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Genomic Analysis of the Multidrug-Resistant *Acinetobacter baumannii* Strain MDR-ZJ06 Widely Spread in China▽

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We previously reported that the multidrug-resistant (MDR) *Acinetobacter baumannii* strain MDR-ZJ06, belonging to European clone II, was widely spread in China. In this study, we report the whole-genome sequence of this clinically important strain. A 38.6-kb AbaR-type genomic resistance island (AbaR22) was identified in MDR-ZJ06. AbaR22 has a structure similar to those of the resistance islands found in *A. baumannii* strains AYE and AB0057, but it contained only a few antibiotic resistance genes. The region of resistant gene accumulation as previously described was not found in AbaR22. In the chromosome of the strain MDR-ZJ06, we identified the gene bla_{oxa-23} in a composite transposon (Tn*2009*). Tn*2009* shared the backbone with other *A. baumannii* transponsons that harbor bla_{oxa-23}, but it was bracketed by two IS*Aba1* elements which were transcribed in the same orientation. MDR-ZJ06 also expressed the *armA* gene on its plasmid pZJ06, and this gene has the same genetic environment as the *armA* gene of the *Enterobacteriaceae*. These results suggest variability of resistance acquisition even in closely related *A. baumannii* strains.

Acinetobacter baumannii is an important opportunistic pathogen that has caused global outbreaks of nosocomial infection (7, 24). *A. baumannii* rapidly increases antibiotic resistance due to the presence of mobile genetic elements, such as insertion sequences (ISs), plasmids, and resistance islands (23). Notably, all of these genetic elements vary even among closely related isolates of *A. baumannii* (1). Resistance islands differ in length and gene content (26). To date, the largest one is AbaR1 in AYE, which contains 45 genes associated with antibiotic, antiseptic, and heavy metal resistance within an 86-kb region (9). In contrast, AbaR2 in ACICU is much shorter (ca. 8.9 kb) and encodes only seven resistance genes, lacking arsenic, mercury, or a tetracycline resistance gene (14).

Our previous studies determined six clones (clones A to F) of imipenem-resistant *A. baumannii* isolates (IRABs) in China by pulsed-field gel electrophoresis (PFGE). Among these clones, clone C has the overwhelming majority of the isolates (160/342) that have been identified in different cities (9/16) (34). Using multilocus sequence typing (MLST) with seven standard housekeeping loci, *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpo*, we assigned MDR-ZJ06, an isolate of clone C, to sequence type (ST) 90 (1-3-3-2-2-62-3), which was clustered into clonal complex 22 (3, 34).

In order to determine the genetic basis of multiple-drug resistance of clone C, in this study we completely sequenced MDR-ZJ06 and performed comparative analyses.

MATERIALS AND METHODS

Bacterial strains and susceptibility test. *A. baumannii* isolate MDR-ZJ06 was isolated on 20 April 2006 from the bloodstream of a patient hospitalized in the intensive care unit of the first affiliated hospital at Zhejiang University in Hangzhou, China. The patient suffered from acute exacerbations of chronic obstructive pulmonary disease, respiratory failure, and ventilator-associated pneumonia (VAP). MDR-ZJ06 was considered to be the pathogen that caused the bloodstream infection. *A. baumannii* strains with the same resistant profile as MDR-ZJ06 were also isolated from sputum specimens twice on 15 April and 23 April, so MDR-ZJ06 might also be the causative agent of VAP. Imipenem and cefoperazone-sulbactam were administered successively until this patient died on 27 April 2006. Susceptibility testing for MDR-ZJ06 was performed using the Etest strip according to the manufacturer's instructions. Results were interpreted according to published recommendations (5a). The breakpoint of tigecycline and rifampin for *A. baumannii* was not available.

High-density pyrosequencing and sequence assembly. The genomic DNA of *A. baumannii* MDR-ZJ06 was prepared using Wizard Genomic DNA purification kits (Promega) according to the manufacturer's instructions. The genomic DNA (3 to 5 μg) was fragmented by nebulization, and DNA fragments were subjected to the complete sequencing work flow of the 454 genome sequencer FLX system (Roche, Basel, Switzerland). Initial assembly was performed by the 454 life Sciences software program newbler. Contigs were aligned to reference genomes to construct the scaffolds, and primer pairs were subsequently designed to close the gaps by sequencing PCR products using the dideoxy-mediated chain termination method (ABI3730; Applied Biosystems, Foster City, CA). Two lanes on an Illumina sequencer (Illumina/Solexa; Illumina Inc., San Diego, CA) were used, both of which were single-end runs of 35 bp, and the acquired short reads were mapped to the MDR-ZJ06 genome by using SOAP software tools (17).

