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Molecular epidemiology and decreased susceptibility to disinfectants in carbapenem-resistant *Acinetobacter baumannii* isolated from intensive care unit patients in central China

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ABSTRACT

Background: Infection with carbapenem-resistant *Acinetobacter baumannii* (CRAB) is an increasing problem for critically ill patients. The srains are frequently resistant to all antibiotics and disinfectants are often used to block the spread of these bacteria, playing an important role in infection control.
Objectives: The aim of this study was to investigate the antibiotic susceptibility, the clonal relationship, disinfectant resistance gene, β-lactamase genes and the disinfectant sensitivity of 82 *A. baumannii* isolates collected at a large hospital in Wuhan, China.
Design: A retrospective basic study.
Methods: Here we investigated 82 *A. baumannii* isolates from intensive care unit patients in a major teaching hospital in China for the distribution of resistance-associated genes and susceptibility to chlorine disinfectant (CLR), benzalkonium bromide (BB) and Chlorhexidine gluconate(CHG). Multi-locus sequence typing (MLST) was applied to explore their genetic evolution relationships.
Results: qacE (30.48%, 25/82) and *qac△E1* (76.82%, 63/82) genes were detected in our study, while none were positive for *qacA/B*, *qacC/D* or *qacG*. The MIC values of CLR were 250 mg/L; The MIC values ranged from 32 to 128 μg/mL for BB; The MIC values ranged from 0.0019% to 0.0078% for CHG. The presence or absence of *qacE* gene has a significant impact($p < 0.05$) on MICs of BB or CHG. All isolates harboured $bla_{OXA-51/23}$ genes, and 98.78% of isolates contained the *ISaba1* insertion sequence. All isolates were classified into 8 sequence types(STs) within clonal complex 92(CC92).
Conclusions: The predominant CRAB strains in our intensive care unit are bla_{OXA-23}-containing *A. baumannii* of CC92. The high prevalence of *qac* genes and reduced susceptibility to disinfectants confirm the need for continued vigilance against nosocomial infections.

Background

Acinetobacter baumannii is an important opportunistic pathogen that causes a multitude of infections associated with significant morbidity and mortality, and it poses a serious threat to patients in intensive care units [1–3]. The success of *A. baumannii* isolates is largely due to its ability to rapidly obtain resistance to antimicrobial therapies and its capability to persist in an abiotic or disinfectant environment [4]. Therefore, this pathogen can easily cause the outbreak of nosocomial infections.

In particular, carbapenem-resistant *A. baumannii* (CRAB) is a serious public health issue [5,6]. The WHO's data on antibiotic-resistant 'priority pathogens' reports CRAB as a kind of bacteria which is in critical need of study and development of new antimicrobials. As shown by the WHO, the mortality, health-care and community burdens of infections caused by CRAB are very serious, and it also brings great challenges such as treatability, transmissibility and preventability in health-care and community setting [7]. China Antimicrobial Resistance Surveillance System (CHINET) reports from 2015 to 2016 indicated that 69.8% of *A. baumannii* isolates were resistant to imipenem and 71.4% were resistant to meropenem [8]. In addition, the rate of drug resistance was 20% higher among *A. baumannii* isolates from intensive care units than among isolates outside the intensive care unit.

Thus disinfection is essential in hospitals to prevent and control *A. baumannii*-related infections. Benzalkonium bromide (BB)

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and Chlorhexidine gluconate (CHG) are commonly used for skin disinfection, oral care and hand disinfection of medical staff worldwide [9]. However, the frequent use of disinfectants has led to strains less susceptible to these agents [10,11]. This may reflect the presence of disinfectant resistance genes, many of which have been found in multi-drug resistant bacteria [12,13]. For example, the gene *qacA,* thought to be plasmid-encoded, encodes resistance to structurally diverse organic cations, such as chlorhexidine and pentamidine [14]. The resistance genes *qacA* and *qacB* encode proteins differing at only six amino acid positions, which therefore offer resistance to different substances [15]. The resistance genes *qacD* and *qacC* are identical, except they are transcribed from different promoters [14]. Many Gram-positive bacteria contain *qacA/B*, *qacC/D* and *qacG* genes [16–18], while many Gram-negative bacteria carry plasmids encoding *qacE* and *qacEΔ1,* associated with resistance to quaternary ammonium compounds(QAC) [12,19].

