathered clinical data of 131 children diagnosed with hospital-associated *A. baumannii* infections from the pediatrics unit of Shengjing Hospital of China Medical University. We obtained 131 isolates of *A. baumannii*, determined the minimal inhibitory concentrations (MICs) for common antibiotics, and measured carbapenemase-encoding genes expression using real-time PCR.

**Results:** We isolated 131 strains, 89 of which were carbapenem-resistant (MIC  $\geq 8$   $\mu\text{g/mL}$ ), and 42 carbapenem-sensitive strains. Univariate analysis identified statistically significant differences between the carbapenem-resistant group and the carbapenem-sensitive group for in-hospital days before infection, previous deep vein catheterization, previous urinary catheterization, previous treatment with a carbapenem (meropenem/imipenem), and expression of oxa-51 and oxa-23. Logistic regression analysis of factors associated with carbapenem-resistant *A. baumannii* infections found significant associations with oxa-23 expression (hazard ratio [HR] 0.005, confidence interval [CI] 95% 0–0.153,  $P = 0.002$ ) and previous carbapenem treatment (HR 0.031 CI 95% 0.1–0.959,  $P = 0.042$ ). Of 131 patients, 27 died within 30 days. Cox regression analysis of factors associated with 30-day mortality from *A. baumannii* infections showed that cephalosporin combined with sulbactam (HR 0.271, CI 95% 0.101–0.723,  $P = 0.009$ ) was associated with 30-day survival.

**Conclusion:** The expression of oxa-23 and the use of carbapenems were independent risk factors for carbapenem resistance. The use of cephalosporins combined with sulbactam was independently associated with 30-day survival. We recommend using cephalosporins combined with sulbactam in children infected with *A. baumannii*.

**Keywords:**  $\beta$ -lactamase gene, carbapenem resistance, *Acinetobacter baumannii*, prognosis, risk factors

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## Introduction

*Acinetobacter baumannii* is an opportunistic pathogen that is frequently found in debilitated inpatients and critically ill patients, especially in intensive care units.<sup>1,2</sup> *A. baumannii* causes multi-system infections, particularly ventilator-associated pneumonia, bloodstream infections, wound infections, and is a significant cause of death.<sup>3,4</sup>

With increasing outbreaks and mortality, current therapeutic options are becoming useless because of drug resistance and the organism's tenacious ability to survive. In 2017, the World Health Organization listed *A. baumannii* as a "priority pathogen" and called on clinicians to find ways to eradicate it.

According to statistics from the European Antibiotic Resistance Surveillance Network in 2018, about one-third of Acinetobacter species are resistant to carbapenem antibiotics. In the United States, the incidence of carbapenem-resistant *A. baumannii* (CRAB) increased from 20.6% in 2002 to 49.2% in 2008. In China, carbapenem resistance increased from 31% in 2005 to 66.7% in 2014.<sup>5,6</sup> The combined incidence of CRAB infection in the intensive care unit (ICU) per 1000 patients was 41.7 cases (95% CI 21.7–78.7).<sup>7</sup> In Asia, studies showed that the fatality rate of carbapenem-resistant *A. baumannii* infection was about 50%.<sup>8</sup>

There is currently no preferred treatment for CRAB which means multi-drug resistant, carbapenem nonsusceptibility is an independent risk factor for death from bacteremia in children.<sup>9</sup> Longer in-hospital stays, antibiotic use, length of stay in the pediatric ICU, and surgery are risk factors for carbapenem-resistant *A. baumannii* infections.<sup>3,10</sup> First-line treatments for CRAB in children include prolonged infusion of meropenem plus fluoroquinolone/aminoglycoside/colistin. Ampicillin-sulbactam/tigecycline are the second-line agents. The combination of first- and the second-line agents appeared to be a reasonable treatment.<sup>11</sup> Emergence of carbapenem nonsusceptibility made colistin and tigecycline the treatment of last resort. It is unknown if colistin is safe in children.<sup>12</sup>

Several mechanisms contribute to carbapenem resistance, including presence of carbapenemases and efflux pumps. The affinity with penicillin-binding protein is low. The expression of outer membrane channel proteins is downregulated or absent. Carbapenemases are among the most important and most frequently observed.<sup>13</sup> There are several carbapenem enzyme classifications; the most common is A, B, C, and D. Class D  $\beta$ -lactamases are also called oxacillinases (OXAs) that hydrolyze oxacillin, the primary mechanism responsible for CRAB.<sup>14</sup> Oxa-58, oxa-23, oxa-24, and oxa-51 like are the most prevalent subgroups worldwide.<sup>15,16</sup> Oxa-23 was associated with mortality according to a recent report from Brazil.<sup>17</sup>