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TABLE 1. Susceptibility profile of strain MDR-ZJ06

Antimicrobial drug	MIC (mg/liter)
Imipenem	>32
Meropenem	>32
Cefepime	128
Ceftazidime	128
Piperacillin	>256
Piperacillin-tazobactam	>256
Ampicillin-sulbactam	128
Cefoperazone-sulbactam	64
Aztreonam	128
Amikacin	>256
Gentamicin	>256
Ciprofloxacin	>32
Minocycline	32
Colistin	0.38
Tigecycline	8
Sulfamethoxazole-trimethoprim	32
Chloramphenicol	256
Rifampin	8

Genome annotation. Gene prediction was performed by two independent software programs, Glimmer and GeneMark (8, 20). Open reading frames (ORFs) that were predicted by both the programs are considered *bona fide* ones, and discrepant ORFs were then manually verified by identification of putative ribosomal binding sites. tRNA genes were predicted using the tRNAscan-SE tools (19). The RNAmmer1.2 software program was used to predict 5S, 16S, and 23S rRNA in full-genome sequences (16). ISs were characterized by using the IS Finder database (www-is.biotoul.fr). The Phage Finder program was used to identified the prophage or prophage remnant on the chromosome (10). Functional classification was performed by aligning predicted proteins to the COG (cluster of orthologous group) database (28). All of the predicted proteins were compared to the nonredundant (nr) protein database of NCBI (www.ncbi.nlm.nih.gov) using BLASTP with a cutoff E-value of ≤1e−4, identity of ≥35%, and coverage length of ≥80%. To further analyze protein functions, protein domains were screened by the InterProScan software program (22).

Comparative genomics. Data used in comparative analysis were downloaded from the NCBI database (ftp://ftp.ncbi.nlm.nih.gov/GenBank/genomes/Bacteria/), including complete genome sequences and annotation of *A. baumannii* isolates AB0057 (GenBank accession no. CP001182), AB307-0294 (CP001172), ATCC 17978 (CP000521), ACICU (CP000863), AYE (CU459141), and SDF (CU468230), as well as the draft assembled genomes of AB0056 (GenBank accession no. ADGZ00000000), AB0058 (ADHA00000000), and AB0059 (ADHB00000000). The bidirectional best-hit (BBH) method and BLASTP algorithm were used to construct the orthologues in different *A. baumannii* isolates, with a cutoff of ≥50% amino acid similarity and ≥80% coverage in length. Other in-house-developed Perl scripts were used for information integration and graphic presentation.

PCR amplification of genetic environment of bla_{oxa-23}. The genetic environment of bla_{oxa-23} was confirmed by PCR amplification and sequence analysis. The three pairs of primers were listed as follows: F1 (5′-GTAATACGGAGCGTCTGACT-3′) and R1 (5′-ACGCTTCTGCATGAGCTTCT-3′), F2 (5′-CAGATGCAGCAGATCCAATG-3′) and R2 (5′-ACCAGGTGCAACTGTTGACT-3′), and F3 (5′-ATCCTGATGCTCGCAATCGT-3′) and R3 (5′-CTGTCTGCGAACACATTCAC-3′). The amplicons were sequenced with an ABI 3730 automatic sequencer using the Sanger chain-termination method.

TABLE 2. *A. baumannii* MDR-ZJ06 genome sequencing

Characteristic for genome assembly	Value
No. of total reads	170,800
No. of assembled reads	166,353
No. of contigs	131
Avg contig size (kb)	34.8
Largest size (kb)	202
No. of PCR for gap filling	2,015
No. of short Solexa reads	7,629,819
No. of Solexa reads mapped to chromosome	2,983,796

TABLE 3. General features of *A. baumannii* MDR-ZJ06 genome and plasmid

Element and characteristic	Value
Chromosome	
Size (bp)	3,991,133
Coding regions (%)	87
G+C content (%)	39
No. of protein-coding sequences	3,887
No. of tRNA genes	69
No. of rRNA operons	6
No. of insertion sequences	41
Plasmid pZJ06	
Size (bp)	20,301
Coding regions (%)	87
G+C content (%)	47.4
No. of protein-coding sequences	29
No. of insertion sequences	6

