Comparative genomic analysis of *Acinetobacter baumannii* clinical isolates reveals extensive genomic variation and diverse antibiotic resistance determinants

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**Abstract**

**Background:** *Acinetobacter baumannii* is an important nosocomial pathogen that poses a serious health threat to immune-compromised patients. Due to its rapid ability to develop multidrug resistance (MDR), *A. baumannii* has increasingly become a focus of attention worldwide. To better understand the genetic variation and antibiotic resistance mechanisms of this bacterium at the genomic level, we reported high-quality draft genome sequences of 8 clinical isolates with various sequence types and drug susceptibility profiles.

**Results:** We sequenced 7 MDR and 1 drug-sensitive clinical *A. baumannii* isolates and performed comparative genomic analysis of these draft genomes with 16 *A. baumannii* complete genomes from GenBank. We found a high degree of variation in *A. baumannii*, including single nucleotide polymorphisms (SNPs) and large DNA fragment variations in the AbaR-like resistance island (RI) regions, the prophage and the type VI secretion system (T6SS). In addition, we found several new AbaR-like RI regions with highly variable structures in our MDR strains. Interestingly, we found a novel genomic island (designated as GI_BJ4) in the drug-sensitive strain BJ4 carrying metal resistance genes instead of antibiotic resistance genes inserted into the position where AbaR-like RIs commonly reside in other *A. baumannii* strains. Furthermore, we showed that diverse antibiotic resistance determinants are present outside the RIs in *A. baumannii*, including antibiotic resistance-gene bearing integrons, the *bla*OXA-23-containing transposon Tn2009, and chromosomal intrinsic antibiotic resistance genes.

**Conclusions:** Our comparative genomic analysis revealed that extensive genomic variation exists in the *A. baumannii* genome. Transposons, genomic islands and point mutations are the main contributors to the plasticity of the *A. baumannii* genome and play critical roles in facilitating the development of antibiotic resistance in the clinical isolates.

**Keywords:** *Acinetobacter baumannii*, Multidrug resistance, Resistance island, SNP, Whole-genome sequencing

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**Background**

*A. baumannii*, an important nosocomial pathogen, is becoming an increasing threat to hospital patients due to its ability to develop multidrug resistance (MDR) [1-3]. Drug resistance in *A. baumannii* is due to a combination of mechanisms, including the expression of β-lactamases, alteration of cell membrane impermeability, and increased expression of efflux pumps [4]. The drug resistance genes of *A. baumannii* isolates are often clustered into antibiotic resistance islands (AbaRs) that interrupt the ATPase gene (*comM*) [5]. For example, a 36-kb resistance island (RI) was found in *A. baumannii* strain AYE (AbaR1) [6], and a shorter RI was identified in the *A. baumannii* strain ACICU (AbaR2) [7]. RIs are thought to emerge from the integration of plasmids or other mobile elements, and some drug-susceptible strains lack these RIs [6]. In addition, plasmid-borne resistance genes have also been reported, e.g., the *bla*OXA-23 gene, which is associated with...
carbapenem resistance, has been identified in clinical A. baumannii isolates around the world [8,9]. Compared with current knowledge regarding antibiotic resistance mechanisms in A. baumannii, less is known regarding the virulence factors in this bacterium [10]. Several studies have focused on characterizing the formation of biofilms, one of the determinants involved in the pathogenesis in A. baumannii. For example, a chaperone-usher pili assembly system (csu locus) has been shown to be involved in attachment and biofilm formation in A. baumannii [11]. Other virulence factors identified include a siderophore-mediated iron acquisition system, Abal autoinducer synthase, the BfmRS two-component regulatory system, the type VI secretion system (T6SS) [12] and lipopolysaccharide (LPS) [13]. The LPS found in A. baumannii, which is composed of lipids, O-antigen, and an outer core (OC) and inner core, has been shown to be a major contributor to the pathogenesis of infection [13]. The OC gene locus contains many genes encoding glycosyltransferase enzymes that catalyze the bonds between sugars in the OC structure [14].

Whole-genome sequencing studies comparing distinct drug-susceptible and MDR strains [1,15] or isolates from a single patient [16] have improved our understanding of the evolution of A. baumannii. To better understand the genomic variation and the antibiotic resistance mechanisms in A. baumannii, here we sequenced eight clinical A. baumannii isolates with various sequence types and drug susceptibility profiles and performed comparative genomic analysis.

**Results**

**Susceptibility profiles, multilocus sequence typing (MLST) and whole-genome sequencing**

The susceptibility profiles for all sequenced strains are shown in Table 1. All 7 MDR strains were resistant to the antibiotics gentamicin (CN), ciprofloxacin (CIP), ceftriaxone (CTR), ceftazidime (CAZ), cefepime (FEP), and tetracycline (TE) but susceptible to polymyxin B (PB). The drug-sensitive strain BJ4 was sensitive or intermediate to all tested antibiotics except CTR.

We found that all 7 MDR strains correspond to global clone II (GC II). The strains BJ2, BJ6, and BJ7 share the same sequence type (ST), namely, ST208, and strains BJ1 and BJ5 share a type (ST191). In addition, strains BJ3 and BJ8 belong to ST218 and ST368, respectively. However, the drug-sensitive strain BJ4 shows a novel sequence type.

The basic whole-genome sequencing statistics are shown in Table 2. Illumina 100 bp paired-end sequencing produced more than 900 Mb of data for each of the eight strains, and the sequencing depth ranged from 239× to 473×. The GC content of the genomes was approximately 38.9%, as expected for the species. The size of the genomes varied from 3.86 to 4.03 Mb.

