OPEN ACCESS Freely available online

PLOS ONE

Characterization of a Novel Plasmid Type and Various Genetic Contexts of *bla*$_{OXA-58}$ in *Acinetobacter* spp. from Multiple Cities in China

Yiqi Fu[1☯], Jingjin Jiang[2☯], Hua Zhou[1], Yan Jiang[3], Ying Fu[3], Yunsong Yu[3]*, Jianying Zhou[1]*

1 Department of Respiratory Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, **2** Department of VIP, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, **3** Department of Infectious Diseases, Sir Run Run Shaw Hospital, College of Medicine, Zhejiang University, Hangzhou, China

Abstract

Background/Objective: Several studies have described the epidemiological distribution of *bla*$_{OXA-58}$-harboring *Acinetobacter baumannii* in China. However, there is limited data concerning the replicon types of *bla*$_{OXA-58}$-carrying plasmids and the genetic context surrounding *bla*$_{OXA-58}$ in *Acinetobacter* spp. in China.

Methodology/Principal Findings: Twelve non-duplicated *bla*$_{OXA-58}$-harboring *Acinetobacter* spp. isolates were collected from six hospitals in five different cities between 2005 and 2010. The molecular epidemiology of the isolates was carried out using PFGE and multilocus sequence typing. Carbapenemase-encoding genes and plasmid replicase genes were identified by PCR. The genetic location of *bla*$_{OXA-58}$ was analyzed using S1-nuclease method. Plasmid conjugation and electrotransformation were performed to evaluate the transferability of *bla*$_{OXA-58}$-harboring plasmids. The genetic structure surrounding *bla*$_{OXA-58}$ was determined by cloning experiments. The twelve isolates included two *Acinetobacter pittii* isolates (belong to one pulsotype), three *Acinetobacter nosocomialis* isolates (belong to two pulsotypes) and seven *Acinetobacter baumannii* isolates (belong to two pulsotypes/sequence types). *A. baumannii* ST91 was found to be a potential multidrug resistant risk clone carrying both *bla*$_{OXA-58}$ and *bla*$_{OXA-23}$. *bla*$_{OXA-58}$ located on plasmids varied from ca. 52 kb to ca. 143 kb. All plasmids can be electrotransformed to *A. baumannii* recipient, but were untypeable by the current replicon typing scheme. A novel plasmid replicase named *rep*Aci10 was identified in *bla*$_{OXA-58}$-harboring plasmids of two *A. pittii* isolates, three *A. nosocomialis* isolates and two *A. baumannii* isolates. Four kinds of genetic contexts of *bla*$_{OXA-58}$ were identified. The transformants of plasmids with structure of IS*6* family insertion sequence (IS*Our1*, IS*1008* or IS*15*)-ΔIS*Aba3*-like element-*bla*$_{OXA-58}$ displayed carbapenem nonsusceptible, while others with structure of intact IS*Aba3*-like element-*bla*$_{OXA-58}$ were carbapenem susceptible.

Conclusion: The study revealed the unique features of *bla*$_{OXA-58}$-carrying plasmids in *Acinetobacter* spp. in China, which were different from that of *Acinetobacter* spp. found in European countries. The diversity of the genetic contexts of *bla*$_{OXA-58}$ contributed to various antibiotics resistance profiles.

Citation: Fu Y, Jiang J, Zhou H, Jiang Y, Fu Y, et al. (2014) Characterization of a Novel Plasmid Type and Various Genetic Contexts of *bla*$_{OXA-58}$ in *Acinetobacter* spp. from Multiple Cities in China. PLoS ONE 9(1): e84680. doi:10.1371/journal.pone.0084680

Editor: J. Ross Fitzgerald, University of Edinburgh, United Kingdom

Received August 19, 2013; **Accepted** November 18, 2013; **Published** January 6, 2014

Funding: This work was funded by research grants from National Natural Science Foundation of China (nos. 81230039 and 81000757) and Science Technology Department of Zhejiang Province (no. 2008C13029-1). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: zjyhz@zju.edu.cn (JZ); yvys119@163.com (YY)

☯ These authors contributed equally to this work.

Introduction

Members of the genus *Acinetobacter* are significant nosocomial pathogens. *Acinetobacter baumannii* and its two close relatives, *Acinetobacter pittii* and *Acinetobacter nosocomialis* account for the majority of *Acinetobacter* infections [1]. A number of reports have detailed the significant increase in resistance of *Acinetobacter* spp. to conventional antibiotics, including carbapenems, the main therapeutic alternative against multidrug resistant *Acinetobacter* infections [2].

