Resistance traits and molecular characterization of multidrug-resistant Acinetobacter baumannii isolates from an intensive care unit of a tertiary hospital in Guangdong, southern China

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Abstract
Purpose This study aims to characterize antimicrobial resistance (AMR) of all the non-duplicated Acinetobacter baumannii strains isolated from an intensive care unit in a tertiary hospital during the period of January 1 to December 31, 2015.
Methods A. baumannii (n = 95 strains) isolated from patients was subjected to antimicrobial susceptibility test (AST) by Vitek 2 Compact system to determine minimum inhibitory concentrations, followed by genotyping by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). Resistance genes of interest were PCR amplified and sequenced.
Results All isolates were qualified as MDR, with a resistance rate of >80% to 8 antimicrobials tested. In terms of beta-lactamase detection, the blaOXA23, blaTEM-1, and armA genes were detected frequently at 92.63%, 91.58%, and 88.42%, respectively. The metallo-β-lactamase genes blaIMP and blaVIM were undetected. Aph (3')-I was detected in 82 isolates (86.32%), making it the most prevalent aminoglycoside-modifying enzyme (AMEs) encoding gene. In addition, ant (3")-I was detected at 30.53%, while 26.32% of the strains harbored an aac (6')-Ib gene. ERIC-PCR typing suggested moderate genetic diversity among the isolates, which might be organized into 10 distinct clusters, with cluster A (n = 86 isolates or 90.53%) being the dominant cluster.
Conclusions All of the A. baumannii strains detected in the ICU were MDR clones exhibiting extremely high resistance to carbapenems and aminoglycosides as monitored throughout the study period. They principally belonged to a single cluster of isolates carrying blaOXA23 and armA co-producing different AMEs genes.

Keywords Acinetobacter baumannii · Antimicrobial resistance · Resistance genes · Healthcare-associated infection · Intensive care unit

Introduction
Acinetobacter baumannii (A. baumannii) is an opportunistic pathogen adept at colonizing and thriving in the hospital environment. In the recent decade, carbapenemase-producing multidrug-resistant (MDR) A. baumannii has emerged as a prominent cause of healthcare-associated infections (HAIs) notably at intensive care units (ICUs), and its incidence seems to be ascending alarmingly in parts of China (He et al. 2011; Li et al. 2018; Bitrian et al. 2012; Behdad et al. 2020). Patients undergoing invasive procedures, immunosuppressive therapy, or treatment with broad-spectrum antibiotics are vulnerable to HAIs caused by A. baumannii, particularly in the contexts of ventilator-associated pneumonia, bacteremia, septicemia, urinary tract, and wound
infections (Bitrian et al. 2012; del Mar et al. 2005; Freire et al. 2016; Gomez-Arrebola et al. 2021).

By virtue of its extraordinary aptitude to survive in the hospital environment and to develop extremely high resistance to an array of common antibiotics including aminoglycoside and carbapenem classes of antibiotics, A. baumannii has become a major challenge to medical care at the ICU (Shimose et al. 2016; Molter et al. 2016; Shamsizadeh et al. 2017). One of the most prevalent sequence types (ST) of epidemic clones in China is ST208, which has gained notoriety for causing outbreaks in local ICUs (Bahador et al. 2015). Analysis on genomic relatedness among clinical isolates can help detect an epidemic strain, which can also offer information on infection diagnosis and anti-infection treatment.

Although substantial efforts have been made over the years in monitoring the epidemiicity and AMR trends of A. baumannii in China, the scope of previous studies tends to be limited to highly populous urban centers in northern and eastern China (Ning et al. 2017; Zhou et al. 2018). In southern China including Guangdong province (population 108.5 million), where the humid subtropical climate indeed favors microbial growth, epidemiological surveys on A. baumannii in HAIs were only with moderate frequencies and again limited to highly populous urban centers in northern and eastern Guangdong. A total of 95 non-duplicated A. baumannii isolates were systematically collected from patients’ samples during the period of January 1 to December 31, 2015. This study had been reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. The study was given a waiver of informed consent on the ground that it focuses only on characterizing bacterial isolates and involves no patient’s information.

Antimicrobial susceptibility

All isolates were first identified by using Vitek 2 Compact system (bioMérieux, France) and their antimicrobial susceptibility profiles obtained by using the Gram negative susceptibility cards (GN16 cards), according to the manufacturer’s instructions. Antimicrobial susceptibility test (AST) results for MICs (minimum inhibitory concentrations) were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI 2015). Confirmed A. baumannii isolates were stored at – 80 °C for subsequent experiments.

