Co-existence of *bla*$_{OXA-23}$ and *bla*$_{VIM}$ in carbapenem-resistant *Acinetobacter baumannii* isolates belonging to global complex 2 in a Chinese teaching hospital

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Abstract

Background: Carbapenem-resistant *Acinetobacter baumannii* (CRAB) have been a challenging concern of health-care associated infections. The aim of the current study was to investigate the molecular epidemiology and clonal dissemination of CRAB isolates in a Chinese teaching hospital.

Methods: Non-duplicate clinical *A. baumannii* isolates were collected from inpatients, and we measured the minimal inhibitory concentrations to determine antimicrobial susceptibility. Polymerase chain reaction (PCR) and sequencing were performed to detect carbapenem-resistance genes and occurrence of transposons among CRAB isolates. Moreover, the genetic diversity among isolates and clonal dissemination were determined by repetitive element PCR-mediated DNA fingerprinting (rep-PCR) and multilocus sequence typing (MLST).

Results: A total of 67 CRAB isolates displayed resistance to most of the antibiotics tested in this study, except tigecycline. We detected *bla*$_{OXA-23}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$, and *bla*$_{VIM}$ genes in 94.0%, 100.0%, 1.5%, and 80.6% of the CRAB isolates, respectively. Nevertheless, 74.6% of the CRAB isolates co-harbored the *bla*$_{OXA-23}$ and *bla*$_{VIM}$. Only one type of transposons was detected: Tn*2008* (79.1%, *53*/67). Although 12 distinctive types (A-L) were determined (primarily A type) ST*195* was the most prevalent sequence type (ST). ST368, ST210, ST90, ST829, and ST136 were also detected, and all belonged to clonal complex 208 (CC208) and global complex 2 (GC2).

Conclusion: The *bla*$_{OXA-23}$ and *bla*$_{VIM}$ genes contributed to the resistance among CRAB isolates collected in our study. Notably, most of the CRAB strains co-harbored *bla*$_{OXA-23}$ and *bla*$_{VIM}$ genes, as well as Tn*2008*, which could contribute to clonal dissemination. The prevalence of such organisms may underlie hospital acquired infections.

Keywords: *Acinetobacter baumannii*; *bla*$_{OXA-23}$ and *bla*$_{VIM}$ genes; rep-PCR; Transposons

Introduction

Acinetobacter baumannii (*A. baumannii*) is prevalent in the hospital environment and is the source of contamination in various clinical settings. *A. baumannii* has emerged as one of the most significant opportunistic pathogens of healthcare-associated infections (HAIs).[1,2] Other than bacteremia and secondary meningitis, *A. baumannii* is responsible for numerous infections of the urinary and respiratory tract, skin, soft tissues, solid organ transplant, and burn wounds.[3-7] During the last decade, rapid emergence of carbapenem-resistant *A. baumannii* (CRAB) has been reported as a global issue. A rapid increase of HAIs caused by CRAB, from 4.0% in 2003 to 62.0% in 2008 ($P < 0.0001$),[8] was reported in Taiwan, China. A US national surveillance study conducted in 2010 reported a prevalence of 44.7% and 49.0% among *A. baumannii* isolates resistant to imipenem and meropenem, respectively.[9] A dramatic increase in the mortality rate, from 16.0% to 76.0%, has been reported in cases of infections caused by CRAB compared to an increase from 5.0% to 53.0% in cases of infections caused by carbapenem susceptible pathogens.[10] Furthermore, high mortality rates recently reported in Taiwan, China (70%), and Korea (79.8%) have been attributed to CRAB infections in the blood, whereas lower mortality (24.5%) has been observed in similar infections where imipenem susceptible *A. baumannii* were involved.[11,12]

As previously described, carbapenemases are responsible for the acquisition of carbapenem-resistance in *A. baumannii*.[13,14] According to the Ambler classification,

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carbapenemases mainly belong to class A, B, and D β-lactamases, and class D β-lactamases are commonly disseminated in *A. baumannii*, which are comprised of OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like, and OXA-23, which is the primary cause of carbapenem resistance in *A. baumannii*.[14,15] Another important type of carbapenemases, plasmid-mediated metallo-β-lactamases (MBLs), which hydrolyze carbapenems and all β-lactams except monobactams, were also increasingly reported in *Acinetobacter spp*. Presently, few reports have been published on the prevalence of MBLs in *A. baumannii* worldwide.[16-18]

In the current study, we investigated if two genes, $bla_{\text{OXA-23}}$ and bla_{VIM}, were co-harbored among CRAB isolates collected from patients admitted to different clinical departments at a Chinese teaching hospital. Furthermore, the molecular epidemiology and clonal dissemination of these isolates were determined.

Methods

Ethics approval

This study was approved by the Ethics Committee of Xiangya Hospital, Changsha, China. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 *Declaration of Helsinki* and its later amendments or comparable ethical standards. Written informed consent was obtained from all individual participants included in the study.

