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Dissemination of bla_{OXA-23} in *Acinetobacter* spp. in China: Main Roles of Conjugative Plasmid pAZJ221 and Transposon Tn*2009*

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Production of the OXA-23 carbapenemase is the most common reason for the increasing carbapenem resistance in *Acinetobacter* spp. This study was conducted to reveal the genetic basis of bla_{OXA-23} dissemination in *Acinetobacter* spp. in China. A total of 63 carbapenem-resistant OXA-23-producing *Acinetobacter* sp. isolates, representing different backgrounds, were selected from 28 hospitals in 18 provinces for this study. Generally, two patterns of plasmids carrying bla_{OXA-23} were detected according to S1-nuclease pulsed-field gel electrophoresis and Southern blot hybridization. A ca. 78-kb plasmid, designated pAZJ221, was found in 23 *Acinetobacter baumannii* and three *Acinetobacter nosocomialis* isolates, while a novel ca. 50-kb plasmid was carried by only two other *A. baumannii* isolates. Three of these isolates had an additional copy of bla_{OXA-23} on the chromosome. Transformation of the two plasmids succeeded, but only pAZJ221 was conjugative. Plasmid pAZJ221 was sequenced completely and found to carry no previously known resistance genes except bla_{OXA-23}. The bla_{OXA-23} gene of the remaining 35 isolates was chromosome borne. The bla_{OXA-23} genetic environments were correlated with Tn*2009* in 57 isolates, Tn*2008* in 5 isolates, and Tn*2006* in 1 isolate. The MIC values for the carbapenems with these isolates were not significantly associated with the genomic locations or the copy numbers of bla_{OXA-23}. Overall, these observations suggest that the plasmid pAZJ221 and Tn*2009* have effectively contributed to the wide dissemination of bla_{OXA-23} in *Acinetobacter* spp. in China and that horizontal gene transfer may play an important role in dissemination of the bla_{OXA-23} gene.

The increasing trend of carbapenem resistance in bacteria worldwide has become a great concern in recent years since it dramatically limits the range of therapeutic alternatives (1, 2). In *Acinetobacter* spp., especially *Acinetobacter baumannii* isolates, the production of carbapenem-hydrolyzing class D β-lactamases, such as OXA-23, OXA-24, and OXA-58, has contributed frequently to carbapenem resistance (1, 3).

The bla_{OXA-23} gene was first identified in an *A. baumannii* isolate from Scotland. However, the progenitor of bla_{OXA-23} was found to be *Acinetobacter radioresistens*, a nonpathogenic and environmental *Acinetobacter* species, which was carbapenem susceptible and thus was considered a silent source of bla_{OXA-23} (4). Now, the bla_{OXA-23} gene is the most common acquired gene for carbapenem resistance among carbapenem-resistant *A. baumannii* (CRAB) (3, 5, 6). Moreover, the bla_{OXA-23} gene has also been detected in non-*baumannii Acinetobacter* spp. (7, 8). Transposable elements have played an essential role in the dissemination of the bla_{OXA-23} gene between different genomic locations within one bacterium or between different isolates (5).

Four main transposons, Tn*2006*, Tn*2007*, Tn*2008*, and Tn*2009*, were found to be associated with the transfer of bla_{OXA-23} in *A. baumannii* (5, 9–11). Besides sharing a common region (OXA-23-ΔATPase), all the transposons have IS*Aba1* upstream from bla_{OXA-23} except Tn*2007*, which is correlated with IS*Aba4* (5, 9–11). Tn*2006* and Tn*2009* were also identified in non-*baumannii* species of *Acinetobacter* (7, 8). Furthermore, the expression of bla_{OXA-23} has probably been enhanced by the strong promoters provided on the upstream insertion sequences of the transposons (12, 13).

The bla_{OXA-23} gene has mostly been identified on plasmids, although a chromosomal location has also been reported (5, 10, 14). The complete genome sequencing of *Acinetobacter* spp. showed that, even for isolates sharing the same genetic background that were collected from the same country and the same time period, the locations of bla_{OXA-23} can be different (10, 15). This phenomenon suggests that frequent horizontal transmission of this carbapenemase-encoding gene is possible, which is distinct from the simple clonal spread we previously anticipated(14). In addition, whether the location of the bla_{OXA-23} gene is influential to the susceptibility of a carbapenem is still unknown.