The worldwide emergence of carbapenem resistant *Acinetobacter* may be attributed to the spread of some risk resistant clones and the horizontal transmission of carbapenemase genes [1,3]. Carbapenem-hydrolyzing class D β-lactamases (CHDLs) are the most concerning carbapenem resistant determinants in *Acinetobacter* spp [1]. OXA-58 is a widely spread CHDL that has been reported in *Acinetobacter* spp. from Europe [4], Argentina [5], Australia [6], the United States [7] and many Asian countries [8]. Though OXA-58 shows only low carbapenem-hydrolyzing activity *in vitro*, the insertion sequence upstream of *bla*$_{OXA-58}$ enhances its transcription greatly and mediates resistance to carbapenems [9–11].

bla$_{OXA-58}$ exists not only in *A. baumannii*, but also in *A. pittii* [12], *A. nosocomialis* [11], *Acinetobacter radioresistens* [13], *Acinetobacter junii*

[6], and *Acinetobacter* phenon 6/ct13TU [14]. bla_{OXA-58} is usually plasmid-borne, which may explain its wide dissemination. It has been reported that OXA-58 producing *A. baumannii* from European countries are associated with carriage of plasmid replicase gene *rep*Aci1 [15]. However, little is known about the replicon types of bla_{OXA-58}-carrying plasmids in *A. baumannii* and non-*baumannii Acinetobacter* spp. outside of Europe.

bla_{OXA-58} is the second most frequently identified CHDL in *A. baumannii* in China. However, the current data is limited to simple epidemiological distribution [16,17]. In this study, we detailed characterized the genetic contexts surrounding bla_{OXA-58} and the replicon typing of the bla_{OXA-58}-carrying plasmids in *Acinetobacter* spp. isolates from multiple cities in China.

Materials and Methods

Bacterial Strains and Antimicrobial Susceptibility Testing

Twelve non-duplicated bla_{OXA-58}-harboring *Acinetobacter* spp. isolates collected from six hospitals in five different cities in China between 2005 and 2010 were analyzed in this study (Table 1). The genomic species identification was performed by sequence analysis of the 16S-23S rRNA intergenic spacer region [18].

Imipenem and ticarcillin-susceptible clinical *A. baumannii* strain LS0148 (imipenem MIC, 0.5 mg/L; ticarcillin MIC, 16 mg/L), deposited in our laboratory, was used as the recipient for plasmid electrotransformation (Table 1). A colistin-resistant mutant strain of *A. baumannii* LS0148 (colistin MIC, 64 mg/L) was used as the recipient for plasmid conjugation.

MICs were determined by the agar dilution method. Interpretation of the results was in accordance with the CLSI 2013 criteria.

All isolates present in this study were stored in the Department of Microbiology, the First Affiliated Hospital, College of Medicine, Zhejiang University. We obtained an exempt status from the Institutional Review Board of the First Affiliated Hospital, College of Medicine, Zhejiang University to use these strains to perform all experiments in this study.

PCR Experiments for the Resistance Genes

PCR assays for the presence of carbapenemase encoding genes (bla_{OXA-51}-like, bla_{OXA-58}-like, bla_{OXA-23}-like, bla_{OXA-40}-like, $bla_{OXA-143}$, bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{NDM}) and ESBL genes (bla_{PER} and bla_{SHV}) were performed as previously reported [19–21].

Pulsed-field Gel Electrophoresis and Multilocus Sequence Typing Analysis

The genetic relationship of the isolates was evaluated by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The results of PFGE were interpreted as Tenover et al. recommended [22]. MLST was carried out using the scheme developed by Bartual et al. with some modifications to the primers of the alleles of *gyrB* and *rpoD* as we previously reported [23,24].

Plasmid Conjugation and Electrotransformation

Plasmid conjugations were performed between OXA-58 producing *Acinetobacter* spp. as donors and a colistin-resistant mutant strain of *A. baumannii* LS0148 as the recipient. The transconjugants were selected on MH agar plates containing ticarcillin (100 mg/L) and colistin (10 mg/L).

The electrical pulse setting of plasmid electrotransformation was 1.8 kV, 25 μF, 200 Ω with Bio-Rad GenePulser Xcell system (Bio-Rad, Shanghai, China). *A. baumannii* strain LS0148 was used as the recipient. The transformants were selected on MH agar plates containing ticarcillin (100 mg/L).

S1 Nuclease-based Plasmid Analysis

The plasmid size and the location of bla_{OXA-58} were analyzed using the S1 nuclease-PFGE method as previously reported [25]. The bacterial-imbedded gel slices were incubated with 10 U S1 nuclease (Takara, Dalian, China) for 40 minutes in 37°C water bath. The digestion products were separated by PFGE using Bio-Rad CHEF Mapper XA system (Bio-Rad, Shanghai, China) with switch times of 2.16S to 63.8S for 18 hours.

Table 1. Basic information, epidemiological features and resistant genes of *Acinetobacter* spp. included in this study[a].

