Identification of antibiotic resistance genes in the multidrug-resistant *Acinetobacter baumannii* strain, MDR-SHH02, using whole-genome sequencing

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Abstract. This study aimed to investigate antibiotic resistance genes in the multidrug-resistant (MDR) *Acinetobacter baumannii* (A. baumanii) strain, MDR-SHH02, using whole-genome sequencing (WGS). The antibiotic resistance of MDR-SHH02 isolated from a patient with breast cancer to 19 types of antibiotics was determined using the Kirby-Bauer method. WGS of MDR-SHH02 was then performed. Following quality control and transcriptome assembly, functional annotation of genes was conducted, and the phylogenetic tree of MDR-SHH02, along with another 5 *A. baumanii* species and 2 *Acinetobacter* species, was constructed using PHYLIP 3.695 and FigTree v1.4.2. Furthermore, pathogenicity islands (PAIs) were predicted by the pathogenicity island database. Potential antibiotic resistance genes in MDR-SHH02 were predicted based on the information in the Antibiotic Resistance Genes Database (ARDB). MDR-SHH02 was found to be resistant to all of the tested antibiotics. The total draft genome length of MDR-SHH02 was 4,003,808 bp. There were 74.25% of coding sequences to be annotated into 21 of the Clusters of Orthologous Groups (COGs) of protein terms, such as ‘transcription’ and ‘amino acid transport and metabolism’. Furthermore, there were 45 PAIs homologous to the sequence MDRSHH02000806. Additionally, a total of 12 gene sequences in MDR-SHH02 were highly similar to the sequences of antibiotic resistance genes in ARDB, including genes encoding aminoglycoside-modifying enzymes [e.g., *aac(3)-Ia*, *ant(2”)-Ia*, *aph(3)ib* and *aph(3”)-Ia*], β-lactamase genes (*bl2b_tem1* and *bl2b_tem1*), sulfonamide-resistant dihydropteroate synthase genes (*sul1* and *sul2*), *catb3* and *tetb*. These results suggest that numerous genes mediate resistance to various antibiotics in MDR-SHH02, and provide a clinical guidance for the personalized therapy of *A. baumanii*-infected patients.

Introduction

*Acinetobacter baumannii (A. baumanii)* is a notable pathogen that causes hospital-acquired infections among immune-compromised patients, accounting for 5% of Gram-negative infections (1). Due to a strong resistance to desiccation and multiple antibacterial agents, the widespread dissemination of multidrug-resistant (MDR) *A. baumanii* strains has been a threat to hospitalized patients in recent years (2).

Antibiotic resistance determinants play pivotal roles in whether or not *A. baumanii* will flourish in the host (3). For instance, the expression of β-lactamase genes [e.g., oxacillinase (OXA)-235 gene, *bla*OXA-235 and *bla*TEM,] has been shown to be involved in antibiotic resistance (4–6). An 86-kb region AbaR resistant to heavy metal and antibiotics has been found in a MDR isolate AYE (7), indicating the important role of AbaR in the spread of *A. baumanii* in hospitals (8,9). Furthermore, other resistance determinants, such as macrolide (*msrA/msrB*), aminoglycoside (e.g., *aacC1*, *armA*, and *aphA1*) and tetracycline [e.g., *tet* (39), *tet* (A), and *tet* (B)] have been identified in various *A. baumanii* isolates (10).

Bacterial whole-genome sequencing (WGS) has enhanced our ability to evaluate antibiotic resistance determinants. For example, a AbaR-type genomic resistance island, AbaR22, has been identified in the MDR *A. baumanii* strain, MDR-ZJ06, via WGS (11). A whole-genome comparison detected 18 putative single nucleotide polymorphisms (SNPs) between 2 pre- and post-therapy *A. baumanii* isolates (12). Furthermore, 10 types of AbaR resistance islands were identified in 2 *A. baumanii* isolates using WGS (13). Despite increased research on antibiotic resistance determinants in *A. baumanii*, the molecular mechanisms of antibiotic resistance in MDR *A. baumanii* are
not yet fully understood, and various antibiotic resistance genes have not been detected.