Our previous study showed that production of the OXA-23 carbapenemase was the predominant mechanism contributing to carbapenem resistance in *Acinetobacter* spp. in China (16, 17). In 2011, we reported the first genome sequence of a multidrug-resistant (MDR) *A. baumannii* strain in China, MDR-ZJ06, which belonged to clonal complex 92 (CC92) (10). In this strain, bla_{OXA-23} is carried on the composite transposon Tn*2009* and is located on the chromosome. However, the genetic characteristics of bla_{OXA-23} in other carbapenem-resistant *Acinetobacter* sp. isolates in China are still unclear. Therefore, this study was performed to investigate the genetic basis of the dissemination of the

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bla_{OXA-23} gene in carbapenem-resistant *Acinetobacter* sp. isolates from different backgrounds in China.

MATERIALS AND METHODS

Bacterial isolates. Between January 2009 and December 2010, 844 nonrepetitive OXA-23-producing CRAB isolates were collected from 28 hospitals in 18 provinces of China (6). Multilocus sequence typing analysis was performed according to Bartual et al. (6, 18). In addition, six OXA-23-producing *Acinetobacter nosocomialis* isolates were detected from 20 carbapenem-resistant non-*baumannii Acinetobacter* spp. in the same period, including *Acinetobacter calcoaceticus* isolates (9 isolates), *A. nosocomialis* (8 isolates), *Acinetobacter soli* (2 isolates), and *Acinetobacter haemolyticus* (1 isolate). All isolates were identified to the species level by PCR amplification of the bla_{OXA-51}-like gene and sequence analysis of the *rpoB* gene (19, 20). According to molecular typing and provincial distributions, 60 *A. baumannii* isolates and 3 *A. nosocomialis* isolates were selected for further study.

Antimicrobial susceptibility testing. Susceptibilities to piperacillin, ampicillin-sulbactam, piperacillin-tazobactam, cefepime, ceftazidime, amikacin, gentamicin, ciprofloxacin, aztreonam, and minocycline (Oxoid, United Kingdom) were determined by the disc diffusion method. The MICs for imipenem and meropenem were determined by the broth microdilution method (Oxoid, United Kingdom). *Escherichia coli* strain ATCC 25922 was used as the quality control. Manipulation and interpretation were in accordance with Clinical and Laboratory Standards Institute (CLSI) 2013 procedures (21).

PFGE and Southern blot analysis. To determine the plasmid location of the bla_{OXA-23} gene, genomic DNA digested with S1-nuclease (TaKaRa, Japan) was electrophoresed on a CHEF-mapper XA pulsed-field gel electrophoresis (PFGE) system (Bio-Rad, USA) for 18 h at 14°C with run conditions of 6 V/cm and pulse times from 2.16 s to 63.8 s. The DNA fragments were transferred to a positive-charged nylon membrane (Millipore, USA) and then hybridized with a digoxigenin-labeled bla_{OXA-23}-specific probe. The fragments then were detected using an NBT/BCIP color detection kit (Roche, Germany) (7). In order to identify plasmid patterns, genomic DNA of isolates with the plasmid-borne bla_{OXA-23} gene, digested with BamHI or EcoRI (TaKaRa, Japan), was separated and hybridized, as described above.

Furthermore, genomic DNA digested by ApaI (TaKaRa, Japan) was electrophoresed and then hybridized, as described above, to reveal the chromosomal locations of the bla_{OXA-23} gene through comparison of hybridization signals with S1-PFGE. ApaI-digested DNA was electrophoresed with a switch time from 5 to 20 s for 20 h. The XbaI-digested DNA of *Salmonella enterica* serotype Braenderup H9812 was electrophoresed as the size marker (22).

TABLE 1 Primers designed in this study

Primer	DNA sequence (5′ to 3′)	Target	Length (bp)
Z5	CGACTTATTTGATGGCTGACG	Tn*2009*	2,431
Z6	CTTGTGGATGCAACTCGGTAT		
Z7	GTAAGGTTGAGCCTGAAGT	Tn*2009*	2,025
Z8	TTTCTTTCCGATGCTTATTCC		
Z9	ATGCTCGCAATCGTTTATCGT	Tn*2009*	1,949
Z10	TCGCCAACTTCTTTGACTTCTG		
Rep-F	ACTCATCAAGGAATAAGACAGC	*repAZJ221*	959
Rep-R	ATCACACTCGCACATACAAT		

PCR amplification and DNA sequencing. PCR mapping was used to detect the occurrence of Tn*2006*, Tn*2007*, Tn*2008*, and Tn*2009*. The common region of those transposons (OXA-23-ΔATPase) was amplified using primers P3 and P5 (23). PCR targeting of IS*Aba1* or IS*Aba4* upstream from bla_{OXA-23} was performed, as previously described (9, 24). However, PCR mapping for Tn*2006* and Tn*2009* was performed using different primers (listed in Table 1).

