Molecular characterization of multidrug resistant strains of Acinetobacter baumannii isolated from pediatric intensive care unit in a Chinese tertiary hospital

Yili Chen¹, Lu Ai¹, Penghao Guo¹, Han Huang¹, Zhongwen Wu¹, Xiaoling Liang² and Kang Liao¹*

Abstract

Background: Acinetobacter baumannii is a nosocomial pathogen which is reported as a major cause of morbidity and mortality in intensive care units (ICUs). However, there is a lack of analysis focused on multidrug-resistant Acinetobacter baumannii (MDRAB) infection among patients from pediatric intensive care unit (PICU) in China. The aim of this study was to investigate the molecular characterization of MDRAB isolated from PICU.

Methods: In this study, 86 isolates of MDRAB were collected from PICU patients, from the First Affiliated Hospital of Sun Yat-sen University. The minimal inhibitory concentrations (MICs) of the isolates against common antibiotics were determined. The carbapenemase-encoding resistance genes and AdeABC-AdeRS efflux system genes of these isolates were detected by PCR. Real-time PCR was performed to determine the relative expression of the relevant efflux pumps.

Results: Among 86 strains of MDRAB, 76.7% (66/86) were carbapenem-resistant A. baumannii (CRAB). All 86 clinical isolates possessed the blaOXA-51 gene. BlaOXA-23 was detected as the second most frequent (90.7%) carbapenemase. Harboring AdeABC efflux pump genes was prevalent among the majority of the MDR isolates. Specially, the distributions of AdeABC-AdeRS efflux system genes in CRAB strains reached up to 90.0%. Compared with those of the CSAB strains, there was a statistically significant increasing distribution of the regulator AdeR and AdeS genes (p < 0.05). Moreover, CRAB strains showed significantly increased expression of AdeB (12.3-fold), but decreased expression of AdeR (3.3-fold) (p < 0.05).

Conclusion: The present study showed a high distribution of multiple genes, mainly the genes of blaOXA-23 and adeABC efflux pump, is responsible to distinct drug-resistance in PICU. It is urgent to strengthen the molecular epidemiological surveillance of pediatric MDRAB isolates to prevent further outbreaks. This study is of significant help for the clinicians to make therapeutic decisions and manage infection control in PICU.

Keywords: Multidrug-resistant, Acinetobacter baumannii, Pediatric intensive care unit, Carbapenemase enzymes, Efflux pump
Background

*Acinetobacter baumannii* has been reported as an epidemiologically and clinically life-threatening nosocomial pathogen causing critical morbidity and mortality, especially in intensive care units (ICUs) [1]. The drug resistance rate against carbapenems of this organism is alarmingly high. It is extensively reported that carbapenem-resistant strains of *Acinetobacter baumannii* (CRAB) have caused the hospital outbreaks worldwide [2–4].

Although, *A. baumannii* was frequently and classically reported as a main hospital-acquired pathogen in adults, it is also a critical pathogen in the pediatric intensive care unit (PICU), because of invasive surgery, severe basic diseases, immunodeficiency, and prolonged hospitalization among the pediatric patients [5, 6]. Several outbreaks of carbapenem-resistant *A. baumannii* in the PICU have been documented in the last decade [7–9].

To increase awareness and improvement of epidemiological surveillance is a significant and critical factor of successful infection control and is particularly recommended in ICUs. To our knowledge, few studies on the molecular characterization of MDRAB isolated from PICU are reported in China. Thus, the present study was conducted to determine the molecular characteristics among the MDRAB strains from PICU in a tertiary hospital in Guangzhou, China, during a 5-year period from January 2013.

Methods

Isolation and identification of bacterial strains

Eighty-six non-repetitive multidrug-resistant *Acinetobacter baumannii* strains were obtained from different PICU patients, in the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China, from January 2013 to December 2017. The isolates were collected from cultures of respiratory tract specimens (80 isolates, including 74 isolates from sputum samples and 6 isolates from nasal secretion samples), blood (5 isolates), and stool (1 isolate). These strains were identified by an automated microbiology analyzer (bioMérieux, Marcy l’Etoile, France) according to the manufacturer’s instructions, and confirmed on blaOXA-51 polymerase chain reaction (PCR). This report was approved by the Clinical Research and Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities for isolates were determined initially using Gram-negative susceptibility (GNS) cards on the Vitek system (bioMérieux, Marcy l’Etoile, France) to designate isolates as either carbapenem-resistant *A. baumannii* (CRAB) (with imipenem MICs ≥8 μg/ml) or carbapenem-susceptible *A. baumannii* (CSAB) (with imipenem MICs ≤2 μg/ml). Susceptibility interpretations were based on CLSI clinical breakpoints (2017; CLSI Document M100-S27). The tested agents included ampicillin/sulbactam, piperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, imipenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and tigecycline. Quality control for the MIC analysis was performed with *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