In the present study, we applied WGS to obtain the whole genomic sequence of the MDR A. baumanii strain, MDR-SHH02, isolated from a patient with breast cancer. Furthermore, the antibiotic resistance of MDR-SHH02 to multiple antibiotics was determined, and potential antibiotic resistance genes in MDR-SHH02 were predicted. The results of our study may enhance our understanding of the molecular mechanisms of antibiotic resistance in MDR A. baumanii, and provide a clinical guidance for the therapy of A. baumanii-infected patients.

Materials and methods

Isolation and identification of A. baumanii strain. The clinical MDR A. baumanii strain, named MDR-SHH02, was isolated from the blood obtained from a 65-year-old woman with terminal-stage breast cancer at Shanghai Sixth People’s Hospital Affiliated to Shanghai Jiaotong University, Shanghai, China. This patient had received a double mastectomy and nearby lymph node excision. After being discharged from the hospital, this patient was hospitalized again due to symptoms of fever, cough (lasting for days) and shortness of breath. During her second hospital administration, she received several antimicrobial therapies, including maxipime, imipenem, methyl-prednisolone, levofloxacin and cefoperazone-sulbactam sodium. A. baumanii was positive in the blood culture and sputum culture. The results of the antimicrobial susceptibility test revealed that the A. baumanii strain was resistant to multiple commonly used antibiotics. This study was approved by the Shanghai Health and Family Planning Commission Foundation (Shanghai, China), and informed consent was obtained from the patient.

The isolated strain was inoculated onto blood agar plates and then incubated in an atmosphere of 5% CO₂ at 35°C for 48 h. Afterwards, this strain was identified using morphological and biochemical tests according to standard methods (14). Colonies with typical morphological and biochemical characteristics of Acinetobacter were cultivated on 5% sheep blood agar and identified using an automated Microscan ® system (Dade Behring, Inc., West Sacramento, CA, USA). The A. baumanii strain was stored at -70°C in skim milk for further analyses.

Antibiotic resistance test for A. baumanii MDR-SHH02. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines (15), disc diffusion assay (DDA) with dry wafers saturated by 19 types of antibiotics, including gentamicin (10 µg/wafer), tobramycin (30 µg/wafer), amikacin (30 µg/wafer), ampicillin-sulbactam (10/10 µg/wafer), ceftazidime (30 µg/wafer), ciprofloxacin (5 µg/wafer), levofloxacin (5 µg/wafer), imipenem (10 µg/wafer), meropenem (10 µg/wafer), piperacillin/tazobactam (100/10 µg/wafer), ticarcillin/clavulanic acid (75/10 µg/wafer), cefepime (30 µg/wafer), cefotaxime (30 µg/wafer), ceftazidime (30 µg/wafer), doxycycline (30 µg/wafer), minocycline (30 µg/wafer), tetracycline (30 µg/wafer), piperacillin(100 µg/wafer) and trimethoprim-sulfa-methoxazole (1.25/23.75 µg/wafer) (all from Oxoid, Ltd., Basingstoke, UK), were carried out using the Kirby-Bauer (KB) method, as previously described (16). Briefly, Mueller-Hinton agar (Oxoid, Ltd.) plates were overlaid with the inocula of the clinical A. baumanii strain, and the turbidity of the inocula was equivalent to the 0.5 McFarland standard. Subsequently, dry wafers saturated by antibiotics were placed on the surface of the agar, and plates were placed in an atmosphere of 5% CO₂ at 35°C. Following 24 h of culture, the diameter of the inhibition zone around each wafer was measured according to the CLSI criteria (15). In this test, Escherichia coli ATCC 25922, ATCC 35218 and Pseudomonas aeruginosa ATCC 27853 obtained from the Clinical Laboratory Center of the Ministry of Health were used as reference strains.