Analysis of bla_{ampC} and *adeB* gene expression by real-time RT-PCR. For gene expression studies, total RNA was prepared using the TRIzol Max method (Invitrogen, Carlsbad, CA). Real-time reverse transcriptase PCR (RT-PCR) was performed using 250 ng of DNase-treated RNA, a PrimeScript RT reagent kit (Takara, Japan), and specific internal bla_{ampC} and *adeB* primer pairs. Expression of the endogenous control 16S rRNA gene was used to normalize data. The *A. baumannii* ATCC 19606 strain was used as the reference strain. Real-time RT-PCRs were carried out using an Opticon 2 real-time PCR detector, and the results were analyzed with the Opticon 2 real-time PCR detection software program.

Nucleotide sequence accession numbers. The *A. baumannii* MDR-ZJ06 chromosome and pZJ06 plasmid sequences were submitted to the GenBank database and can be found under accession numbers CP001937 and CP001938, respectively.

RESULTS AND DISCUSSION

Susceptibility profiles. As shown in Table 1, we found that *A. baumannii* MDR-ZJ06 was resistant to carbapenems, cephalosporins, penicillins, β-lactamase inhibitor combinations, aminoglycosides, quinolones, chloramphenicol, trimethoprim-sulfamethoxazole, and minocycline. It was susceptible only to colistin. The MIC values of tigecycline and rifampin were both 8 mg/liter.

Genome annotation and comparative analysis. The chromosome and plasmid of *A. baumannii* MDR-ZJ06 were completely sequenced and quality promoted by high-throughput sequencing. The sequencing process is summarized in Table 2. *Ab initio* methods and comparative analysis were both used to

TABLE 4. Comparison of conserved proteins between different *A. baumannii* strains

Strain	No. of proteins conserved						
	ZP6	ACICU	AYE	AB0057	AB307 0294	SDF	ATCC 17978
ZP6	3,887	3,312	3,038	3,062	2,979	2,245	2,065
ACICU		3,667	3,022	3,059	2,962	2,195	2,115
AYE			3,607	3,207	3,117	2,219	2,075
AB0057				3,790	3,124	2,160	2,104
AB307 0294					3,451	2,128	2,083
SDF						2,913	1,563
ATCC 17978							3,351

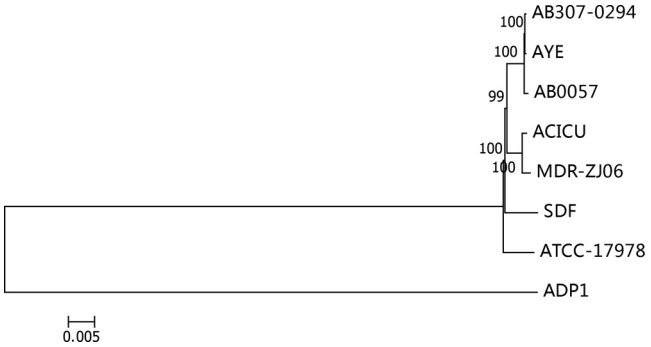

FIG. 1. Phylogenetic analyses of *Acinetobacter baumannii* isolates. The phylogenetic tree of eight sequenced *Acinetobacter baumannii* genomes was constructed according to the orthologous proteins in multigenomes. The 1,200 protein sequences conserved among the sequenced *Acinetobacter baumannii* genomes were aligned using the software program MUSCLE, and the neighbor-joining tree was constructed using the MEGA4 program. The bootstrap was set to 1,000 replicates with the seed 24,054. The genome of *Acinetobacter baylyi* ADP1 was used as the outgroup to root the tree. The scale unit is substitutions per site.

identify the coding regions, and the followed function category showed us the genome features of MDR-ZJ06 that were similar to those of other sequenced *A. baumannii* strains.

The genetic features of the chromosome and plasmid of MDR-ZJ06 are summarized in Table 3. Its genome and plasmid sizes are approximately 4 million and 20,000 bp, respectively. The genome and plasmid were predicted to encode 3,887 and 29 proteins, respectively.