**Phylogenetic analysis of A. baumannii isolates**

A maximum-likelihood tree of the 8 sequenced genomes and 16 reported A. baumannii complete genomes were created based on core SNPs from whole-genome alignment (Figure 1). The phylogenetic tree showed that the previously sequenced strains and all of the 7 MDR clinical isolates belonging to GC II formed a clade, while strains AB307-0294, AYE, and AB0057, which belong to GC I, grouped together. The BJ1 and BJ5 strains are closely related, while strains BJ2, BJ6, and BJ7 form another closely related group. Interestingly, strain BJ4, the drug-sensitive strain, is distinct from all of the sequenced MDR strains, which may indicate that it has a unique origin compared with other drug-resistant strains.

| Table 1 Antimicrobial susceptibility profiles. R, resistant; I, intermediate; S, susceptible |
|-----------------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Antimicrobial classes                          | Antimicrobial drugs | MIC (mg/L) and Susceptibility |
|                                               | BJ1 | BJ2 | BJ3 | BJ4 | BJ5 | BJ6 | BJ7 | BJ8 |
| Aminoglycosides                                | Gentamicin (CN) | ≥8, R | ≥8, R | ≥8, R | ≤1, S | ≥8, R | ≥8, R | ≥8, R |
|                                               | Amikacin (AK)   | ≤16, S | >32, R | >32, R | ≤2, S | >32, R | ≤16, S | ≤16, S |
| Antipseudomonal carbapenems                    | Imipenem (IPM) | ≥8, R | ≥8, R | ≥8, R | ≤1, S | ≥8, R | ≥8, R | ≥8, R |
|                                               | Ciprofloxacin (CIP) | ≥2, R | ≥2, R | ≥2, R | ≤0.25, S | ≥2, R | ≥2, R | ≥2, R |
| Antipseudomonal fluoroquinolones               | Lavo-ofloxacin (LEV) | ≥4, R | >4, R | >4, R | ≤0.25, S | >4, R | >4, R | >4, R |
| Antipseudomonal penicillins and β-lactamase inhibitors | Piperacillin-tazobactam (TZP) | ≥64/4, R | >64/4, R | >64/4, R | ≤4, I | =64/4, R | >64/4, R | >64/4, R |
| Extended-spectrum cephalosporins                | Ceftriaxone (CTR) | ≥32, R | ≥32, R | ≥32, R | 8, R | ≥32, R | ≥32, R | ≥32, R |
|                                               | Ceftazidime (CAZ) | ≥16, R | >16, R | >16, R | 4, I | >16, R | >16, R | >16, R |
|                                               | Cefepime (FEP)  | ≥16, R | >16, R | >16, R | 2, S | >16, R | >16, R | >16, R |
| Polymyxins                                     | Polymyxin B (PB) | ≤2, S | ≤2, S | ≤2, S | ≤2, S | ≤2, S | ≤2, S | ≤2, S |
| Tetracyclines                                  | Tetracycline (TE) | =8, I | >8, R | >8, R | ≤4, S | >8, R | >8, R | >8, R |
Table 2 Sequencing statistics for the *A. baumannii* isolates

| Strain | Raw data (Mb) | Sequencing depth (X) | Scaffold number | N50 length | Mean length | Max length | Full length (bp) | GC (%) |
|--------|---------------|---------------------|-----------------|------------|-------------|------------|------------------|--------|
| BJ1    | 979           | 246                 | 44              | 203665     | 90399       | 458403     | 3977574          | 38.9%  |
| BJ2    | 959           | 239                 | 52              | 164054     | 77219       | 364231     | 4015419          | 38.9%  |
| BJ3    | 1540          | 390                 | 35              | 236595     | 112903      | 460149     | 3951629          | 38.9%  |
| BJ4    | 1666          | 420                 | 54              | 150346     | 73375       | 433944     | 3962927          | 38.9%  |
| BJ5    | 1895          | 473                 | 47              | 211748     | 85260       | 458351     | 4007238          | 38.9%  |
| BJ6    | 1079          | 268                 | 56              | 133586     | 71990       | 364278     | 4031457          | 38.9%  |
| BJ7    | 961           | 239                 | 54              | 133586     | 74623       | 364278     | 4029682          | 38.9%  |
| BJ8    | 1193          | 309                 | 56              | 170785     | 68971       | 466960     | 3862420          | 39.0%  |

*A. baumannii* core and unique genes

We compared the gene contents of the 8 genomes with other *A. baumannii* reference genomes using the PanOCT analysis software [17], which utilizes conserved gene neighborhood (CGN) and frameshift detection in a weighted scoring scheme and the BLAST score ratio to effectively generate non-paralogous gene clusters. We found that the pan-genome continued to expand after the compilation of 24 genomes, whereas the number of core genes remained relatively stable with the addition of new strains (Figure 2A). The size of the pan-genome was 8245 genes, and there are 1902 genes (core) shared among the 24 isolates (Figure 2B). The number of unique genes ranges from 7 in strain BJ1 to 552 in strain SDF (Table 3). Many of these unique genes are hypothetical, transposon-related and phage-related genes. Detailed information regarding orthologous groups and singletons of the strains is provided in Additional file 1: Table S1. The large number of unique genes in these genomes likely indicates frequent horizontal gene transfer events in *A. baumannii*. Hierarchical clustering of these strains based on gene content yields a dendrogram (Figure 2B) that is similar to the core SNP-based phylogenetic tree (Figure 1) in which strains from GC I form one group and strains from GC II form another group.

We further analyzed the core and unique genes according to the various classes of the Clusters of Orthologous Groups (COGs) (Figure 2C). We found that core genes were significantly enriched in genes belonging to class J (Translation, ribosomal structure and biogenesis; P value = 1.53e-09) and class F (Nucleotide transport and metabolism; P value = 0.0008301). In contrast, unique genes were significantly enriched in class L (Replication, recombination and repair; P value < 2.2e-16), class V (Defense mechanisms; P value = 1.868e-09), and class M (Cell wall/membrane/envelope biogenesis; P value = 0.0005701).

To compare protein sequence evolution rates between the MDR isolates and the drug-sensitive isolates, we measured the nonsynonymous substitution rate (Ka or dN) in 1902 orthologous genes. We previously showed that this rate is a relatively consistent parameter for defining fast-evolving and slow-evolving protein-coding genes [18]. The fast-evolving genes we identified among the MDR isolates include many outer membrane proteins and stress-related proteins; one of these proteins is a phenazine biosynthetic PhzF-like protein that serves as an enzyme essential for phenazine synthesis. Phenazines are pigments, and many exhibit broad-spectrum antibiotic activity against bacteria, fungi, and parasites and can contribute to the ecological competence of the
strains [19]. In contrast, the identified slow-evolving genes include many conserved hypothetical proteins and metabolism-related proteins. For example, SbmA, which is involved in the prokaryotic internalization of antimicrobial peptides (AMPs), was identified as a slow-evolving gene [20].