A. baumannii possesses multiple resistance mechanisms, including through acquisition of resistance determinants, mutations in genes encoding pore proteins, outer membrane impermeability and overexpression of the efflux pump [20]. These bacteria can acquire carbapenem resistance through expression of genes encoding class B metallo-β-lactamases (MBLs) or carbapenem-hydrolyzing class D β-lactamases (CHDLs). The $bla_{OXA\text{-}51\text{-}like}$ CHDL gene is chromosomal, while the CHDL genes $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$ and $bla_{OXA\text{-}143\text{-}like}$ are encoded on plasmids [21]. These OXA β-lactamase genes have rapidly spread to epidemic strains of *A. baumannii,* which has a great impact on the infectivity of the global *A.baumannii* [22].

Based on the above information, we could get a clear overview about the *qac* gene and carbapenem-resistant genes in *A. baumannii*. Therefore, this study aimed to investigate the prevalence of *qac* and carbapenem-resistant genes in CRAB isolated from the intensive care unit in Wuhan University People's Hospital and the susceptibility of *A. baumannii* to Chlorine disinfectant(CLR), BB and CHG.

Materials and methods

Clinical sample collection & microbial identification

From the beginning of January 2017 to the end of January 2018, 82 *A. baumannii* isolates were obtained from intensive care patients in the People's Hospital of Wuhan University (4000 of beds, more than 5 million outpatient admissions/per year). All isolates were identified by the BD Phoenix™ 100 system(BD, United States) and confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry(Bruker Daltonics, United States), with ≥2.00 the threshold for correct species identification [23]. *A. baumannii* isolates were further confirmed based on PCR amplification of the *ropB* gene [24]. PCR products were sequenced by Jinkairui Biotechnology (Wuhan, China) and sequences were aligned using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antimicrobial susceptibility testing

The susceptibility of 82 *A. baumannii* isolates to antibiotics and disinfectants was determined by the disk diffusion method and broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018) [25]. Drug sensitive papers were purchased from OXOID company (United Kingdom), disinfectants CLR, BB and CHG from Macklin Biochemical Technology Co., Ltd (Shanghai, China). The CLR used in our study is a kind of disinfectant effervescent tablet (effective chlorine content 250 mg/per tablet) containing trichloroisocyanuric acid as the main component. Susceptibility of isolates to tigecycline was tested using the E-test method, and minimal inhibitory concentrations (MICs) were interpreted using the tigecycline MIC thresholds defined for Enterobacteriaceae by the US Food and Drug Administration (≤2 μg/mL, sensitive; 4 μg/mL, intermediate; ≥8 μg/mL, resistant). Susceptibility of isolates to other antibiotics was conducted using the disk diffusion method. Carbapenem MIC results were acquired for each *A. baumannii* isolate using the BD PhoenixTM 100 system and the results were confirmed by the disk diffusion method. All procedures and clinical thresholds were consistent with guidelines of the CLSI. Quality control strains during susceptibility testing were *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (National Clinical Testing Center).

Biocide stock solutions of 4000 mg/L CLR, 1024 μg/mL BB, and 4% CHG in deionized water were separately prepared and stored at 4 °C. MICs of disinfectants were determined by the broth microdilution method according to the CLSI (2018) [25]. Since there was no standard breakpoints available for disinfectants against *A. baumannii*, we tested a 2 fold dilutions from 4000 to 1.95 mg/L (CLR), 1024 to 0.5 μg/mL (BB), 4% to 0.0019% (CHG). A standard bacterial concentration of McFarland Standard 0.5 (1.5×10^8 CFU/mL) [26] was prepared, and then was further diluted at 1:100 with LB broth(Sangon Biotech, Shanghai, China). Briefly 100 μL of bacterial suspension was added to the twelve gradient distribution wells with 100 μL CLR or BB or CHG. The 96-well plates were placed in a 37 °C incubator for 18 h and then susceptibility was interpreted. The concentration with no visible growth was defined the MIC. The isolates were considered to be reduced in susceptibility if their MIC was found to be 2-fold higher than that for the *A. baumannii* susceptible strain [27].

PCR of resistance genes

Multiplex PCR was used to detect the presence of several common CHDL-encoding resistance genes in CRAB isolates: $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$, and $bla_{OXA\text{-}143\text{-}like}$. The amplification program consisted of 5 min at 94 °C, followed by 30 cycles of 25 s at 94 °C, 40 s at 52 °C, 50 s at 72 °C and finally 6 min at 72 °C. PCR was also performed against several MBL-encoding resistance genes (bla_{NDM}, bla_{IMP}, bla_{VIM}), transposase gene *tpn513,* insertion sequence ISaba1, as well as genes encoding resistance to quaternary ammonium compounds (*qacA/B*, *qacC/D*, *qacE*, *qacΔE1*, *qacG)*. Amplification conditions were 5 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 40 s at 58 °C, and 40 s at 72 °C, then 10 min at 72 °C. PCR primers are given in Table 3. PCR products were analyzed using 1.8% agarose gel electrophoresis, and PCR products were confirmed by DNA sequencing.