Gene content was comparatively analyzed between completely sequenced *A. baumannii* strains (Table 4). Sequence analyses indicated that among the sequenced genomes of *A. baumannii* strains, the genome of MDR-ZJ06 is closest in sequence to that of ACICU, another isolate of the European clone II (ECII) strain. Between MDR-ZJ06 and ACICU, approximately 90% of total genes are conserved. Phylogenetic analysis also revealed their closest relationship (Fig. 1). Comparative analysis between all the completely sequenced or the draft assembled genomes derived 371 ECII lineage-specific genes, most of which were of unknown function. One hundred twenty-three of these genes were common between strains ACICU and MDR-ZJ06, including transposase and prophage genes and those involved in cell wall/membrane/envelope biogenesis.

Resistance island. Genome analyses revealed that MDR-ZJ06 has a genomic island (AbaR22) that is inserted into the ATPase gene *comM* (Fig. 2). AbaR22 contains 40 genes within a 38.683-kb region. The flanked sequences at both ends of AbaR22, including the identical 5-bp direct repeat (5′-ACCGC-3′), were highly similar to those of AbaR1 and the genomic island in *A. baumannii* SDF (AbaG1) (9).

A 16.3-kb backbone of AbaR-type resistance islands, termed Tn*6019*, has been derived from comparative analysis (25). AbaR22 was basically made of transposon Tn*6021*. AbaR22 contains two copies of Tn*6021*, which was identified in the antibiotic-susceptible strain ATCC 17978 in the same location (26). Tn*6021* of AbaR22 contains the *sul1* resistance genes (ABZJ_00240 and ABZJ_00259) and genes corresponding to universal stress proteins (*uspA*) (ABZJ_00239 and ABZJ_00258), which share more than 94% amino acid identity with those of ATCC 17978. In addition to *sul1* and *uspA*, transposases encoded within Tn*6021* and two putative proteins (ABZJ_00234 and ABZJ_00235) with unknown function were conserved among AbaR22 and the resistance islands in AYE (AbaR1), AB0057 (AbaR3), and ATCC 17978. The tetracycline efflux pump and its regulator genes, *tetA* and *tetR*, whose products share the amino acid similarity of ca.46% and 53% with AbaR1 and AbaR3, were also found in AbaR22. However, StrA and StrB were conserved only between AbaR22 and AbaR1, showing ca. 97% amino acid similarity.

Additionally, AbaR22 contains a truncated Tn*5393*-like

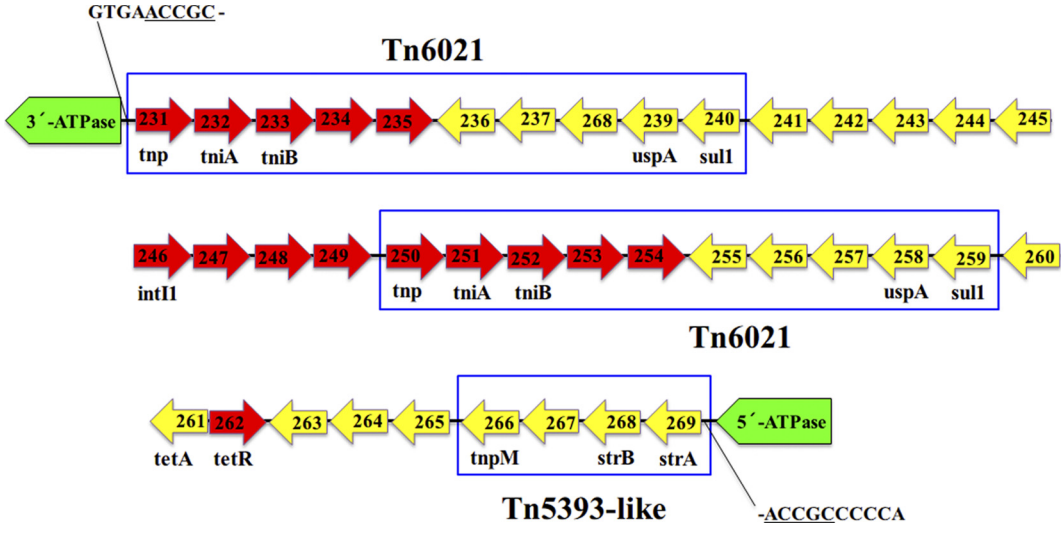

FIG. 2. The gene structure of the resistance island AbaR22 in MDR-ZJ06. AbaR22 is inserted into the ATPase gene, and the sequence underlined is the direct repeat. Predicted genes in AbaR22 are displayed as forward/reverse arrows, whose direction is identical to the gene transcription direction. The numbers in the arrows represent the gene locus tags, e.g., 231 is the abbreviated form of ABZJ_00231. The regions marked by the rectangle frames are the transposons (or remnant).