Identification of the drug resistance genes

Bacterial DNA was extracted from *A. baumannii* isolates by boiling. PCR was performed using TaKaRa Ex Taq (Takara Bio Inc., Otsu, Japan). All PCR primers targeting resistance genes and mobile elements used in this study are listed in Table 1. PCR was performed in a 25 μL reaction mixture containing 1 μL primer, 1 μL Taq polymerase, 3 μL DNA template, 3.0 mM MgCl2, 2.5 μL 10 × buffer, 0.2 mM dNTPs, and nuclease-free water. Amplification conditions consisted of denaturation at 94 °C for 5 min and 30 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 30 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. PCR products were detected in 2% agarose gel.

RNA expression of efflux pump genes

The expression of efflux pumps of AdeABC-AdeRS was quantified. The total RNA of isolates was extracted with TRIzol extraction (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. The relative expression of the efflux pumps was determined using real-time PCR by SYBR® Premix Ex TaqTM (Takara Bio Inc., Otsu, Japan) on the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument (Life Technologies Corporation, Foster City, CA). The RT-PCR reaction mixture was prepared in a volume of 20 μL comprised of 3 μL of cDNA, 10 μL 2 × SYBR® Premix Ex Taq II (Tli RNaseH Plus), 0.4 μL 50 × ROX Reference Dye or Dye II and 0.8 μL of each primer. The PCR reaction was carried out under the following conditions: 1 cycle of 30 s at 95 °C, 40 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, and elongation at 72 °C for 30 s, with one final cycle of 5 min at 72 °C. Reactions were repeated in triplicate and the fold changes in expression of these genes were calculated relative to the level of housekeeping gene 16S rDNA using the comparative C_T method (2^ΔΔCT method).

Statistical analysis

Data are described using the mean and standard deviation (mean ± s). Categorical variables were compared using Fisher’s exact test. A p value of 0.05 was considered statistically significant. Statistical Package for the Social Sciences (SPSS) for Windows Version 16.0 (SPSS Inc.; Chicago, IL, USA) was used in this study (Fig. 1).
showed a flow chart for representing this research methods).

**Results**

**Antimicrobial susceptibility profile**

The findings of the antibiotic susceptibility testing for all isolates were showed in Table 2. Among the 86 MDRAB strains, 76.7% (66/86) were CRAB, which showed 100% resistant to many of the tested antimicrobial agents, except tigecycline.