MLST

Multi-locus sequence typing was performed using specific primers and amplification conditions from the *A. baumannii* MLST (Oxford) databases (https://pubmlst.org/abaumannii/). Sequence types (STs) were defined based on nucleotide sequences of seven housekeeping genes (*gltA, gyrB, gdhB, recA, cpn60, gpi* and *rpoD)*. The Sequence types of our isolates were compared to those in the database using eBURST 3 (http://eburst.mlst.net/) in order to identify clonal complexes.

Statistical analysis

The data in the study were non-continuous variables and did not obey the normal distribution. Therefore, differences in the distribution of MIC values towards disinfectants between *qac* genes positive and negative isolates were assessed using Kruskal–Wallis

Table 1
Clinical information about 82 CRAB isolates and their sources.

Patient gender	n (%)	Isolate source	n (%)	Department	n (%)	Age, yr	n (%)
Male	65 (79.26)	Sputum	36 (43.90)	Critical care medicine	32 (39.02)	16–30	5 (6.09)
Female	17 (20.73)	Throat swab	18 (21.95)	RICU	24 (29.26)	31–60	37 (45.12)
		Bronchial lavage fluid	8(9.75)	NICU	23 (28.04)	61–97	40 (48.78)
		Urine	7 (8.53)	CICU	3 (3.65)		
		Alveolar lavage fluid	6(7.31)				
		Blood	3 (3.65)				
		Catheter tip	2 (2.43)				
		Bile	1 (1.21)				
		Cerebrospinal fluid	1 (1.21)				

CRAB, carbapenem-resistant *A. baumannii*; RICU, respiratory intensive care unit; NICU, neurological intensive care unit; CICU, cardiac intensive care unit.

Table 2
Antimicrobial susceptibility profiles of 82 CRAB isolates.

Antibiotic	Antibiotic susceptibility, n (%)			Inhibition zone diameter[a], mm		
	S	I	R	S	I	R
Amikacin	3 (3.65)	0 (0)	79 (96.34)	≥17	15–16	≤14
Gentamicin	1 (1.21)	0 (0)	81 (98.78)	≥15	13–14	≤12
Sulfamethoxazole/trimethoprim	2 (2.43)	0 (0)	80 (97.56)	≥16	11–15	≤10
Cefoperazon/sulbactam	48 (58.53)	14 (17.07)	20 (24.39)	≥21	16–20	≤15
Minocycline	57 (69.51)	19 (23.17)	6 (7.31)	≥16	13–15	≤12
				MIC (μg/mL)		
Tigecycline	78 (95.12)	2 (2.43)	2 (2.43)	≤2	4	≥8

I, intermediate; MIC, minimal inhibitory concentration; R, resistant S, sensitive.
[a] From the Clinical Laboratory Standards Institute (CLSI, 2018).

Table 3
Correlation between *qac* gene and MIC values of disinfectants.

qac genes	N[a]	CLR (mg/L)		BB (μg/mL)		CLX (%)	
		MIC range	MIC50	MIC range	MIC50	MIC range	MIC50
Susceptible	1	250	–	32	–	0.0019	–
qac△*E1*(+)	63	250	250	32–128	64	0.0019–0.0078	0.0039
qac△*E1*(−)	19	250	250	32–64	64	0.0019–0.0078	0.0039
qacE(+)	25	250	250	64–128	64	0.0019–0.0078	0.0078
qacE(−)	57	250	250	32–64	64	0.0019–0.0078	0.0039
U value[*]	–	–		571.5		538.5	
P value[*]	–	–		0.68		0.437	
U value[**]	–	–		471.5		231.0	
P value[**]	–	–		0.001		0.000	

[a] Number of isolates.
[*] Statistics and P value of nonparametric tests for *qac*△*E1* (+) and *qac*△*E1* (−) groups.
[**] Statistics and P values of nonparametric tests for *qacE*(+) and *qacE*(−) groups.

nonparametric test. We used $\alpha = 0.05$ (2 tailed) for all statistical tests. Statistical analysis were conducted using SPSS20.0 software.