DNA preparation, library construction and sequencing. The genomic DNA of A. baumanii MDR-SHH02 was extracted using a bacterial genomic DNA purification kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer’s instructions. The Illumina sequencing library was then prepared using the Nextera™ DNA Sample Preparation kit (Illumina®-Compatible). Paired-end dual index 2x90 bp sequencing was fulfilled following the Illumina HiSeq 2000. Sequencing was performed by Beijing Genomics Institute (BGI; Shenzhen, China). The sequencing data were uploaded to the public database the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) under the BioProject PRJNA256112 with BioSample accession no. SAMN02991371.

Quality control. For the raw sequencing data, the reads were cleaned by removing the empty reads, adapter sequences and reads with q <10% using the SeqPrep program (https://github.com/jstjohn/SeqPrep) and Sickle (https://github.com/najoshi/sickle). In addition, the reads were trimmed by discarding the reads containing >30% bases with a Q-value <=20 in the 3' terminal, as well as reads with adapter sequences (the length of overlapping sequences between adaptor and read was at least >15 bp, and the number of mismatch bases was <3 bp).

Genome assembly. The clean reads were assembled using the short oligonucleotide analysis package SOAPdenovo (version 2.04; http://soap.genomics.org.cn/). To determine whether the GC content has a significant effect on sequencing randomness or not, the GC content and average depth of the genomic sequence were calculated without repetition as a unit of 500 bp.

Genome annotation. Genes in the assembled genomic sequence were predicted using Glimmer 3.0 (http://www.ccb.mast.duke.edu/software/glimmer/) (17), which is a system for identifying genes in DNA sequences of microorganisms, particularly bacteria, archaea and viruses.

Furthermore, tRNA and rRNA [5S, 16S, and 23S] RNA in the genome sequence were searched using TRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/) (18) and RNAmer 1.2 (http://www.ews.dtu.dk/services/RNAmer/) (19), respectively.

Additionally, tandem repeat sequences and clustered regularly interspaced short palindromic repeats (CRISPR) in the genomic sequence were predicted using the Tandem Repeat Finder (http://tandem.bu.edu/trf/trf.html) and CRISPR Finder (http://crispr.u-psud.fr/Server/) software, respectively.
Insertion sequences (ISs) were characterized using the IS Finder database (https://www-is.biotoul.fr/), and the parameter e was set as 1e-5, identity set as 35%. Besides, protein domains associated with the genomic sequence were predicted using the InterPro database (https://www.ebi.ac.uk/interpro/), and the parameter was set as -apl PlamA.

**Functional annotation of genes.** Sequence alignment of the amino acid sequences of genes to the Cluster of Orthologous Groups (COGs) of proteins database (http://www.ncbi.nlm.nih.gov/COG/) (20) was performed using the Basic Local Alignment Search Tool (BLASTP; version 2.0) program from NCBI (E-value ≤10−4) (21). We also performed sequence alignment of the amino acid sequences of genes to the NCBI non-redundant (NR) database (E-value ≤10−10, identity score ≥35%, and coverage length ≥80%). If the amino acid sequence of a gene was aligned to multiple sequences in the databases, the optimal result was retained.

**Construction of phylogenetic tree.** Based on the NCBI 16S rRNA gene database, 16S rRNA gene sequences of 5 *A. baumanii* strains, including ATCC 17978, ATCC 19606, CIP 70.34, DSM 30007 and *A. baumanii* JCM 68415, as well as 2 species belonging to *Acinetobacter* (A. *haemolyticus* ATCC 17906 and A. *bereniceae* ATCC 17924), were used to construct the phylogenetic tree, along with MDR-SHH02. Briefly, multiple sequence alignment was performed using ClustalW-2.1 (22). Subsequently, the software package PHYLIP 3.695 (http://evolution.genetics.washington.edu/phylip.html), along with the bootstrap algorithm, was used to construct the maximum likelihood phylogenetic tree, and the phylogenetic tree was visualized by FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

**Prediction of pathogenicity islands (PAIs).** The Pathogenicity Island database (PAIDB; http://www.paidb.re.kr/about_paidb.php), which is a web-based user-friendly resource and widely used for detecting PAIs in newly sequenced genomes (23), was utilized to predict PAIs in the genomic sequence of MDR-SHH02.