Plasmid extraction, sequencing, and analysis. Plasmid DNA was extracted using a Qiagen plasmid midi kit (Qiagen, Germany). The plasmid DNA was further sequenced using HiSeq 2000 (Illumina, Inc., USA) technology following the 2× 100-bp paired-end protocol. The derived reads were assembled using the Velvet program version 1.1 (25). Gaps were filled by primer walking using plasmid DNA as a template. The plasmid sequence was annotated by the RAST server (26), and all of the predicted proteins were further compared against the NCBI nonredundant protein database using the BLASTP program. The CGview server was used to generate a circular map of plasmid pAZJ221 (27). In addition, plasmid types were identified by PCR-based replicon typing according to the *A. baumannii* PCR-based replicon typing (AB-PBRT) method (28).

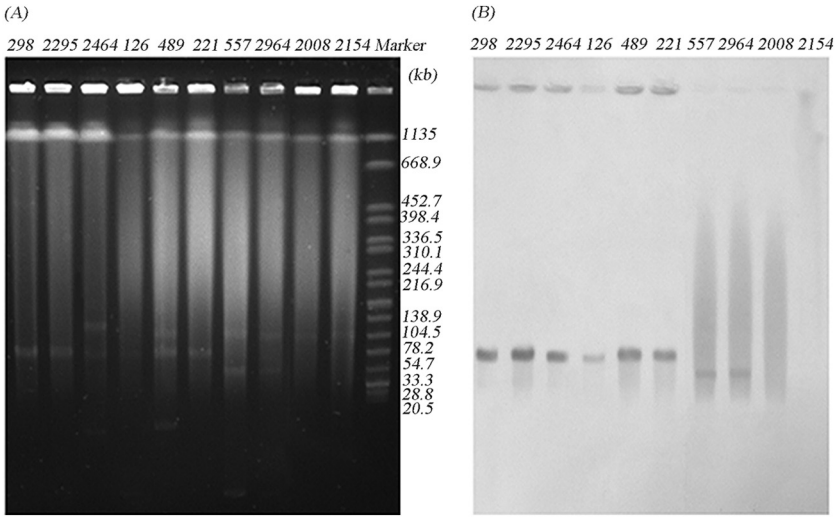

FIG 1 Analysis of the localization of bla_{OXA-23} using the S1 nuclease-PFGE method. Shown are PFGE profiles after S1 nuclease digestion (A) and Southern blot hybridization with a bla_{OXA-23} probe (B). The 10 isolates displayed were clustered into one of four groups: *Acinetobacter nosocomialis* isolates (298, 2295, and 2464), *A. baumannii* isolates carrying the 78-kb plasmid (126, 489, and 221), *A. baumannii* isolates carrying the 50-kb plasmid (557 and 2964), and *A. baumannii* isolates of the same clone with chromosomal bla_{OXA-23} (2008 and 2154). *Salmonella enterica* serotype Braenderup strain H9812 DNA digested by XbaI was used as a molecular marker (in kb).

