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ARTICLE

Prevalence of carbapenem-hydrolyzing class D β-lactamase genes in *Acinetobacter* spp. isolates in China

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Abstract In order to assess the prevalence of carbapenem-hydrolyzing class D β-lactamase genes in *Acinetobacter* spp. isolates in China, we conducted a polymerase chain reaction (PCR)-based surveillance of OXA-type β-lactamase gene clusters for a total of 2,880 *Acinetobacter* spp. isolates collected from 23 Chinese provinces. All isolates were tested for susceptibility to 12 antimicrobial agents and showed high rates of resistance to all these agents except minocycline. We also found that the vast majority of carbapenem-resistant *Acinetobacter* spp. were OXA-23-like-producing isolates, predominantly *Acinetobacter baumannii* isolates. Besides, $bla_{\text{OXA-58}}$-like and $bla_{\text{OXA-24}}$-like genes were detected in 32 and 11 isolates, respectively, involving many provinces throughout China. Furthermore, these two carbapenem-resistance determinants were located on transferable plasmids in most cases, indicating an emerging threat for both OXA-58-like- and OXA-24-like-producing *Acinetobacter* spp. isolates in China. Interestingly, a novel homologue of the $bla_{\text{OXA-143}}$ gene was identified in a susceptible *Acinetobacter pittii* isolate. Overall, these observations suggest that the $bla_{\text{OXA-23}}$-harboring *A. baumannii* isolates are the most frequent carbapenem-resistant *Acinetobacter* spp. in China, and the $bla_{\text{OXA-24}}$-like and $bla_{\text{OXA-58}}$-like genes have emerged as potential threats of hospital outbreaks of multidrug-resistant *Acinetobacter* spp.

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Introduction

In recent years, carbapenem resistance in species of the genus *Acinetobacter* has increased worldwide, and the rapid spread of carbapenem-resistant *Acinetobacter baumannii* (CRAB) poses a severe threat to public health [1, 2].

Some reports identified Ambler class A carbapenemases and metallo-β-lactamases (MBLs) in *Acinetobacter* spp., but carbapenem resistance in these species has mostly been associated with the production of five main groups of carbapenem-hydrolyzing class D β-lactamases (CHDLs), namely, OXA-23-like, OXA-24-like, OXA-51-like, OXA-58-like, and OXA-143-like enzymes [2]. The OXA-23-like enzyme now contributes to carbapenem resistance in *A. baumannii* throughout the world [3–5]. The OXA-24-like and OXA-58-like enzymes are often involved in hospital outbreaks of CRAB in some European countries, such as Spain, France, Belgium, Italy, and Greece [4, 6]. The OXA-143-like enzyme is the first representative of a novel subgroup of CHDLs, and a high prevalence of OXA-143-producing *Acinetobacter* isolates has been reported in Brazil [7, 8].

Regarding the current situation in China, our group previously reported clonal dissemination of CRAB harboring $bla_{\text{OXA-23}}$-like among different cities [9], while OXA-24 and OXA-58 were found in several carbapenem-resistant *Acinetobacter* spp. isolates by Wang et al. in a small-scale molecular epidemiological study in 2007 [10]. However, the distribution of bla_{OXA} and their contribution to the high carbapenem resistance rates of *Acinetobacter* spp. in the mainland of China are still unclear. In this study, we collected 2,880 *Acinetobacter* spp. isolates across different geographical regions of China to assess the prevalence of CHDLs and studied the clonal relationship and genomic environment of these CHDLs genes.

Materials and methods

Bacterial strains and susceptibility testing

A total of 2,880 sequential clinical *Acinetobacter* spp. isolates (up to a maximum of 50 per hospital) were collected from 67 hospitals representing 23 provinces in China from January 2009 to December 2010 (Fig. 1). Each hospital undertook collection for three consecutive months. Isolates were obtained from bile (n=20), blood (n=71), catheter (n=26), exudate (n=192), lavage (n=53), puncture fluid (n=41), respiratory tract (n=68), skin (n=2), sputum (n=2,276), tissue (n=16), urine (n=104), and wound (n=11). All isolates were identified to the genus level using Vitek GNI+cards (bioMérieux, Marcy l'Etoile, France). *A. baumannii* were confirmed by polymerase chain reaction (PCR) detection of the $bla_{\text{OXA-51}}$-like gene. 16S-23S rRNA gene intergenic spacer (ITS) sequencing and partial RNA polymerase β-subunit (*rpoB*) sequencing were performed for the identification of the other *Acinetobacter* species, as previously described [11, 12]. Antimicrobial susceptibility testing was performed by the disk diffusion method and Etest strips, and the results of susceptibility testing were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2012 guidelines [13].