| Table 1 Primer sequences of the target genes |
|---------------------------------------------|
| Gene  | Primer Sequence(5′→3′) | Size (bp) | Reference |
| IMP   | F: ATGAGCAAGTATGTCAGTGTTAT | 741 | [10] |
|       | R: TTAGTGTGATTTTGTTATGTTGTT |           |         |
| KPC   | F: TCGCCGCTCAGTTCTGCTGCTC | 965 | [10] |
|       | R: CGGCAGGACTCCTTCAAGGCTAA |           |         |
| NDM-1 | F: TACCGCGATGTTCCCTCAACGGCA | 457 | [10] |
|       | R: GGGCCAGTCCTCCACCGGTA |           |         |
| VIM   | F: GGTCGATATGCAACCCGAGATTT | 636 | [10] |
|       | R: CGGCAGTGAGCGATTTCGTC |           |         |
| IMI   | F: CTGGTCTGATGCATGTTCC | 440 | [10] |
|       | R: CTACCGCATAACTGGTGTTG |           |         |
| SPM   | F: CTGCTTGGATTCATGGGCGC | 783 | [10] |
|       | R: CTTTTCCGAGCGCTTACAT |           |         |
| OXA-51| F: TCCAAATCAACGCGCTTCAAAA | 639 | [10] |
|       | R: TGGAGGCTGAAACCCCATC |           |         |
| OXA-23| F: ACTTGTCTATGTTGTTGCTTCAT | 797 | [10] |
|       | R: TTTTGCTTTGATTGAGCCTTA |           |         |
| OXA-24| F: CAGTGCATGCATGCATCAATT | 702 | [10] |
|       | R: TCTAAGTTGAGCGAGAAAG |           |         |
| OXA-58| F: AAGTATGGGCGTGTGCGTGCTG | 599 | [10] |
|       | R: CCCCTGCGGTCTCTCATAAC |           |         |
| ISAba1-OXA-51 | F: CAGCAATTCGAGAATTGTG | 1200 | [10] |
|       | R: CTCTTGTTGTGTTGC |           |         |
| AdeA  | F: GAGGTGGCAGAACGTAAGGTC | 113 | [11] |
|       | R: GCTAGACGCTGACGATACTGAGC |           |         |
| AdeB  | F: TACCGCGATTTACCTTTGCGAGA | 250 | [11] |
|       | R: GTTCATAGTGGTGGTTAAACGCA |           |         |
| AdeC  | F: ACAATCGATATCCTCGTGACT | 361 | [11] |
|       | R: TAGAAGAATCGTTATGAGTTG |           |         |
| AdeR  | F: ACTACGATATTGGACGACCATT | 447 | [12] |
|       | R: GCCTGCGATTAAGCAAGATT |           |         |
| AdeS  | F: TTGGTACTGCACGTGTATCAT | 544 | [12] |
|       | R: AGTGGGATCTTTAGTGGTTCT |           |         |

Distribution of the carbapenemase and AdeABC-AdeRS efflux system genes

The distribution of carbapenemase and efflux pump genes in drug-resistant strains was shown in Table 3. Of note, \( \text{bla}_{\text{OXA-51}} \) genes were present in all 86 MDR \( A. \ baumannii \) isolates. \( \text{bla}_{\text{OXAS-23}} \) was detected the second most frequently (90.7%) of carbapenemase. The \( \text{ISAba}-\text{OXA-51}, \text{bla}_{\text{OXA-24}}, \text{bla}_{\text{OXA-58}} \), and \( \text{NDM}-1 \) genes were detected in 23.3% (20/86), 22.1%(19/86), 3.49% (3/86), and 3.49% (3/86) of the 86 MDR isolates, respectively. Among the CRAB strains, there was a statistically significant increasing distribution of \( \text{bla}_{\text{OXA-24}}, \text{bla}_{\text{OXA-58}}, \) and \( \text{ISAba}-\text{OXA-51}(p < 0.05) \). The majority of the MDR isolates were found to harbor \( \text{AdeABC} \) efflux pump genes. Notably, the distributions of \( \text{AdeABC}-\text{AdeRS} \) efflux system genes among CRAB strains were more than 90.0%, and compared with the CSAB strains, there was a statistically significant increasing distribution of the regulator \( \text{AdeR} \) and \( \text{AdeS} \) genes\((p < 0.05) \). Specifically, there was a higher distribution of co-harboring \( \text{AdeABC-C-AdeRS} \) efflux system genes and \( \text{bla}_{\text{OXAS-23}} \) among the CRAB strains (Table 4).

Relative expression levels of the AdeABC-AdeRS efflux system genes

The level of expression of the \( \text{AdeABC-AdeRS} \) efflux system genes was measured by qRT-PCR (Table 5). It was found that, compared with those of CSAB strains, CRAB strains showed significantly increased expression of \( \text{AdeB}(12.3\text{-fold}) \), but decreased expression of \( \text{AdeR} \) (3.3-fold). However, there was no significant difference of the relative expression of \( \text{AdeA}, \text{AdeC} \) or \( \text{AdeS} \) between the two groups.
Discussion

In this study, we found there was a 76.7% prevalence of imipenem-resistance among the MDRAB in PICU from our hospital. Tigecycline proved to be the most active antibiotics. Even though effective and safe therapeutic options are limited against MDRAB strains to PICU patients, combination therapy is still the potential choice to fight against carbapenem resistance in MDRAB.