Strain	Species	Hospital (Cities)	Year	PFGE type	ST	allelic profiles[b]	bla_{OXA} genes	ESBL genes
AP04	*A. pittii*	HZ (Hangzhou)	2009	A	ND	–	bla_{OXA-58}	Neg
AP25	*A. pittii*	TZ (Taizhou)	2009	A	ND	–	bla_{OXA-58}	Neg
AN113	*A. nosocomialis*	WZ (Wenzhou)	2009	B	ND	–	bla_{OXA-58}	Neg
AN116	*A. nosocomialis*	WZ (Wenzhou)	2009	B	ND	–	bla_{OXA-58}	Neg
AN119	*A. nosocomialis*	WZ (Wenzhou)	2009	C	ND	–	bla_{OXA-58}	Neg
WA3	*A. baumannii*	WHC (Wuhan)	2008	E	363	51-54-49-11-48-25-4	bla_{OXA-58}, bla_{OXA-51}	bla_{PER-1}
WA8	*A. baumannii*	WHC (Wuhan)	2008	E	363	51-54-49-11-48-25-4	bla_{OXA-58}, bla_{OXA-51}	bla_{PER-1}
WH8144	*A. baumannii*	WH (Wuhan)	2010	D	91	22-15-13-12-4-62-2	bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-51}	Neg
JH01	*A. baumannii*	JH (Jinhua)	2005	D	91	22-15-13-12-4-62-2	bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-51}	Neg
JH02	*A. baumannii*	JH (Jinhua)	2005	D	91	22-15-13-12-4-62-2	bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-51}	Neg
AB212	*A. baumannii*	JH (Jinhua)	2009	D	91	22-15-13-12-4-62-2	bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-51}	Neg
AB222	*A. baumannii*	JH (Jinhua)	2009	D	91	22-15-13-12-4-62-2	bla_{OXA-58}, bla_{OXA-23}, bla_{OXA-51}	Neg
LS0148	*A. baumannii*	LS (Lishui)	2005	ND	20	1-15-13-12-4-12-2	bla_{OXA-51}	Neg

[a]Abbreviations: HZ, Hangzhou First hospital; TZ, Taizhou Hospital; WZ, The First Affiliated Hospital of Wenzhou Medical College; WHC, Wuhan Children Hospital; WH, Wuhan Tongji Hospital; JH, Jinhua Center Hospital; LS, Lishui People Hospital; ND: not defined; Neg: negative; Pos: positive.
[b]Seven loci in the order of *gltA-gyrB-gdhB-recA-cpn60-gpi-rpoD*.
doi:10.1371/journal.pone.0084680.t001

The separated DNA was transferred to a positive charged Nylon membrane (Millipore, Shanghai, China) and hybridized with a digoxigenin-labled bla_{OXA-58} probe. The detection of hybrids was performed using enzyme immunoassay and NBT/BCIP coloration according to the manufacturer's instruction (Roche, Shanghai, China).

PCR-based Plasmid Replicon Typing

The plasmid replicase genes were detected by multiplex PCR scheme developed by Bertini et al [26]. The novel replicase gene *rep*Aci10 was detected by a single PCR with primers designed in this study (Forward primer: 5′-TAGGACGTCAAGCATCTTA-3′; backward primer: 5′-TCGCTATCAAGAAGATCAC-3′).

Cloning Experiments

The genetic contexts of bla_{OXA-58} were determined by cloning and sequencing experiments. The plasmids or total DNA were digested by EcoRI or SacI. The digested fragments were inserted into corresponding sites of pET28a, and the ligation mixture was used for transformation. Transformants were selected on MH agar containing ampicillin 50 mg/L and kanamycin 50 mg/L. The bla_{OXA-58}-containing inserts were fully or partially sequenced to obtain the context of bla_{OXA-58}.

Nucleotide Sequence Accession Numbers

The novel insertion sequence IS*Aba20* has been submitted to the IS Finder Database (http://www-is.biotoul.fr/). The nucleotide sequences surrounding bla_{OXA-58} of AN119, AP04, WA3 and WH8144 are deposited in the GenBank database under accession no. JQ241789 to JQ241792 respectively.

Results

Species Identification and Antimicrobial Susceptibility Profiles

The 12 OXA-58-producing *Acinetobacter* spp. isolates were assigned to three genomic species: *A. baumannii* (seven isolates), *A. nosocomialis* (three isolates) and *A. pittii* (two isolates), and showed various resistance profiles (Table 1 and 2). The five non-*baumanii Acinetobacter* displayed imipenem and meropenem susceptible. On the contrary, all of the *A. baumannii* isolates were imipenem and meropenem resistance. In general, the *A. baumannii* isolates were more frequently resistant to broad-spectrum cephalosporins, ampicillin/sulbactam, aminoglycosides, ciprofloxacin and minocycline than the five non-*baumanii Acinetobacter*.

Molecular Epidemiology of the OXA-58-producing *Acinetobacter* spp.

PFGE identified five pulsotypes among the 12 OXA-58-producing *Acinetobacter* spp. isolates (Table. 1). Two *A. pittii* isolates from different hospitals showed a same pulsotype. Three *A. nosocomialis* isolates from a single hospital belonged to two pulsotypes. Seven *A. baumannii* isolates were divided into two pulsotypes, corresponding to two sequence types (ST91 and ST363). *A. baumannii* ST91 were identified from two hospitals in different cities (Wuhan and Jinhua). Moreover, ST91 were detected in *A. baumannii* collected from Jinhua Center Hospital in 2005 and 2009, implying probable endemic in this hospital.

Distribution of Resistance Genes

The *A. pittii* and *A. nosocomialis* were negative for other carbapenemase genes and ESBLs. Intrinsic bla_{OXA-51} was detected in all *A. baumannii* isolates. All *A. baumannii* ST91 isolates carried another CHDL gene bla_{OXA-23}. bla_{PER-1} was detected in WA3 and WA8 (Table 1).