SNPs among A. baumannii strains

The number of SNPs among the 7 MDR strains with distinct STs ranged from 920 to 2675 (Additional file 2: Table S2). The strains with the same STs showed fewer SNPs, ranging from 74 to 196. Among the 74 putative SNPs identified between BJ6 and BJ7, only 12 (16%) were nonsynonymous mutations; these SNPs were located within genes coding for outer membrane receptor for monomeric catechols, dihydropteroate synthase, fatty acid desaturase, and a putative RND superfamily exporter. We also found similar nonsynonymous mutations within all of the genes mentioned above between BJ1 and BJ5.

To identify SNP regions clustered among the 7 MDR strains, SNP density was estimated throughout the genomes using a sliding window of 5 kb. The resulting SNP density map shows a non-random distribution, with many regions having elevated SNP density (Additional file 3: Figure S1). One large region of elevated SNP density is around the origin of replication of the genome and the K locus, as reported by Snitkin et al. [21]. We also found other SNP clusters containing genes involved in heme
The homologs of protein sequences to the virulence factors in the VFDB Putative virulence genes were identified by aligning ORF Database (VFDB) Virulence genes identified in the Virulence Factors type transport system, etc.

### Table 3 Orthologous clusters in the A. baumannii pan-genome

| Strain        | Total | Non-core | % Non-core | Unique | % Unique |
|---------------|-------|----------|------------|--------|----------|
| BJ1           | 3808  | 1906     | 50.1%      | 7      | 0.2%     |
| BJ2           | 3846  | 1944     | 50.5%      | 32     | 0.8%     |
| BJ3           | 3747  | 1845     | 49.2%      | 10     | 0.3%     |
| BJ4           | 3767  | 1865     | 49.5%      | 408    | 10.8%    |
| BJ5           | 3833  | 1931     | 50.4%      | 9      | 0.2%     |
| BJ6           | 3870  | 1968     | 50.9%      | 8      | 0.2%     |
| BJ7           | 3869  | 1967     | 50.8%      | 9      | 0.2%     |
| BJ8           | 3651  | 1749     | 47.9%      | 27     | 0.7%     |
| 1656_2        | 3715  | 1813     | 48.8%      | 159    | 4.3%     |
| AB0057        | 3790  | 1888     | 49.8%      | 182    | 4.8%     |
| AB307_0294    | 3458  | 1556     | 45.0%      | 76     | 2.2%     |
| AOUU          | 3667  | 1765     | 48.1%      | 70     | 1.9%     |
| ATCC_17978    | 3787  | 1885     | 49.8%      | 507    | 13.4%    |
| AYE           | 3607  | 1705     | 47.3%      | 183    | 5.1%     |
| BJAB07104     | 3755  | 1853     | 49.3%      | 32     | 0.9%     |
| BJAB0715      | 3848  | 1946     | 50.6%      | 247    | 6.4%     |
| BJAB0868      | 3703  | 1801     | 48.6%      | 35     | 0.9%     |
| D1279779      | 3388  | 1486     | 43.9%      | 105    | 3.1%     |
| MDR_TJ        | 3704  | 1802     | 48.7%      | 28     | 0.8%     |
| MDR_ZJ06      | 3860  | 1958     | 50.7%      | 58     | 1.5%     |
| SDF           | 2913  | 1011     | 43.7%      | 552    | 18.9%    |
| TCDC_AB0715   | 3851  | 1949     | 50.6%      | 178    | 4.6%     |
| TTYH_1        | 3680  | 1778     | 48.3%      | 130    | 3.5%     |
| ZW85_1        | 3465  | 1563     | 45.1%      | 136    | 3.9%     |

utilization, arginine and proline metabolism, the ABC-type transport system, etc.

### Virulence genes identified in the Virulence Factors Database (VFDB)
Putative virulence genes were identified by aligning ORF protein sequences to the virulence factors in the VFDB (Additional file 4: Table S3). The homologs of clpP (ATP-dependent Clp protease proteolytic subunit), alaD (aldehyde dehydrogenase), xcpR (general secretion pathway protein E), ureA (urease alpha subunit), tviB (Vi polysaccharide biosynthesis protein), pilG (twitching motility protein), pilH (twitching motility protein), htpB (60 K heat shock protein), sodB (superoxide dismutase) and manB (phosphomannomutase) were present in all of the A. baumannii strains. The homologs of pilC, pilT, and pilU were absent in SDF but present in the other strains. In addition, the homologs of bpiB (putative acetyltransferase), VC0817 (putative transposase), SF2983 (transposase of Tn10) and katB (catalase-peroxidase) were exclusively present in ATCC 17978, BJ4, AYE and D1279779, respectively.

Large genomic variants among A. baumannii strains
We compared the genomes of our 8 A. baumannii strains with the reference genome of A. baumannii MDR-TJ, a multidrug resistance strain belonging to GC II group [22]. We identified many highly variable regions (Figure 3); specifically, the following regions on the MDR-TJ genome are missing or have low identity with our strains: from 982 to 1,034 kb, 1,343 to 1,363 kb, 1,364 to 1,400 kb, 1,575 to 1,617 kb, 2,460 to 2,500 kb, 3,672 to 3,710 kb and 3,798 to 3,894 kb.