Drug resistance mechanisms of MDRAB are sophisticated, including the production of enzymes that act as intrinsic drug resistance, the pathogen's amazing ability to obtain resistance determinants horizontally, accident changes of outer membrane permeability, and the role of efflux systems [12, 13]. For example, mainly due to producing various carbapenemase enzymes such as class B metallo-β-lactamases (MBLs) and class D oxacillinases (OXAs), A. baumannii developed its resistance to carbapenems.

Table 2: Antimicrobial susceptibility (%) of 86 strains of MDR Acinetobacter baumannii isolates

| Antibiotics agents          | CRAB (n = 66) | CSAB (n = 20) | Total (n = 86) |
|-----------------------------|---------------|---------------|---------------|
| ampicillin                  | 0 (0/66)      | 0 (0/20)      | 0 (0/86)      |
| ampicillin/sulbactam        | 0 (0/66)      | 0 (0/20)      | 0 (0/86)      |
| piperacillin/tazobactam     | 0 (0/66)      | 80.0 (16/20)  | 18.6 (16/86)  |
| cefotaxime                  | 0 (0/66)      | 0 (0/20)      | 0 (0/86)      |
| ceftazidime                 | 0 (0/66)      | 10.0 (2/20)   | 2.33 (2/86)   |
| cefepime                    | 0 (0/66)      | 30.0 (6/20)   | 6.98 (6/86)   |
| aztreonam                   | 0 (0/66)      | 30.0 (6/20)   | 6.98 (6/86)   |
| imipenem                    | 0 (0/66)      | 100 (20/20)   | 23.3 (20/86)  |
| amikacin                    | 0 (0/66)      | 100 (20/20)   | 23.3 (20/86)  |
| gentamicin                  | 0 (0/66)      | 80.0 (16/20)  | 18.6 (16/86)  |
| ciprofloxacin               | 0 (0/66)      | 75.0 (15/20)  | 17.4 (15/86)  |
| levofloxacin                | 0 (0/66)      | 80.0 (16/20)  | 18.6 (16/86)  |
| trimethoprim/sulfamethoxazole | 0 (0/66)  | 75.0 (15/20)  | 17.4 (15/86)  |
| tigecycline                 | 100 (66/66)   | 100 (20/20)   | 100 (100/100) |

Table 3: Distribution of Carbapenemase genes and Ade efflux pump genes in the 86 MDRAB isolates in this study

| Gene              | CRAB (n = 66) | Prevalence rate | CSAB (n = 20) | Prevalence rate | P value | Total (N/%) |
|-------------------|---------------|-----------------|---------------|-----------------|---------|-------------|
| IMP               | 0             | 0               | 0             | 0               | –       | 0           |
| KPC               | 0             | 0               | 0             | 0               | –       | 0           |
| NDM-1             | 3             | 4.54            | 0             | 0               | 0.52    | 3 (3.49)    |
| VIM               | 0             | 0               | 0             | 0               | –       | 0           |
| IMI               | 0             | 0               | 0             | 0               | –       | 0           |
| SPM               | 0             | 0               | 0             | 0               | –       | 0           |
| OXA-51            | 66            | 100             | 20            | 100             | –       | 86 (100)    |
| OXA-23            | 60            | 90.9            | 18            | 90.0            | 0.92    | 78 (90.7)   |
| OXA-24            | 19            | 28.8            | 0             | 0               | 0.02    | 19 (22.1)   |
| OXA-58            | 3             | 4.54            | 0             | 0               | 0.07    | 3 (3.49)    |
| ISaBA-OXA-51      | 20            | 30.3            | 0             | 0               | 0.01    | 20 (23.3)   |
| AdeA              | 61            | 92.4            | 18            | 90.0            | 0.71    | 79 (91.9)   |
| AdeB              | 65            | 98.5            | 18            | 90.0            | 0.54    | 83 (96.5)   |
| AdeC              | 61            | 92.4            | 15            | 75.0            | 0.10    | 76 (88.4)   |
| AdeR              | 60            | 90.9            | 10            | 50.0            | 0.04    | 70 (81.4)   |
| AdeS              | 61            | 92.4            | 11            | 55.0            | 0.04    | 72 (83.7)   |
efficiency of OXA-carbapenemases for the hydrolysis of carbapenems is lower than that of MBL (100–1000 times lower), it is important to consider them as potential hazards because their expression can be regulated by upstream insertion of IS elements such as \textit{ISAba1}. [14] \textit{BlaOXA-23}, \textit{blaOXA-24} and \textit{blaOXA-58} are most common OXA-type genes detected within clinical MDRAB isolates, typically among Asia-Pacific region[15–18]. It is reported that the \textit{blaOXA-23} gene is much more prevalent, while the \textit{blaOXA-58} gene was rarely found in MDRAB isolates [18]; in addition, MBLs SIM, IMP, and VIM-producing \textit{A. baumannii} isolates are also worth noting [19].