Detection of antimicrobial resistance genes

Whole genomic DNA was extracted by using TIANamp Bacteria DNA kit (Tiangen Biotech, China), according to the manufacturer’s instructions. Detection of antimicrobial resistance (AMR) genes by PCR amplification was carried out with specific primers (Lin et al. 2015; Chen et al. 2010) (see details in Table 1) to screen for the following genes of interest: extended-spectrum β-lactamases (ESBLs) encoding gene (blaTEM-1, blaSHV), metallo-β-lactamases encoding genes (blaIMP, blaVIM-2, blaNDM-1), OXA carbapenemases encoding genes (blaOXA23, blaOXA24, blaOXA58), aminoglycoside-modifying enzyme (AME) encoding genes (aac(6’)-Ib, ant(3’)-I, aph(3’)-I), and 16 s rRNA methylase encoding gene (armA) were detected. For PCR amplification, the following thermal cycling conditions were adopted: initial denaturation at 94 °C for 3 min, followed by 30 cycles (94 °C for 1 min, 58–62 °C for 1 min, and 72 °C for 1 min), and a final extension step of 8 min at 72 °C. PCR products were separated by electrophoresis (at 100 V through a 1% agarose gel in 0.5 × TBE running buffer), stained with ethidium bromide, and observed under ultraviolet light. Identity of all PCR products was confirmed by DNA sequencing (Beijing Genomics Institute, BGI).

Materials and methods

Research settings and bacterial isolates

This study was conducted at the ICU of a tertiary-level teaching hospital affiliated to the Shantou University Medical College (SUMC) in Shantou City in Guangdong, a populous province in southern China. The hospital (1816 inpatient beds) serves the Chaoshan metropolitan area in eastern Guangdong. A total of 95 non-duplicated A. baumannii isolates were systematically collected from patients’ samples during the period of January 1 to December 31, 2015. This study had been reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. The study was given a waiver of informed consent on the ground that it focuses only on characterizing bacterial isolates and involves no patient’s information.

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Table 1  Primer sequences used in this study for detecting resistance genes

| Target gene | Sequence 5’→3’ | Annealing temp. (°C) | Ampli-con size (bp) |
|-------------|----------------|----------------------|---------------------|
| blaTEM-1    | ACCAGAAGACGCTGGTGAAA TGAACCCCCCTGCTTGATAG | 57 | 724 |
| BlaSHV      | TTATCTCCTGTAGGGCACC GATTTGCTATTTGCTCGG | 55 | 795 |
| blaIMP      | ATTTGAGAAGATTTGAGAAGCCTTAAACGCTGCTCCATGT | 56 | 621 |
| blavIM-2    | AGTCTCCAGGCATCTTCAT CAAACCACCATAGAGCA | 57 | 505 |
| blaKDR-1    | GGTTCGGGATCTGGTTTC CCGAAATGCGATCGACGATC | 55 | 621 |
|blaOXA23     | TTTCGGTTGTACGGTTCA AACAGGCCCCTTTGGGTTT | 57 | 646 |
|blaOXA24     | GITTCTCTACGTCATGCATTTCATCT CCAACCCAGTCAACCAACTT | 55 | 664 |
|blaOXA58     | CCACTCGGCTTTTTCCTACGA TCTCACCAGCTTCTCTTGGCAT | 57 | 837 |
|aac(6’)-Ib  | TTGCGATGCTCATAGTGGGCTA CTCGAAATGCGCTGGGTTT | 57 | 482 |
|ant(3’)-I    | GCCATACAGCGATTTGATTG | 58 | 306 |
|aph(3’)-I   | AAGCAACGCTATGTTCTGTCAT | 58 | 333 |
|armA        | TGAAAGGTGTGTTCCATCTTGTA TCAATCCCTACAACCCTCGAATCA | 57 | 669 |

Genotyping of isolates

For determination of genetic relatedness of the isolates, enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) was performed with primer ERIC2 (5’-AAG TAAGTGACTGGGGTTGAGG-3’) (Bahador et al. 2015) to amplify the conserved sequences of bacterial strains, by using the following thermal cycling conditions: initial denaturation at 94 °C for 5 min, 4 cycles (94 °C for 1 min, 26 °C for 1 min, 72 °C for 1 min), then 40 cycles (94 °C for 30 s, 40 °C for 30 s, and 72 °C for 1 min), and extension at 72 °C for 5 min. To resolve the PCR products, each PCR product was analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Results for ERIC-PCR banding patterns were appraised by the software Quantity One (version 4.6.2) and scored as absent (0) or present (1) to construct a dendrogram according to the unweighted pair group (UPGMA) method, using the software NTSYS-pc (version 2.10e). Isolates with more than 90% similarity were considered as belonging to the same cluster.