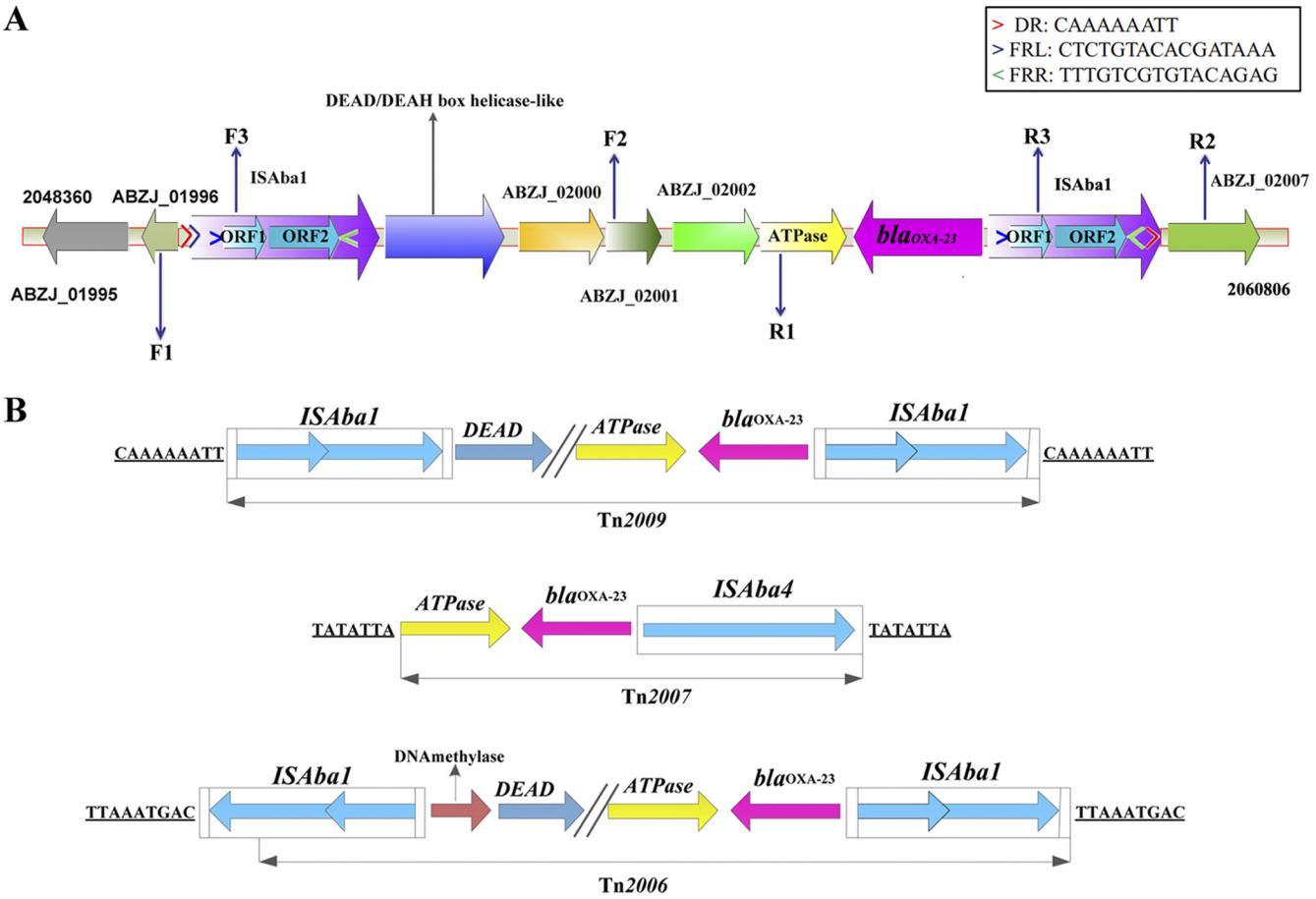

FIG. 3. Genetic structure of Tn*2009* in *Acinetobacter baumannii* MDR-ZJ06. The detailed structure of Tn*2009* (A) and a comparison of bla_{oxa-23}-containing transposons (B) are shown. DR, direct repeat; FRL/FRR, invert repeat. Primer pairs (F1 and R1, F2 and R2, and F3 and R3) were used to determine the structure. The underlined sequences are the direct repeats flanking the insertion segments.

transposon, which is highly similar to that in AbaR1. In this location, AbaR22 and AbaR1 contain the homologues of the aminoglycoside resistance genes *strAB* (ABZJ_00268 and ABZJ_00269) and the transposase gene *tnpM* (ABZJ_00261), whose products share more than 97% and 66% amino acid identity, respectively.