Molecular detection of resistance genes

Isolates were screened for the presence of bla_{OXA} genes, including $bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-24}}$-like, $bla_{\text{OXA-51}}$-like, $bla_{\text{OXA-58}}$-like, and $bla_{\text{OXA-143}}$-like genes, by multiplex PCR

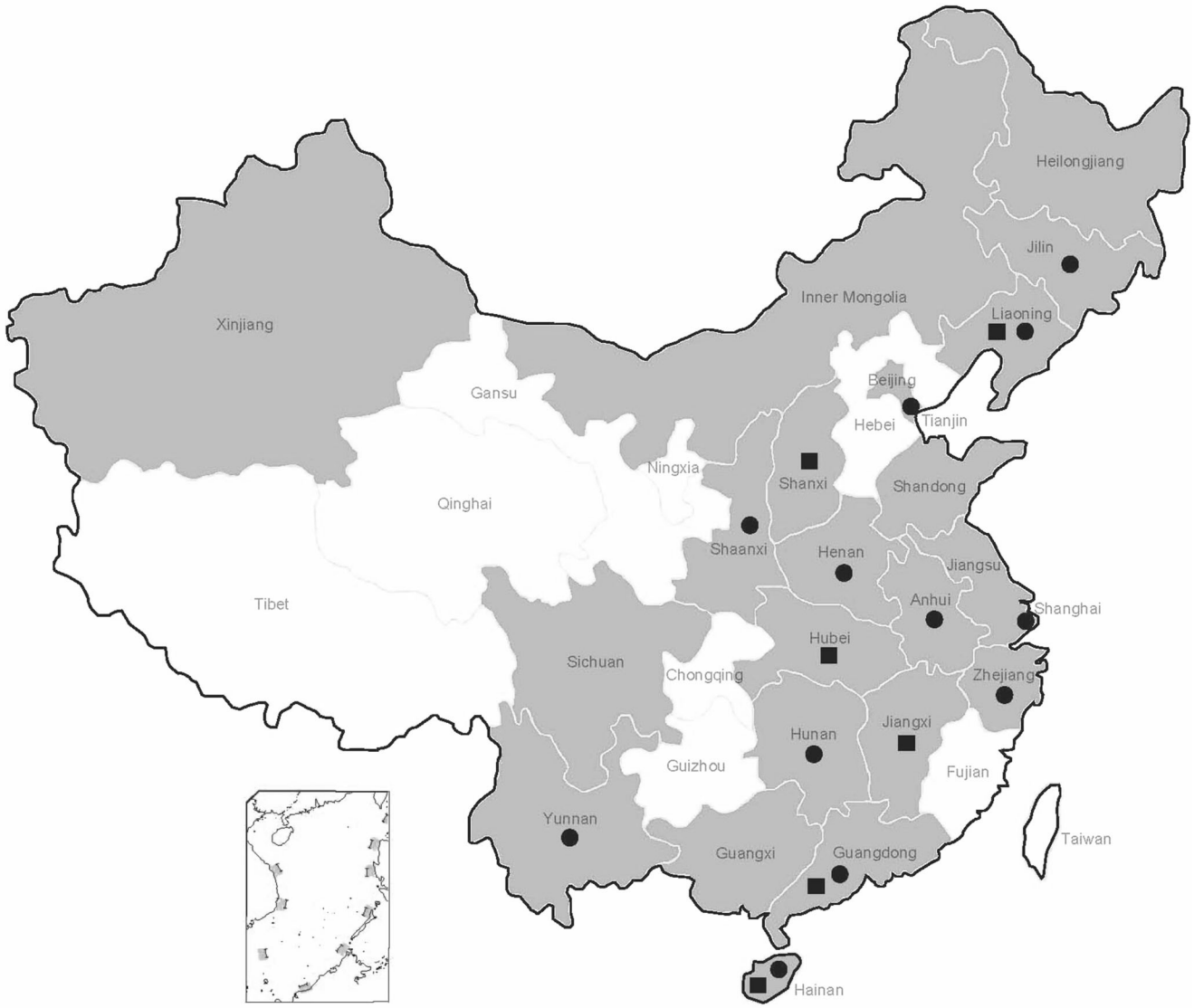

Fig. 1 Distribution of carbapenem-hydrolyzing class D β-lactamases (CHDLs) in *Acinetobacter* spp. in China. OXA-24-like-producing isolates are denoted with *squares* and OXA-58-like-producing isolates are denoted with *circles*. The provinces covered in this study are highlighted in *gray* and OXA-23-like-producing isolates were detected in all these provinces

using published primers and PCR parameters [14]. PCR screening was performed for additional MBLs genes of the bla_{OXA-24}-like and bla_{OXA-58}-like isolates, including bla_{IMP}-like, bla_{VIM}-like, bla_{SIM-1}, and bla_{NDM-1} [15, 16].

Plasmid analysis and Southern blot

Genomic DNA was digested with S1 nuclease and separated by pulsed-field gel electrophoresis (PFGE) with a switch time from 2.16 to 63.8 s for a 20-h runtime. Then, the DNA fragments were transferred to nylon membranes (Millipore, Billerica, MA, USA), hybridized with digoxigenin-labeled bla_{OXA-24}-like-specific probes, and detected using an NBT/BCIP color detection kit (Roche Applied Science, Mannheim, Germany).

Plasmid DNA was extracted with the Qiagen Midi Kit (Qiagen, Hilden, Germany). The transformation of plasmids was performed using electroporation and *Acinetobacter baylyi* ADP1 as the recipient. Transformants were selected on agar plates containing meropenem (1 mg/L) and confirmed by PCR analysis.

MLST

Multilocus sequence typing (MLST) was carried out using seven standard housekeeping loci (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*) as described by Fu et al. and Bartual et al. [3, 17]. The novel sequence types (STs) unassigned were numbered N1, N2, N3, etc. consecutively.