The region from 982 to 1,034 kb was predicted to be the prophage locus. The sequence of strain BJ1 in this locus is highly similar to that of the reference genome, while other strains have variable sequences in this region (low protein identity or missing). Interestingly, an ISabaI-associated deletion of approximately 20 kb in a region of adhesion genes (csuE) from 1,343 to 1,363 kb was absent from the strain BJ2 and from the previously reported reference strain MDR-ZJ06 [23]. The region from 1,364 to 1,400 kb encompasses a cluster of genes involved in iron acquisition. The region from 1,575 to 1,617 kb was predicted to be the second prophage locus. The approximately 40-bp region from 2,460 to 2,500 kb, which encodes the entire type VI secretion system (T6SS), was completely absent from strain BJ4. The region from 3,672 to 3,710 kb, which encodes the entire AbaR-like genomic island (RIs), was also completely absent from strain BJ4. A more detailed analysis of this island is shown in Figure 4. The region from 3,798 to 3,894 kb contains many highly divergent genes, including several membrane proteins, stress-related proteins, and efflux pumps. Specifically, the region from 3,869 to 3,894 kb encompasses a series of genes encoding the O-antigen component of LPS.

In addition, the above-mentioned variable regions are always accompanied by several insertion elements, which may assist the integration of resistant and pathogenesis-related genes and facilitate the transfer of drug resistance and pathogenic genes among strains. In addition, IS elements may enhance drug-resistance and virulence by promoting gene expression [24,25]. Furthermore, the CRISPR (clustered regularly interspaced short palindromic repeats) systems, which were identified in the genomes of three GC I strains (AYE, AB0057 and AB307-0294), were not present in any of the 8 sequenced strains.

### AbaR-like resistance islands (RIs)
We compared the sequences of AbaR-like RIs in each A. baumannii isolate and found a series of variation events at this locus (Figure 4). AbaR-like RIs inserted in the comM gene were identified in all 7 MDR strains: AbaRBJ2 and AbaRBJ3 shared the same structure, AbaRBJ6 and AbaRBJ7 shared a structure, and AbaRBJ1 and AbaRBJ5 shared a structure. In addition, AbaRBJ8, AbaRBJ2 and AbaRBJ3 were novel AbaR-like RIs, while the remaining regions have
been previously reported [23,26]. AbaR-like RIs were not found in strain BJ4, which may partly explain its susceptibility to antibiotics. AbaR$_{BJ8}$ shares the same backbone with AbaR$_{MDR-TJ}$ and consists of two Tn6022 transposons and 3 resistance-related regions (RR) (Figure 4). RR1a inserted at the 3’-end of the island contains IS$_{Aba1A}$ (mobile element), sul2 (conferring sulfonamide resistance), glmM (phosphoglucomamine mutase) and tnsA (transposase protein A); RR2 located between the two copies of Tn6022 bears the antibiotic resistance gene sul2; RR3 at the 5’-end of AbaR$_{BJ8}$ contains four resistance genes: tetA and tetR (conferring tetracycline resistance), strA and strB (conferring streptomycin resistance). There was also a cluster of ORFs inserted between Tn6022 and RR2; this cluster is designated “ORFs” in Figure 4. Compared with AbaR$_{BJ9}$, the RR1a region and a part of the 3’-end of Tn6022 in AbaR$_{MDR-TJ}$ were absent. AbaR$_{BJ2}$ and AbaR$_{BJ3}$ had a truncated RR1a (with a tnsAΔ) designated as RR1b; the rest of the structure was identical to that of AbaR$_{BJ8}$. AbaR$_{BJ6}$ and AbaR$_{BJ7}$ included Tn6022Δ1 and RR3 segments, while AbaR$_{BJ1}$ and AbaR$_{BJ5}$ only contained the Tn6022Δ1 segment without resistance genes.

Comparative analysis of AbaR-like RIs in the 7 MDR strains and the other 7 GC II isolates was also performed (Figure 4). One major distinction among the RIs in the GC II strains was the presence or absence of a second Tn6022 copy. Tn6022 or Tn6022Δ1 were mutual segments that existed in all of these strains, while the three resistance regions (RR1, RR2 and RR3) and ORFs were deleted or truncated in certain strains. In AbaR$_{BJAB07104}$, AbaR$_{TCDC-AB0715}$ and AbaR$_{1656-2}$, large fragments of inserted segments were found: the vertical arrow in AbaR$_{BJAB07104}$ indicates the insertion of Tn6206 and the tra system specific to this island [27]; the arrow in AbaR$_{1656-2}$ indicates an inserted RR3a segment containing the resistance genes per-1 and strA (tupA-tupA2-gst-per-1-tupA1-insB-IS3-strA) but not the tetA(B) and tetR(B) regions of RR3 [28]; and the arrow in AbaR$_{TCDC-AB0715}$ indicates a large segment specific to this strain containing six IS26 elements and multiple resistance genes [29].

Interestingly, the comM region of the drug-sensitive strain BJ4 was interrupted by a novel genome island (GI, designated GI$_{BJ4}$). This island was 29.3 kb in length and contained no antibiotic resistance gene (Figure 5). Rather, five metal-resistance genes, including cueR, zntA,
arsR, cccD and xre were identified within this island. Furthermore, 12 orfs of unknown function were present in GI_{BJ4}, including a tnsA gene encoding an endonuclease domain protein, an int gene encoding an integrase core domain protein, the transposon-related tniB and tniQ genes and an inserted IS1236 segment.

**Other mobile elements containing resistance genes**

Class 1 integron is an important factor for the horizontal transfer of resistance genes in *A. baumannii*, especially the aminoglycoside resistance genes [30]. Six of our MDR strains contained a class 1 integron, and their gene cassette arrays were as follows: the integron of BJ1 harbored a gene cassette array of \( aacC1 \)-orfP-orfP-orfQ-aadA1; the integrons of BJ2, BJ6 and BJ7 included gene a cassette array of \( aacC1 \)-orfA-orfB-aadA1; and the integrons of BJ3 and BJ5 had a gene cassette array of \( aacA4 \)-catB8-aadA1. Among these gene cassettes, \( aacC1 \), \( aadA1 \) and \( aacA4 \) are aminoglycoside resistance genes; catB8 is group B chloramphenicol acetyltransferase; and

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**Figure 4** Genetic structures of AbaR-like RIs in seven MDR strains and seven previously reported GC2 strains. Green rectangles indicate resistance region RR1; red rectangles indicate resistance region RR2; grey rectangles indicate resistance region RR3; orange rectangles indicate Tn6022 and Tn6022Δ; and blue rectangles indicate a cluster of orfs encoding proteins with unknown function. The dashed black lines denote deletions. The vertical arrows in AbaR_{BJAB07104}, AbaR_{1656-2} and AbaR_{TCDC-AB0715} indicate insertions of segments specific to these strains. RR1: tniC, tniA, tniBΔ, IS\( _{Aba1} \), sul2, glmM (phosphoglucosamine mutase), and tnsA (transposase protein A); RR2: tniCb, mIaB, tniB2, IS\( _{Aba1} \), sul2, and CRΔ; RR3: tetA(B), tetR(B), CR (IS\( _{Vsa3} \)), strB, strA, and orf4b.