In summary, AbaR22 in MDR-ZJ06 is similar to the resistance islands in European clone I (ECI) strains and was divergent from AbaR2 in ECII strain ACICU. With a few genes (such as *strA*, *strB*, *sul1*, *tetA*, and *tetR*) associated with antibiotic resistance being found, AbaR22 clearly lacks the multiple antibiotic resistance regions as previously described (26).

bla_{oxa-23} and its genetic environment. The bla_{oxa-23} gene, associated with carbapenems resistance, has been identified in *A. baumannii* isolates around the world (5). Several genetic structures surrounding bla_{oxa-23}, such as the plasmid-mediated composite transposons Tn*2006* and Tn*2007* and the genomic island AbaR4 (2, 6), have been found in *A. baumannii* isolates. In MDR-ZJ06, chromosome location of bla_{oxa-23} was previously predicted from the results of Southern blotting and conjugation (34) and was confirmed in this study. Genome analyses revealed that bla_{oxa-23} is located in an 8.3-kb transposon (designated Tn*2009*) that is inserted into a cluster of genes involved in P pilus assembly, with a 9-bp target site duplication (5′-CAAAAAATT-3′). Tn*2009* is flanked by two IS*Aba1* elements, in both of which a 16-bp inverted repeat was found (5′-CTCTGTACACGATAAA-3′) (Fig. 3). Interestingly, two copies of IS*Aba1* in Tn*2009* are transcribed in the same orientation, whereas those in Tn*2006* and AbaR4 are opposite (6).

Compared to the sequence of Tn*2006*, there is an additional 2-kb segment in Tn*2009* between the truncated DEAD/DEAH box helicase-like gene and the ATPase gene, encoding three putative proteins. Notably, the DEAD/DEAH box helicase-like gene and these hypothetical ORFs are all absent in Tn*2007* and AbaR4.

Plasmid pZJ06 and the *armA* gene. Plasmid pZJ06 contains a 16S rRNA methylase gene (*armA*), an aminoglycoside 3′-phosphotransferase gene [*aph(3′)-I*], a macrolide efflux protein-coding gene (*mel*), and a class I integron, which contained aminoglycoside acetyltransferase (*aacC1*) and adenyltransferase (*aadA1*) (Fig. 4). pZJ06-carried *armA* is located in Tn*1548*, and this kind of structure was also identified in a plasmid of *A. baumannii* (EU014811), as well as a variety of plasmids of the *Enterobacteriaceae*, such as pKT51748 of *Klebsiella pneumoniae* (GenBank accession no. FJ715937), pMUR050 of *Escherichia coli* (AY522431), pCOP1 of *E. coli* (FJ187822), pCTX-M3 of *Citrobacter freundii* (AF550415), and pKSMA0710 of *Serratia marcescens* (FJ917355). The *armA* ge-