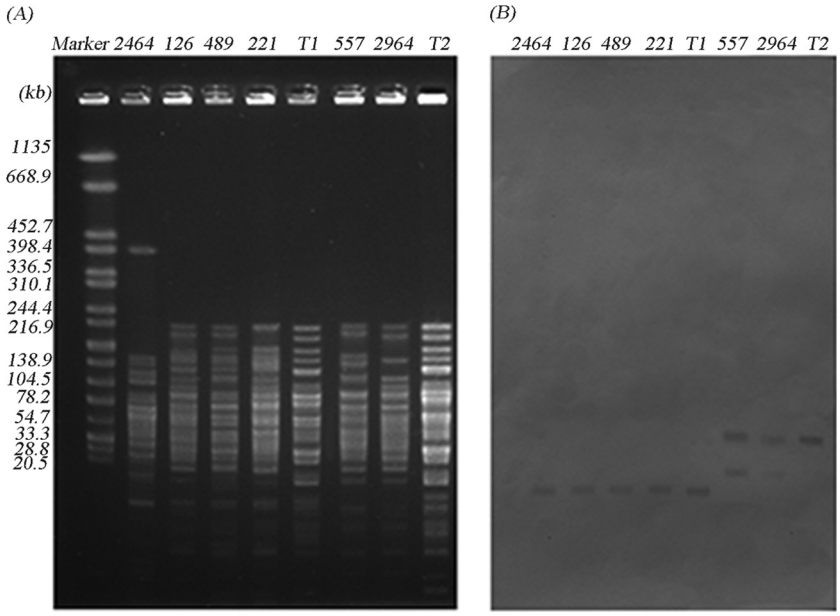

FIG 2 PFGE files of BamHI-digested genomic DNA of isolates with plasmid-borne bla_{OXA-23} (A) and Southern blot hybridization with a bla_{OXA-23} probe (B). T1 and T2 indicate the transformants associated with the 78-kb and 50-kb plasmids, respectively. The two isolates carrying the 50-kb plasmid have an additional copy of bla_{OXA-23} on the chromosome; thus, the donors have one more hybridization signal than the transformants. Detailed information of other isolates is given in the Fig. 1 legend. *Salmonella enterica* serotype Braenderup strain H9812 DNA digested by XbaI was used as a molecular marker (in kb).

Transferability of the bla_{OXA-23} gene. Filter mating was performed using a spontaneous rifampin-resistant mutant of *Acinetobacter baylyi* ADP1 (ADP1-rif^r) as the recipient strain (7). The transconjugants were selected based on growth on agar supplemented with imipenem (2 mg/liter) and rifampin (256 mg/liter).

The plasmid DNA was extracted as previously described and then electrotransformed into *A. baumannii* ATCC 17978 (7). The transformants were selected on agar plates containing imipenem (2 mg/liter). The transconjugants and transformants were confirmed as bla_{OXA-23} positive by PCR analysis.

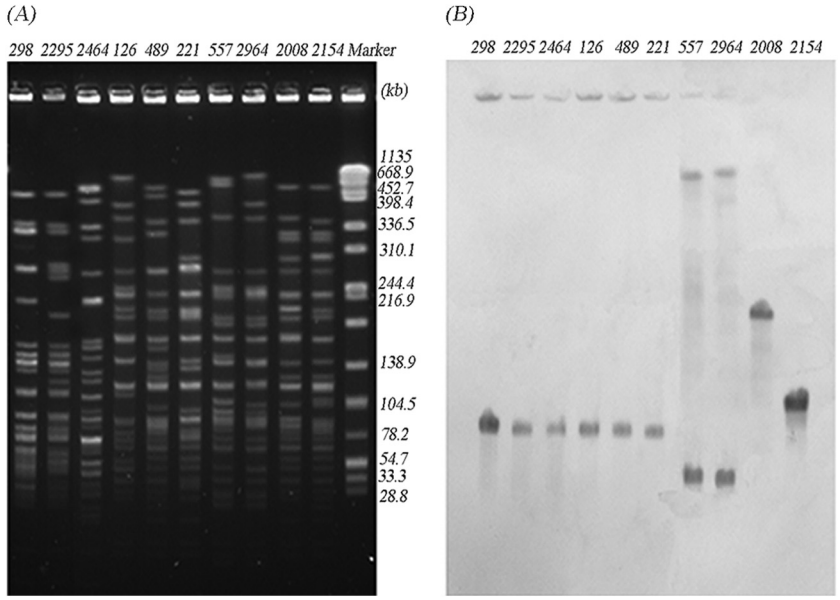

FIG 3 Analysis of the localization of bla_{OXA-23} using the ApaI-PFGE method. Shown are PFGE profiles after ApaI digestion (A) and Southern blot hybridization with a bla_{OXA-23} probe (B). The 10 isolates displayed are in the same order as in Fig. 1. *Salmonella enterica* serotype Braenderup strain H9812 DNA digested by XbaI was used as a molecular marker (in kb).