There is a lack of study of this problem in children, particularly those in the pediatric ICU (PICU). Nevertheless, knowledge of antibiotic resistance and the risk factors for CRAB infections can guide treatment strategies. Therefore, in the present study, we reported findings a group of 131 children in the PICU with hospital-

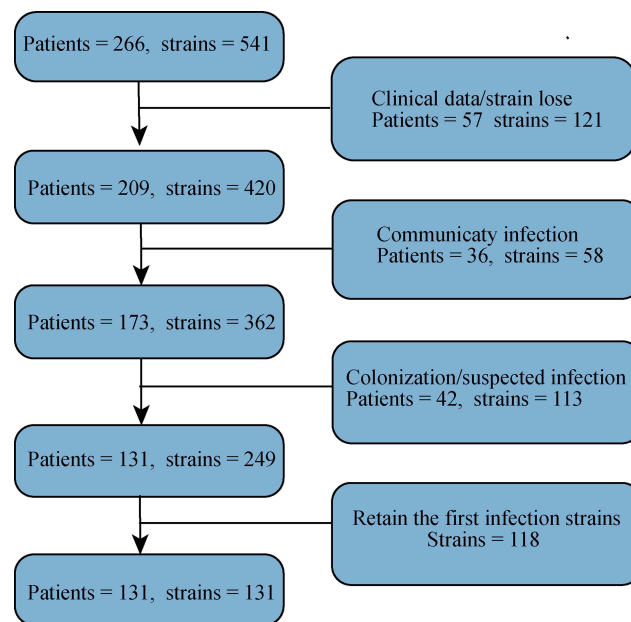
acquired infections to determine which OXA genes participate in carbapenem-resistance and evaluate if gene expression is associated with mortality in the PICU.

## Materials and Methods

### Study Population and Eligibility Criteria

This retrospective study included 131 pediatric patients infected by *A. baumannii* in the PICU of Shengjing Hospital affiliated with China Medical University from January 2012 to December 2018. After the study was approved by the Research Ethics Committee (2019PS431K), we obtained consent from the hospital and the medical records of the patients considered for inclusion. There were a total of 266 patients and 541 strains from 2012 to 2018. To better explore the causes of carbapenem resistance and evaluate the outcomes of children as early as possible, considered the following inclusion criteria in Figure 1: 1) > 1 month and < 13 years old; 2) *A. baumannii* cultured from any sample, with clinical signs and symptoms consistent with hospital-acquired infection; and 3) retention of the first confirmed infection strain. Exclusion criteria are as follows in Figure 1; 1) absence of clinical data or loss of bacteria caused by improper preservation; 2) suspected infection strains: when culture at least two kinds of micros including *A. baumannii*, cannot clarify which one or both play a role; or colonies; and 3) community-acquired infection. We collected the basic clinical data of the patients with confirmed *A. baumannii* infections for the first time before drug sensitivity testing and Real-time reverse-transcription Polymerase Chain Reaction (Real-time PCR) testing of infected strains. These data included age, sex, underlying diseases, invasive procedures (mechanical ventilation, deep venous catheterization, thoracic puncture), changes in c-reactive protein levels, white blood cell counts within 24 hours after clinical diagnosis of infection and 48 hours before infection diagnosis ( $\Delta$ ), fever at culture time, and previous carbapenem treatment (meropenem/imipenem). The strain was stored at  $-80^{\circ}\text{C}$ , removed, resuscitated, and inoculated. The strains were transferred on blood plate culture medium using the plate marking method, and the strains were identified again using a VITEK-2 compact instrument.

The patients were divided into a carbapenem-resistant group and a carbapenem sensitive-group to determine the effect of oxa-23/24/51/58 gene expression on drug resistance as the primary outcome, and to explore the effect of



**Figure 1** Exclusion criteria and inclusion criteria.

gene expression on 30-day mortality as the secondary outcome.