The Plasmid Localization of bla_{OXA-58}

The bla_{OXA-58}-probe hybridized with plasmid bands of different sizes, from ca. 52 kb to 143 kb. Isolates with same pulsotypes generally possessed same plasmid location of bla_{OXA-58}, except AP04 and AP25 (Table 2).

The Transferability of bla_{OXA-58}-carrying Plasmids

While plasmid conjugation ultimately failed, bla_{OXA-58}-carrying plasmids were successfully electro-transferred from all *Acinetobacter* spp. isolates to the recipient strain.

PCR detection of transformants found bla_{PER-1} and bla_{OXA-23} were not co-transferred with bla_{OXA-58}, suggesting these genes are not colocalized on a single plasmid.

The results of antimicrobial susceptibility testing are presented in Table 2. Electrotransformation of bla_{OXA-58}-harboring plasmids into recipient strain LS0148 resulted in high resistance to ticarcillin (>256 mg/L) and increased MICs of imipenem and meropenem (2 to 32 folds), but transformants retained similar MICs of cefepime, ceftazidime and cefotaxime when compared with that of the original LS0148 strain. The transformants of *A. nosocomialis* AN119 and all *A. baumannii* displayed higher MICs of imipenem and meropenem than transformants of *A. pittii* isolates and remaining *A. nosocomialis* isolates. Transformants TWH8144, TJH01, TAB212 also showed gentamicin and amikacin resistance, implying potential aminoglycosides resistant determinants are colocalized with bla_{OXA-58} on the same plasmid.

Identification a Novel Plasmid Replicase Gene

Further investigation of the bla_{OXA-58}-containing clone fragment of strain WA3 identified a novel plasmid replication protein gene (Figure. 1). This replication protein belonged to Rep-3 superfamily group. It shared similarity with two replication proteins deposited in GenBank database: *Acinetobacter* sp. RUH2624 (ZP_05826577; 100% amino acid identity) and *A. radioresistens* SH164 (ZP_06073941; 73% amino acid identity). We have designated this novel replicase gene as *rep*Aci10 herein. Of the available *A. baumannii* replicase genes in the current replicon typing scheme [26], *rep*Aci5 was most similar to *rep*Aci10 (66% nucleotide identity). Therefore, *rep*Aci10 should be assigned as a novel homolog group (GR20). No iteron was identified upstream of *rep*Aci10.

Using the current PCR-based replicon typing scheme of *A. baumannii* [26], only GR8 was detected in strain JH01 and JH02 from the 12 OXA-58 producing *Acinetobacter* spp. (Table 2). GR3 and GR7 are the intrinsic plasmid *rep* genes of recipient strain LS0148. No other replicase genes were detected in the transformants except for the intrinsic plasmid replicase genes of LS0148 (GR3 and GR7), suggesting the bla_{OXA-58}-carrying plasmids do not belong to any previously known replicon group. The novel replicase gene *rep*Aci10 was detected in *A. pittii* (AP04, AP25), *A. nosocomialis* (AN113, AN116 and AN119), *A. baumannii* (WA3, WA8) and their transformants (Table 2).

Genetic Contexts of bla_{OXA-58}

Four kinds of genetic contexts of bla_{OXA-58} were identified among 12 *Acinetobacter* spp. (Figure. 1). Structure A included two *A. pittii* isolates (AP04, AP25) and two *A. nosocomialis* isolates (AN113 and AN116). Structure B encompassed all *A. baumannii* ST91 isolates of WH8144, JH01, JH02, AB212 and AB222. Structure C encompassed *A. baumannii* isolates WA3 and WA8. Structure D

Table 2. The sizes and replicon types of bla_{OXA-58}-harboring plasmids, genetic contexts of bla_{OXA-58}, and MICs (mg/L) of represented strains[a].

Isolates[b]	Plasmid size (kb)	*rep* gene group[c]	Genetic contexts of bla_{OXA-58}[d]	IPM	MEM	FEP	CAZ	CTX	SAM	TZP	TIC	GEN	AMK	MIN	CIP
AP04	93	*aci10*	A	0.5	0.5	4	4	16	4	32	>256	64	2	<0.125	<0.125
AP25	52	*aci10*	A	1	0.5	2	4	16	2	16	>256	2	2	<0.125	<0.125
AN113	143	*aci10*	A	0.5	1	4	4	16	1	16	>256	>256	4	4	0.5
AN116	143	*aci10*	A	1	2	16	8	32	16	128	>256	>256	4	4	0.25
AN119	76	*aci10*	D	4	2	4	8	16	4	64	>256	128	4	0.5	0.25
WA3	101	*aci10*	C	>32	8	256	>256	>256	64	256	>256	64	16	<0.125	<0.125
WH8144	55	–	B	>32	>32	128	32	64	128	>256	>256	>256	256	64	64
JH01	55	GR8	B	>32	>32	256	128	256	128	>256	>256	>256	>256	64	16
AB212	55	–	B	>32	>32	32	64	128	32	>256	>256	>256	>256	32	16
LS0148	–	[GR3, GR7]	–	0.5	0.5	2	4	16	2	16	16	1	2	8	16
TAP04	93	*aci10*, [GR3, GR7]	A	2	2	4	8	16	4	64	>256	16	2	8	32
TAP25	52	*aci10*, [GR3, GR7]	A	2	1	4	8	16	4	64	>256	4	4	8	32
TAN113	143	*aci10*, [GR3, GR7]	A	1	1	4	8	16	2	32	>256	>256	4	4	32
TAN116	143	*aci10*, [GR3, GR7]	A	1	2	4	4	16	2	16	>256	>256	4	4	32
TAN119	76	*aci10*, [GR3, GR7]	D	16	16	4	8	16	16	256	>256	64	4	8	32
TWA3	101	*aci10*, [GR3, GR7]	C	16	8	4	8	16	16	256	>256	1	2	8	16
TWH8144	55	[GR3, GR7]	B	8	4	4	8	16	8	128	>256	256	128	8	16
TJH01	55	[GR3, GR7]	B	8	4	4	8	16	8	128	>256	256	128	8	16
TAB212	55	[GR3, GR7]	B	16	4	4	8	16	8	256	>256	256	128	8	16