PCR mapping and primer walking for the genetic context of bla_{OXA-58}-like

Primers (IS*6*F 5′-CGTAAGCCGTCTTCATGGAT-3′ and IS*Aba3*R 5′-CTTCTGAAGCTACGCCTAAT-3′) were designed to determine the presence of IS*6* family-ΔIS*Aba3*-like-bla_{OXA-58}-like-IS*Aba3* structure in bla_{OXA-58}-like-harboring isolates according to the sequences previously deposited in GenBank under nucleotide sequence accession number GU327621 [18].

The plasmids extracted from the ten plasmid-mediated bla_{OXA-24}-like-harboring isolates were partially sequenced on both strands by primer walking.

Results

Distribution of bla_{OXA}-like genes and antimicrobial susceptibility testing

Multiplex PCRs for the detection of the five OXA carbapenemase gene groups were performed with all 2,880 *Acinetobacter* spp. isolates. bla_{OXA-51}-like genes were found in 2,197 isolates, all of which were presumptively identified as *A. baumannii* in this study. bla_{OXA-23}-like, bla_{OXA-24}-like, bla_{OXA-58}-like, and $bla_{OXA-143}$-like genes were detected in 1,316, 11, 32, and 1 isolates, respectively.

The resistance rates of these 2,880 *Acinetobacter* spp. isolates to the antimicrobial agents tested were about 50 %, except for minocycline (11.6 %) (Table 1). A total of 1,399 (48.6 %) and 1,442 (50.1 %) isolates were resistant to imipenem and meropenem, respectively, while 1,354 (47.0 %) isolates were resistant to both.

OXA-51 group

Among the 2,880 *Acinetobacter* spp. isolates, 2,197 (76.3 %) possessed bla_{OXA-51}-like genes and were identified presumptively as *A. baumannii*. The rates of resistance to β-lactams, aminoglycosides, and quinolones in bla_{OXA-51}-like-positive isolates were significantly higher than those in bla_{OXA-51}-like-negative isolates (Table 1). 62.6 % (1,375 isolates) of the *A. baumannii* isolates were non-susceptible to carbapenems.