Statistics analysis

Statistical analysis on antimicrobial susceptibility rates was analyzed by WHONET 5.6 software.

Results

Isolate characteristics and resistance rates

In this study, a total of 95 non-duplicative A. baumannii strains were isolated from ICU patients. Strains from male patients evidently outnumbered those from females at a ratio of 65 (68.42%) to 30 (31.58%). Affected patients had a mean age of 61.93 ± 1.87 years (range of 7 to 89 years old). The major isolation sites were sputum (n = 91), puncture fluid (n = 2), and stool (n = 2). As shown in Table 2, AST results suggested that all isolates could be qualified as multidrug-resistant (MDR) A. baumannii, which were highly resistant to 8 antibiotics including cefepime (FEP), ceftriaxone (CRO), imipenem (IPM), gentamicin (GEN), tobramycin
(TOB), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT), and piperacillin/tazobactam (TZP) while levofloxacin (LVX) might not be deemed any more efficient than the abovementioned agents against A. baumannii, as it had a notable rate of intermediate-level resistance (37.90%) as shown in Fig. 1.

Genotypic patterns in ERIC-PCR analysis

ERIC-PCR was used to compare the genetic relatedness among the A. baumannii isolates. All PCR banding patterns ranging from 550 to 2000 bp were analyzed by the NTSYS software to construct a dendrogram, as shown in Fig. 2. In general, 86 (or 90.53%) of the analyzed A. baumannii strains belonged to a major cluster A, while the remaining 9 isolates exhibited substantially different banding patterns, additionally designated as isolates B, C, D, E, F, G, H, J, and K. In a longitudinal analysis, strains belonging to cluster A were detectable throughout the study period in 2015, indicating that members of this cluster correspond to the major clone causing the epidemic of MDR A. baumannii at our hospital.

Determination of antimicrobial resistance genes

Analysis on AMR genes suggests that the A. baumannii isolates included in this study had high carriage rates for some specific AMR genes. Among the 95 strains, 87 (91.58%) were tested positive for the ESBL encoding gene blaTEM-1. In terms of detection of carbapenemase genes, 88 strains (92.63%) were found to harbor the blaOXA23 gene. Gene armA, a member of 16S rRNA methylases, was detected in 84 isolates (88.42%), while the most prevalent AME encoding gene aph (3′)-I was found in 82 isolates (86.32%). In comparison, 29 (30.53%) and 25 (26.32%) of the isolates harbored the ant (3″)-I and aac (6′)-Ib genes, respectively. In further analysis, the genes blaSHV, blaIMP, blaVIM-2, blaNDM-1, blaOXA24, and blaOXA58 were undetected in any of the A. baumannii strains (Table 3).

Through genotyping and detection of resistance genes, we classified the 95 isolates of A. baumannii in this study, as shown in Table 3. Cluster A could be categorized into 9 subtypes on the basis of different AMR gene combinations. The most prevalent subtype in cluster A was subtype Ai, comprising 55 (63.95%) isolates expressing the genes blaOXA23, blaTEM-1, armA, and aph (3′)-I. Among subtype Ai isolates, 38 (44.19%) were non-susceptible to all of the antibiotics tested. Twenty-two (25.58%) isolates in group Aii harbored 6 different genes, non-susceptible to cefepime and ceftriaxone. The aminoglycoside resistance genes aph (3′)-I and ant (3″)-I were only detected in subtypes Aviii and Aix, but surprisingly they were susceptible to gentamycin and tobramycin. The rest of the isolates showed various combinations of AMR genes, presumably giving rise to different resistance patterns.

Discussion

ERIC-PCR, a genotyping method premised on amplification of conserved regions of genomic DNA, has the advantage of facile instrumentation and reliability comparable to pulsed field gel electrophoresis (PFGE). It has been proven useful for determining genomic relationship across strains with heterogeneous backgrounds (Cartelle Gestal et al. 2016; Ece et al. 2015). In the present study, a dendrogram based on ERIC-PCR results identifies 1 cluster (cluster A) and other 9 distinct isolates, suggesting that a single dominant clone of MDR A. baumannii prevailed in the ICU in 2015 (Jan. to Dec.). In terms of resistance phenotype, strains in cluster A were consistently more non-susceptible to all tested antibiotics than other strains. By using ERIC-PCR as a genotyping method, Ning and coworkers reported carbapenem-resistant clones of A. baumannii spreading at an ICU in western China (Ning et al. 2017). Chen and coworkers also described