TABLE 2 Features of the bla_{OXA-23} gene

Species	Molecular pattern	Isolate no.	Province[a]	Transposon	Location	Copy no.
A. baumannii	ST75 (CC92)	6	XJ, BJ	Tn*2009*	Plasmid, 78 kb	1
			HB	Tn*2009*	Plasmid, 50 kb	1
					Chromosome	1
			GD, SH	Tn*2009*	Chromosome	1
			SC	Tn*2008*	Chromosome	1
	ST76 (CC92)	1	HLJ	Tn*2009*	Chromosome	1
	ST88 (CC92)	1	SC	Tn*2008*	Chromosome	1
	ST90 (CC92)	2	SH, HB	Tn*2009*	Chromosome	1
	ST91	3	XJ, ZJ, HN1	Tn*2009*	Chromosome	1
	ST92 (CC92)	7	XJ, BJ, HLJ, NMG	Tn*2009*	Plasmid, 78 kb	1
			HB, ZJ	Tn*2009*	Chromosome	1
			SC	Tn*2008*	Chromosome	1
	ST118 (CC92)	1	SX1	Tn*2009*	Plasmid, 78 kb	1
	ST136 (CC92)	1	GD	Tn*2009*	Plasmid, 50 kb	1
					Chromosome	1
	ST137 (CC92)	1	HLJ	Tn*2009*	Plasmid, 78 kb	1
	ST138 (CC92)	7	LN, SC, HLJ	Tn*2009*	Plasmid, 78 kb	1
			XJ	Tn*2009*	Plasmid, 78 kb	1
					Chromosome	1
			ZJ, HB, BJ	Tn*2009*	Plasmid, 78 kb	1
	ST189 (CC92)	1	XJ	Tn*2009*	Plasmid, 78 kb	1
	ST223 (CC92)	1	HN1	Tn*2009*	Plasmid, 78 kb	1
	ST254	1	GD	Tn*2009*	Chromosome	2
	ST346 (CC92)	1	SX1	Tn*2009*	Chromosome	2
	ST365 (CC92)	1	GD	Tn*2006*	Chromosome	1
	ST381 (CC92)	4	GD, SC, ZJ	Tn*2009*	Chromosome	1
			HB	Tn*2009*	Chromosome	2
	ST395 (CC92)	1	ZJ	Tn*2009*	Chromosome	1
	ST492 (CC92)	1	AH	Tn*2009*	Chromosome	1
	ST517	1	GD	Tn*2008*	Chromosome	1
	ST520	2	BJ, HLJ	Tn*2009*	Plasmid, 78 kb	1
	ST522	1	XJ	Tn*2009*	Chromosome	1
	ST523 (CC92)	1	XJ	Tn*2009*	Chromosome	1
	ST524	1	HN1	Tn*2009*	Plasmid, 78 kb	1
	ST525 (CC92)	1	SX	Tn*2009*	Plasmid, 78 kb	1
	ST526	1	NMG	Tn*2009*	Plasmid, 78 kb	1
	ST527 (CC92)	1	HN	Tn*2009*	Plasmid, 78 kb	1
	ST528	1	SH	Tn*2009*	Chromosome	1
	ST529	1	JS	Tn*2009*	Chromosome	1
	ST531	1	GD	Tn*2008*	Chromosome	2
	ST532 (CC92)	1	BJ	Tn*2009*	Plasmid, 78 kb	1
	ST533 (CC92)	1	GD	Tn*2009*	Chromosome	1
	ST668	1	SD	Tn*2009*	Plasmid, 78 kb	1
	ST669 (CC92)	1	HLJ	Tn*2009*	Plasmid, 78 kb	1
	ST670 (CC92)	1	HN1	Tn*2009*	Chromosome	1
	ST671	1	JX	Tn*2009*	Chromosome	1
	ST736 (CC92)	1	SH	Tn*2009*	Chromosome	1
A. nosocomialis	PFGE clone A	1	BJ	Tn*2009*	Plasmid, 78 kb	1
	PFGE clone B	1	BJ	Tn*2009*	Plasmid, 78 kb	1
	PFGE clone C	1	HN1	Tn*2009*	Plasmid, 78 kb	1

[a] AH, Anhui; BJ, Beijing; GD, Guangdong; HB, Hubei; HLJ, Heilongjiang; HN, Hunan; HN1, Henan; LN, Liaoning; JS, Jiangsu; JX, Jiangxi; NMG, Inner Mongolia; SC, Sichuan; SD, Shandong; SH, Shanghai; SX, Shanxi; SX1, Shaanxi; XJ, XinJiang; ZJ, Zhejiang.

Nucleotide sequence accession number. The nucleotide sequence of plasmid pAZJ221 has been submitted to the EMBL/GenBank database under the accession number KM922672.

RESULTS AND DISCUSSION

Bacterial isolates and susceptibility testing. The 60 OXA-23-producing CRAB isolates that were selected for further study belonged to 36 different sequence types (STs) (6). Forty-five of the CRAB isolates were grouped into CC92, and ST92, ST138, and ST75 were the most common STs. The other 15 CRAB isolates were of diverse genetic backgrounds, and only 3 of them belonged to the same CC (CC254). The three OXA-23-producing carbapenem-resistant *A. nosocomialis* (CRAN) isolates, which were col-