Colonization was defined as positive culture without clinical signs of infection patients. Hospital-acquired infections were defined as infections that were not present at admission and occur within 48–72 hours after admission or up to 6 weeks after discharge, not during the incubation period.<sup>18</sup> Infection was defined as cultured strains with relevant clinical symptoms and signs, and other infections excluded according to the definitions of infection in the intensive care unit.<sup>19</sup> Sepsis and severe pneumonia were defined according to international definitions.<sup>20,21</sup> Intra-abdominal infections: Patients typically present with rapid-onset abdominal pain and signs of local and systemic inflammation (pain, tenderness, fever, tachycardia, and/or tachypnea). Hypotension and hypoperfusion signs such as oliguria, acute alteration of mental status, and lactic acidosis are indicative of ongoing organ failure.<sup>22</sup> Bacterial Meningitis: signs of meningeal irritability, fever, poor feeding, lethargy, irritability, seizure, with vomiting, photophobia, headache, and neck stiffness, positive CSF (Cerebrospinal fluid) Gram stain, CSF leukocyte count of at least 1000 cells per mm<sup>3</sup>, CSF protein level of at least 0.8 g per liter, peripheral blood leukocyte count of at least 10,000 cells per mm<sup>3</sup>,<sup>23,24</sup> Urinary tract infection: clinical symptoms: fever, dysuria, urgency, and costovertebral angle tenderness and so on, a growth of more than 10<sup>8</sup> colony-forming

units (CFU) per liter (10<sup>5</sup> per mL) of a unique bacterium is regarded most frequently as the cutoff between contamination and UTI.<sup>25</sup>

## Antimicrobial Susceptibility Testing

We tested susceptibility of *A. baumannii* isolates according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints against amikacin/gentamicin, sulbactam and cefoperazone, cefepime/ceftazidime/ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, compound sulfamethoxazole, and ciprofloxacin/levofloxacin/tetracycline/tigecycline (Supplementary Table 2). We performed the E-TEST method based on those defined by the Clinical and Laboratory Standards Institute.<sup>26–30</sup> Carbapenem-non-susceptible *A. baumannii* was defined as isolates that exhibited in vitro resistance to imipenem or meropenem based on the E-TEST method (minimum inhibitory concentration [MIC] ≥ 8 µg/mL). *A. baumannii* was identified using the Vitek 2 System (France). The strains were *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853.

## Real-Time PCR Method

The Real-time reverse-transcription Polymerase Chain Reaction (Real-time PCR) method was as follows. We added appropriate amounts of sample to 1 mL TRIzol (9108, Takara Bio, Inc.), added 200 UL chloroform and mixed and incubated for 15 min. The mixture was

centrifuged at 4 °C, 14,000 g for 15 min, after which we transferred the water phase to fresh test tubes, added 500 L isopropanol to precipitate, incubated on ice for 10 min, and centrifuged at 14,000 g for 15 min at 4 °C. The purity of the extracted RNA was tested (NanoPhotometer), and the samples with absorbance values between 1.8 and 2.0 were reverse transcribed. The unqualified samples underwent the procedure again. Extracted RNA was then reverse transcribed into cDNA using the PrimeScriptRT reagent kit with gDNA Eraser (RR047A, Takara Bio, Inc.). The reverse transcribed products were quantified using real-time PCR by 16s rRNA. Each targeted cDNA (2 µL) was amplified using the TB Green PCR Core kit (RR820A, Takara Bio, Inc.) via the ABI 7500 system, and the following primers: OXA-24 forward, 5'-CCTTGACATAACCGATTACCT-3' and reverse, 5'-CAGTCAACCAACCTACCTGTGG-3'; OXA-58 forward, 5'-ATATCAAGAATTGGCACGTCGT-3' and reverse, 5'-TGTAATTGTCAAAGGCCCTTTC-3'; OXA-23 forward, 5'-TCCCAGTCTATCAGGAACTTGC-3' and reverse, 5'-GGCGTAACCTTTAATGGTCCTA-3'; OXA-51 forward, 5'-TCCAACAAGGCCAACTCAAC-3' and reverse, 5'-CTTCTGTGGTGGTTGCCTTATG-3'; 16srRNA forward, 5'-ATTAATGCAACTGCTCAACAAGC' and reverse, 5'-ATGTCTGCTAAGTGGCAAGTTC-3'. The gene expression levels of the target gene and standard Baumannian ATCC19606 were compared. The reaction conditions were as follows: 95 °C pre-incubation 5 min, annealing 40 cycles: 95 °C for 3s, 60 °C for 20s. Then, 95 °C 15 s, 60 °C 15 s, 15 s, and 95 °C 15 s. The results were expressed as 2-delta CT value, and multiple changes of expression levels of these genes and the expression level of the housekeeping gene 16S were compared.