**Identification of antibiotic resistance genes.** To identify potential antibiotic resistance genes in the genomic sequence of MDR-SHH02, sequence alignment of the protein sequences of antibiotic resistance genes in the Antibiotic Resistance Genes database (ARDB; http://ardb.cbc.b.umd.edu/) (24) and MDR-SHH02 genomic sequence was conducted using BLASTP (E-value ≤10−10, identity score ≥90%, and coverage length ≥80%).

**Results**

**Antibiotic-resistance of *A. baumanii* MDR-SHH02.** The antibiotic-resistance assay revealed that the diameter of the inhibition zone for 17 types of antibiotics on MDR-SHH02 plates was 6 mm, apart from levofloxacin and minocycline (diameter, 10 mm) (Table I). According to the CLSI criteria, MDR-SHH02 was resistant to all of the tested antibiotics.

**Assembly and annotation of the genomic sequence of *A. baumanii* MDR-SHH02.** During the genome assembly, a total of 85 scaffolds were generated, and the scaffold N50 length was 131,822 bp. The total draft genome length of MDR-SHH02 was 4,003,808 bp, with 38.99% of GC content. There were 3,787 coding sequences, 62 tRNA sequences and 3 rRNA sequences in the genomic sequence. Moreover, 2 CRISPR and 36 tandem repeat sequences, as well as 29 ISs were predicted in the genomic sequence (Table II).

Furthermore, numerous protein domains were predicted in the genomic sequence of MDR-SHH02, such as the AcrB/AcrD/AcrF family (Table III).

**Functional annotation of the genomic sequence of *A. baumanii* MDR-SHH02.** According to the COG annotation, 74.25% (2,812/3,787) of coding sequences were annotated into 21 COG terms, which were divided into 3 categories: information storage of processing, cellular processes and signaling, and metabolism. Apart from the category of poorly characterized, most of the coding sequences were annotated into ‘transcription’ (number of coding sequences, 261) and ‘amino acid transport and metabolism’ (number of coding sequences, 261).

| Antibiotic name          | Diameter of inhibition zone on MDR-SHH02 plate (mm) | Antibiotic-resistance of MDR-SHH02 |
|--------------------------|------------------------------------------------------|-----------------------------------|
| Ampicillin-sulbactam     | 6                                                    | R                                 |
| Ceftazidime              | 6                                                    | R                                 |
| Ciprofloxacin            | 6                                                    | R                                 |
| Levofloxacin             | 10                                                   | R                                 |
| Imipenem                 | 6                                                    | R                                 |
| Meropenem                | 6                                                    | R                                 |
| Gentamicin               | 6                                                    | R                                 |
| Tobramycin               | 6                                                    | R                                 |
| Amikacin                 | 6                                                    | R                                 |
| Piperacillin/tazobactam  | 6                                                    | R                                 |
| Ticaricillin/clavulanic acid | 6                                                | R                                 |
| Cefepime                 | 6                                                    | R                                 |
| Cefotaxime               | 6                                                    | R                                 |
| Ceftriaxone              | 6                                                    | R                                 |
| Doxycycline              | 6                                                    | R                                 |
| Minocycline              | 10                                                   | R                                 |
| Tetracycline             | 6                                                    | R                                 |
| Piperacillin             | 6                                                    | R                                 |
| Trimethoprim-sulfamethoxazole | 6                                | R                                 |

Diameter of inhibition zone <15 mm is determined as drug-resistant (R); diameter of inhibition zone between 15 and 17 mm is determined as intermediate (I); and diameter of inhibition zone >17 mm is determined as drug-sensitive (S). *A. baumannii, Acinetobacter baumannii.*
sequences, 250) (Fig. 1A). The distribution of COG categories in the genomic sequence of *A. baumannii* MDR-SHH02 is shown in Fig. 1B.

**Analysis of the phylogenetic tree.** Based on the 16S rRNA gene sequences of *A. baumannii* in NCBI, the phylogenetic tree revealed that the genomic sequence of MDR-SHH02 was most similar to the 16S rRNA gene sequence of *A. baumannii* ATCC 17978 (Fig. 2).