Bacterial isolates

Non-repetitive *A. baumannii* isolates were collected consecutively from inpatient units between January 1st and December 31st in 2016 at Xiangya Hospital Central South University, a teaching hospital with a 3500-bed capacity located in Changsha, Hunan Province, China. Isolates were collected from different types of specimens from one patient or from the same specimen, but the interval collection time was at least 1 week. All isolates were identified by MALDI-TOF-MS (Bruker Daltonics GmbH, Bremen, Germany) and confirmed by polymerase chain reaction (PCR) by detecting 16S rRNA.[19]

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) were measured using 10 representative antimicrobial agents, including imipenem, amikacin, cotrimoxazole, piperacillin/tazobactam, ceftazidime, gentamicin, cefepime, ciprofloxacin, tigecycline, and minocycline. The MICs were determined using VITEK 2 Compact (BioMérieux, Missouri, France), except for tigecycline, which was measured using *E* test (BioMérieux). *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* (*E. coli*) ATCC 25922 were used as the control organisms. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[20] For tigecycline, interpretive criteria was based on the United States Food and Drug Administration, where isolates having MIC ≥8, 4, and ≤2 μg/mL were considered resistant, intermediate, and sensitive, respectively.[21]

Phenotypic tests for MBLs production

MBLs production was performed using the double-disc synergy test (DDST) and the imipenem-EDTA combined disc test (CDT). In the DDST method, isolates (turbidity was adjusted to 0.5 McFarland) were cultured on Mueller-Hinton agar plates as recommended by the CLSI. After drying, two discs (one disc contained imipenem [10 μg], and the other contained only 10 μL of 0.5 mol/L EDTA [Sigma Chemicals, USA]) were placed on the culture plate at 20 mm from the center of the discs. After overnight incubation, the presence of even a small synergistic inhibition zone was interpreted as positive.[22] The CDT was carried out as follows: two discs (one disc contained imipenem [10 μg] and 5 μL of 0.5 mol/L EDTA [Sigma Chemicals], and the other disc contained only imipenem [10 μg]) were placed about 20 mm apart on a Mueller-Hinton agar plate inoculated with each test strain. The result was considered positive, meaning there was MLB hydrolysis activity, when the zone diameter around the imipenem-EDTA disc was >7.0 mm of the imipenem alone disc.[23]

PCR and sequencing of carbapenemase genes

PCR was conducted to detect the presence of carbapenemase genes in 67 CRAB isolates. $bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-24}}$-like, $bla_{\text{OXA-51}}$-like, $bla_{\text{OXA-58}}$-like, bla_{IMP}, bla_{VIM}, bla_{SPM}, and bla_{NDM} were detected using previously described primers.[24] All PCR products were sequenced by Sangon Biotech (Sangon Biotech, Shanghai, China) and analyzed for similarity searches with the BLAST website (https://blast.ncbi.nlm.nih.gov/blast.cgi).

Repetitive element PCR-mediated DNA fingerprinting (rep-PCR)

Extraction of bacterial genomic DNA and rep-PCR was performed with the GeneAmp PCR System 2720 (Applied Biosystems ABI, USA) using previously described methods. The primers were (REP1: 5'-IIIGCGCCGICATCAGGC-3', REP2: 5'-ACGTCTTATCAGGCCTAC-3'; I represents hypoxanthine).[25,26] PCR-banding patterns were analyzed and interpreted using NTsys-2.10 software (Exeter Software, Stauket, NY, USA) for rep-PCR types.[26]

Multilocus sequence typing (MLST)

MLST was performed for molecular typing of 67 CRAB isolates by sequencing seven housekeeping genes of *A. baumannii* (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) as previously described.[27] Primer sequences are available at http://pubmlst.org/abaumannii/. The sequencing results were compared to the sequence types (STs) available on online databases at http://pubmlst.org/databases/. For investigation of genetic relationships and clonal complexes (CCs), all sequence data of the isolates included in this study were analyzed with eBURST.

Genetic environment of the blaOXA-23 gene

Genetic characterization of bla_{OXA-23}-carrying transposons, Tn*2006*, Tn*2007*, Tn*2008*, and Tn*2009*, was conducted using PCR and sequencing with previously described primers.[28] The presence of insertion sequence IS*Aba1* upstream of transposons was also confirmed by PCR using previously described primers (F: 5′-CATTGGCATTAAACTGAGGAGAAA-3′, R: 5′-TTGGAAATGGGGGAAAACGAA-3′).[29]

Results

Samples and bacterial isolates

A total of 67 CRAB isolates were collected from 67 inpatients, including 47 male and 20 female patients. Approximately 70.1% (47/67) of the CRAB isolates were identified in patients from the intensive care unit (ICU), and the remaining isolates were acquired from patients in the emergency (9.0%), burn (6.0%), pancreatic surgery (3.0%), nose and skull base surgery (3.0%), infectious diseases (3.0%), rehabilitation (3.0%), brain trauma specialist (1.5%), and cerebrovascular surgery (1.5%) departments. The proportions of specimens included in this study were 35.8% each for blood and cerebrospinal fluid, followed by 11.9%, 7.5%, 7.5%, and 1.5% for pleural effusion, tissue block, ascitic fluid, and bile, respectively, as shown in Figure 1.

Antimicrobial susceptibility testing and detection of carbapenemase genes

The MICs of 10 antimicrobial agents were determined against all clinical *A. baumannii* isolates. The resistant rates of imipenem, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, ceftazidime, gentamicin, amikacin, cefepime, ciprofloxacin, and minocycline were 100.0%, 53.7%, 98.5%, 95.5%, 86.6%, 77.6%, 98.5%, 83.6%, and 67.1%, respectively. Although only two isolates showed resistance to tigecycline, 40.3% of CRAB isolates exhibited an intermediate phenotype to tigecycline. Details of isolate susceptibility are shown in Table 1.

The DDST and imipenem-EDTA CDT were used to determine the prevalence of MBLs among CRAB; 77.6% of the isolates were positive for DDST, while 76.1% isolates were positive for CDT as shown in Figure 1.

bla_{OXA-23}, bla_{OXA-51}, and bla_{VIM} genes were detected in 94.0%, 100.0%, and 80.6% of the 67 CRAB isolates, respectively, whereas only one isolate carried the bla_{OXA-58} gene. None of these isolates harbored bla_{OXA-24}, bla_{IMP}, bla_{SPM}, and bla_{NDM}. Notably, 74.6% of the CRAB isolates carried both the bla_{OXA-23} and bla_{VIM} genes [Figure 1]. Moreover, isolates harboring bla_{VIM} genes were positive in the DDST and CDT analysis, except for two isolates (CS24 and CS53).