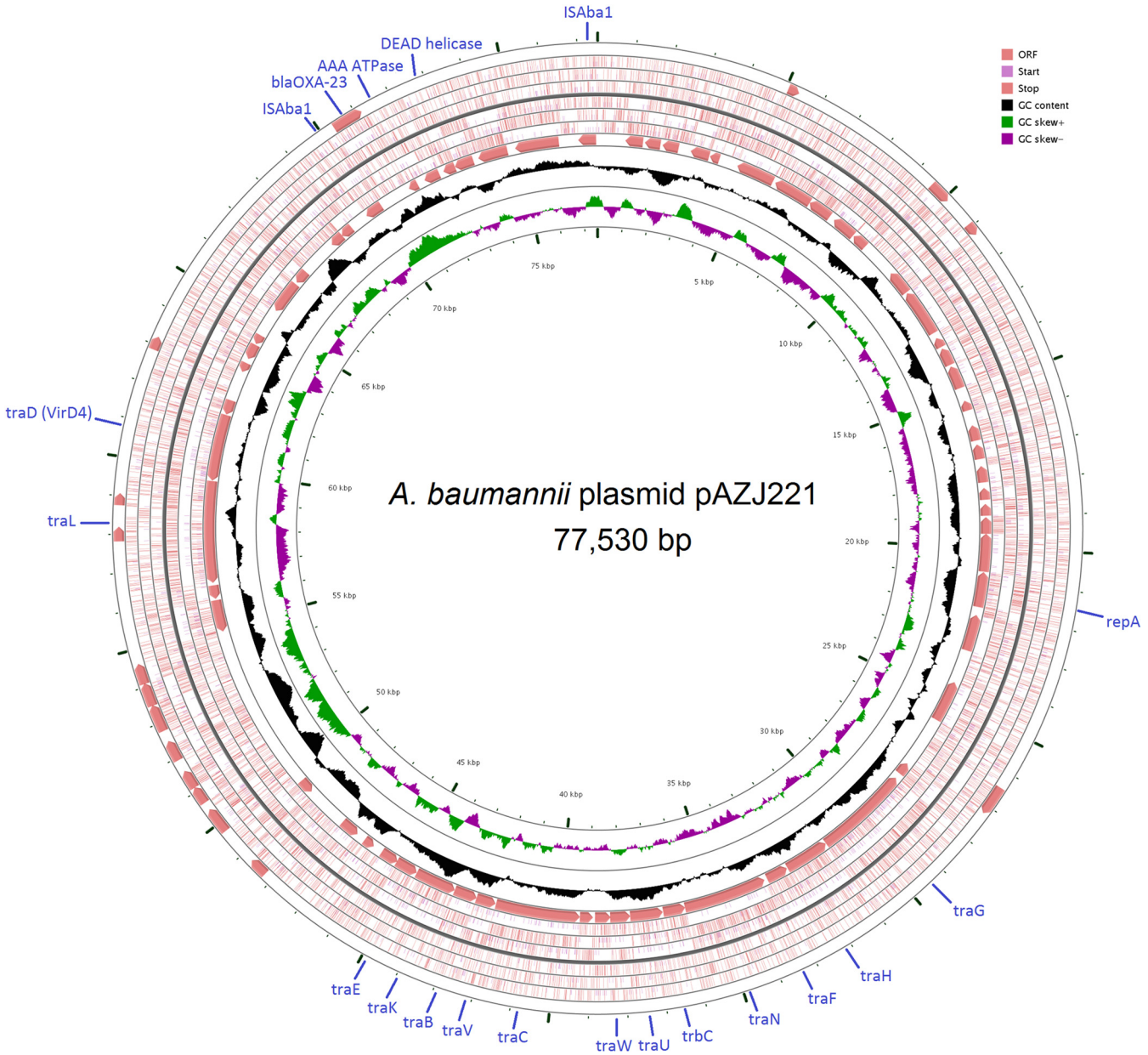

FIG 4 Circular map of plasmid pAZJ221. The two inner circles indicate the G+C content plotted against the average G+C content of 34.03% (black circle) and GC skew information (green and purple circles). The outer circles display the open reading frames (ORFs) in opposite orientations. Regions related to conjugation, replication, and Tn*2009* are marked in blue.

lected from three hospitals in two provinces, belonged to three different PFGE patterns and were included in this study.

Among the 63 isolates, the MIC values for meropenem ranged from 32 to 256 mg/liter, and the imipenem MICs ranged from 16 to 128 mg/liter. All of the isolates showed high resistance rates (>90%), except to ceftazidime (82.5%), to the penicillins, monobactams, quinolones, and extended-spectrum cephalosporins. The rates of resistance to gentamicin and amikacin were 85.7% and 71.4%, respectively. Minocycline (66.7% susceptibility) was the most active antimicrobial tested.