**Analysis of PAIs.** During the process of bacterial infection, PAIs play pivotal roles in the evolution of pathogens and...
the development of diseases. In the genomic sequence of MDR-SHH02, a total of 45 PAIs homologous to the sequence MDRSHH02000806 were detected (Table IV). Most of the PAIs were previously identified from *Escherichia coli* [e.g., locus of enterocyte effacement (LEE)] and *Pseudomonas* (e.g., PAPI-1 and T-PAI).

**Screening of antibiotic resistance genes.** To reveal the genes relevant to the antibiotic resistance of MDR-SHH02, sequence alignment of the protein sequences of antibiotic resistance genes in ARDB and MDR-SHH02 genomic sequence was performed. Based on the selection criteria, a total of 12 gene sequences (e.g., MDRSHH02002408, MDRSHH02000600 and MDRSHH02000597) in MDR-SHH02 were highly similar to the resistance genes in ARDB.

**Table II.** The results of genome assembly and annotation of *A. baumannii* MDR-SHH02.

| Feature                        | Statistics          |
|--------------------------------|---------------------|
| Length of total draft genome length (bp) | 4,003,808          |
| No. of scaffolds                | 85                  |
| Length of scaffold N50 (bp)     | 131,822             |
| GC content (%)                  | 38.99               |
| No. of coding sequences         | 3,787               |
| No. of tRNAs                    | 62                  |
| No. of rRNAs                    | 3                   |
| No. of CRISPR                   | 2                   |
| No. of tandem repeat sequences  | 36                  |
| No. of insertion sequences      | 29                  |

*A. baumannii, Acinetobacter baumannii; CRISPR, clustered regularly interspaced short palindromic repeats.*

**Table III.** The top 10 predicted protein domains in the genomic sequence of *A. baumannii* MDR-SHH02.

| Gene ID            | Protein domain                | P-value  |
|--------------------|-------------------------------|----------|
| MDRSHH02002231     | AcrB/AcrD/AcrF family         | <1.0E-300|
| MDRSHH02000985     | Monomeric isocitrate dehydrogenase | <1.0E-300|
| MDRSHH02003056     | AcrB/AcrD/AcrF family         | <1.0E-300|
| MDRSHH02000840     | AcrB/AcrD/AcrF family         | <1.0E-300|
| MDRSHH02003594     | AcrB/AcrD/AcrF family         | <1.0E-300|
| MDRSHH02000667     | AcrB/AcrD/AcrF family         | <1.0E-300|
| MDRSHH02000153     | Phosphoenolpyruvate carboxylase | 2.70E-292|
| MDRSHH02000199     | Urocanase                     | 5.20E-287|
| MDRSHH02000831     | Phosphoenolpyruvate carboxykinase | 2.70E-279|
| MDRSHH02003134     | AcrB/AcrD/AcrF family         | 5.50E-266|

*A. baumannii, Acinetobacter baumannii.*

![Figure 2. Whole-genome phylogeny of the genomes of *Acinetobacter baumannii* (A. baumannii) MDR-SHH02 and 5 sequenced *A. baumannii* genomes. The phylogenetic tree was rooted with 2 genomes of *Acinetobacter* species.](image-url)
to the sequences of antibiotic resistance genes in ARDB, such as \textit{aac(3)-Ia}, \textit{aac(6')-Ib}, \textit{ant(2'')-Ia} and \textit{aph(3')-Ia}. According to the antibiotics that were resistant by the 12 gene sequences, MDR-SHH02 was resistant to multiple antibiotics, such as
Table V. The potential gene sequences in *A. baumannii* MDR-SHH02 relevant to antibiotic resistance.