OXA-23 group

bla_{OXA-23}-like genes were detected in 1,316 isolates, which covered all provinces included in this study, and 98.3 % (1,294 isolates) were *A. baumannii*. The resistance rates of these bla_{OXA-23}-like-harboring isolates to the 12 antimicrobial agents tested were extremely high, with a range from 86.1 to 99.2 %, except for minocycline (Table 1). In particular, the resistance rate of these bla_{OXA-23}-like-positive isolates to imipenem and meropenem was above 95 %.

OXA-24 group

bla_{OXA-24}-like genes were detected in 11 isolates obtained from six provinces, which were identified as *A. baumannii* (n=6), *A. pittii* (n=4), and *A. baylyi* (n=1) (Table 2). Isolate A1429 possessed bla_{OXA-24} and the other isolates possessed bla_{OXA-72} genes. In addition, all these isolates were resistant to meropenem and no MBL gene was detected. The six bla_{OXA-24}-like-positive *A. baumannii* isolates were classified into five STs. Interestingly, three isolates (A2485, A2503, and A2706) belonged to CC92, had multidrug resistance phenotypes to all β-lactams, aminoglycosides, and quinolones tested, and co-carried bla_{OXA-23}-like genes (Table 2).

Southern blot hybridization indicated that the bla_{OXA-24}-like genes were located on different small plasmids (<20.5 kb), except A2706 (data not shown). The plasmids carrying bla_{OXA-24}-like genes from the ten isolates were successfully transferred to *A. baylyi* ADP1. The transformants exhibited at least 30-fold higher minimum inhibitory concentration (MIC) values for carbapenems compared with those for

Table 1 Antibiotic susceptibilities of *Acinetobacter* spp. isolates

Antimicrobials	Percentage of isolates that were resistant				
	Total (*n*=2,880)	OXA-51+ (*n*=2,197)	OXA-51− (*n*=683)	OXA-23+(*n*=1,316)	OXA-23− (*n*=1,564)
GEN	63.0	74.0	27.5	94.4	36.6
AMK	56.5	68.9	16.8	88.2	29.9
IMP	48.6	58.9	15.4	96.9	8.1
MEM	50.1	61.0	15.1	97.3	10.3
CIP	63.9	76.7	22.6	97.9	35.2
MIN	11.6	14.0	3.8	17.6	6.5
CAZ	62.2	74.7	21.8	95.4	34.1
CTX	65.3	77.4	26.2	98.0	37.7
FEP	56.0	67.5	19.2	94.3	23.9
TZP	62.3	75.6	19.8	99.1	31.4
PRL	65.9	77.8	27.7	99.2	38.0
SAM	51.9	61.8	20.4	86.1	23.2

GEN gentamicin; *AMK* amikacin; *IMP* imipenem; *MEM* meropenem; *CIP* ciprofloxacin; *MIN* minocycline; *CAZ* ceftazidime; *CTX* cefotaxime; *FEP* cefepime; *TZP* piperacillin/tazobactam; *PRL* piperacillin; *SAM* ampicillin/sulbactam

ADP1, and there were minor discrepancies in the susceptibility to minocycline, aminoglycosides, fluoroquinolones, and the other β-lactams between transformants and the recipient (Table 2). After performing PCR detection for the transformants, we found that no $bla_{\text{OXA-23}}$-like gene was co-transferred to the recipient from donor strains A2485 and A2503. The electrotransformations of the $bla_{\text{OXA-24}}$-like gene in A2706 to the recipient failed in repeated attempts. Of note, analysis of the plasmid sequences showed that the $bla_{\text{OXA-24}}$-like genes were flanked by XerC/XerD-like recombination sites in all isolates (GenBank accession no. JX968505).

OXA-58 group

Thirty-two *Acinetobacter* spp. isolates obtained from ten provinces had the $bla_{\text{OXA-58}}$-like genes (Table 3). Among these $bla_{\text{OXA-58}}$-like-gene-positive isolates, five *A. baumannii*, one *A. haemolyticus*, and one *A. pittii* were non-susceptible to carbapenems and showed variable susceptibilities to other β-lactams. The six $bla_{\text{OXA-58}}$-like-positive *A. baumannii* isolates were classified into three STs. Four isolates from one hospital in Henan had the same sequence type (ST91) and co-harbored $bla_{\text{OXA-23}}$-like genes. No MBL gene was detected in these 32 isolates.