In our study, all of the 86 clinical isolates possessed the chromosomally encoded enzyme gene \textit{blaOXA-51}. Among these MDR isolates, such a high frequency of \textit{blaOXA-51} gene detected is likely to suggest that an intrinsic drug resistance mechanism plays an important role on the multidrug resistance. However, further quantitative assay is necessary to value the contribution of \textit{blaOXA-51} gene in the mechanism of multidrug resistance of these isolates.

The widespread prevalence of \textit{blaOXA-23} gene in \textit{Acinetobacter spp.} has been recently reported, that the plasmids pAZJ221 and Tn2009 were likely to play a key role on horizontally transferring \textit{blaOXA-23} gene [20]. Hu et al. investigated 17 cases of \textit{Acinetobacter baumannii} infection in a neonatal intensive care unit (NICU), and \textit{blaOXA-23} was detected in all of the isolates, suggesting that the detection of the \textit{blaOXA-23} gene might be of significant help in neonatology decision-making on managing nosocomial infection [21]. Consistent with these findings, the present study showed that \textit{blaOXA-23} was detected most frequently (90.7%) of carbapenemase among the 86 MDRAB from PICU. Notably, we showed 18 strains among the 20 CSAB strains displayed imipenem susceptibility. To our knowledge, the OXA-23 enzyme has a high affinity to hydrolyse imipenem, however, there have been reports on occurrence of \textit{blaOXA-23} gene in imipenem-susceptible \textit{Acinetobacter baumannii} [22]. Imipenem susceptibility may be explained by the absence of the \textit{ISAba1} element upstream of the \textit{blaOXA-23} gene, since \textit{ISAba1} has promoter sequences to up-regulate \textit{blaOXA-23}. Therefore, the \textit{blaOXA-23} gene may be silently transmitted in hospital settings, highlighting the undetected threat to the carbapenemase gene pool.

In addition to the production of carbapenemase, efflux pumps systems also have presented important roles in the carbapenem resistance of MDRAB to antibiotics, especially the presence of \textit{AdeABC} multidrug efflux pump [23–25]. Reports from other studies had indicated that the expression of \textit{AdeABC} efflux pumps were obviously influenced by \textit{AdeRS} [26, 27]. Either the point mutations in \textit{AdeRS} or by the insertion sequence (IS) \textit{Aba-1} insertion upstream of the \textit{AdeABC} operon would result in over-expression of the \textit{AdeABC} efflux pump [28]. Remarkably, single point mutations in \textit{AdeS} (Thr153Met) and \textit{AdeR} (Pro116Leu) are believed to be in a close relationship with \textit{AdeABC} over-expression [29], and, as a result, these changes would lead to resistance against lots of antibiotics, including \beta-lactams, chloramphenicol, tetracyclines, aminoglycosides, and fluoroquinolones [30].

In the present study, the \textit{AdeABC-AdeRS} efflux systems were widely distributed among these isolates. Specifically, in the 66 CRAB strains, the distributions of \textit{AdeABC-AdeRS} efflux system genes were more than 90.0%. The CRAB group had a statistically significant increased expression of \textit{AdeB} gene, but decreased expression of \textit{AdeR}, but there was no significant difference of the relative expression of \textit{AdeS}. These results indicated the \textit{AdeABC-AdeRS} efflux systems might be potential cause of carbapenem resistance, with the over-expression of \textit{AdeB} gene. Moreover, the down-regulated expression of \textit{AdeR} gene might contribute to this process. Though our study did not show significant different expression on \textit{AdeS} between the two groups, the research by Srinivasan et al [29] has displayed that \textit{AdeS} could confer resistance to some antibiotics. Since bacterial isolates collected in the present study were from a single clinical center, further investigation about the role of \textit{AdeS} gene on carbapenem resistance is warranted.