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**Figure 5** Structure of genomic islands in the BJ4 strain. The structure was drawn to scale according to the sequences of the genomic island in the genome and showed that the comM gene was interrupted by a novel genome island (designated as GI_{BJ4}). This island was 29.3 kb in length and did not contain any antibiotic resistance genes.
orf P, orfQ, orfA and orfB encode proteins of unknown function. In addition, all of the found integrase protein sequences were 100% identical.

We also identified a blaOXA-23-containing transposon Tn2009 in all sequenced strains, except for BJ4 and BJ8. This result was consistent with the antimicrobial susceptibility testing. Tn2009 has been previously described in three Chinese A. baumannii strains: MDR-ZJ06, AB16 and MDR-TJ (in the pABT1plasmid) [22,23,30]. Genomic analysis revealed that Tn2009 was flanked by two directed repeats of ISAba1 elements at both ends, and the class D ß-lactamase gene blaOXA-23 existed at the 3’-end and was adjacent to the ISAba1 element. IS elements represent another source of variability among A. baumannii isolates, and the insertion of ISAba1 might play a significant role in the expression of blaOXA-23 [24,25].

Comparative analysis of antibiotic resistance genes
A comparative analysis of antibiotic resistance genes was performed on the 8 sequenced strains and 16 reference strains, among which the BJ4, D1279779, AB307-0294, ATCC17978 and SDF strains are antibiotic susceptible (Table 4). There are four types of ß-lactamase in all of these strains, including class A ß-lactamase, class C ß-lactamase, class D ß-lactamase and the metallo-ß-lactamase superfamily. The four types of ß-lactamase are encoded by various types of genes and are responsible for much of the multidrug resistance of these strains. Among the 8 sequenced strains in this study, the ampC, metallo-ß-lactamase superfamily gene, putative class A ß-lactamase gene and blaOXA-66 existed in all of the 7 MDR strains. The tem-1 and putative class C ß-lactamase gene were shared by six of the seven MDR strains. However, per-1 is unique to BJ4, and blaOXA-69 is unique to BJ4. The per-1 gene also exists in MDR strain 1656-2. Per-1 is an extended-spectrum ß-lactamase, and its induction might be responsible for resistance to all cephalosporins and cause difficulties in treating infections [31].

Resistance to aminoglycosides is primarily mediated by aminoglycoside-modifying enzymes (AMEs), which include three types: aminoglycoside N-acetyltransferase, aminoglycoside O-phosphotransferase, and aminoglycoside O-adenyllyltransferase. These three types of AME genes, especially the aacA4, aacC1, strA, strB, aphA1 and aadA1 genes, are commonly found in the 7 sequenced MDR BJ4 strains and the 12 reference MDR strains. In contrast, the sequenced non-MDR strain BJ4 and the 4 reference non-MDR strains contain fewer AMEs genes: BJ4, D1279779, AB307-0294 and SDF each contain one apha1 gene, and ATCC17978 does not contain an AME gene. Mutations in the gyrA (Ser83Leu) and parC (Ser80Ile) genes are responsible for quinolone resistance in A. baumannii. In this analysis, all of the MDR strains except ZW85-1 contained a mutation in gyrA gene, while the parC gene mutation was only present in nine of the MDR strains. This result might indicate that gyrA (Ser83Leu) plays a more important role than does parC (Ser80Ile) in fluoroquinolone resistance. Among the 19 MDR strains, only 10 include the group A or group B chloramphenicol acetyltransferase genes. There may be other resistance mechanisms, such as efflux pumps, that contribute to chloramphenicol resistance in these MDR strains. The sulfonamide-resistant dihydropteroate synthase genes were present in all of the analyzed MDR strains, while in the 5 non-MDR strains, only ATCC17978 contained a sul2 gene.

The RND (resistance-nodulation-division) family efflux pump, consisting of the adeA, adeB and adeC genes, was present in most of these strains. This efflux pump requires the coexistence of all three genes (adeA, adeB and adeC) to function properly. The antibiotic-susceptible strains D1279779, ATCC17978 and SDF do not contain a functional AdeABC efflux pump. All of the MDR strains except BJAB0715 contain intact adeA, adeB and adeC genes, which might play a role in their antibiotic resistance. Some efflux pump genes belonging to the MFS (major facilitator superfamily) were also identified in several of the MDR strains, including tetA, tetB and tetG, which encode tetracycline efflux proteins, and cmlA and cmlA5, which encode chloramphenicol efflux proteins.

Discussion
In this study, we used whole-genome sequencing methods to characterize genomic variations and antibiotic resistance mechanisms in clinical A. baumannii isolates with various sequence types and drug susceptibility profiles. Although the isolates are closely related, we identified significant genetic differences and a high degree of genomic plasticity in these strains. Pan-genomic analysis of the 8 A. baumannii isolates and the other 16 complete genomes revealed that A. baumannii genomes were highly heterogeneous with respect to gene content and possessed a series of unique genes; these results are similar to those of previous studies [15,32]. The unique genes are enriched in COG class L (Replication, recombination and repair), class V (Defense mechanisms), and class M (Cell wall/membrane/envelope biogenesis), which suggests that these genes are critical for A. baumannii survival or are closely associated with the ability of the bacteria to adapt to challenging niches.