## Statistical Analysis

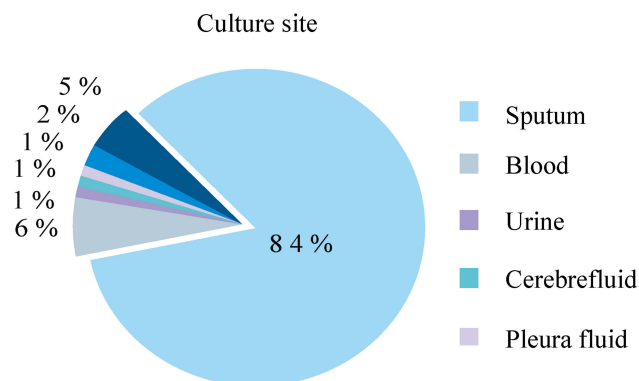
The primary outcomes were risk factors of carbapenems resistance. The Secondary outcomes were risk factors related to 30-day mortality. The Chi-square test or Fisher exact test was used to analyze the categorical variables. The 2-tailed *t*-test and Mann–Whitney test were used to analyze the continuous variables. Logistic regression analysis was used to determine the risk factors for carbapenem resistance. Kaplan–Meier curves and univariate analysis were used to evaluate the proportional hazard hypothesis. Cox regression analysis was used in multivariate analysis, using SPSS 21.0 statistical software for data processing. Differences with *P* < 0.05 were statistically significant.

## Results

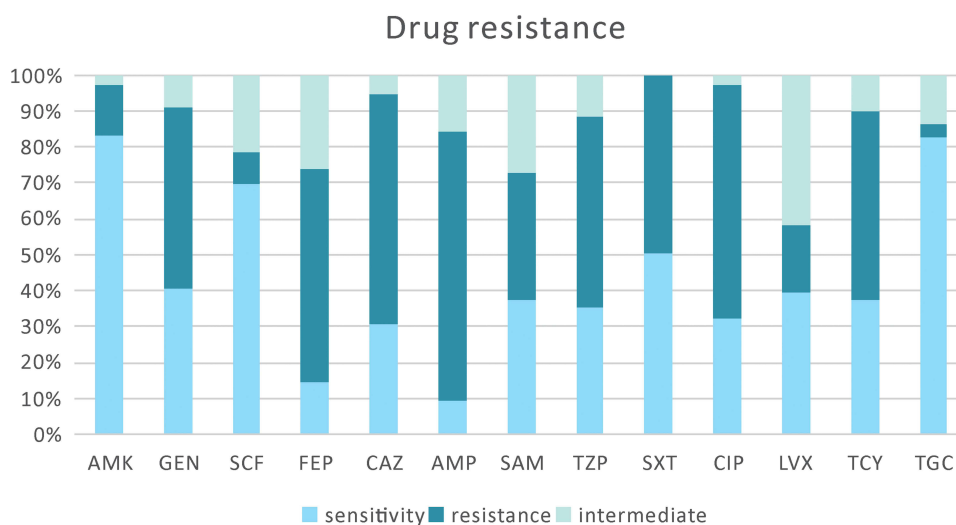
There were 131 patients (57 girls and 74 boys) aged from 1 month to 13 years. Of these 60 patients had underlying diseases: 68 patients with severe pneumonia, 17 with sepsis before infection, 12 with shock before infection, four with multiple organ dysfunction syndrome (MODS) before infection, 14 patients with sepsis after infection, 12 patients with shock after infection, and eight patients with MODS after infection. Twenty-seven patients died within 30 days after infection. Figure 2 shows there were 131 strains, 110 sputum samples, eight blood cultures, one urine culture, one cerebral fluid culture, two pleural fluid culture, three ascites cultures, and six bronchoalveolar lavage fluid samples. Of these 89 strains were carbapenem-resistant and 42 were carbapenem-sensitive.

Figure 3 shows that 67.94% of the isolates were resistant to carbapenems, followed by ceftazidime (64.12%). High susceptibility was detected for amikacin (83.21%) and tigecycline (82.44%), followed by cefoperazone sulbactam (69.47%). The sensitivity rate of gentamicin was 40.46% and that of levofloxacin was 39.69%.