Homology analysis

Based on rep-PCR results, 12 (A-L) genetically different profiles were observed among 67 CRAB isolates. The 56.6% of the isolates were linked to the type A profile. However, type A and I were further divided into three subtypes. Similarly, type H, J, and K were distributed into two subtypes, whereas type B into G and L was not divided into subtypes. A large number of isolates from each profile was derived from the ICU samples [Figure 1].

Among the 67 CRAB isolates, six STs – ST195 (41.8%), ST368 (13.4%), ST210 (3.0%), ST90 (3.0%), ST829 (11.9%), and ST136 (3.0%) – were detected by MLST. The eBURST analysis indicated that all STs belonged to the CC208 clonal complex and originated from the CC92 clonal complex, corresponding to global clone 2 (GC2) [Figure 2].

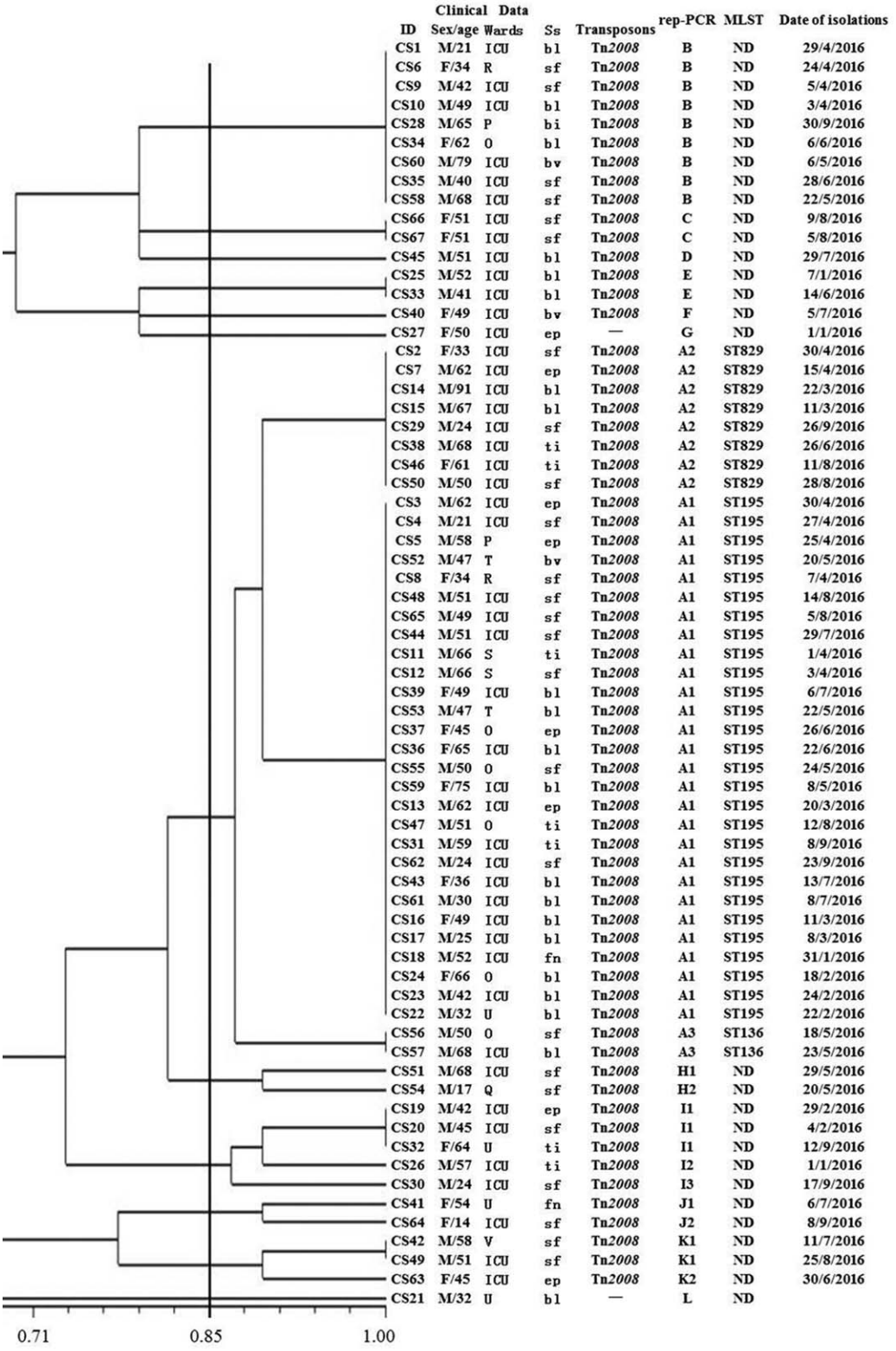
Genetic environment of the blaOXA-23 gene

Among the four common transposons tested in this study, only Tn*2008* was identified in 53 isolates by PCR analysis. Nevertheless, insertion sequence IS*Aba1* located upstream of the transposons was characterized among all the CRAB isolates.