Location of the *bla*$_{OXA-23}$ gene. *Acinetobacter radioresistens* was found to be the progenitor of *bla*$_{OXA-23}$, and plasmids played an essential role in the mobilization process of *bla*$_{OXA-23}$ from *A. radioresistens* to *A. baumannii* (4). By S1-nuclease digestion and Southern blot analysis, the *bla*$_{OXA-23}$ gene was found to be plasmid encoded in 25 CRAB isolates and in all 3 CRAN isolates in this study. According to the hybridization signals of CRAB, the *bla*$_{OXA-23}$ gene was located on ca. 78-kb plasmids in 23 isolates and on ca. 50-kb plasmids in 2 isolates, assigned into 15 STs and 2 STs, respectively. Interestingly, three CRAN isolates were found to be associated with the same ca. 78-kb plasmid as well (Fig. 1). Furthermore, digestion by BamHI (Fig. 2) or EcoRI (not shown) clas-

TABLE 3 Antibiotic susceptibilities of representative isolates with plasmid-borne bla_{OXA-23} and their transconjugants and/or transformants

Isolate	Antibiotic[a] susceptibility by:											
	MIC (mg/liter)		Inhibition zone (mm)									
	MEM	IPM	ATM	CAZ	FEP	PRL	SAM	TZP	AK	CIP	CN	MH
A221[b]	128	64	6	6	6	6	6	6	6	6	6	16
A221-ADP1-rif^r	64	64	18	23	14	6	6	6	26	29	27	27
A221-17978	64	32	13	19	10	6	6	6	20	27	19	23
A2964[b]	64	64	6	6	6	6	6	9	6	10	6	14
A2964-17978	64	64	13	18	12	6	6	11	20	24	19	22
ADP1-rif^r	0.094	0.094	18	25	25	24	24	28	27	30	27	24
ATCC 17978	0.38	0.25	15	18	20	20	20	24	21	25	18	21

[a] MEM, meropenem; IPM, imipenem; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; PRL, piperacillin; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; AK, amikacin; CIP, ciprofloxacin; CN, gentamicin; MH, minocycline.
[b] A221 and A2964 carried the 78- and 50-kb plasmids, respectively.

sified these plasmids into two distinct patterns, suggesting that these bla_{OXA-23}-carrying plasmids belonged to only two plasmid types.

To reveal the chromosomal location of bla_{OXA-23}, ApaI-PFGE and hybridization were performed on all 63 isolates. Through comparison with the hybridization signals of S1-PFGE, ApaI-PFGE and hybridization with the probe of bla_{OXA-23} revealed that 38 isolates harbored the bla_{OXA-23} gene on the chromosome (Fig. 3). Chromosomal locations of bla_{OXA-23} make it less likely for the bacteria to lose carbapenem resistance. Therefore, clonal spread might make the dissemination of bla_{OXA-23} wider. Moreover, the carbapenem resistance of *Acinetobacter* spp. will still exist for a long period of time, even without the antimicrobial selective pressure found in hospital settings. In particular, four of the isolates harbored two chromosomal copies of the bla_{OXA-23} gene on different fragments. Three isolates had one copy on the chromosome and another copy on a plasmid; in two of these three isolates, the 50-kb plasmid carried bla_{OXA-23}, which had an additional hybridization band compared to their transformants examined through BamHI/EcoRI-PFGE and hybridization (Fig. 2). In the 45 CC92 isolates, the PFGE bands were similar, as expected, but the bla_{OXA-23} hybridization signals appeared at different bands, ranging from ca. 33.3 kb to 336.5 kb. Different insertion sites of bla_{OXA-23} on the chromosomal fragments can be considered another piece of evidence that supports horizontal gene transfer instead of clonal spread. Details about the locations and genetic surroundings of bla_{OXA-23} are shown in Table 2.

The carbapenem resistance of isolates with different locations or copy numbers of bla_{OXA-23} was further studied. The 63 isolates were divided into three groups according to the locations of the bla_{OXA-23} gene, i.e., plasmid, chromosome, and both locations (plasmid and chromosome). The MIC_{50} and MIC_{90} values for meropenem and imipenem fluctuated between 64 and 128 mg/liter in the three groups. In addition, for isolates with only one copy of the bla_{OXA-23} gene, the MIC_{50} and MIC_{90} values for the two carbapenems tested were 64 and 128 mg/liter, respectively. For isolates with two copies of the bla_{OXA-23} gene, the MIC_{50} and MIC_{90} for meropenem were 64 and 128 mg/liter, respectively; however, the two values for imipenem were the same (64 mg/liter). These facts revealed that the carbapenem susceptibility was not significantly different among isolates carrying bla_{OXA-23} on different genetic locations. Moreover, the increased number of copies of bla_{OXA-23} does not obviously change the carbapenem susceptibility. Different locations or different copies of bla_{OXA-23} may only be pieces of evidence revealing the evolution process.