### Table 4 The distribution of co-harbouring genes among the 86 MDRAB strains

| Co-harbouring gene | CRAB (n = 66) | CSAB (n = 20) | Total |
|--------------------|--------------|--------------|-------|
|                    | N            | Prevalence rate | N     | Prevalence rate | P value | (N/%) |
| AdeABC + blaOXA23  | 61           | 92.4          | 15    | 75             | 0.032   | 76 (88.4) |
| AdeABC - RS + blaOXA23 | 60           | 85.7          | 10    | 50             | 0.048   | 70 (81.4) |

### Table 5 Relative expression levels of the \textit{AdeABC-AdeRS} efflux system genes in MDR \textit{Acinetobacter baumannii} isolates

| Efflux system gene | MDRAB (n = 86) | CRAB (n = 66) | CSAB (n = 20) |
|--------------------|---------------|--------------|--------------|
| \textit{AdeA}     | 2.07 ± 0.88   | 2.13 ± 0.35  |
| \textit{AdeB}     | 2.83 ± 0.39*  | 0.23 ± 0.50  |
| \textit{AdeC}     | 1.77 ± 0.67   | 1.66 ± 1.21  |
| \textit{AdeR}     | 1.27 ± 1.01*  | 4.23 ± 1.52  |
| \textit{AdeS}     | 1.80 ± 2.50   | 1.12 ± 1.24  |

*\(p < 0.05\)
Studies about *A. baumannii* isolates co-harboring oxacillinase and efflux pump genes are accumulating. For example, in a hospital of Korea, it is proved that the expression both of *blaOXA-23* gene and *AdeABC* efflux pump genes were responsible for acquiring carbapenem resistant MDRAB isolates [31]. Hu et al. have indicated that among the imipenem resistant *A. baumannii* isolates, the production of carbapenemase carrying *blaOXA-51/blaOXA-66* genes could contribute to the intrinsic resistance to imipenem, but the drug efflux pump systems were much more responsible for the widespread dissemination of imipenem-resistant *A. baumannii* [32]. It is believed that the OXA-type carbapenemases can be fortified when increased expression of efflux pumps are present.

Our study had the following limitations. First, the isolates were collected from one centre, and the sample size was small, which might result in a special tendency of the molecular characteristics of MDRAB. Second, except *AdeABC*, other efflux systems have not been investigated in the present study. Further studies on how the *AdeABC-RS* system influences the decreasing carbapenem-susceptibility against MDRAB are needed.

**Conclusion**

The present study showed high distributions of multiple genes, mainly the genes of *blaOXA-23/blaOXA-51* carbapenemase and *AdeABC* efflux pump system, indicating a potential threat of MDRAB in PICU. Therefore, to develop an effective guidance to prevent hospital-acquired infections caused by MDRAB in ICUs is critical. All-around interventions such as hand hygiene, environmental cleaning, contact isolation precautions, and active surveillance are significantly important to reduce the incidence of *A. baumannii* infections.

**Abbreviations**

CRAB: Carbapenem-resistant *A. baumannii*; CSAB: Carbapenem-susceptible *A. baumannii*; MBLs: Metallo-β-lactamases; MDRAB: Multidrug-resistant Acinetobacter baumannii; MICS: Minimal inhibitory concentrations; OXAs: Oxacillinases; PCR: Polymerase chain reaction; PICU: Pediatric intensive care unit

**Acknowledgements**

None.

**Funding**

This study was not funded by any sponsor or financial institution.

**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Authors’ contributions**

YLC was responsible for designing experiments, interpreting data and writing the manuscript. LA participated in drawing tables and helping analyse the data. PHG, HH and ZWW participated in strain collection and literature searching. XLL was responsible for operating the relevant PCR experiment, acquisition of experimental data, and interpretation of data. KL was fully responsible for designing the research and gave final approval of the version to be published. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

This report was approved by the Clinical Research and Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University. Written informed consent was obtained from each participant’s legal guardian. All the bacterial isolates in this study were isolated prior to this study.

**Consent for publication**

Not applicable since there are no details on individuals reported within the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Publisher’s Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Author details**

1Department of Laboratory Medicine, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, Guangdong, China. 2Gaoyao People’s Hospital, Zhaoqing 526040, Guangdong, China.