Results

A. Baumannii isolates and antimicrobial susceptibility

A total of 82 *A. baumannii* isolates were obtained from 65 male patients and 17 from female patients; mean age of all patients was 59.06 (±18.02) years, ranging from 16 to 97 (Table 1). Most *A. baumannii* isolates were collected from patients older than 60 years. Isolates were collected mostly from the following units at our hospital: Department of Critical Care Medicine (32, 39.02%), respiratory intensive care unit (RICU; 24, 29.26%), neurological intensive care unit (NICU; 23, 28.04%). Isolates came from sputum (36, 43.90%), throat swab (18, 21.95%), bronchial lavage fluid (8, 9.75%), urine (7, 8.53%), alveolar lavage fluid(6, 7.31%), blood (3, 3.65%) and so on (Table 1).

Antimicrobial susceptibility of all isolates is presented in Table 2: all met the clinical definition of multi-drug resistance by showing resistance to more than three classes of antibiotics; [28] all isolates (100%) were resistant to carbapenems (imipenem and meropenem). The isolates varied in their resistance to the following antibiotics: 81 (98.78%) were resistant to gentamicin, 80 (97.56%) to sulfamethoxazole/trimethoprim, 79 (96.34%) to amikacin, 20 (24.39%) to cefoperazone/sulbactam, 6 (7.31%) to minocycline, and 2 (2.43%) to tigecycline. All isolates showed resistance to the remaining antibiotics (Table 2).

Biocide susceptibility

The MIC results of *A. baumannii* susceptible strain for CLR, BB and CHG were 250 mg/L, 32 μg/mL and 0.0019%, respectively. The MIC results of 82 CRAB for CLR were 250 mg/L, MICs for BB ranged from 32 to 128 μg/mL, and MICs for CHG ranged from 0.0019% to 0.0078% (Table 3).

Detection of resistance-associated genes

All isolates contained not only the chromosomal $bla_{OXA\text{-}51}$ gene but also the plasmid-borne $bla_{OXA\text{-}23}$ gene. No isolate contained $bla_{OXA\text{-}24}$, $bla_{OXA\text{-}58}$, or $bla_{OXA\text{-}143}$ genes; the MBL-encoding genes(bla_{NDM}, bla_{IMP}, bla_{VIM}) and the gene *tpn513* were also not detected in our study. All isolates except isolate 19 contained the

Table 4
Primer sequences for detection of resistance genes in *Acinetobacter baumannii*.

Target gene	Primer sequence (5'-3')	Reference
bla_{OXA-51}	F: TAA TGC TTT GAT CGG CCT TG R: TGG ATT GCA CTT CAT CTT GG	Woodford, et al. [55]
bla_{OXA-23}	F: GAT CGG ATT GGA GAA CCA GA R: ATT TCT GAC CGC ATT TCC AT	Woodford, et al. [55]
bla_{OXA-24}	F: GGT TAG TTG GCC CCC TTA AA R: AGT TGA GCG AAA AGG GGA TT	Woodford, et al. [55]
bla_{OXA-58}	F: AAG TAT TGG GGC TTG TGC TG R: CCC CTC TGC GCT CTA CAT AC	Woodford, et al. [55]
$bla_{OXA-143}$	F: TGG CAC TTT CAG CAG TTC CT R: TAA TCT TGA GGG GGC CAA CC	Higgins, et al. [60]
bla_{NDM}	F: GAA TGT CTG GCA GCA CAC TT R: TTG GCC TTG CTG TCC TTG AT	Chen, et al. [46]
bla_{IMP}	F: GGC AGT CGC CCT AAA ACA AA R: TAG TTA CTT GGC TGT GAT GG	Lee, et al. [61]
bla_{VIM}	F: GTT GAT GTC CTT CGG GCG G R: TGC TTG ACA ACT CAT GAA CGG C	Yum, et al. [62]
qacA/B	F: GCA GAA AGT GCA GAG TTC G R: CCA GTC CAA TCA TGC CTG	Babaei, et al. [12]
qacC/D	F: GGC TTT TCA AAA TTT ATA CCA TCC T R: ATG CGA TGT TCC GAA AAT GT	Sidhu, et al. [63]
qacG	F: TCGCCTACGCAGTTTGGT R: AACGCCGCTGATAATGAA	Xiao-min, et al. [53]
qacE	F: GCG AAG TAA TCG CAA CAT CC R: GCC CCA TAC CTA CAA AGC C	Babaei, et al. [12]
qac△E1	F: TAG CGA GGG CTT TAC TAA GC R: ATT CAG AAT GCC GAA CAC CG	Xiao-min, et al. [53]
ISaba1	F: AAT GAT TGG TGA CAA TGA AG R: ATG CAG CGC TTC TTT GCA GG	Xiao-min, et al. [53]
tpn513	F: ATG TCG CTG GCA AGG AAC GC R: GGG TTC GCT GCG AGG ATT GT	Xiao-min, et al. [53]

Table 5
Distribution of resistance genes among the sequence types (STs) determined by multi-locus sequence typing.