Supplementary Table 1 shows Beta-lactamase gene expression. Among them, 14 strains of oxa-51 gene were not detected, 22 strains of oxa-23 gene were not detected, 25 strains of oxa-24 gene were not detected, and only oxa-58 gene was expressed 10 strains. In the CRAB group, 91% of the strains detected the OXA-23 gene, 93% of the strains detected OXA-51, and 81% of the strains detected OXA-24; in the non-CRAB, 67% of the strains detected OXA. Among 23 genes, OXA-51 was detected in 81% of the strains, and OXA-24 gene expression was detected in 83% of the strains.



**Figure 2** The different Specimen culture site including sputum, blood culture, urine culture, cerebra fluid, pleural fluid, ascites and bronchoalveolar lavage fluid from patients.



**Figure 3** Resistance of *Acinetobacter baumannii*. Drug resistance about *Acinetobacter baumannii* includes amikacin/gentamicin, sulbactam and cefoperazone, cefepime/ceftazidime/ampicillin, ampicillin/sulbactam, piperacillin /tazobactam, compound sulfamethoxazole, and ciprofloxacin/levofloxacin/tetracycline/tigecycline.

Univariate analysis of the CRAB vs non-CRAB groups in Table 1 revealed the following: in-hospital days before infection (14 (18) vs 9 (13),  $P = 0.024$ ), previous deep vein catheterization (40VS7 catheter,  $P = 0.001$ ), previous urinary catheterization (56VS19 catheter,  $P = 0.042$ ), fever (60vs21,  $P = 0.04$ ), previous carbapenem (meropenem/imipenem) (30 vs 6,  $P = 0.017$ ), oxa-24 (0.04 (0.33)vs 0.05(0.31),  $P = 0.305$ ), oxa-51 (0.9 (2.05)vs 0.10 (0.30),  $P < 0.000$ ) and oxa-23 (0.29 (1.23)vs 0.00 (0.05),  $P < 0.000$ ). The oxa-58-like gene was only detected in ten strains. Logistic regression analysis of factors associated with CRAB indicated that oxa-23 (HR = 0.005, CI 95% 0–0.153,  $P = 0.002$ ) and previous carbapenem treatment (meropenem/imipenem) (HR = 0.031, CI 95% 0.1–0.959,  $P = 0.042$ ) is shown in Table 2.

Univariate analysis showed in Table 3 that there was no difference in age and sex expression between death group and survival group within 30 days. Differences between survivors and non-survivors were genetic metabolic diseases (12 vs 6,  $P = 0.041$ ), sepsis after infection (4 vs 4,  $P = 0.02$ ), shock after infection (6 vs 6,  $P = 0.015$ ), MODS after infection (8 vs 6,  $P = 0.029$ ),  $\Delta$ CRP (mg/L) (3.34 (67) vs  $-1.04$  (18),  $P = 0.012$ ), albumin (g/L) ( $31.3 \pm 5.04$  vs  $34.43 \pm 4.46$ ,  $P = 0.008$ ), AST ( $\mu\text{mol/L}$ ) ( $66.6 \pm 68.3$  vs  $33.06 \pm 22.42$ ,  $P = 0.037$ ), ALT ( $\mu\text{mol/L}$ ) ( $129.91 \pm 393.29$  vs  $44.88 \pm 83.93$ ,  $P =$

0.017), and bilirubin ( $\mu\text{mol/L}$ ) ( $8.43 \pm 6.19$  vs  $6.64 \pm 3.81$   $p = 0.004$ ). However, sulbactam was used in combination with cephalosporins (6 vs 50,  $P = 0.023$ ) was a protect factor for 30-day survivors. The expression of oxa-23, oxa-24, oxa-58, oxa-51 showed no significant difference between the those who survived and those who died: oxa-51 (0.47 (1.95) vs 0.11 (1.44),  $P = 0.65$ ), oxa-24 (0.06 (0.73) vs 0.08 (2.41), oxa-23 (0.28 (2.4) vs 0.15 (0.22),  $P = 0.181$ ).

Cox regression analysis of factors associated with 30-day mortality showed that sulbactam in combination with cephalosporins (HR = 0.271, CI 95% 0.095–0.712,  $P = 0.009$ ) was associated with 30-day survival in Table 4.

## Discussion

To the best of our knowledge, this is the largest study about children of *A. baumannii* in northern China. We found that previous meropenem/imipenem therapy and oxa-23-like gene expression were independent risk factors for carbapenem resistance. Interestingly, carbapenem resistance was not associated with 30-day mortality. We explored the risk factors for 30-day mortality and found that  $\Delta$ CRP, sepsis, albumin, transaminases, total bilirubin, and cephalosporins combined with sulbactam therapy were significant in univariate analysis. Only cephalosporins combined with sulbactam therapy were significant in Cox regression analysis.