**Received:** 2 May 2018 **Accepted:** 8 November 2018

**Published online:** 04 December 2018

**References**

1. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis. 2010;2(3):291–304.

2. Almaghrabi MK, Joseph M, Assiry MM, Hamid ME. Multidrug-resistant Acinetobacter baumannii: an emerging health threat in Aseer region, Kingdom of Saudi Arabia. Can J Infect Dis Med Microbiol. 2018;2018:9182747.

3. Ramette A, Kronenberg A. Prevalence of carbapenem-resistant Acinetobacter baumannii from 2005 to 2016 in Switzerland. BMC Infect Dis. 2018;18(1):159.

4. Soltanifar B, Heidari H, Ebrahim-Saraie HS, Hadi N, Mardaneh J, Motamedifar M. Molecular characteristics of multiple and extensive drug-resistant Acinetobacter baumannii isolates obtained from hospitalized patients in southwestern Iran. Infec Med. 2018;26(1):67–76.

5. Ozdemir H, Kendirli T, Ergun H, Ciftci E, Tapsiz A, Guriz H, Aysev D, Ince E, Dogru U. Nosocomial infections due to Acinetobacter baumannii in a pediatric intensive care unit in Turkey. Turk J Pediatr. 2011;53(3):255–60.

6. Cai XF, Sun JM, Bao LS, Li WB. Risk factors and antibiotic resistance of pneumonia caused by multidrug resistant Acinetobacter baumannii in pediatric intensive care unit. World J Emerg Med. 2012;3(3):202–7.

7. Jin ZP, Cheng YB, Wang QS, Wang Q, Ge YJ. Drug resistance of Acinetobacter baumannii isolated from children in the pediatric intensive care unit. Zhongguo Dang Dai Er Ke Za Zhi. 2012;14(3):229–30.

8. Kapoor K, Jain S, Jajoo M, Dubkhil S, Dabas V, Manchanda V. Risk factors and predictors of mortality in critically ill children with extensively-drug resistant Acinetobacter baumannii infection in a pediatric intensive care unit. Iran J Pediatr. 2014;24(5):569–74.

9. Huang YC, Su LH, Wu TL, Leu HS, Hsieh WS, Chang TM, Lin TY. Outbreak of Acinetobacter baumannii bacteraemia in a neonatal intensive care unit: clinical implications and genotyping analysis. PEDIATR INFECT DIS J. 2002;21(12):1105–1109.

10. Pagonarichkli S, Tribuddharat C, Chuancharoen R. Distribution and expression of the Ade multidrug efflux systems in Acinetobacter baumannii clinical isolates. Can J Microbiol. 2016;62(9):794–801.

11. Ni W, Han Y, Zhao J, Wei C, Cui J, Wang R, Liu Y. Tigecycline treatment experience against multidrug-resistant Acinetobacter baumannii infections: a systematic review and meta-analysis. Int J Antimicrob Agents. 2016;47(2):107–116.

12. Pannek S, Higgins PG, Steinke P, Jonas D, Akova M, Bohnert JA, Seifert H, Kern WV. Multidrug efflux inhibition in Acinetobacter baumannii: comparison between 1-(1-naphthylmethyl)-piperazine and phenyl-arginine-beta-naphthylamide. J Antimicrob Chemother. 2006;57(5):970–974.
13. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect. 2006;12(9):826–836.

14. Segal H, Nelson EC, Elshis BG. Genetic environment and transcription of ampC in an Acinetobacter baumannii clinical isolate. Antimicrob Agents Chemother. 2004;48(2):612–614.

15. Yang YS, Lee YT, Wang YC, Chiu CH, Kuo SC, Sun JR, Yin T, Chen TL, Lin JC, Fung CP, et al. Molecular epidemiology of carbapenem non-susceptible Acinetobacter nosocomialis in a medical center in Taiwan. INFECT GENET EVOL. 2015;31:305–311.

16. Ruan Z, Chen Y, Jiang Y, Zhou H, Zhou Z, Fu Y, Wang H, Wang Y, Yu Y. Wide distribution of CC92 carbapenem-resistant and OXA-23-producing Acinetobacter baumannii in multiple provinces of China. Int J Antimicrob Agents. 2013;42(4):322–328.