| Gene ID         | Resistance gene type from ARDB                                                                 | Resistance gene class                                      | Antibiotics                                      |
|-----------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------------------|------------------------------------------------|
| MDRSHH02002408  | **aac(3)-Ia** Aminoglycoside N-acetyltransferase, which modifies aminoglycosides by acetylation | Aminoglycoside                                             | Astromycin, gentamicin, sisomicin                |
| MDRSHH02000600  | **aac(6')-Ib** Aminoglycoside N-acetyltransferase, which modifies aminoglycosides by acetylation| Aminoglycoside                                             | Amikacin, dibekacin, isepamicin, netilmicin, sisomicin, tobramycin |
| MDRSHH02000597  | **ant(2'')-Ia** Aminoglycoside O-nucleotidylyltransferase, which modifies aminoglycosides by adenyllylation | Aminoglycoside                                             | Dibekacin, gentamicin, kanamycin, sisomicin, tobramycin |
| MDRSHH02000611  | **ant(3'')-Ia** Aminoglycoside O-nucleotidylyltransferase, which modifies aminoglycosides by adenyllylation | Aminoglycoside                                             | Spectinomycin, streptomycin                      |
| MDRSHH02001946  | **aph33ib** Aminoglycoside O-phosphotransferase, which modifies aminoglycosides by phosphorylation | Aminoglycoside                                             | Streptomycin                                     |
| MDRSHH02002406  | **aph(3')-Ia** Aminoglycoside O-phosphotransferase, which modifies aminoglycosides by phosphorylation | Aminoglycoside                                             | Gentamicin b, kanamycin, neomycin, paromomycin, lividomycin, ribostamycin streptomycin |
| MDRSHH02001945  | **aph(6)-Id** Aminoglycoside O-phosphotransferase, which modifies aminoglycosides by phosphorylation | Aminoglycoside                                             | Gentamicin b, kanamycin, neomycin, paromomycin, lividomycin, ribostamycin streptomycin |
| MDRSHH02002408  | **bl2b_tem, bl2b_tem1** Class A β-lactamase. This enzyme breaks the β-lactam antibiotic ring open and deactivates the molecule's antibacterial properties | Aminoglycoside                                             | Cephalosporin, penicillin, cephalosporin i, cephalosporin ii |
| MDRSHH02000599  | **cath3** Group B chloramphenicol, acetyltransferase which can inactivate chloramphenicol. Also referred to as xenobiocytic acetyltransferase | Aminoglycoside                                             | Chloramphenicol                                  |
| MDRSHH02000610  | **sul1** Sulfonamide-resistant dihydropteroate synthase, which can not be inhibited by sulfonamide | Sulfonamide                                               | Sulfonamide                                     |
| MDRSHH02001738  | **sul2** Sulfonamide-resistant dihydropteroate synthase, which can not be inhibited by sulfonamide | Sulfonamide                                               | Sulfonamide                                     |
| MDRSHH02001941  | **tetb** Major facilitator superfamily transporter, tetracycline efflux pump                  | Sulfonamide                                               | Tetracycline                                     |

*A. baumannii*, *Acinetobacter baumannii*; ARDB, Antimicrobial Resistance Genes Database; DDA, disc diffusion assay. The antibiotics in bold are resisted by *A. baumannii* MDR-SHH02 in the DDA.

Discussion

In the present study, we reported the draft genomic sequence of the clinical MDR *A. baumannii* strain, MDR-SHH02, and predicted one gene sequence homologous to 45 PAIs and 12 potential gene sequences relevant to antibiotic resistance. The antibiotic-resistance assay and the high similarity between the 12 gene sequences in MDR-SHH02 and the sequences of antibiotic resistance genes in ARDB, revealed that MDR-SHH02 was resistant to multiple antibiotics.

According to the prediction of PAIs, the gene sequence MDRSHH02000806 was homologous to 45 PAIs, such as LE and PAPI-1. LE PAIs were previously identified from multiple enteropathogenic *Escherichia coli* strains, and they are highly conserved in gene order and nucleotide sequence (25,26). PAPI-1 was previously identified from the *P. aeruginosa*...
strain, PA14, and it contributes directly and synergistically along with PAPI-2 to the virulence of PA14 (27). Therefore, the virulence of MDR-SHH02 may be due to the presence of MDRSHH02000806 homologous to these PAs.