**Table 1** Comparison Between Carbapenem-Resistant Group and Carbapenem-Sensitive Group

	Carbapenem-Resistant Group (89)	Carbapenem-Sensitive Group (43)	P
Male sex	51	22	0.679
Age (month)	47.65+-46.13	34.48+-43.46	0.122
Previous in-hospital days	14 (18)	9 (13)	0.024
Basic disease	41	19	0.92
Congenital heart disease	14	8	0.678
Inherited metabolic disease	12	6	0.941
Severe pneumonia	45	23	0.753
Sepsis before	13	4	0.419
Shock before	8	4	1
MODS before	3	1	1
MODS after	9	5	0.994
Sepsis after	10	2	0.231
Shock after	7	1	0.405
Glasgow at time of admission in picu	12 (4)	12 (2)	0.321
Mechanical Ventilation without 24 hours in PICU	46	23	0.846
WBC without 24 hours in PICU	10.28 (8.56)	10.8 (7.91)	0.786
PLT without 24 hours in PICU	256.75+-161.13	273.65 (130.32)	0.524
Previous Mechanical Ventilation	81	37	0.571
CRRT	9	4	1
Previous Deep vein catheterization	40	7	0.001
Previous Thoracentesis	15	4	0.237
Previous Catheterization	56	19	0.042
Fever when culture	60	21	0.04

(Continued)

**Table 1** (Continued).

	Carbapenem-Resistant Group (89)	Carbapenem-Sensitive Group (43)	P
WBC (10 <sup>9</sup> )	10.6 (7.62)	11.06 (8.7)	0.488
ΔWBC (10 <sup>9</sup> )	0.57+—6.62	-0.49+—8.04	0.442
ΔCRP (mg/L)	-1.1 (23)	2.05 (15)	0.225
ΔPCT (ng/mL)	0 (1)	0.01 (2)	0.953
oxa-51 like gene	0.90 (2.05)	0.10 (0.30)	0.000
oxa-23 like gene	0.29 (1.23)	0.00 (0.05)	0.000
oxa-24 like gene	0.04 (0.33)	0.05 (0.31)	0.305
Previous ≥3 antibiotic therapy	64	24	0.066
Previous carbapenem (meropenem/ imipenem) therapy	30	6	0.017
IgG (g/L)	6.23 (5.37)	9.02 (8.44)	0.313
IgM (g/L)	0.77 (0.52)	0.71 (0.79)	0.729
IgA (g/L)	0.55 (0.79)	0.40 (0.68)	0.158
T cell (/ul)	1014 (1935)	1694 (1235)	0.710
B cell (/ul)	324 (536)	666 (520)	0.699
NK cell (/ul)	122 (152)	154 (159)	0.177
CD4 cell (%)	33.2 (21)	32 (10)	0.699
CD8 cell (%)	20.8 (13)	18 (13)	0.177
30-day mortality	19	8	0.714

**Note:** The meaning of the numbers in the parentheses is Interquartile range.

Our carbapenem resistance rate was 67.94%. Amikacin and tigecycline had the highest sensitivity, followed by cefoperazone/sulbactam. Amikacin is an aminoglycoside that is ototoxic and nephrotoxic in children; tigecycline can increase mortality.<sup>31</sup> Therefore, these treatments are not recommended. The low frequency of use may be the cause of sensitivity. A study showed that carbapenem-resistant *A. baumannii* infection was an independent risk factor for death from bacteremia in children.<sup>9</sup> For children infected with *A. baumannii*, 7-day and 30-day mortality rates were 18.96% and 35.1%, respectively. Carbapenem resistance was an independent risk factor for 30-day mortality (18 vs 26, P =

**Table 2** Logistic Regression Analysis About Risk Factors Associated with Carbapenem Resistance

	HR	95% C.I.	P
Previous in-hospital days	1.004	0.979–1.03	0.747
Previous Deep vein catheterization	0.401	0.131–1.228	0.109
Previous Catheterization	0.815	0.295–2.251	0.693
Fever when culture	0.677	0.256–1.793	0.433
Previous carbapenem (meropenem/imipenem) therapy	0.31	0.1–0.959	0.042
oxa-23 like gene	0.005	0–0.153	0.002
oxa-51 like gene	0.805	0.567–1.143	0.225