17. Lee Y, Kim YR, Kim J, Park YJ, Song W, Shin JH, Uhm Y, Lee K, Lee SH, Cho JH, et al. Increasing prevalence of blaOXA-23-carrying Acinetobacter baumannii and the emergence of blaOXA-182-carrying Acinetobacter nosocomialis in Korea. Diagn Microbiol Infect Dis. 2013;77(2):160–163.

18. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among Acinetobacter spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. J Antimicrob Chemother. 2009;63(1):55–59.

19. Safari M, Mozaffari NA, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in Acinetobacter baumannii strains isolated from patients of intensive care units (ICU). SAUDI J BIOL SCI. 2015;22(4):424–429.

20. Liu LL, Li SJ, Ruan Z, Fu Y, Fu YQ, Wang YF, Yu YS. Dissemination of blaOXA-23 in Acinetobacter spp. in China: main roles of a conjugative plasmid pAZ221 and transposon Tn2009. Antimicrob Agents Chemother. 2015;59(4):1998–2005.

21. Hu Z, Wang Z, Liu D, Chen P, Wang H, Chen Y, Zhao X, Shi Y. Clinical and molecular microbiological characteristics of carbapenem-resistant Acinetobacter baumannii strains in an NICU. PEDIATR INT. 2011;53(6):867–872.

22. Carvalho KR, Carvalho-Assef AP, Santos LG, Pereira MJ, Asensi MD. Occurrence of blaOXA-23 gene in imipenem-susceptible Acinetobacter baumannii. Mem Inst Oswaldo Cruz. 2011;106(4):505–506.

23. Rumbo C, Gato E, Lopez M, Ruiz DAC, Fernandez-Cuenca F, Martinez-Martinez L, Vila J, Pachon J, Cesneros J, Rodriguez-Bano J, et al. Occurrence of efflux pumps, porins, and beta-lactamases to multidrug resistance in clinical isolates of Acinetobacter baumannii. Antimicrob Agents Chemother. 2013;57(11):5247–5257.

24. Wieczorek P, Sacha P, Hauchold T, Zorawski M, Krawczyk M, Tryniszewka E. Multidrug resistant Acinetobacter baumannii—the role of AdeABC (RND family) efflux pump in resistance to antibiotics. Folia Histochem Cytobiol. 2008;46(3):257–267.

25. Coyne S, Couvralin P, Perichon B. Efflux-mediated antibiotic resistance in Acinetobacter spp. Antimicrob Agents Chemother. 2011;55(3):947–953.

26. Yoon EJ, Courvalin P, Grillot-Courvalin C. RND-type efflux pumps in multidrug-resistant clinical isolates of Acinetobacter baumannii: major role for AdeABC overexpression and AdeRS mutations. Antimicrob Agents Chemother. 2013;57(7):2989–2995.

27. Marchand I, Darnier-Piollte L, Couvralin P, Lambert T. Expression of the RND-type efflux pump AdeABC in Acinetobacter baumannii is regulated by the AdeABC two-component system. Antimicrob Agents Chemother. 2004;48(9):3298–3304.

28. Mugnier PD, Poirel L, Nordmann P. Functional analysis of insertion sequence ISAba1, responsible for genomic plasticity of Acinetobacter baumannii. J BACTERIOL. 2009;191(7):2414–2418.

29. Srinivasan VB, Rajamohan G, Gebruyers WA. Role of AbeS, a novel efflux pump of the SMR family of transporters, in resistance to antimicrobial agents in Acinetobacter baumannii. Antimicrob Agents Chemother. 2009;53(12):5312–5316.

30. Magné S, Couvralin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in Acinetobacter baumannii strain BM4545. Antimicrob Agents Chemother. 2001;45(12):3375–3380.

31. Lee Y, Yum JH, Kim CK, Yang DJ, Jeon EH, Jeong SH, Ahn JY, Lee K. Role of OXA-23 and AdeABC efflux pump for acquiring carbapenem resistance in Acinetobacter baumannii strain carrying the blaOXA-66 gene. ANNI CLIN LAB SCI. 2010;40(1):43–48.

32. Hu WS, Yao SM, Fung CP, Hsieh YP, Liu JP, Lin JF. An OXA-66/OXA-51-Like Carbapenemase and Possibly an Efflux Pump Are Associated with Resistance to Imipenem in Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy. 2007;51(11):3844–3852.