| Antibiotics | Resistance | Intermediate | Susceptible | MIC range | MIC50 | MIC90 |
|-------------|------------|--------------|-------------|-----------|-------|-------|
|             | n          | Rate (%)     | n           | Rate (%)  |       |       |
| FEP         | 89         | 93.68        | 0           | 0.00      | 6     | 6.32  |
|             |            | 1–64         | 64          |           |       |
| CRO         | 88         | 92.63        | 7           | 7.37      | 0     | 0.00  |
|             |            | 1–64         | 64          |           |       |
| IPM         | 89         | 93.68        | 0           | 0.00      | 6     | 6.32  |
|             |            | 1–16         | 16          |           |       |
| GEN         | 85         | 89.47        | 2           | 2.11      | 8     | 8.42  |
|             |            | 1–16         | 16          |           |       |
| TOB         | 82         | 86.32        | 0           | 0.00      | 13    | 13.68 |
|             |            | 1–16         | 16          |           |       |
| LVX         | 51         | 53.68        | 36          | 37.90     | 8     | 8.42  |
|             |            | 0.25–8       | 4           |           |       |
| CIP         | 90         | 94.74        | 0           | 0.00      | 5     | 5.26  |
|             |            | 0.25–4       | 4           |           |       |
| SXT         | 77         | 81.05        | 0           | 0.00      | 18    | 18.95 |
|             |            | 1–16         | 16          |           |       |
| TZP         | 79         | 83.16        | 4           | 4.21      | 12    | 12.63 |
|             |            | 4–28         | 128         |           |       |

FEP cefepime, CRO ceftriaxone, IPM imipenem, GEN gentamycin, TOB tobramycin, CIP ciprofloxacin, SXT trimethoprim/sulfamethoxazole, TZP piperacillin/tazobactam
a major epidemic strain spreading at different hospital units in Hunan province of southern China (Chen et al. 2016). In our study, the spread of A. baumannii strains in the ICU lasted for a substantial period and their resistance rates to antibiotics were extremely high. We found that among the 9 subtypes of strains within cluster A, the most frequent type of AMR gene combination was blaOXA23 -blaTEM1 -aph (3')-I-armA. Strains harboring this gene combination could be routinely isolated throughout the study period, suggesting the existence of entrenched extrinsic factors favoring their spread. Cross-transmission and contamination within the ward environment might underpin this process, which calls for greater awareness for monitoring and timely disinfection of the ward environment (Protano et al. 2019).

In our study, we found that multidrug-resistant Acinetobacter baumannii (MDRAB) strains simultaneously
Fig. 2 Dendrogram depicting genetic relationships of *A. baumannii* isolates.
Table 3  Classification of MDR A. baumannii isolates based upon ERIC-PCR and genotypic profiles

| Cluster | n (%) | Subtype (n) | Resistance genes<sup>a</sup> | Resistance patterns (R+I)<sup>b</sup> |
|---------|-------|------------|-------------------------------|--------------------------------------|
| A       | 86 (90.35) | Ai (55) | blaoXA23, blaTEM-1, armA, aph(3′)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT (40) |
|         |        | Aii (22) | blaoXA23, blaTEM-1, armA, aph(3′)-I, aac(6′)-Ib, ant(3″)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT (20) |
|         |        | Aiii (2) | blaoXA23, blaTEM-1, armA, aac(6′)-Ib, ant(3″)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP |
|         |        | Aiv (1) | blaoXA23, blaTEM-1, armA, ant(3″)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT |
|         |        | Av (1) | blaoXA23, blaTEM-1, aph(3′)-I, ant(3″)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT |
|         |        | Avii (2) | blaoXA23, blaTEM-1, armA, aac(6′)-Ib, ant(3″)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT |
|         |        | Avii (1) | blaoXA23, blaTEM-1, aph(3′)-I | FEP, CRO, IPM, LVX, CIP |
|         |        | Aiv (1) | blaoXA23, ant(3″)-I | FEP, CRO, IPM, SXT |
| B       | 1 (1.05) | – | blaoXA23, armA | CRO |
| C       | 1 (1.05) | – | – | CRO, GEN, LVX, CIP, SXT |
| D       | 1 (1.05) | – | – | CRO, CIP |
| E       | 1 (1.05) | – | – | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT |
| F       | 1 (1.05) | – | blaTEM-1 | FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT |
| G       | 1 (1.05) | – | blaoXA23, blaTEM-1, armA, aph(3′)-I | FEP, CRO, IPM, GEN, TOB, LVX, CIP |
| H       | 1 (1.05) | – | aph(3′)-I | CRO |
| I       | 1 (1.05) | – | – | CRO |
| J       | 1 (1.05) | – | – | CRO, CIP, SXT |

<sup>a</sup>All isolates were tested negative for blasHV, blasVIM-2, blasNDM, blasOXA58, and blaoXA24.