**Abbreviations:** HR, hazard ratio; CI, confidence interval.

0.034).<sup>9</sup> Punpanich et al reported that the 30-day mortality in children with *A. baumannii* infection and bacteremia was 26.1%; carbapenem resistance was 4.76 (1.5–14.32),  $P = 0.005$ .<sup>32</sup> We found that there was no significant difference in 30-day mortality between the carbapenem-resistant group and the carbapenem-sensitive group; the case-fatality rate was only 20.61% (carbapenem-resistant group vs carbapenem-sensitive group: 19 vs 8,  $P = 0.714$ ). First, they studied bloodstream infections and no patients were in the pediatric intensive care unit. Second, we showed that cephalosporin/sulbactam for *A. baumannii* reduced the 30-day mortality rate. In our study, nearly half of the children with *A. baumannii* infection and were given appropriate treatment.

In summary, the differences in infection sites, departments, medication protection, and even regional differences may account for our low mortality rate and drug resistance with no difference in 30-day fatality rate between groups.

Sulbactam is a  $\beta$ -amidase inhibitor that has synergistic effects with other carbapenems. We found that cefoperazone/sulbactam was used most often (31/56). In a study of carbapenem-resistant *A. baumannii* infection-related hospital-acquired pneumonia, the 30-day survival rate among those treated with cefoperazone/sulbactam was 95.1%; for those treated without cefoperazone/sulbactam, the rate was 73.3%.<sup>33</sup> Chi et al found that the use of cefoperazone/sulbactam had a protective effect on the 30-day mortality rate, and the mortality rate of children receiving cefoperazone/sulbactam treatment was lower than that of patients receiving tigecycline treatment (35.7% vs 51.9%,  $P =$

0.001).<sup>34</sup> The results of pharmacokinetic studies suggested that imipenem is more suitable than meropenem for severely ill patients.<sup>35,36</sup> Cefoperazone/sulbactam combined with imipenem/cilastatin is recommended as routine treatment.

D- $\beta$ -lactamase is closely related to carbapenem resistance and can hydrolyze carbapenem enzymes. The subgroups of oxa-23, oxa-24, oxa-51, and oxa-58 are prevalent worldwide, especially oxa-23.<sup>37</sup> Liu et al detected oxa-23 expression in 28 hospitals in 18 provinces in China.<sup>38</sup> A recent study suggested oxa-23 has a role in promoting sulbactam resistance.<sup>39</sup> Many studies suggested that carbapenem resistance was related to oxa-23 expression, and the mortality rate of children with carbapenem resistance was high.<sup>9,40</sup> An intensive care unit study in Brazil showed that oxa-23-producing *A. baumannii* strains belonged to the ST79 (CC79) clonal group, and patients infected or colonized with these isolates had high mortality rates (34.6%).<sup>17</sup> 14 strains in carbapenem-sensitive group absence oxa-23 expression. We found that the expression of oxa-23 was significantly different in the carbapenem-resistant group and the sensitive group ( $P = 0.002$ ). Nevertheless, there was no significant difference in the 30-day mortality rate related to oxa-23 on further exploration. There are many factors that affect the mortality rate, and it is possible that the expression of a single gene plays a limited role.

## Conclusions

In general, resistance to carbapenems is closely related to the expression of the oxa-23 gene. The expression of