The seven carbapenem-non-susceptible $bla_{\text{OXA-58}}$-like-harboring *Acinetobacter* spp. isolates were selected to evaluate the transferability of $bla_{\text{OXA-58}}$-like genes. The $bla_{\text{OXA-58}}$-like-harboring plasmids extracted from these isolates were successfully transferred to the recipient strain ADP1 using electrotransformation, except isolate A1323. The transformants exhibited increased MIC values (16- to>340-fold) for carbapenems compared to the ADP1 strain (Table 3). The PCR performed for transformants confirmed that no other CHDLs genes were co-transferred with $bla_{\text{OXA-58}}$ to the recipient, suggesting that they did not co-exist with $bla_{\text{OXA-58}}$ in the same plasmid.

The structure of IS*6* family-ΔIS*Aba3*-like-$bla_{\text{OXA-58}}$-like, which could increase the transcription level of the $bla_{\text{OXA-58}}$-like gene, has been described in different *Acinetobacter* spp. isolates [18, 19]. In this study, PCR mapping was performed to identify the genetic sequences surrounding the $bla_{\text{OXA-58}}$-like genes in the 32 $bla_{\text{OXA-58}}$-like-harboring isolates. The seven carbapenem-non-susceptible $bla_{\text{OXA-58}}$-like-harboring isolates gave positive results in PCR detection based on primers located on sequences of IS*6* family-ΔIS*Aba3*-like-$bla_{\text{OXA-58}}$-like structure (GenBank accession no. JX968506). IS*Aba3* elements were identified downstream of the $bla_{\text{OXA-58}}$-like genes in all isolates.

OXA-143 group

Only one isolate named A1254 was positive for the $bla_{\text{OXA-143}}$-like gene. Sequencing of the amplicon (564 bp) obtained from A1254 identified a fragment that shared 96 % nucleotide identity with $bla_{\text{OXA-143}}$ (GenBank accession no. JX968504). Isolate A1254 was identified as *A. pittii* using *rpoB* sequencing and was susceptible to carbapenems, cephalosporins, aminoglycosides, and quinolones. The S1-digested plasmid DNA and Southern blot hybridization with $bla_{\text{OXA-143}}$-like-specific probe gave a negative result, indicating that this homologous gene might be located on the chromosome.

Discussion

Carbapenem-resistant *A. baumannii* has become one of most troublesome pathogens throughout the world in the past decade [1, 2, 20]. According to the antimicrobial susceptibility

Table 2 Characteristics of 11 *bla*$_{OXA-24}$-like-harboring *Acinetobacter* spp. isolates, one *bla*$_{OXA-143}$-like-harboring *Acinetobacter* spp. isolates, and transformants

Strain	MEM	IMP	GEN	TZP	PRL	CIP	MIN	AMK	SAM	CAZ	CTX	FEP	Specimen	Origin	ST	Species	Other CHDLs
A352	>32	8	S	S	I	S	S	S	S	S	I	S	Sputum	Liaoning	ND	*A. pittii*	Neg
A1283	>32	>32	S	R	R	S	S	S	S	S	I	S	Sputum	Hainan	N17	*A. baumannii*	*bla*$_{OXA-51}$-like
A1343	32	8	S	I	I	S	S	S	S	S	I	S	Sputum	Hainan	N18	*A. baumannii*	*bla*$_{OXA-51}$-like
A1429	>32	>32	R	R	R	R	I	R	S	R	R	R	Secretions	Guangdong	N19	*A. baumannii*	*bla*$_{OXA-51}$-like
A2485	>32	>32	R	R	R	R	S	R	R	R	R	R	Sputum	Shanxi	92	*A. baumannii*	*bla*$_{OXA-23}$-like, *bla*$_{OXA-51}$-like
A2503	>32	>32	R	R	R	R	S	R	R	R	R	R	Sputum	Shanxi	92	*A. baumannii*	*bla*$_{OXA-23}$-like, *bla*$_{OXA-51}$-like
A2584	>32	>32	S	R	R	S	S	S	S	S	I	S	Sputum	Jiangxi	ND	*A. pittii*	Neg
A2587	>32	16	S	R	R	S	S	S	S	S	I	S	Sputum	Jiangxi	ND	*A. pittii*	Neg
A2702	>32	8	S	S	S	S	S	S	S	S	I	S	Sputum	Jiangxi	ND	*A. baylyi*	Neg
A2706	>32	>32	R	R	R	R	S	R	R	R	R	R	Sputum	Jiangxi	381	*A. baumannii*	*bla*$_{OXA-51}$-like
A2949	>32	32	S	R	R	S	S	S	S	S	I	S	Sputum	Hubei	ND	*A. pittii*	Neg
A1254	2	2	S	S	S	S	S	S	S	S	S	S	Sputum	Zhejiang	ND	*A. pittii*	Neg
ADP1-A352	4	1	S	S	S	S	S	S	S	S	I	S	–	–	–	–	Neg
ADP1-A1283	4	2	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A1343	4	4	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A1429	>32	32	S	R	R	S	S	S	S	S	I	S	–	–	–	–	Neg
ADP1-A2485	>32	>32	S	R	R	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2503	>32	>32	S	R	R	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2584	4	4	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2587	4	4	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2702	4	2	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2949	8	4	S	S	S	S	S	S	S	S	I	S	–	–	–	–	Neg
ADP1	0.064	0.064	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg

MEM meropenem; *IMP* imipenem; *GEN* gentamicin; *TZP* piperacillin/tazobactam; *PRL* piperacillin; *CIP* ciprofloxacin; *MIN* minocycline; *AMK* amikacin; *SAM* ampicillin/sulbactam; *CAZ* ceftazidime; *CTX* cefotaxime; *FEP* cefepime; *S* susceptible; *I* intermediate; *R* resistant; *ND* not done; *Neg* negative

Table 3 Characteristics of 32 bla_{OXA-58}-harboring *Acinetobacter* spp. isolates and transformants

Strain	MEM	IMP	TZP	PRL	SAM	CAZ	CTX	FEP	GEN	AMK	CIP	MIN	Species	Origin	ST	IS*6* upstream	Other CHDLs
A119	0.25	0.5	I	R	R	R	R	R	S	R	S	S	*A. junii*	Anhui	ND	Neg	Neg
A249	2	2	R	R	R	R	R	R	R	R	S	S	*A. pittii*	Liaoning	ND	Neg	Neg
A332	1	1	S	R	S	S	I	S	R	R	S	S	*A. pittii*	Liaoning	ND	Neg	Neg
A545	1	4	R	R	R	R	R	R	R	S	R	S	*A. pittii*	Guangdong	ND	Neg	Neg
A599	4	4	R	R	S	S	I	S	S	S	S	S	*A. pittii*	Zhejiang	ND	Neg	Neg
A601	0.5	1	I	R	S	S	I	S	R	S	S	S	*A. pittii*	Zhejiang	ND	Neg	Neg
A875	1	1	S	I	S	S	I	S	R	S	S	S	*A.nosocomialis*	Zhejiang	ND	Neg	Neg
A972	0.5	1	S	R	S	S	I	S	R	S	S	S	*A.nosocomialis*	Zhejiang	ND	Neg	Neg
A999	1	2	I	R	S	S	I	S	R	R	S	S	*A. pittii*	Zhejiang	ND	Neg	Neg
A1214	0.25	1	I	R	R	R	R	R	I	S	S	S	*A. junii*	Zhejiang	ND	Neg	Neg
A1272	0.125	0.5	I	R	I	R	R	R	S	R	I	S	*A. junii*	Hainan	ND	Neg	Neg
A1323	>32	>32	R	R	R	R	R	R	R	R	S	S	*A. haemolyticus*	Hainan	ND	Pos	bla_{OXA-23}-like
A1363	2	1	R	R	I	R	R	R	R	R	I	S	*A. baumannii*	Hainan	N20	Neg	bla_{OXA-51}-like
A1416	1	1	S	R	S	S	I	S	R	S	S	S	*A.nosocomialis*	Guangdong	ND	Neg	Neg
A1426	4	2	I	R	I	R	R	R	R	R	I	S	*A.nosocomialis*	Guangdong	ND	Neg	Neg
A1428	0.5	1	I	R	R	R	R	R	R	R	S	S	*A.nosocomialis*	Guangdong	ND	Neg	Neg
A1543	2	2	R	R	R	R	R	R	R	R	R	S	*A. pittii*	Tianjin	ND	Neg	Neg
A1604	0.5	0.5	I	R	S	S	I	S	R	S	S	S	*A. pittii*	Yunnan	ND	Neg	Neg
A1784	2	4	I	R	S	S	I	S	R	S	S	S	*A. pittii*	Jilin	ND	Neg	Neg
A1788	1	1	I	R	I	R	R	R	R	I	S	S	*A. pittii*	Jilin	ND	Neg	Neg
A1793	0.5	0.5	I	R	I	R	R	R	R	R	S	S	*A.nosocomialis*	Jilin	ND	Neg	Neg
A1796	1	0.5	I	R	S	S	I	S	R	S	S	S	*A. pittii*	Jilin	ND	Neg	Neg
A1968	0.5	1	R	R	R	R	R	R	R	S	S	S	*A. pittii*	Shaanxi	ND	Neg	Neg
A1989	0.5	2	I	R	R	R	R	R	R	S	S	S	*A. junii*	Shaanxi	ND	Neg	Neg
A2195	0.5	2	S	I	S	S	S	S	R	S	S	S	*A. junii*	Tianjin	ND	Neg	Neg
A2445	>32	>32	R	R	R	I	R	R	R	R	R	R	*A. baumannii*	Henan	91	Pos	bla_{OXA-51}-like, bla_{OXA-23}-like
A2460	>32	>32	R	R	I	I	R	R	R	R	R	R	*A. baumannii*	Henan	91	Pos	bla_{OXA-51}-like, bla_{OXA-23}-like
A2466	>32	>32	R	R	R	I	R	R	R	R	R	R	*A. baumannii*	Henan	91	Pos	bla_{OXA-51}-like, bla_{OXA-23}-like