Discussion

A. baumannii is a significant opportunistic pathogen associated with HAIs. *A. baumannii* has the ability to adapt to its environment and acquire drug resistance genes from its surroundings. As such, *A. baumannii* can lead to multiple drug-resistant (MDR) and even extensively drug-resistant (XDR) isolates in the hospital setting.[30] Carbapenemase production is considered the primary underlying cause of the CRAB, oxacillinase (OXA), especially OXA-23.[14] However, MBLs are another common carbapenemase in the *Acinetobacter calcoaceticus-A. baumannii* complex (ACB), but few reports of MBL prevalence in *A. baumannii* have been found. In New Delhi in 2009, Metallo-β-lactamases (NDM, common MBL) was first discovered in *Klebsiella pneumoniae*. Since then, it has disseminated to other species including *A. baumannii*, *E. coli*, and *Enterobacter cloacae*.[31] However, another MBL gene, bla_{VIM}, has rarely been identified in *A. baumannii*.[32,33] In this study, we identified MDR *A. baumannii* isolated in patients from different clinical departments of a teaching hospital. Moreover, we investigated the molecular characteristics and clonal dissemination of these isolates.

In the current study, three types of OXA genes were detected, including OXA-23 (94.0%), OXA-51 (100.0%), and OXA-58 (1.5%), in the CRAB isolates. The occurrence of OXA-23 was considered the main reason of carbapenem resistance in *A. baumannii*.[14] CRAB isolates carrying-bla_{OXA-23} genes are disseminated worldwide due to the transposons. Five transposons have been identified, Tn*2006*, Tn*2007*, Tn*2008*, Tn*2008*B, and Tn*2009*, among bacterial isolates; however, Tn*2006* and Tn*2008* were globally disseminated,[34] and Tn*2006* was the most prevalent transposon in China.[35] In contrast, we identified Tn*2008*, rather than Tn*2006*, in 79.1% of CRAB isolates, in accordance with Chen *et al*,[28] indicating that Tn*2008* could be involved in the transmission of the bla_{OXA-23} gene among CRAB isolates, and also closely



ID	Clinical Data: Sex/age	Wards	Ss	Transposons	rep-PCR	MLST	Date of isolations
CS1	M/21	ICU	bl	Tn*2008*	B	ND	29/4/2016
CS6	F/34	R	sf	Tn*2008*	B	ND	24/4/2016
CS9	M/42	ICU	sf	Tn*2008*	B	ND	5/4/2016
CS10	M/49	ICU	bl	Tn*2008*	B	ND	3/4/2016
CS28	M/65	P	bi	Tn*2008*	B	ND	30/9/2016
CS34	F/62	O	bl	Tn*2008*	B	ND	6/6/2016
CS60	M/79	ICU	bv	Tn*2008*	B	ND	6/5/2016
CS35	M/40	ICU	sf	Tn*2008*	B	ND	28/6/2016
CS58	M/68	ICU	sf	Tn*2008*	B	ND	22/5/2016
CS66	F/51	ICU	sf	Tn*2008*	C	ND	9/8/2016
CS67	F/51	ICU	sf	Tn*2008*	C	ND	5/8/2016
CS45	M/51	ICU	bl	Tn*2008*	D	ND	29/7/2016
CS25	M/52	ICU	bl	Tn*2008*	E	ND	7/1/2016
CS33	M/41	ICU	bl	Tn*2008*	E	ND	14/6/2016
CS40	F/49	ICU	bv	Tn*2008*	F	ND	5/7/2016
CS27	F/50	ICU	ep	—	G	ND	1/1/2016
CS2	F/33	ICU	sf	Tn*2008*	A2	ST829	30/4/2016
CS7	M/62	ICU	ep	Tn*2008*	A2	ST829	15/4/2016
CS14	M/91	ICU	bl	Tn*2008*	A2	ST829	22/3/2016
CS15	M/67	ICU	bl	Tn*2008*	A2	ST829	11/3/2016
CS29	M/24	ICU	sf	Tn*2008*	A2	ST829	26/9/2016
CS38	M/68	ICU	ti	Tn*2008*	A2	ST829	26/6/2016
CS46	F/61	ICU	ti	Tn*2008*	A2	ST829	11/8/2016
CS50	M/50	ICU	sf	Tn*2008*	A2	ST829	28/8/2016
CS3	M/62	ICU	ep	Tn*2008*	A1	ST195	30/4/2016
CS4	M/21	ICU	sf	Tn*2008*	A1	ST195	27/4/2016
CS5	M/58	P	ep	Tn*2008*	A1	ST195	25/4/2016
CS52	M/47	T	bv	Tn*2008*	A1	ST195	20/5/2016
CS8	F/34	R	sf	Tn*2008*	A1	ST195	7/4/2016
CS48	M/51	ICU	sf	Tn*2008*	A1	ST195	14/8/2016
CS65	M/49	ICU	sf	Tn*2008*	A1	ST195	5/8/2016
CS44	M/51	ICU	sf	Tn*2008*	A1	ST195	29/7/2016
CS11	M/66	S	ti	Tn*2008*	A1	ST195	1/4/2016
CS12	M/66	S	sf	Tn*2008*	A1	ST195	3/4/2016
CS39	F/49	ICU	bl	Tn*2008*	A1	ST195	6/7/2016
CS53	M/47	T	bl	Tn*2008*	A1	ST195	22/5/2016
CS37	F/45	O	ep	Tn*2008*	A1	ST195	26/6/2016
CS36	F/65	ICU	bl	Tn*2008*	A1	ST195	22/6/2016
CS55	M/50	O	sf	Tn*2008*	A1	ST195	24/5/2016
CS59	F/75	ICU	bl	Tn*2008*	A1	ST195	8/5/2016
CS13	M/62	ICU	ep	Tn*2008*	A1	ST195	20/3/2016
CS47	M/51	O	ti	Tn*2008*	A1	ST195	12/8/2016
CS31	M/59	ICU	ti	Tn*2008*	A1	ST195	8/9/2016
CS62	M/24	ICU	sf	Tn*2008*	A1	ST195	23/9/2016
CS43	F/36	ICU	bl	Tn*2008*	A1	ST195	13/7/2016
CS61	M/30	ICU	bl	Tn*2008*	A1	ST195	8/7/2016
CS16	F/49	ICU	bl	Tn*2008*	A1	ST195	11/3/2016
CS17	M/25	ICU	bl	Tn*2008*	A1	ST195	8/3/2016
CS18	M/52	ICU	fn	Tn*2008*	A1	ST195	31/1/2016
CS24	F/66	O	bl	Tn*2008*	A1	ST195	18/2/2016
CS23	M/42	ICU	bl	Tn*2008*	A1	ST195	24/2/2016
CS22	M/32	U	bl	Tn*2008*	A1	ST195	22/2/2016
CS56	M/50	O	sf	Tn*2008*	A3	ST136	18/5/2016
CS57	M/68	ICU	bl	Tn*2008*	A3	ST136	23/5/2016
CS51	M/68	ICU	sf	Tn*2008*	H1	ND	29/5/2016
CS54	M/17	Q	sf	Tn*2008*	H2	ND	20/5/2016
CS19	M/42	ICU	ep	Tn*2008*	I1	ND	29/2/2016
CS20	M/45	ICU	sf	Tn*2008*	I1	ND	4/2/2016
CS32	F/64	U	ti	Tn*2008*	I1	ND	12/9/2016
CS26	M/57	ICU	ti	Tn*2008*	I2	ND	1/1/2016
CS30	M/24	ICU	sf	Tn*2008*	I3	ND	17/9/2016
CS41	F/54	U	fn	Tn*2008*	J1	ND	6/7/2016
CS64	F/14	ICU	sf	Tn*2008*	J2	ND	8/9/2016
CS42	M/58	V	sf	Tn*2008*	K1	ND	11/7/2016
CS49	M/51	ICU	sf	Tn*2008*	K1	ND	25/8/2016
CS63	F/45	ICU	ep	Tn*2008*	K2	ND	30/6/2016
CS21	M/32	U	bl	—	L	ND	