Phylogenetic analysis showed that the drug-susceptible isolate BJ4 was distinct from the other MDR strains, and its closer relationship with the AB0057 and AYE MDR isolates offers another perspective on the origins and acquisition of antibiotic resistance determinants. In addition, the close relationship among strains BJ2, BJ6, and BJ7 indicated these strains may come from a common ancestor. The csuE deletion in strain BJ2 suggested that this
| Drug class                      | Enzyme class, description          | Coding gene | BJ1   | BJ2   | BJ3   | BJ4   | BJ5   | BJ6   | BJ7   | BJ8   | AYE   | ACICU | TYTH-1 | AB0715 |
|--------------------------------|------------------------------------|-------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|---------|
| β-Lactamases                   | class A β-lactamase                | tem-1       | 1     | 1     | 0     | 0     | 1     | 1     | 1     | 1     | 0     | 0     | 1      |                     |
|                                | per-1                              |             | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0      |                     |
|                                | veb                                |             | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0      |                     |
|                                | putative class A β-lactamase       |             | 1     | 1     | 1     | 0     | 1     | 1     | 1     | 0     | 1     | 1     | 1      |                     |
|                                | class C β-lactamase                | ampC        | 1     | 1     | 1     | 1     | 2     | 1     | 1     | 1     | 3     | 1     | 2      |                     |
|                                | putative class C β-lactamase       |             | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 0     | 1      |                     |
|                                | class D β-lactamase                | bla_DCA-23  | 1     | 1     | 0     | 1     | 1     | 1     | 0     | 0     | 0     | 0     | 0      |                     |
|                                |                                     | bla_DCA-66  | 1     | 1     | 0     | 1     | 1     | 1     | 1     | 0     | 1     | 0     | 1      |                     |
|                                |                                     | bla_DCA-49  | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 0     | 1     | 0     | 0      |                     |
|                                |                                     | bla_DCA-10  | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0      |                     |
|                                |                                     | bla_DCA-90  | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |                     |
|                                |                                     | bla_DCA-109 | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      |                     |
|                                | metallo-β-lactamase superfamily    |             | 2     | 2     | 1     | 1     | 2     | 2     | 2     | 2     | 1     | 0     | 2      |                     |
|                                | Aminoglycosides                    | aacA4       | 0     | 0     | 1     | 0     | 1     | 0     | 0     | 0     | 0     | 1     | 1      | 1                  |
|                                |                                     | aacC1       | 1     | 1     | 0     | 0     | 0     | 1     | 1     | 0     | 1     | 0     | 0      | 1                  |
|                                |                                     | aac3ia      | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0      | 0                  |
|                                | aminoglycoside O-phosphotransferase| strA        | 0     | 1     | 1     | 0     | 0     | 1     | 1     | 1     | 1     | 0     | 1     | 1                  |
|                                |                                     | strB        | 0     | 1     | 1     | 0     | 0     | 1     | 1     | 1     | 1     | 1     | 0     | 1     |
|                                |                                     | aphA1       | 0     | 1     | 1     | 1     | 1     | 0     | 1     | 1     | 1     | 1     | 1     | 3                  |
|                                |                                     | aph3via     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0                  |
|                                | aminoglycoside O-adenylyltransferase| aadA1       | 1     | 1     | 1     | 0     | 1     | 1     | 1     | 1     | 0     | 2     | 0     | 1     |
|                                |                                     | ant2ia      | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0                  |
|                                | Chloramphenicol                     | catA1       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0     | 0      |                     |
|                                | group A chloramphenicol acetyltransferase|             |       |       |       |       |       |       |       |       |       |       |                     |
|                                |                                     | catB8       | 0     | 0     | 1     | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 0      | 0                  |
|                                |                                     | catB3       | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1      | 1                  |
|                                | group B chloramphenicol acetyltransferase|             |       |       |       |       |       |       |       |       |       |       |                     |
|                                | Sulfonamides                        | sul1        | 1     | 1     | 1     | 0     | 1     | 1     | 1     | 1     | 3     | 1     | 1      | 2                  |
|                                |                                     | sul2        | 0     | 1     | 1     | 0     | 0     | 0     | 1     | 0     | 0     | 1     | 0      | 1                  |
|                                | Fluoroquinolones                    | gyrA        | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R                |
|                                | parC                               | parC        | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R    | 1R                |
|                                | Efflux pumps                        | tetA        | 0     | 1     | 1     | 0     | 0     | 1     | 1     | 0     | 1     | 0     | 0      | 0                  |
|                                | MFS (major facilitator superfamily) | tetB        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 1                  |
|                                | family                             | tetG        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 1     | 0      | 0                  |
|                                | RND (resistance-nodulation-division)| crmA        | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 1     | 0     | 0      | 0                  |
|                                | adeA                               | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |                     |
|                                | adeB                               | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |                     |
|                                | adeC                               | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1      |                     |
Table 4 Antimicrobial resistance-associated genes in the 8 BJ strains and 16 reference strains. 0 = absent, 1 = present, 1R = present and resistant (bearing a point mutation), 1S = present but sensitive (no point mutation)