[a]IPM, imipenem; MEM, meropenem; FEP, cefepime; CAZ, ceftazidime; CTX, cefotaxime; SAM, ampicillin/sulbactam; TZP, piperacillin/tazobactam; TIC, ticarcillin; GEN, gentamicin; AMK, amikacin; MIN, minocycline; CIP, ciprofloxacin.
[b]The isolates with names starting with alphabet T- were transformants;
[c]Brackets indicate that GR3 and GR7 replicases are present in the recipient strain LS0148.
[d]The alphabet corresponded to four kinds of genetic structure displayed in Figure 1.
doi:10.1371/journal.pone.0084680.t002

included *A. nosocomialis* isolate AN119. The most notable difference was the IS elements located upstream of bla_{OXA-58}. In structure A, an intact IS*Aba3*-like element was exclusively present upstream of bla_{OXA-58}. However, in structure B, C and D, the IS*Aba3*-like element upstream of bla_{OXA-58} was truncated at a same position (58 bp downstream of the start codon of the transposase gene of IS*Aba3*-like element) by IS*Our1*, IS*1008* and IS*15* respectively. All of the latter three IS elements belong to the IS*6* family. The transformants of the plasmids with the structure of IS*6* family-ΔIS*Aba3*-like-bla_{OXA58} displayed a much higher increase in imipenem MICs (16–32 folds) than those with intact IS*Aba3*-like-bla_{OXA58} (2–4 folds) (Table 2).

IS*Aba3*, *araC1* (putative transcriptional regulator gene) and *lysE* (putative threonine efflux protein gene) were identified downstream of bla_{OXA-58} in all isolates. However, the *araC1* and *lysE* in structure B were disrupted by IS*Aba20* and Tn*aphA6* respectively. IS*Aba20* is a novel insertion sequence of IS*3* family, and is 1199 bp long with two ORFs. The insertion of IS*Aba*20 into *araC1* generated two 4-bp direct repeats (CTTA). Tn*aphA6* was a composite transposon, comprising an aminoglycoside *O*-phosphotransferase gene, *aphA6*, and two flanked IS*Aba125* of same orientation. Tn*aphA6* was inserted into *lysE* and generated two 3-bp target site duplications (CTG).

It has been reported that the acquisition of bla_{OXA-58} is usually associated with recombination events characterized by the presence of two 27-bp sequences named Re27-1 and Re27-2 [9]. In structure A, we identified a similar Re27-1 sequence located 8 bp downstream of the intact IS*Aba3*-like element (5′-ATTTAACATAATGGCTGTTATACGAAA-3′), and an imperfect Re27-2 sequence (5′-ATTTAACATAATGGTGGTTATACGCAA-3′) was just adjacent to the downstream of *lysE*. In structure D, a pair of 29-bp imperfect probable recombination points were identified 748 bp downstream of IS*15* element (5′-ATTTAACATAATGGTGGTTATGCGAAGTC-3′) and adjacent to *lysE* (5′-ATTTAACATAATGGGCGTTATGCGAAGTC-3′). In structure B and C, we failed to find pairs of Re27-like regions.

Discussion

Previous studies reported that European clone II lineage OXA-23-producing *A. baumannii* CC92 was the most popular carbapenem-resistant clone in China [24,27]. The OXA-58 producing *A. baumannii* of European clone II has been reported in Italy [28], Greece [29] and China [30]. However, only *A. baumannii* ST91 and ST363 were identified in this study without any European clone II lineage isolates. We have showed ST91 strains contain both bla_{OXA-23} and bla_{OXA-58}, and possess multidrug resistance to carbapenems, broad-spectrum cephalosporins, aminoglycosides, ampicillin/sulbactam, minocycline, and ciprofloxacin. Moreover, ST91 was detected in two cities. Therefore, we speculate ST91 is a potential risk multidrug resistant clone that is widely present in China. A larger scale epidemiological investigation would be necessary to fully elucidate the true distribution of ST91 in China.