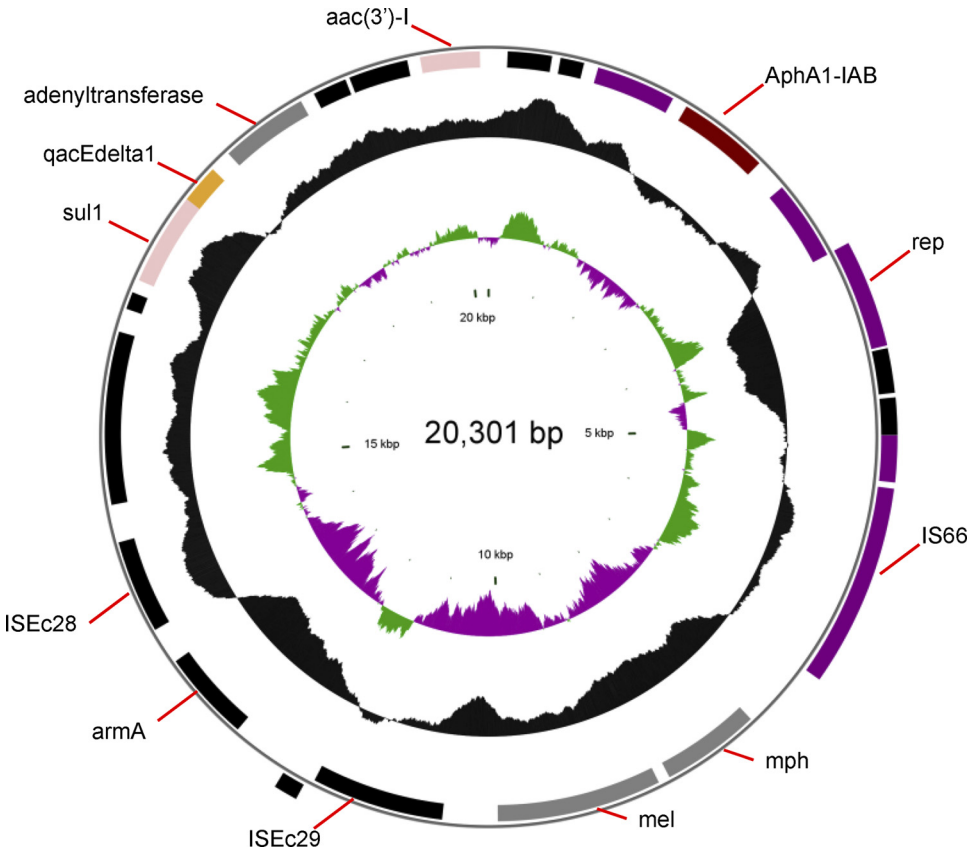

FIG. 4. Circular map of plasmid pZJ06. The two outer circles represent ORFs in the plus (outside) and minus (inside) orientations, respectively. The two inner circles represent the G+C content plotted against the average G+C content of 47.4% (black circle) and GC skew information (green and purple circles).

netic environment of MDR-ZJ06 is also identical to those of *Klebsiella oxytoca* and *K. pneumoniae*, which were isolated from the same region as MDR-ZJ06 (15, 33).

Posttranscriptional rRNA methylation by 16S rRNA methylase has been associated with a high level of resistance to aminoglycosides in the *Enterobacteriaceae* (12, 31). About 66.4% of IRABs in China are *armA* positive and are resistant to aminoglycosides (32). The present work found that plasmid-carried *armA* is present in a dominant clone of IRABs in China, suggesting that the plasmid confers the widespread ArmA methylase-mediated aminoglycoside resistance in *A. baumannii* isolates. According to the methods described by Bertini et al. (4), the replicon of pZJ06 was classified in the Rep-3 group of *A. baumannii* with a similarity of ca. 80%, suggesting that pZJ06 may be particularly associated with *A. baumannii*. Nevertheless, the Tn*1548*-harboring *armA* gene in pZJ06 is identical to that of plasmids of members of the *Enterobacteriaceae* isolated from the worldwide regions, suggesting the possibility of Tn*1548*-mediated horizontal transfer of the *armA* gene between plasmids of the *Enterobacteriaceae* and *Acinetobacter* spp.

Other resistance mechanisms in MDR-ZJ06. Our genome analyses have also identified some other genes associated with resistance to β-lactams, aminoglycosides, quinolones, tetracyclines, chloramphenicol, and sulfonamide, including a cephalosporinase gene, an aminoglycoside degradation enzyme-coding gene, a 16S rRNA methylase gene, multidrug efflux genes, and others (Table 5).

IS elements are associated with the genome gain/loss of genes, especially resistant genes. Apart from IS elements, IS*Aba1* could also provide strong promoters to upregulate the expression of antibiotic resistance genes (21, 27, 29). IS*30*, IS*Aba6*, and IS*Aba1* were found upstream of bla_{ampC} in *A. baumannii* isolates ACICU, SDF, and AYE, respectively. In this study, IS*Aba1* was found to be present in the same location in MDR-ZJ06. IS*Aba1* has been shown to play a role in upregulation of bla_{ampC} in *A. baumannii* isolates (13). We found that bla_{ampC} expression was more than 26-fold higher in MDR-ZJ06 than in ATCC 19606 (data not shown).