<sup>b</sup>R+I resistant and intermediate.

carrying the blaoXA23 gene and multiple aminoglycoside resistance genes are apparently spreading in southern China. The carriage of blaoXA23 carbapenemases in A. baumannii has been documented worldwide and blaoXA23 was one of the most prevalent carbapenemase genes detected in Chinese hospitals (Ruan et al. 2013; Shoja et al. 2017). While the prevalence of A. baumannii co-expressing aminoglycoside resistance genes and carbapenemase genes has been reported in eastern China (Wang et al. 2016), to the best of our knowledge, there have been no studies on the epidemicity of A. baumannii co-carrying AMR genes against aminoglycosides and carbapenemases in southern China. It is noteworthy that the blatem-1 gene was the most prevalent ESBL gene in the present study, which differs from a previous study, where blactx-m was reported to be the predominant ESBL gene (Mahamat et al. 2016).

Aminoglycoside resistance of A. baumannii has been reported with increasing frequency in China in recent years (Gao et al. 2017; Jiang et al. 2014; Lin et al. 2015). In a study on A. baumannii from Jiangsu province, China, the most prevalent AMEs were identified as aac(3′)-I and aac(6′)-Ib (Wen et al. 2014). The resistance rates for GEN and TOB in this study were 89.47% and 86.32%, respectively (Table 2), and the most representative aminoglycoside resistance gene combination in the present study was armA-aph(3′)-I (58.95%). Interestingly, the isolate exhibited susceptibility to both GEN and TOB with only armA gene being detected. The most prevalent of the AMEs was aph(3′)-I (86.32%), followed by ant(3″)-I (30.53%), with 84 (88.42%) of the strains carrying armA. In addition, high levels of aminoglycoside resistance co-occurring with carbapenem resistance have been reported in epidemic clones of A. baumannii from western China (Lin et al. 2015). The imipenem resistance rates of A. baumannii were extremely high in China and numerous studies have raised concerns over the emergence and spread of imipenem-resistant A. baumannii in hospitals (Neves FC et al. 2016). Resistance rates for imipenem reported in different Chinese ranged from 58 to 100% (Jiang et al. 2016; Zong et al. 2008; Ji et al. 2014; Wu et al. 2015). Our current results suggested that efficacy of carbapenemases as treatment for MDR-AB infections seemed to be fast diminishing, especially in ICU contexts. A growing body of literature documents blaoXA23 as a predominant carbapenemase genotype among epidemic clones in China (Chen et al. 2017;
Thummeepak et al. 2016) and outbreaks caused by bla\textsubscript{OXA23} producing \textit{A. baumannii} paralleled those occurring worldwide (Neves et al. 2016; Hammoudi et al. 2015; Novovic et al. 2015; Koh et al. 2007; Martins et al. 2009). In this present study, we found that 88 of the \textit{A. baumannii} strains (92.63\%) harbored a bla\textsubscript{OXA23} gene, suggestive of a level of prevalence seen in other parts of China (Ana Kovacic et al. 2017). Collectively, we proposed that the presence of bla\textsubscript{OXA23} gene could be a cardinal molecular determinant of carbapenem resistance in our study.

**Conclusion**

In this study, we described the resistance traits and genetic relatedness of MDR \textit{A. baumannii} strains with high resistance that prevailed at the ICU of a teaching tertiary hospital in the Chaoshan area of Guangdong province, a populous yet epidemiologically overlooked region in southern China. Those strains highly resistant to carbapenem and aminoglycoside including imipenem and gentamycin/tobramycin may be associated with the carriage of bla\textsubscript{OXA23} and AME genes as determined in PCR assays. A single cluster A of epidemic clones seemed to dominate the spread of MDR \textit{A. baumannii} in the ICU of our hospital. Surveillance work in this study represents a first step towards a better understanding of MDR \textit{A. baumannii} as a causative agent in ICUs, which calls for greater attention to continued monitoring and rational use of antibiotics.

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**Author contribution** Yuan-Chun Huang designed the experiments, carried out the study, interpreted the data, and reviewed the manuscript. Zuo-Ran Chen and Hui-Wu Guo designed and performed the experiments and wrote the paper. Jun Liu, Mao-Zhang Fu, Ying-Kun Qiu, and Qing Pan collected and analyzed the data. All the authors read and approved the final manuscript.

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**Code availability** Not applicable.

**Declarations**

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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