| Drug class          | 16S-2 | ZW85-1 | D1279779 | AB0057 | AB307-0294 | ATCC17978 | MDR-TJ | MDR-ZJ06 | BJAB07104 | BJAB0868 | BJAB0715 | SDF |
|---------------------|-------|--------|----------|--------|------------|-----------|--------|----------|-----------|-----------|-----------|-----|
| β-Lactamases        | 0     | 0      | 1        | 0      | 0          | 0         | 0      | 1        | 0         | 0         | 0         | 1   |
|                     | 1     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 1     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 2     | 1      | 1        | 0      | 0          | 0         | 0      | 0        | 1         | 1         | 1         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 1   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 1         | 1         | 1         | 1   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 1         | 1         | 1         | 0   |
|                     | 0     | 0      | 1        | 0      | 0          | 0         | 0      | 0        | 0         | 1         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 1         | 0   |
| Aminoglycosides     | 0     | 0      | 0        | 0      | 0          | 0         | 1      | 1        | 1         | 1         | 1         | 0   |
|                     | 1     | 0      | 0        | 1      | 0          | 0         | 1      | 1        | 0         | 0         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 1   |
|                     | 2     | 0      | 0        | 0      | 0          | 0         | 1      | 1        | 1         | 1         | 1         | 1   |
|                     | 1     | 0      | 0        | 0      | 0          | 0         | 1      | 1        | 1         | 1         | 1         | 0   |
|                     | 1     | 1      | 2        | 1      | 0          | 1         | 2      | 2        | 2         | 0         | 1         | 0   |
|                     | 0     | 1      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 1         | 0   |
|                     | 2     | 0      | 0        | 1      | 0          | 0         | 2      | 2        | 1         | 1         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
| Chloramphenicol     | 0     | 0      | 0        | 1      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 1        | 0         | 0         | 0         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 1      | 1        | 1         | 1         | 1         | 0   |
| Sulfonamides        | 1     | 0      | 2        | 0      | 0          | 0         | 2      | 2        | 1         | 1         | 1         | 0   |
|                     | 1     | 1      | 0        | 0      | 0          | 1         | 1      | 0        | 1         | 1         | 1         | 0   |
| Fluoroquinolones    | 1R    | 1S     | 1S       | 1R     | 1S         | 1R        | 1S     | 1R       | 1R        | 1R        | 1R        | 1S  |
|                     | 1S    | 1S     | 1S       | 1R     | 1S         | 1S        | 1S     | 1S       | 1S        | 1S        | 1S        | 1S  |
| Efflux pumps        | 0     | 0      | 0        | 1      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 0     | 1      | 0        | 0      | 0          | 0         | 1      | 1        | 1         | 1         | 1         | 0   |
|                     | 0     | 0      | 0        | 0      | 0          | 0         | 0      | 0        | 0         | 0         | 0         | 0   |
|                     | 1     | 1      | 1        | 1      | 2         | 1         | 1      | 1        | 1         | 0         | 0         | 0   |
|                     | 1     | 1      | 1        | 1      | 1         | 1         | 1      | 1        | 1         | 1         | 0         | 0   |
|                     | 1     | 1      | 1        | 1      | 0         | 1         | 1      | 1        | 1         | 0         | 0         | 0   |
|                     | 1     | 0      | 0        | 1      | 1         | 0         | 1      | 1        | 1         | 1         | 1         | 0   |
loss may have occurred after the ancestral strain entered the hospital, followed by the mixing of strains with and without csuE.

A comparison of the gene content-based dendrogram with the core SNP tree revealed a similar clustering relationship. The slight difference in tree topology is primarily driven by (i) lateral gene transfer and (ii) IS-mediated phage- and plasmid-associated gene gain and loss. The CRISPR repeat elements, which are involved in a complex mechanism that inhibits invasive phage and plasmid DNA, were not present in any of the eight strains, which may partly explain the widespread distribution of phage- and plasmid-related genes and the extensive genomic plasticity among *A. baumannii* isolates.

Many regions associated with IS-mediated deletions, including a deletion of the entire T6SS region, have been shown to be involved in interbacterial interactions [12]. We found that the T6SS region is conserved in all 7 of the analyzed MDR *A. baumannii* isolates. As antibiotic therapy appears to reduce interbacterial competition, this result is consistent with the hypothesis that the MDR phenotype is conferred by antibiotic resistance genes, indicating that the T6SS regions are less important [33]. The surface polysaccharide loci are highly variable among the 8 strains, which is consistent with a previous report that these regions are significant sources of variability within *A. baumannii* strains [14]. We also found that the OC locus (from 558 to 566 kb in Figure 3) was less variable and was highly conserved among the MDR strains, but this region was almost completely missing from the drug-sensitive strain BJ4. In addition, virulence gene analysis showed that a total of 10 putative virulence genes were present in all *A. baumannii* genomes, suggesting that these genes may play significant roles in the pathogenesis of *A. baumannii* infection.

AbaR-like RIs inserted in the comM gene were identified in all 7 of the analyzed MDR strains but not in the drug-sensitive strain BJ4. This isolate contained a 29.3-kb new GI with five metal-resistance genes but no antibiotic resistance genes (Figure 5). Therefore, we suggested that GIs inserted into the comM gene are not always associated with antibiotic resistance, and their function might be related to the adaption of the strain to its survival niche. We also found that the RI is highly variable in composition and is not the only contributor to the MDR phenotype. Resistance genes in other mobile elements are found outside the RIs, and they are able to contribute to drug resistance in each strain examined. Among the seven MDR strains, only strain BJ8 did not contain the *bla* 

Antimicrobial susceptibility testing indicated that, compared with the 7 MDR strains, the drug-sensitive strain BJ4 shows low-level resistance to piperacillin-tazobactam (TZP), ceftriaxone (CTR) and ceftazidime (CAZ). We hypothesize that this type of low-level resistance is likely caused by the four β-lactamase genes carried by this strain (Table 4). In addition, the aphA1 gene identified in BJ4 encodes resistance to kanamycin but not to gentamicin, amikacin or netilmicin [34]. This result may explain why this strain is susceptible to gentamicin and amikacin. The class D β-lactamase gene *bla* 

SNPs are another important source of genetic variation and may contribute to drug resistance and pathogenesis in *A. baumannii* [37]. Both phylogenic and SNP analysis indicated that the drug-sensitive isolate BJ04 is genetically distinct from other MDR *A. baumannii* strains. In addition, only a small proportion of SNPs are nonsynonymous among closely related clinical MDR *A. baumannii* strains with the same STs, indicating that these strains may undergo purifying selection on a genome-wide scale. Furthermore, mutation hotspots between MDR strains were identified in several genes associated with drug resistance, e.g., genes encoding dihydropteroate synthase, a target for sulfonamide antibiotics, and the putative RND superfamily exporter genes, which encode multidrug efflux pumps [38].