A class 1 integron, including the integrase gene (ABZJ_01295) and three resistance genes, *aac(6)-Ib* (ABZJ_01297), *catB8* (ABZJ_01299), and *aadA1* (ABZJ_01300), was also found in the chromosome of MDR-ZJ06, in addition to AbaR22 and the plasmid. These genetic elements may also play a role in the drug resistance of MDR-ZJ06.

Multidrug efflux pumps and porins may play roles in *A. baumannii* antimicrobial resistance (30). In this study, our genome analyses revealed the porins CarO (ABZJ_03018) and OprD-like proteins (ABZJ_00224, ABZJ_01013, ABZJ_01357, and ABZJ_02177), but these genes were complete and not disrupted. The coding genes of transporters AdeABC, AdeIJK, AdeT, and AbeM were also in MDR-ZJ06. The

TABLE 5. Antimicrobial resistance-associated genes in MDR-ZJ06 genome

Antimicrobial class	Enzyme class/family	Coding gene(s)	Locus tag(s); genetic location
β-Lactamases	Intrinsic cephalosporinase	*bla*$_{adc-30}$	ABZJ_02776; chromosome
	Class D OXA enzymes	*bla*$_{oxa-23}$	ABZJ_02004; chromosome
		bla$_{oxa-66}$	ABZJ_01736; chromosome
	Enzymatic degradation	*aac(2′)-Ib*	ABZJ_00200; chromosome
		aph(6)-Id	ABZJ_00268; chromosome
		aph(3″)-Ib	ABZJ_00269; chromosome
		aac(6)-Ib	ABZJ_01297; chromosome and integron association
Aminoglycosides	Enzymatic degradation	*aadA1*	ABZJ_01300; chromosome and integron association
		aadA1	pABZJ_00026; plasmid and integron association
		aacC1	pABZJ_00029; plasmid and integron association
	16S rRNA gene methylase	*aphA1-IAB*	pABZJ_00004; plasmid and integron association
		armA	pABZJ_00017; plasmid
Tetracyclines	Efflux	*tetA*	ABZJ_00261; chromosome
Quinolone	DNA gyrase mutations	*gyrA* (Ser-Ler) mutation at position 83	ABZJ_02465; chromosome
Chloramphenicol	Acetyltransferase	*catB6*	ABZJ_01329; chromosome
		catB6	ABZJ_01299; chromosome and integron association
Sulfonamide	Dihydropteroate synthase	*sul1*	ABZJ_03130, ABZJ_01302, ABZJ_00240, ABZJ_00259; chromosome
Efflux pumps	RND family	*adeABC*	ABZJ_02017, ABZJ_02018, ABZJ_02019; chromosome
		adeIJK	ABZJ_03188, ABZJ_03189, ABZJ_03190; chromosome
		abeM	ABZJ_00435; chromosome
		adeT	ABZJ_03885; chromosome

AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in *Acinetobacter calcoaceticus-A. baumannii* complex (30). The overexpression of the *adeB* gene was also detected in strain MDR-ZJ06 (data not shown), which might result in the increased MIC to tigecycline. The roles of effluxes and porins in drug resistance in MDR-ZJ06 need to be further defined.

The production of pili is essential for biofilm formation by this clinical strain (11). These pili are the products of the *csuA*/*BABCDE* operon. Interestingly, the *csuA*/*B* operon, which is present in the *A. baumannii* strains ACICU, ATCC 19606, AB0057, AYE, and ATCC 17978, was not found in MDR-ZJ06. The P pilus assembly protein PapD, a homolog of the staphylococcal biofilm-associated protein that is conserved among *Acinetobacter* strains, was found in MDR-ZJ06 (ABZJ_01996), indicating that this protein may play a role in MDR-ZJ06 adhesion and its biofilm formation (18).

Conclusions. Genomic analyses reveal that MDR-ZJ06, a widespread *A. baumannii* isolate in China, is genetically closest to the strain ACICU among completely sequenced *A. baumannii* isolates, indicating a worldwide spread of ECII. Accumulation of resistance genes was not detected in the genomic island AbaR22 in MDR-ZJ06, whereas some drug resistance genes are present in IS elements and the plasmid, suggesting that IS elements and the plasmid but not AbaR22 appear to be important in acquisition of resistance genes in this strain. This work may lay an important molecular foundation for future study of the mechanism of drug resistance in *A. baumannii*.

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