**Table 3** Univariate Analysis Comparing Survivors and Non-Survivors at 30 Days from Infection Onset

	Survival (104)	Death (27)	P
Sex	46	14	0.343
Congenital heart disease	18	4	0.877
Inherited metabolic disease	12	6	0.041
Severe pneumonia	62	16	0.35
Sepsis before	12	5	0.445
Shock before	11	1	0.221
MODS before	2	2	0.072
Sepsis after	8	6	0.029
Shock after	6	6	0.015
MODS after	4	4	0.02
Glasgow when admission in picu	12 (3)	12 (4)	0.791
WBC without 24 hours in PICU	10.85 (8.4)	9.5 (8.7)	0.238
PLT without 24 hours in PICU	273.57+-154.06	218.27+-135.72	0.312
WBC ( $10^9$ )	10.6 (6.6)	10.7 (10)	0.837
$\Delta$ WBC ( $10^9$ )	0.23+-8	0.28+-9	0.921
$\Delta$ CRP (mg/L)	-1.04 (18)	3.34 (67)	0.012
Albumin (g/L)	34.43+-4.46	31.3+-5.04	0.008
AST ( $\mu$ mol/L)	33.06+-22.42	66.6+-68.3	0.037
ALT ( $\mu$ mol/L)	44.88+-83.93	129.91+-393.29	0.017
Total Bilirubin ( $\mu$ mol/L)	6.64+-3.81	8.43+-6.19	0.004
Creatinine ( $\mu$ mol/L)	20.10+-10.25	21.66+-6.76	0.456
Urea nitrogen (mmol/L)	4.045 (4.38)	5.07 (3.99)	0.806
oxa-51	0.47 (1.95)	0.11 (1.44)	0.65
oxa-24	0.06 (0.73)	0.08 (2.41)	0.712
oxa-23	0.28 (2.4)	0.15 (0.22)	0.181
IgG (g/L) (93:32)	7.8+-4.91	10.62+-7.05	0.368
IgM (g/L) (93:32)	0.76+-0.44	0.73+-0.52	0.722
IgA (g/L) (93:32)	0.39 (0.59)	0.23 (0.45)	0.324
T cell (/ul)	1435.48+-1407.57	1215.88+-1112.34	0.0929
B cell (/ul)	724.84+-661.55	748.35+-770.91	0.786
NK cell (/ul)	205.98+-226.72	232.76+-342.80	0.387
CD4 cell (%)	30.17+-13.40	30.71+-14.14	0.475
CD8 cell (%)	19.82+-10.06	22.31+-10.69	0.833
Cephalosporins combined with sulbactam (Cefoperazone /Sulbactam:31/56)	50	6	0.023

**Notes:** the meaning of the numbers in the parentheses is Interquartile range.( $\Delta$ )=value of 24 hours after clinical diagnosis of infection- value of 48 hours before infection diagnosis.

**Table 4** Cox Regression Analysis About Risk Factors Associated with 30-Day Mortality

	HR	95.0% CI	P
MODS after	3.1	0.602–15.971	0.176
Shock after	2.81	0.969–8.143	0.057
Sepsis after	1.42	0.284–7.088	0.669
Cephalosporins combined with sulbactam	0.26	0.095–0.712	0.009
ALT	1	0.999–1.001	0.749
AST	1	0.995–1.005	0.905
Total Bilirubin (μmol/L)	1.012	0.983–1.043	0.42
Albumin (g/L)	0.935	0.845–1.035	0.193
ΔCRP (mg/L)	1.004	0.996–1.013	0.296

**Abbreviations:** HR, hazard ratio; CI, confidence interval.

oxa-23 and the use of carbapenems are independent risk factors for carbapenem resistance. Cephalosporins combined with sulbactam are independently related to the 30-day mortality. *A. baumannii* were sensitive to tigecycline (82.44%) and amikacin (83.21%); however, they are not recommended for use in children because of ototoxicity and nephrotoxicity. We recommend using cephalosporins combined with sulbactam in children infected with *A. baumannii*. However, this study is a retrospective one, and it is impossible to distinguish which enzyme inhibitor is the best. There is a need to further explore treatment strategies for *A. baumannii* infections.

## Abbreviations

MODS, multiple organ dysfunction syndrome; Real-time PCR, real-time reverse-transcription polymerase chain reaction; *A. baumannii*, *Acinetobacter baumannii*; ALT, alanine aminotransferase; AST, aspartate transaminase; CRP, C-reaction protein; PLT, platelet; WBC, white blood cell; Procalcitonin; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; T cell, T-lymphocyte; B cell, B-lymphocyte; NK cell, natural killer cell; CRRT, continuous renal replacement therapy; PICU, pediatric intensive care unit; CSF, cerebrospinal fluid; AMK, amikacin; GEN, gentamicin; SCF, sulbactam and cefoperazone; FEP, cefepime; CAZ, ceftazidime; AMP, ampicillin; SAM, ampicillin/sulbactam; TZP, piperacillin sodium/tazobactam sodium; SXT,

compound sulfamethoxazole; CIP, ciprofloxacin; LVX, levofloxacin; TCY, tetracycline; TGC, tigecycline.

## Ethical Consideration

The ethical approval was obtained from the Ethical Review Committee of Shengjing Hospital Affiliated to China Medical University. Since we reviewed the secondary data and obtained the clinical director's informed consent, the patient's informed consent was not required. Strict confidentiality during the data collection process, data processing and report writing process. The research was also conducted in accordance with the Declaration of Helsinki.

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## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, agreed to the submitted journal, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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