Figure 1: Dendrogram based on rep-PCR profiles with clinical features and molecular characterization of carbapenemases and MLST. +, positive; –, negative; ND, not determined; Ss, specimens; RDs, resistance determinants; bl, blood; bi, bile; bv, blood vessel; ti, tissue; sf, cerebrospinal fluid; ep, empyema pleural; fn, aspirate fine-needle; O, emergency department; P, pancreatic biliary surgery; Q, cerebrovascular surgery; R, nose and skull base surgery; S, infectious diseases department; T, the department of rehabilitation; U, burns surgery; V, brain trauma specialist; A-L, rep-PCR types; rep-PCR: Repetitive element PCR-mediated DNA fingerprinting; MLST: Multilocus sequence typing.

related to the molecular epidemiological characteristics of the strains in this area of China. Tn*2008*B is the most recently identified structure containing bla_{OXA-23}. To date, Tn*2008*B was only reported in China;[36] however, we did not detect Tn*2008*B in our study. In addition, we found that IS*Aba1* was located upstream of the bla_{OXA-23} gene, which could facilitate the mobilization of bla_{OXA-23} and provide promoters for its expression.[37] The bla_{OXA-51} gene is unique to *A. baumannii* and may be used as a marker to identify this species.[38] Moreover, some previous studies reported that OXA-51 carbapenemases had minimal ability to hydrolyze carbapenems when IS*Aba1* was upstream of the bla_{OXA-51} gene.[39] In contrast, we showed in this study that 88.9% of the CRAB isolates carried IS*Aba1* upstream of the bla_{OXA-51} gene. Hence, there may be contribution of OXA-51 in CRAB. Isolates carrying $bla_{OXA-24/58}$-like genes are typically resistant to carbapenems. Fortunately, they have not been commonly identified in China. In the current study, only one isolate carried OXA-58. Our results also demonstrated that bla_{OXA-24}, bla_{NDM}, bla_{SPM}, and bla_{IMP} were not expressed in our isolates. However, due to their plasmid location, the distribution of these genes in *A. baumannii* should be monitored early.

Notably, MBLs, which are rarely reported in *A. baumannii*,[16,17] widely existed in the CRAB isolates included in this study, and 80.6% of these CRAB isolates harbored bla_{VIM} genes and produced MBLs confirmed by DDST and CDT, which have been considered the standardized methods for detecting MBL production.[40] Notably, 74.6% of the CRAB isolates co-harbored bla_{OXA-23} and bla_{VIM}. However, the globally disseminated NDMs, including *K. pneumoniae*, *A. baumannii*, *E. coli* and *E. cloacae*,[31] were not detected in any of the isolates included in this study.

We analyzed 67 CRAB isolates using rep-PCR, which has been widely used in molecular typing of *A. baumannii*.[41] Additionally, 12 distinctive types of profiles obtained by rep-PCR indicated that genetically diversified CRAB isolates are prevalent in our teaching hospital. Three European clones (EC I, II, and III) of *A. baumannii* have been reported;[42] EC I (GC1) and EC II (GC2) are globally propagated.[43] Of particular interest, six STs (ST195, ST368, ST210, ST90, ST829, and ST136) were found by MLST, all of which belong to CC208 and are generally linked to GC2. Although ST195 (41.8%) was the predominant ST in the current study, ST92 is among the most prevalent CRAB isolates in China.[44] We did not detect ST92 in the current study; we detected ST208 using the Institute Pasteur scheme (compared to ST92; Oxford scheme). Actually, ST92 may not exist.[45]

Table 1: Antimicrobial susceptibility of 67 CRAB isolates (%).