Genetic environment of the bla_{OXA-23} gene. In this study, we found that 54 of 60 CRAB and all three CRAN isolates were associated with Tn*2009*, while five CRAB isolates were correlated with Tn*2008*. Tn*2009* was detected in all 18 provinces included in this study, while Tn*2008* was found only in the Beijing, Sichuan, and Guangdong provinces. In addition, one isolate of ST365, collected from the Guangdong province, carried chromosome-borne bla_{OXA-23} on Tn*2006*. Tn*2007* was not detected in these isolates.

Tn*2009* and Tn*2008* have played dominant roles in the dissemination of bla_{OXA-23} in China (10, 29). However, Tn*2006* has been the most common transposon associated with OXA-23-producing *A. baumannii* in many countries (5). The genetic structures of Tn*2009* and Tn*2006* were similar, but Tn*2009* has an additional 2-kb segment between the DEAD/DEAH box helicase gene and the ATPase gene, and the orientations of IS*Aba1* are different (10). In this study, the dissemination of Tn*2008* was limited in three provinces, which might be due to its chromosomal location. In addition, the target site duplications were different among those transposons; thus, the bla_{OXA-23} gene can be inserted into different locations in isolates with the same genetic background (10). These facts suggest that isolates of *Acinetobacter* spp. acquire carbapenem resistance independently under antimicrobial selective pressure in different environments. Moreover, the detection of common transposable elements in isolates of different molecular patterns revealed the horizontal transfer of the bla_{OXA-23} gene.

Plasmid analysis. As the most common vehicle carrying the bla_{OXA-23} gene in China, the ca. 78-kb plasmid designated pAZJ221 was sequenced and then analyzed. *In silico* analysis showed that the plasmid pAZJ221 was 77,530 bp in size and contained 108 open reading frames (ORFs), with an average G+C content of 34.03%. The plasmid consisted of two main regions, a complete array of genes associated with conjugation and the composite transposon Tn*2009* (Fig. 4). Moreover, this plasmid did not contain cleavage sites for the restriction endonuclease ApaI. Further analysis indicated that this plasmid shared 99% nucleotide identity with a previously described plasmid, pABTJ1, which was carried by *A. baumannii* MDR-TJ of global clone 2, isolated from Tianjin, China (30). These two plasmids were associated with the same replicase gene, which was designated *repAZJ221* in this study, and shared 67% identity with the replicase gene of

pACICU2 (30). No resistance genes other than bla_{OXA-23} were found in pAZJ221.

Neither of the plasmids were classified into any previously known replicon group using the current scheme; therefore, a pair of primers, which were added to the AB-PBRT system, was designed in this study to amplify a 959-bp region of the replicase gene of pAZJ221. Furthermore, all isolates carrying the ca. 78-kb plasmid were confirmed as carrying the same replicase gene, *repAZJ221*. To detect *A. baumannii* resistance plasmids more fully, the replicase gene associated with pAZJ221, which may represent a new homology group (GR20), was added into the multiplex 4 of the AB-PBRT system. Moreover, we were still unable to type the 50-kb bla_{OXA-23}-carrying plasmid, which indicates that it represented a new replicon group.

Transferability of the bla_{OXA-23} gene. The plasmid pAZJ221 was successfully transferred to *A. baylyi* ADP1-rif^r through conjugation, while transfer of the 50-kb plasmid failed. The two plasmids were successfully transformed into *A. baumannii* ATCC 17978 through electroporation. Except for susceptibility to ceftazidime, all transformants and transconjugants had a β-lactam resistance pattern consistent with their donors, with imipenem and meropenem MICs of ≥32 mg/liter (Table 3). These isolates were susceptible to the aminoglycosides and quinolones tested. Thus, from the susceptibility profile of the transformants, we deduced that the 50-kb plasmid might also carry no resistance genes except bla_{OXA-23}. Furthermore, the ca. 50-kb plasmid was detected in two isolates, probably indicating that the mobility of this nonconjugative plasmid was limited.

In conclusion, the conjugative plasmid pAZJ221 and Tn*2009* may play important roles in the intra- and interspecies transfer process and further integration of bla_{OXA-23} in China. Such transposable elements might make the dissemination of bla_{OXA-23} easier.

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