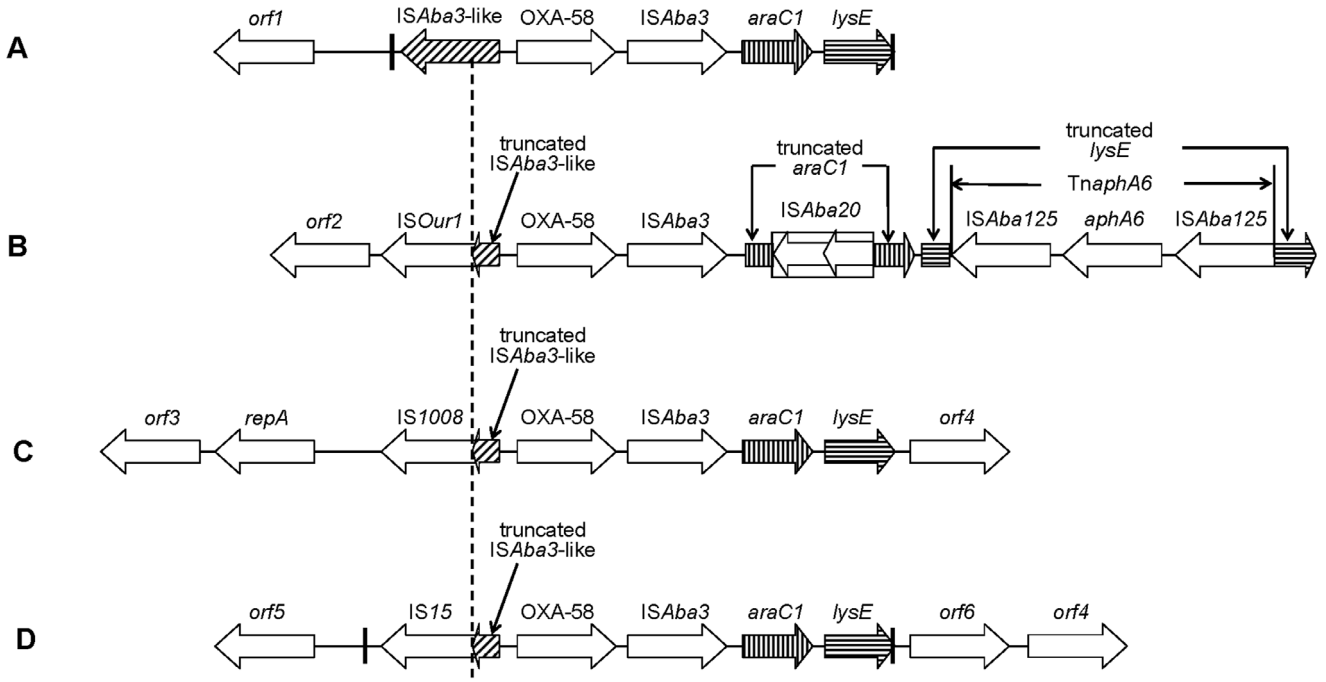

Figure 1. Schematic map of the genetic contexts of bla_{OXA-58}. Structure A (AP04, AP25, AN113 and AN116); Structure B (WH8144, JH01, JH02, AB212 and AB222); Structure C (WA3 and WA8); Structure D (AN119). The dash line indicates the truncated positions of IS*Aba3*-like element. The thick vertical lines indicate the Re27 recombination points. The location and orientation of primers are indicated by arrows and numbers, being consistent with Table 2. *orf1*, DNA-binding response regulator gene; *orf2*, putative exodeoxyribonuclease VII large subunit gene; *orf3*, ParA family protein gene; *orf4*, putative inner membrane protein gene; *orf5*, putative chromate transporter gene; *orf6*, putative cytoplasmic protein gene. GenBank accession No.: A, JQ241790; B, JQ241792; C, JQ241791; D, JQ241789. The figure is not to scale.
doi:10.1371/journal.pone.0084680.g001

Gentamicin and amikacin resistance were observed in transformants of *A. baumannii* ST91. The analysis of nucleotide sequence around bla_{OXA-58} identified an aminoglycoside *O*-phosphotransferase gene, *aphA6*. The gentamicin and amikacin resistance gene *aphA6* was first reported in *A. baumannii* in 1988 [31]. Nigro et al. recently reported *aphA6* located in a potential transposon Tn*aphA6*, flanked by two copies of IS*Aba125* [32]. An identical transposon was identified in our study and Tn*aphA6* was inserted into a putative threonine efflux protein gene *lysE*. It should be noticed that the susceptible *A. baumannii* could develop carbapenem and amikacin resistance simultaneously via the bla_{OXA-58} and *aphA6* co-harboring plasmid.