Antibiotics	R	I	S
Tigecycline	3.0	40.3	56.7
Imipenem	100.0	0.0	0.0
Trimethoprim/sulfamethoxazole	53.7	0.0	46.3
Piperacillin/tazobactam	98.5	1.5	0.0
Ceftazidime	95.5	1.5	3.0
Gentamicin	86.6	4.5	8.9
Amikacin	77.6	0.0	22.4
Cefepime	98.5	0.0	1.5
Ciprofloxacin	83.6	4.5	11.9
Minocycline	67.1	11.9	21.0

CRAB: Carbapenem-resistant *Acinetobacter baumannii;* R: resistant; S: sensitive; I: intermediate.

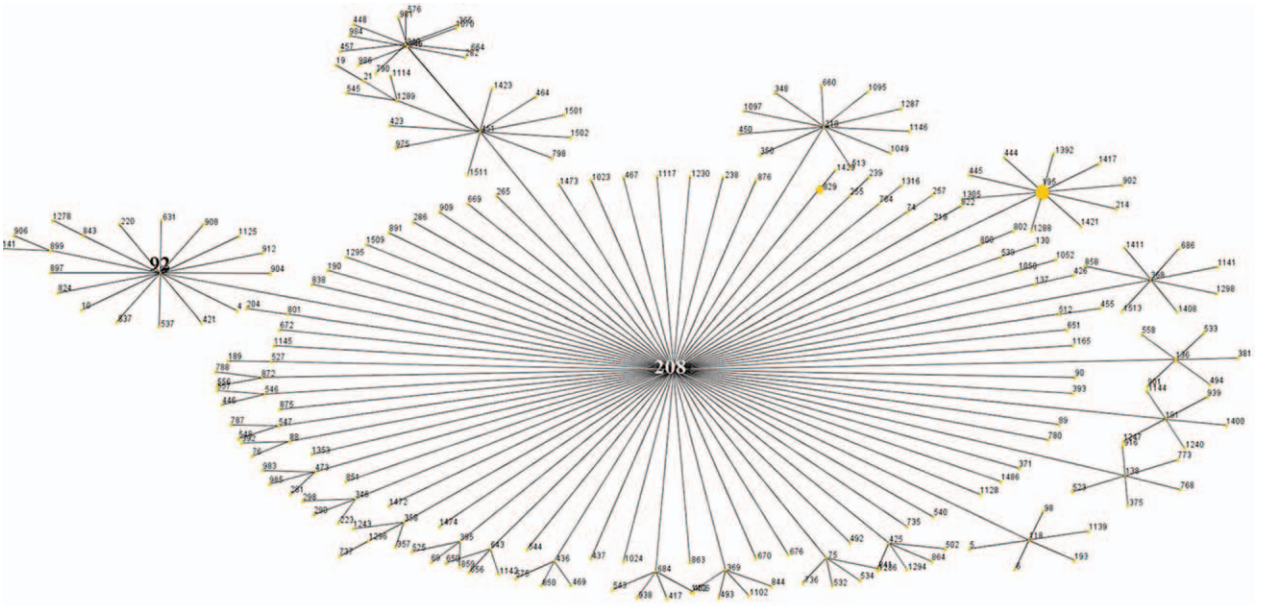


Figure 2: Identification of clonal complex by eBURST analysis of CRAB isolates. ST195, ST368, ST210, ST90, ST829, and ST136, all of which were detected in this study, belong to the CC208 clonal complex. CRAB: Carbapenem-resistant *Acinetobacter baumannii.*

There are also some limitations in this study. We did not analyze the plasmid where the drug resistance gene is located. In addition, we only studied the CRAB isolated from our hospital and did not include other hospitals in our region.

In summary, bla_{OXA-23}, bla_{OXA-51}, and bla_{VIM} may be responsible for CRAB. ST195 was the most prevalent clonal complex found in our teaching hospital and could disseminate in this area of China. Remarkably, the clonal dissemination of GC2 isolates co-harboring bla_{OXA-23} and bla_{VIM} genes among *A. baumannii* strains may be responsible for the rapid acquisition of carbapenem resistance. Furthermore, the detection of Tn*2008* suggests that dissemination of bla_{OXA-23} might be facilitated by these transposons. Therefore, some infection control measures should be reinforced to reduce the further spread of *A. baumannii*, including rapid identification of bla_{OXA-23}, hand hygiene, and environmental disinfection.

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Conflicts of interest

None.