Table 3 (continued)

Strain	MEM	IMP	TZP	PRL	SAM	CAZ	CTX	FEP	GEN	AMK	CIP	MIN	Species	Origin	ST	IS*6* upstream	Other CHDLs
A2471	>32	>32	R	R	R	I	R	I	R	R	R	R	*A. baumannii*	Henan	91	Pos	bla_{OXA-51}-like, bla_{OXA-23}-like
A2754	16	>32	R	R	R	R	R	R	R	R	R	S	*A. baumannii*	Hunan	110	Pos	bla_{OXA-51}-like
A2790	0.5	0.5	I	R	S	S	I	S	R	S	S	S	*A. pittii*	Shanghai	ND	Neg	Neg
A2856	8	>32	R	R	R	S	I	S	R	S	S	S	*A. pittii*	Shanghai	ND	Pos	Neg
ADP1	0.125	0.125	S	S	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2445	4	16	R	R	S	S	S	S	R	S	S	S	–	–	–	–	Neg
ADP1-A2460	>32	>32	R	R	S	S	S	S	R	S	S	S	–	–	–	–	Neg
ADP1-A2466	8	16	R	R	S	S	S	S	R	I	S	S	–	–	–	–	Neg
ADP1-A2471	16	8	R	R	S	S	S	S	R	I	S	S	–	–	–	–	Neg
ADP1-A2754	2	2	R	R	S	S	S	S	S	S	S	S	–	–	–	–	Neg
ADP1-A2856	>32	>32	R	R	R	S	S	S	S	S	S	S	–	–	–	–	Neg

MEM meropenem; *IMP* imipenem; *TZP* piperacillin/tazobactam; *PRL* piperacillin; *SAM* ampicillin/sulbactam; *CAZ* ceftazidime; *CTX* cefotaxime; *FEP* cefepime; *GEN* gentamicin; *AMK* amikacin; *CIP* ciprofloxacin; *MIN* minocycline; *S* susceptible; *I* intermediate; *R* resistant; *ND* not done; *Neg* negative; *Pos* positive