Bertini et al. reported the bla_{OXA-58} harboring plasmids could be classified into various groups, including GR2 (Aci1), GR3 (Aci3 and Aci7), GR4 (Aci4) and GR5 (Aci5) [26]. Using the same typing scheme, Towner reported that OXA-58 producing *A. baumannii* from European countries were commonly associated with Aci1, Aci3, Aci4, and AciX [15]. However, the bla_{OXA-58}-carrying plasmids in this study did not belong to any known replicon groups, and a novel replicase gene *rep*Aci10 was identified This suggests that the spread of bla_{OXA-58} in China may be mediated by unique plasmids being different from those of Europe. Meanwhile, the plasmids of *rep*Aci10 are viable in different genomic species of *Acinetobacter* and may contribute to horizontal transmission of resistance genes. However, the replicase genes of the plasmids of *A. baumannii* ST91 remain unknown and further complete plasmid sequencing is in process.

The acquisition of bla_{OXA-58} is associated with a recombination event at site of Re27 sequence [9]. However, the pairs of Re27 sequence around bla_{OXA-58} were absent in partial isolates in this study, suggesting it may have been lost during plasmid evolution.

It is speculated that the insertion of other IS element into IS*Aba3*-like could generate a hybrid promoter to enhance the transcription of bla_{OXA-58} and mediate greater carbapenem resistance than the intact IS*Aba3*-like element as previously reported [9–11,33]. In this study, for plasmids that the IS*Aba3*-like element was disrupted by IS*Our1*, IS*1008* or IS*15*, their corresponding transformants showed a high increase in imipenem MICs (16–32 folds), while for plasmids that the IS*Aba3*-like element was intact, the imipenem MICs of their corresponding transformants were only slightly increasing (2–4 folds). The special structure of IS*6* family-ΔIS*Aba3*-like-bla_{OXA-58} is different from IS*Aba2*, IS*18*, IS*Aba125*, IS*Aba1* and IS*Aba825* that is usually inserted into IS*Aba3*-like in *Acinetobacter* spp. from Europe [9,34,35].

In conclusion, the genetic background of OXA-58-producing *Acinetobacter* spp. in China was diverse, and the multidrug resistant *A. baumannii* ST91 is a potential risk clone. The STs of *A. baumannii*, replicon typing of bla_{OXA-58}-harboring plasmids and genetic contexts of bla_{OXA-58} were distinct from those of Europe, implying the unique evolution and transmission pattern of bla_{OXA-58} in *Acinetobacter* spp. in China.

Acknowledgments

We are grateful to Dr Christopher Weier (Johns Hopkins University School of Medicine) for his comments and revision on the manuscript.

Author Contributions

Conceived and designed the experiments: Yiqi Fu JJ YY JZ. Performed the experiments: Yiqi Fu JJ HZ Ying Fu. Analyzed the data: Yiqi Fu JJ YJ. Contributed reagents/materials/analysis tools: YJ Ying Fu YY JZ. Wrote the paper: Yiqi Fu YY JZ.