References

1. Balkhy HH, Bawazeer MS, Kattan RF, Tamim HM, Al Johani SM, Aldughashem FA, *et al*. Epidemiology of *Acinetobacter spp*.-associated healthcare infections and colonization among children at a tertiary-care hospital in Saudi Arabia: a 6-year retrospective cohort study. Eur J Clin Microbiol Infect Dis 2012;31:2645–2651. doi: 10.1007/s10096-012-1608-8.
2. El-Shazly S, Dashti A, Vali L, Bolaris M, Ibrahim AS. Molecular epidemiology and characterization of multiple drug-resistant (MDR) clinical isolates of *Acinetobacter baumannii*. Int J Infect Dis 2015;41:42–49. doi: 10.1016/j.ijid.2015.10.016.
3. Roca I, Espinal P, Vila-Farres X, Vila J. The *Acinetobacter baumannii* oxymoron: commensal hospital dweller turned pan-drug-resistant menace. Front Microbiol 2012;3:148. doi: 10.3389/fmicb.2012.00148.
4. Kempf M, Rolain JM, Azza S, Diene S, Joly-Guillou ML, Dubourg G, *et al*. Investigation of *Acinetobacter baumannii* resistance to carbapenems in Marseille hospitals, south of France: a transition from an epidemic to an endemic situation. APMIS 2013;121:64–71. doi: 10.1111/j.1600-0463.2012.02935.x.
5. Yuan X, Liu T, Wu D, Wan Q. Epidemiology, susceptibility, and risk factors for acquisition of MDR/XDR Gram-negative bacteria among kidney transplant recipients with urinary tract infections. Infect Drug Resist 2018;11:707–715. doi: 10.2147/IDR.S163979.
6. Gao F, Ye Q, Wan Q, Liu S, Zhou J. Distribution and resistance of pathogens in liver transplant recipients with *Acinetobacter baumannii* infection. Ther Clin Risk Manag 2015;11:501–505. doi: 10.2147/TCRM.S82251.
7. Nie XM, Huang PH, Ye QF, Wan QQ. The distribution, drug resistance, and clinical characteristics of *Acinetobacter baumannii* infections in solid organ transplant recipients. Transplant Proc 2015;47:2860–2864. doi: 10.1016/j.transproceed.2015.09.037.
8. Su CH, Wang JT, Hsiung CA, Chien LJ, Chi CL, Yu HT, *et al*. Increase of carbapenem-resistant *Acinetobacter baumannii* infection in acute care hospitals in Taiwan: association with hospital antimicrobial usage. PLoS One 2012;7:e37788. doi: 10.1371/journal.pone.0037788.
9. Queenan AM, Pillar CM, Deane J, Sahm DF, Lynch AS, Flamm RK, *et al*. Multidrug resistance among *Acinetobacter spp*. in the USA and activity profile of key agents: results from CAPITAL Surveillance 2010. Diagn Microbiol Infect Dis 2012;73:267–270. doi: 10.1016/j.diagmicrobio.2012.04.002.
10. Lemos EV, de la Hoz FP, Einarson TR, McGhan WF, Quevedo E, Castaneda C, *et al*. Carbapenem resistance and mortality in patients with *Acinetobacter baumannii* infection: systematic review and meta-analysis. Clin Microbiol Infect 2014;20:416–423. doi: 10.1111/1469-0691.12363.
11. Lee HY, Chen CL, Wu SR, Huang CW, Chiu CH. Risk factors and outcome analysis of *Acinetobacter baumannii* complex bacteremia in critical patients. Crit Care Med 2014;42:1081–1088. doi: 10.1097/CCM.0000000000000125.
12. Kim SY, Jung JY, Kang YA, Lim JE, Kim EY, Lee SK, *et al*. Risk factors for occurrence and 30-day mortality for carbapenem-resistant *Acinetobacter baumannii* bacteremia in an intensive care unit. J Korean Med Sci 2012;27:939–947. doi: 10.3346/jkms.2012.27.8.939.
13. Vijayakumar S, Gopi R, Gunasekaran P, Bharathy M, Walia K, Anandan S, *et al*. Molecular characterization of invasive carbapenem-resistant *Acinetobacter baumannii* from a tertiary care hospital in South India. Infect Dis Ther 2016;5:379–387. doi: 10.1007/s40121-016-0125-y.
14. Al Atrouni A, Hamze M, Jisr T, Lemarie C, Eveillard M, Joly-Guillou ML, *et al*. Wide spread of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* belonging to clonal complex II in different hospitals in Lebanon. Int J Infect Dis 2016;52:29–36. doi: 10.1016/j.ijid.2016.09.017.
15. Chen Z, Liu W, Zhang Y, Li Y, Jian Z, Deng H, *et al*. Molecular epidemiology of carbapenem-resistant *Acinetobacter spp*. from XiangYa Hospital, in Hunan Province, China. J Basic Microbiol 2013;53:121–127. doi: 10.1002/jobm.201100420.
16. Huang YM, Zhong LL, Zhang XF, Hu HT, Li YQ, Yang XR, *et al*. NDM-1-Producing *Citrobacter freundii, Escherichia coli,* and *Acinetobacter baumannii* identified from a single patient in China. Antimicrob Agents Chemother 2015;59:5073–5077. doi: 10.1128/AAC.04682-14.
17. Cicek AC, Saral A, Iraz M, Ceylan A, Duzgun AO, Peleg AY, *et al*. OXA- and GES-type beta-lactamases predominate in extensively drug-resistant *Acinetobacter baumannii* isolates from a Turkish University Hospital. Clin Microbiol Infect 2014;20:410–415. doi: 10.1111/1469-0691.12338.
18. Chen F, Wang L, Wang M, Xie Y, Xia X, Li X, *et al*. Genetic characterization and in vitro activity of antimicrobial combinations of multidrug-resistant *Acinetobacter baumannii* from a general hospital in China. Oncol Lett 2018;15:2305–2315. doi: 10.3892/ol.2017.7600.
19. Vaneechoutte M, Dijkshoorn L, Tjernberg I, Elaichouni A, de Vos P, Claeys G, *et al*. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J Clin Microbiol 1995;33:11–15.
20. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, document M100-26. Wayne: Clinical and Laboratory Standards Institute; 2016.
21. Jones RN, Ferraro MJ, Reller LB, Schreckenberger PC, Swenson JM, Sader HS. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter spp*. J Clin Microbiol 2007;45:227–230. doi: 10.1128/JCM.01588-06.
22. Rizvi M, Fatima N, Rashid M, Shukla I, Malik A, Usman A, *et al*. Extended spectrum AmpC and metallo-beta-lactamases in Serratia and Citrobacter spp. in a disc approximation assay. J Infect Dev Ctries 2009;3:285–294.
23. Kotwal A, Biswas D, Kakati B, Singh M. ESBL and MBL in Cefepime Resistant *Pseudomonas aeruginosa*: an update from a rural area in Northern India. J Clin Diagn Res 2016;10:DC09–DC11. doi: 10.7860/JCDR/2016/18016.7612.
24. Joshi PR, Acharya M, Kakshapati T, Leungtongkam U, Thummeepak R, Sitthisak S. Co-existence of bla_{OXA-23} and bla_{NDM-1} genes of *Acinetobacter baumannii* isolated from Nepal: antimicrobial resistance and clinical significance. Antimicrob Resist Infect Control 2017;6:21. doi: 10.1186/s13756-017-0180-5.
25. Bou G, Cervero G, Dominguez MA, Quereda C, Martinez-Beltran J. Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in *A. baumannii* is not due solely to the presence of beta-lactamases. J Clin Microbiol 2000;38:3299–3305.

26. Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, *et al*. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter spp*. J Clin Microbiol 1997;35: 3071–3077.
27. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5:e10034. doi: 10.1371/journal.pone.0010034.
28. Chen Y, Gao J, Zhang H, Ying C. Spread of the bla_{OXA-23}-containing Tn2008 in carbapenem-resistant *Acinetobacter baumannii* isolates grouped in CC92 from China. Front Microbiol 2017;8:163. doi: 10.3389/fmicb.2017.00163.
29. Ruiz M, Marti S, Fernandez-Cuenca F, Pascual A, Vila J. Prevalence of IS(Aba1) in epidemiologically unrelated *Acinetobacter baumannii* clinical isolates. FEMS Microbiol Lett 2007;274:63–66. doi: 10.1111/j.1574-6968.2007.00828.x.
30. Li YJ, Pan CZ, Fang CQ, Zhao ZX, Chen HL, Guo PH, *et al*. Pneumonia caused by extensive drug-resistant *Acinetobacter baumannii* among hospitalized patients: genetic relationships, risk factors and mortality. BMC Infect Dis 2017;17:371. doi: 10.1186/s12879-017-2471-0.
31. Hammerum AM, Littauer P, Hansen F. Detection of Klebsiella pneumoniae co-producing NDM-7 and OXA-181, *Escherichia coli* producing NDM-5 and *Acinetobacter baumannii* producing OXA-23 in a single patient. Int J Antimicrob Agents 2015;46:597–598. doi: 10.1016/j.ijantimicag.2015.07.008.
32. Ikonomidis A, Ntokou E, Maniatis AN, Tsakris A, Pournaras S. Hidden VIM-1 metallo-beta-lactamase phenotypes among *Acinetobacter baumannii* clinical isolates. J Clin Microbiol 2008;46:346–349. doi: 10.1128/JCM.01670-07.
33. Purohit M, Mendiratta DK, Deotale VS, Madhan M, Manoharan A, Narang P. Detection of metallo-beta-lactamases producing *Acinetobacter baumannii* using microbiological assay, disc synergy test and PCR. Indian J Med Microbiol 2012;30:456–461. doi: 10.4103/0255-0857.103770.
34. Lee MH, Chen TL, Lee YT, Huang L, Kuo SC, Yu KW, *et al*. Dissemination of multidrug-resistant *Acinetobacter baumannii* carrying bla_{OXA-23} from hospitals in central Taiwan. J Microbiol Immunol Infect 2013;46:419–424. doi: 10.1016/j.jmii.2012.08.006.
35. Lee HY, Chang RC, Su LH, Liu SY, Wu SR, Chuang CH, *et al*. Wide spread of Tn2006 in an AbaR4-type resistance island among carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Taiwan. Int J Antimicrob Agents 2012;40:163–167. doi: 10.1016/j.ijantimicag.2012.04.018.
36. Nigro SJ, Hall RM. Structure and context of Acinetobacter transposons carrying the oxa23 carbapenemase gene. J Antimicrob Chemother 2016;71:1135–1147. doi: 10.1093/jac/dkv440.
37. Lopes BS, Amyes SG. Role of ISAba1 and ISAba125 in governing the expression of blaADC in clinically relevant *Acinetobacter baumannii* strains resistant to cephalosporins. J Med Microbiol 2012;61:1103–1108. doi: 10.1099/jmm.0.044156-0.
38. Heritier C, Poirel L, Fournier PE, Claverie JM, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49: 4174–4179. doi: 10.1128/AAC.49.10.4174-4179.2005.
39. Nowak P, Paluchowska P, Budak A. Distribution of bla_{OXA} genes among carbapenem-resistant *Acinetobacter baumannii* nosocomial strains in Poland. New Microbiol 2012;35:317–325.
40. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2008;26:233–237.
41. Pasanen T, Koskela S, Mero S, Tarkka E, Tissari P, Vaara M, *et al*. Rapid molecular characterization of *Acinetobacter baumannii* clones with rep-PCR and evaluation of carbapenemase genes by new multiplex PCR in Hospital District of Helsinki and Uusimaa. PLoS One 2014;9:e85854. doi: 10.1371/journal.pone.0085854.
42. van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Paauw A, van den Broek P, *et al*. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res Microbiol 2004;155:105–112. doi: 10.1016/j.resmic.2003.10.003.
43. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233–238. doi: 10.1093/jac/dkp428.
44. Huang L, Sun L, Yan Y. Clonal spread of carbapenem resistant *Acinetobacter baumannii* ST92 in a Chinese Hospital during a 6-year period. J Microbiol 2013;51:113–117. doi: 10.1007/s12275-013-2341-4.
45. Hamidian M, Nigro SJ, Hall RM. Problems with the Oxford multilocus sequence typing scheme for *Acinetobacter baumannii*: do sequence type 92 (ST92) and ST109 exist? J Clin Microbiol 2017;55:2287–2289. doi: 10.1128/JCM.00533-17.

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