testing results, the carbapenem resistance rates of *Acinetobacter* spp. were high in China, with 48.6 % (1,399/2,880) and 50.1 % (1,442/2,880) being resistant to imipenem and meropenem, respectively. The results of multiplex PCR showed that 76.3 and 45.7 % of these isolates were positive for bla_{OXA-51}-like and bla_{OXA-23}-like genes, respectively, while 44.9 % (1,294/2,880) of the isolates also harbored bla_{OXA-51}-like and bla_{OXA-23}-like genes. Moreover, more than 95 % of bla_{OXA-23}-like-harboring isolates were resistant to carbapenems, which indicated that the high prevalence of OXA-23-producing *A. baumannii* was the predominant reason for high carbapenem resistance rates of *Acinetobacter* spp. in China. According to the results of the MLST analysis for the carbapenem-resistant *A. baumannii* we previously reported, it is determined that OXA-23-producing CC92 isolates have disseminated throughout hospitals in China and played an important role contributing to the high prevalence of carbapenem-resistant *Acinetobacter* spp. in this country [3, 21].

Comparing with the bla_{OXA-23}-like genes, the bla_{OXA-24}-like genes were less frequently identified in *Acinetobacter* spp. in China, as only 11 bla_{OXA-24}-like-positive isolates were detected. These bla_{OXA-24}-like-harboring isolates were resistant to carbapenems and most had multidrug-resistance phenotypes. Furthermore, the bla_{OXA-24}-like genes were located on small plasmids in most isolates. Moreover, analysis of the genetic environment showed that the bla_{OXA-24}-like genes were flanked by XerC/XerD-like sites, which were considered to be responsible for the mobilization of the bla_{OXA-24} gene [22]. Outbreaks of multidrug-resistant *A. baumannii* harboring bla_{OXA-24}-like genes were reported in USA, Spain, and sporadic isolates were reported in China [10, 23–25]. Although our surveillance showed that the bla_{OXA-24}-like-harboring *Acinetobacter* spp. were less prevalent in China, the plasmid location and bla_{OXA-24} mobilization cassette (Xer system) identified in these isolates were similar to the outbreak isolates in Europe and USA, indicating that these bla_{OXA-24}-like genes have the capacity to disseminate among different *Acinetobacter* species in hospital environments and have the potential to cause outbreaks of carbapenem-resistant *Acinetobacter* spp. in China.

In contrast to the resistant phenotypes observed in bla_{OXA-24}-like-harboring isolates, 25 out of 32 bla_{OXA-58}-like-harboring *Acinetobacter* spp. isolates remained susceptible to carbapenems. This might be explained by the low-level expression of bla_{OXA-58}-like genes in these isolates, due to the insertions of the IS*6* family, which enhance the transcription of the bla_{OXA-58} gene and mediate resistance to carbapenem, and were identified upstream of the bla_{OXA-58}-like genes in all carbapenem-resistant isolates [18]. OXA-58 is often associated with hospitals outbreaks in European countries and the United States [4]. It appears from our surveillance that the rate of carbapenem-non-susceptible *Acinetobacter* spp.

associated with bla_{OXA-58}-like genes is still rather low in China. However, the dissemination of this group of CHDLs is also worrisome because of the widespread distribution of these genes in *Acinetobacter* species and the genetic structure we found in the carbapenem-non-susceptible isolates, which contribute to the mobility and expression of bla_{OXA-58}-like genes.

Some *Acinetobacter* species intrinsically possess chromosomal genes encoding CHDLs [26, 27]. For example, *A. baumannii* carries bla_{OXA-51}-like, *A. radioresistens* bla_{OXA-23}-like, and *A. lwoffii* $bla_{OXA-134}$-like genes. We identified a $bla_{OXA-143}$ homologue in a susceptible *A. pittii* isolate. Though the entire DNA sequence of this $bla_{OXA-143}$-like gene has not yet been obtained, this finding indicates that the distribution of bla_{OXA} genes is much wider than previously assumed.

In conclusion, this is a large-scale study to characterize the distribution of CHDLs in *Acinetobacter* spp. in China. Our results indicate that the bla_{OXA-23}-harboring *A. baumannii* isolates are the most frequent carbapenem-resistant *Acinetobacter* spp. in China, predominantly CC92 isolates, and the bla_{OXA-24}-like and bla_{OXA-58}-like genes have emerged as potential threats of hospital outbreaks of multidrug-resistant *Acinetobacter* spp.

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