In this study, we discovered a set of gene sequences that were highly similar to the sequences of multiple genes encoding aminoglycoside-modifying enzymes (AMEs), including 2 aminoglycoside N-acetyltransferase genes [aac(3)-Ia and aac(6′)-Ib], 2 aminoglycoside O-nucleotidyltransferase genes [ant(2″)-Ia and ant(3″)-Ia], and 3 aminoglycoside O-phosphotransferase genes [aph(3′′)3ib, aph(3′)-Ia and aph(6)-Id]. The expression of AMEs enables bacteria to catalyze the modification of amino and hydroxyl groups on sugar moieties, such as aminoglycosides (28), which is a major cause of aminoglycoside resistance in many bacteria (29). The majority of aminoglycoside-resistant Acinetobacter isolates have the ability of enzymatic modification of aminoglycosides by acetyltransferases, nucleotidyltransferases and/or phosphotransferases (30). Previous studies have reported the prevalence of multiple AME genes (e.g., aac(3)-Ia, and aac(6′)-Ib) in a set of A. baumannii isolates that are resistant to various aminoglycosides (e.g., amikacin, gentamicin and tobramycin) (31,32). Besides, Aph(6)-Id and ant(3″)-Ia have been detected in the A. baumannii strain, MRSN 12227, which is resistant to various antibiotics, such as amikacin, tobramycin and cefotaxime (33). Another study reported that ant(2″)-Ia present in a group of A. baumannii isolates (62.6%) was associated with resistance to the tested aminoglycosides (amikacin, tobramycin and gentamicin) (34). Furthermore, in this study, we found that MDR-SHH02 was resistant to 19 antibiotics, such as several types of aminoglycosides (amikacin, gentamicin and tobramycin), indicating that the resistance of MDR-SHH02 to aminoglycosides likely resulted from the coding sequences highly similar to AME genes. However, aph33ib has not been previously detected in A. baumannii isolates, and it is worthy of further study. For example, following knockout and complementation of the gene sequence that is highly similar to aph33ib, the resistance of MDR-SHH02 to aminoglycosides is determined.

In this study, several gene sequences in MDR-SHH02 had a high similarity to Class A β-lactamase genes (bl2b_tem and bl2b_tem1), group B chloramphenicol acetyltransferase gene (catb3), sulfonamide-resistant dihydroyterotide synthase genes (sul1 and sul2) and tetracycline efflux pump gene (tetB). The gene bl2b_tem has been detected in Staphylococcus aureus (35), and bl2b_tem1 was detected in a series of marine bacteria, such as Pelagibacter, Polaribacter and Roseobacter (36). However, there is no evidence to support that bl2b_tem and bl2b_tem1 are carried by A. baumannii isolates. All other genes (catb3, sul1, sul2 and tetB) have been found in A. baumannii isolates (37-40).

Despite the aforementioned results, there were still several limitations to this study. The association of MDRSHH02000806 with the virulence of MDR-SHH02 needs to be validated in further studies. Besides, the associations between the 12 gene sequences similar to AME genes and the antibiotic resistance of MDR-SHH02 are also needs to be confirmed in further studies. We aim to conduct such experiments in our future studies.

In conclusion, this study fulfilled the draft genomic sequence of the clinical MDR A. baumannii strain, MDR-SHH02, and 12 gene sequences in MDR-SHH02 had a highly similarity to the sequences of genes encoding AMEs [e.g., aac(3)-Ia, ant(2″)-Ia, aph33ib and aph(3′)-Ia], β-lactamase genes (bl2b_tem and bl2b_tem1), sulfonamide-resistant dihydroyterotide synthase genes (sul1 and sul2), catb3 and tetb. Of these genes, aph33ib, bl2b_tem and bl2b_tem1 were potential new antibiotic resistance genes. Furthermore, the antibiotic-resistance assay revealed that MDR-SHH02 was resistant to multiple antibiotics, such as amikacin, gentamicin and tobramycin. These findings were expected to enrich the data of antibiotic resistance genes in MDR A. baumannii, and provide a clinical guidance for the personalized therapy of A. baumannii-infected patients.

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