Conclusions

In this study, we used whole-genome sequencing to identify genetic variants in *A. baumannii* isolates. We performed comparative genomic analysis of 8 clinical *A. baumannii* isolates with 16 available complete *A. baumannii* genomes in the NCBI database. Our results shed new light on the importance of genomic variations, especially transposon-related and/or phage-related gene variations, in the evolution of *A. baumannii*. Furthermore, we suggest that the MDR *A. baumannii* strains harbor diverse antibiotic resistance mechanisms. Future studies focused on a larger sample of *A. baumannii* isolates from various hospitals and lineages are necessary to
better understand the rapid development of antibiotic resistance in *A. baumannii*.

**Methods**

**Bacterial isolates and antimicrobial susceptibility testing**
The *A. baumannii* strains BJ1 to BJ8 were isolated from the 306th Hospital of People’s Liberation Army in Beijing, China. Identification of the isolates and antimicrobial susceptibility testing were performed using the bioMérieux VITEK-2 AST-GN13 system following the manufacturer’s instructions. The minimum inhibitory concentration (MIC) of 11 antimicrobial agents was determined according to the recommendations given by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2011) [1]. The reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

**DNA extraction, whole-genome sequencing, and annotation**
Genomic DNA was extracted using the TIANamp bacteria DNA kit (Tiangen Biotech (Beijing) Co., Ltd.) according to the manufacturer’s instructions. The genomic DNA was fragmented by ultrasonication, and the DNA fragments were subjected to the whole-genome sequencing workflow of the Illumina HiSeq 2000 system. Genome assembly was carried out by SOAPdenovo (http://soap.genomics.org.cn). The detailed methods for genome assembly and annotation were described in another study [39]. To close gaps within the AbaR-like RIs and integrorns, primer pairs were designed at the end of each gap using the genomes as templates. The PCR products were sequenced with an ABI 3730 automated DNA sequencer, using the genomes as templates. The PCR products were then extracted the aligned sequences by comparing them with the read coverage of the SNP site was greater than five; and 3) the count of the all of the mapped best bases was more than twice the count of all of the mapped second best base. From all of the SNPs identified in the sequenced genome sequences, the SNP density was calculated throughout the MDR-TJ genome using a sliding-window size of 5 kb. This window was moved at steps of 1 kb at a time, and the SNP number within each window size was counted. The construction of an SNP clustering map was performed using Circos [42].

**Comparative genomics analysis**
Genomic data used in comparative analysis were downloaded from the NCBI ftp server, including complete genome sequences of *A. baumannii* isolates MDR-ZJ06 (CP001937.1), MDR-TJ (CP003500.1), BJAB0715 (CP003847.1), AB1656-2 (CP001921.1), AB0057 (CP001182.1), AB307-0294 (CP001172.1), ACICU (CP000863.1), ATCC 17978 (CP000521.1), AYE (CU459141.1), BJAB 07104 (CP003846.1), BJAB0868 (CP003849.1), D1279779 (CP003967.1), TCDC-AB0715 (CP002522.2), TY TH-1 (CP003856.1), ZW85-1 (CP006768.1), SDF (CU468230), and ADP1 (NC_005966.1). Multiple sequence alignments of the *A. baumannii* genomes were performed with Mugsy [43]. The phylogenetic tree was constructed using dnaml from the PHYLIP package [44] based on SNPs from the whole-genome alignment, and the genome of Acinetobacter sp. ADP1 was used as the outgroup. An all-against-all BLASTP search between every pair of protein sequences from each strain was performed. Orthologs were identified using PanOCT [17] with the BLASTP output (Identity 80%; Aligned length 30%; E-value < 1e^−5). The map for core and pan-genome calculations in *A. baumannii* isolates was performed using PanGP [45]. The heatmap figure was generated using the R package pheatmap [46]. The map of ORF comparisons among *A. baumannii* genomes was constructed using Circos [42]. As shown in the phylogenetic analysis, the strain SDF was genetically the most distant from our strains; this strain is therefore more suitable for analyzing the nonsynonymous substitution rate and thereby defining the rapidly and slowly evolving protein-coding genes. We aligned the amino acid sequences of SDF with our sequenced strains, estimating the nonsynonymous substitution rates of orthologs based on the NG method using KaKs_Calculator 2.0 [47].

COG annotation was performed using the BLAST software against the COG database. COG enrichment analysis was determined using Fisher’s exact test by comparing the prevalence of a target group of genes assigned to a specific COG category to the prevalence of genes in the whole genome assigned to that COG category. To identify possible virulence factors, the Virulence Factors Database (VFDB) [48] was aligned to the ORF protein sequences and filtered with 60% identity and 90% aligned length. To search the antibiotic resistance genes,
the protein-coding sequences were aligned against the Antibiotic Resistance Database (ARDB) [49,50] using the similarity thresholds recommended in ARDB. PHAST [51] was used to identify the putative prophages in Acinetobacter genomes. ISs were identified using the IS Finder database (http://www.is.biotoul.fr) [52]. The detection of CRISPR loci in 8 sequenced draft genome sequences was performed using CRISPRFinder [42].

Nucleotide Sequence Accession Numbers
The genome sequences of A. baumannii strains from BJ1 to BJ8 reported in this study have been deposited in GenBank under accession numbers JPLF00000000, JPLG00000000, JPLH00000000, JPLI00000000, JPLJ00000000, JPLK00000000, JPLL00000000, and JPLM00000000, respectively.

Additional files

Additional file 1: Table S1. Orthologous groups and unique CDS. Detailed information for the A. baumannii pan-genome analysis used in this study. "1" and "0" indicate that the gene is present and absent, respectively, from the individual strain.

Additional file 2: Table S2. Comparison of pair-wise SNP numbers between various strains using MDR-TI as a reference.

Additional file 3: Figure S1. SNP density map constructed using Circos. The red bars indicate regions with significantly high SNP density. The scale bar within the circle indicates the number of SNPs.

Additional file 4: Table S3. Putative virulence genes identified in the 8 sequenced strains and 16 reference strains using VFDB. "1" and "0" indicate that the gene is present and absent, respectively, from the individual strain.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
YFH and BLZ conceived and designed the study; YYZ, FL, YY, and NL contributed to the individual strain. All of the authors read and approved the final manuscript.

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