References

1. Peleg AY, Seifert H, Paterson DL (2008) *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 21: 538–582.
2. Karageorgopoulos DE, Falagas ME (2008) Current control and treatment of multidrug-resistant *Acinetobacter baumannii* infections. Lancet Infect Dis 8: 751–762.
3. Woodford N, Turton JF, Livermore DM (2011) Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 35: 736–755.
4. Poirel L, Marque S, Heritier C, Segonds C, Chabanon G, et al. (2005) OXA-58, a novel class D β-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 49: 202–208.
5. Coelho J, Woodford N, Afzal-Shah M, Livermore D (2006) Occurrence of OXA-58-like carbapenemases in *Acinetobacter* spp. collected over 10 years in three continents. Antimicrob Agents Chemother 50: 756–758.
6. Peleg AY, Franklin C, Walters LJ, Bell JM, Spelman DW (2006) OXA-58 and IMP-4 carbapenem-hydrolyzing β-lactamases in an *Acinetobacter junii* blood culture isolate from Australia. Antimicrob Agents Chemother 50: 399–400.
7. Castanheira M, Wanger A, Kruzel M, Deshpande LM, Jones RN (2008) Emergence and clonal dissemination of OXA-24- and OXA-58-producing *Acinetobacter baumannii* strains in Houston, Texas: report from the SENTRY Antimicrobial Surveillance Program. J Clin Microbiol 46: 3179–3180.
8. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN (2009) Emergence and widespread dissemination of OXA-23, −24/40 and −58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. J Antimicrob Chemother 63: 55–59.
9. Poirel L, Nordmann P (2006) Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene $bla_{\text{OXA-58}}$ in *Acinetobacter baumannii*. Antimicrob Agents Chemother 50: 1442–1448.
10. Chen TL, Wu RC, Shaio MF, Fung CP, Cho WL (2008) Acquisition of a plasmid-borne $bla_{\text{OXA-58}}$ gene with an upstream IS*1008* insertion conferring a high level of carbapenem resistance to *Acinetobacter baumannii*. Antimicrob Agents Chemother 52: 2573–2580.
11. Chen TL, Chang WC, Kuo SC, Lee YT, Chen CP, et al. (2010) Contribution of a plasmid-borne $bla_{\text{OXA-58}}$ gene with its hybrid promoter provided by IS*1006* and an IS*Aba3*-like element to beta-lactam resistance in acinetobacter genomic species 13TU. Antimicrob Agents Chemother 54: 3107–3112.
12. Marti S, Sanchez-Cespedes J, Blasco MD, Ruiz M, Espinal P, et al. (2008) Characterization of the carbapenem-hydrolyzing oxacillinase oxa-58 in an *Acinetobacter* genospecies 3 clinical isolate. Antimicrob Agents Chemother 52: 2955–2958.
13. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Deshpande LM, et al. (2009) Codetection of $bla_{\text{OXA-23}}$-like gene ($bla_{\text{OXA-133}}$) and $bla_{\text{OXA-58}}$ in *Acinetobacter radioresistens*: report from the SENTRY antimicrobial surveillance program. Antimicrob Agents Chemother 53: 843–844.
14. Marti S, Sanchez-Cespedes J, Blasco MD, Espinal P, Ruiz M, et al. (2008) Characterization of the carbapenem-hydrolyzing oxacillinase OXA-58 in an *Acinetobacter* phenon 6/ct13TU clinical isolate. Diagn Microbiol Infect Dis 61: 468–470.
15. Towner KJ, Evans B, Villa L, Levi K, Hamouda A, et al. (2011) Distribution of intrinsic plasmid replicase genes and their association with carbapenem-hydrolyzing class D β-lactamase genes in European clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 55: 2154–2159.
16. Wang H, Guo P, Sun H, Yang Q, Chen M, et al. (2007) Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. Antimicrob Agents Chemother 51: 4022–4028.
17. Zhou H, Yang Q, Yu YS, Wei ZQ, Li LJ (2007) Clonal spread of imipenem-resistant *Acinetobacter baumannii* among different cities of China. J Clin Microbiol 45: 4054–4057.
18. Chang HC, Wei YF, Dijkshoorn L, Vaneechoutte M, Tang CT, et al. (2005) Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. J Clin Microbiol 43: 1632–1639.
19. Adams-Haduch JM, Paterson DL, Sidjabat HE, Pasculle AW, Potoski BA, et al. (2008) Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob Agents Chemother 52: 3837–3843.
20. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H (2009) OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in *Acinetobacter baumannii*. Antimicrob Agents Chemother 53: 5035–5038.
21. Chen Y, Zhou Z, Jiang Y, Yu Y (2011) Emergence of NDM-1-producing *Acinetobacter baumannii* in China. J Antimicrob Chemother 66: 1255–1259.
22. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, et al. (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33: 2233–2239.
23. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, et al. (2005) Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 43: 4382–4390.
24. Fu Y, Zhou J, Zhou H, Yang Q, Wei Z, et al. (2010) Wide dissemination of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* clonal complex 22 in multiple cities of China. J Antimicrob Chemother 65: 644–650.
25. Barton BM, Harding GP, Zuccarelli AJ (1995) A general method for detecting and sizing large plasmids. Anal Biochem 226: 235–240.
26. Bertini A, Poirel L, Mugnier PD, Villa L, Nordmann P, et al. (2010) Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. Antimicrob Agents Chemother 54: 4168–4177.
27. He C, Xie Y, Fan H, Kang M, Tao C, et al. (2011) Spread of imipenem-resistant *Acinetobacter baumannii* of European clone II in Western China. Int J Antimicrob Agents 38: 257–260.
28. D'Arezzo S, Capone A, Petrosillo N, Visca P, Ballardini M, et al. (2009) Epidemic multidrug-resistant *Acinetobacter baumannii* related to European clonal types I and II in Rome (Italy). Clin Microbiol Infect 15: 347–357.
29. Gogou V, Pournaras S, Giannouli M, Voulgari E, Piperaki ET, et al. (2011) Evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages: a 10 year study in Greece (2000–09). J Antimicrob Chemother 66: 2767–2772.
30. Zhang JP, Zhu W, Tian SF, Chu YZ, Chen BY (2010) Molecular characteristics and resistant mechanisms of imipenem-resistant *Acinetobacter baumannii* isolates in Shenyang, China. J Microbiol 48: 689–694.
31. Martin P, Jullien E, Courvalin P (1988) Nucleotide sequence of *Acinetobacter baumannii aphA-6* gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. Mol Microbiol 2: 615–625.
32. Nigro SJ, Post V, Hall RM (2011) Aminoglycoside resistance in multiply antibiotic-resistant *Acinetobacter baumannii* belonging to global clone 2 from Australian hospitals. J Antimicrob Chemother 66: 1504–1509.
33. Boo TW, Crowley B (2009) Detection of $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-23}}$-like genes in carbapenem-susceptible *Acinetobacter* clinical isolates: should we be concerned? J Med Microbiol 58: 839–841.
34. Ravasi P, Limansky AS, Rodriguez RE, Viale AM, Mussi MA (2011) IS*Aba825*, a functional insertion sequence modulating genomic plasticity and $bla_{\text{OXA-58}}$ expression in *Acinetobacter baumannii*. Antimicrob Agents Chemother 55: 917–920.
35. Evans BA, Hamouda A, Towner KJ, Amyes SG (2010) Novel genetic context of multiple $bla_{\text{OXA-58}}$ genes in *Acinetobacter* genospecies 3. J Antimicrob Chemother 65: 1586–1588.