MLST[a]	Isolate(s)	Resistance elements present (n)[b]		
		Genes encoding CHDLs	Genes associated with disinfectant resistance	Insertion sequence ISaba1
ST540	Total of 30: 2,3,5,6,9,10,12,17,21,23,26,32,34,36,37,39,40,44,45,46,47,48,56,63,66,69,70,72,75,76	OXA-23 (30) OXA-51(30)	qacE (13) qac△E1 (30)	ISaba1 (30)
ST195	Total of 18: 7,14,19,24,29,33,50,55,57,58,62,64,65,68,73,74,78,80	OXA-23 (18) OXA-51(18)	qacE (2) qac△E1 (5)	ISaba1 (17)
ST208	Total of 15: 11,20,27,30,31,38,41,43,49,59,67,77,79,81,82	OXA-23 (15) OXA-51(15)	qacE (4) qac△E1 (10)	ISaba1 (15)
ST191	Total of 11: 1,4,13,15,22,28,42,52,53,54,71	OXA-23 (11) OXA-51(11)	qacE (5) qac△E1 (11)	ISaba1 (11)
ST369	8,16,25,51	OXA-23 (4) OXA-51(4)	qacE (1) qac△E1 (4)	ISaba1 (4)
ST469	60,61	OXA-23 (2) OXA-51 (2)	qacE (0) qac△E1 (2)	ISaba1 (2)
ST381	18	OXA-23 (1) OXA-51 (1)	qacE (0) qac△E1 (1)	ISaba1 (1)
ST136	35	OXA-23 (1) OXA-51 (1)	qacE (0) qac△E1 (0)	ISaba1 (1)

[a] All STs belong to clonal complex 92.
[b] The n values refer to the number of isolates positive for the resistance element, out of the total number of isolates shown in the "Isolate(s)" column.

ISaba1 insertion sequence, and in all cases it was upstream of the $bla_{OXA-51/23}$ genes (Table 5).

Among the *qac* genes screened by PCR, 63 isolates (76.82%) contained *qac△E1* and 25 (30.48%) contained *qacE*. These two genes confer resistance to quaternary ammonium compounds such as benzalkonium chloride or chlorhexidine. The resistance genes *qacA/B*, *qacC/D* and *qacG* were not detected in any isolates (Table 5).

MLST

The isolates fell into eight STs (Fig. 1). ST540 was the most prevalent type, accounting for 30 isolates (36.58%), followed by ST195 (18, 21.95%), ST208 (15, 18.29%), ST191 (11, 13.41%), ST369 (4, 4.87%), ST469 (2, 2.43%), ST381 (1, 1.21%) and ST136 (1, 1.21%) (Table 5). All eight STs belonged to clonal complex 92, based on eBURST analysis, and the STs generally clustered into international clone II [20,29]. These STs were closely related genetically because they shared five or six of seven housekeeping genes tested, differing only in one gene (*gpi*) or two (*gyrB, gpi*).

Discussion

A major cause of morbidity and mortality rates among critically ill patients is infection acquired while they are in the intensive care unit [30], primarily with multi-drug resistant pathogens such as CRAB. The increasing emergence of CRAB brings considerable challenges to preventing and control infection outbreaks, particularly in large countries such as China. In an effort to build an evidence base to support health care policymaking, the present study investigated the distribution of genetic elements associated with resistance to antibiotics and disinfectants in *A. baumannii* isolates.

During the study period, a total of 82 non-repetitive *A. baumannii* strains was collected and they were all confirmed as CRAB isolates by antimicrobial susceptibility testing. Next study showed that *qacE* and *qac△E1* genes were present in 30.48% and 76.82%, respectively, of CRAB isolates and there was no *A. baumannii* isolates harbored *qacA/B*, *qacC/D*, *qacG* genes. The correlation between *qacA/B*, *qacC/D*, *qacG* genes and reduced sensitivity to biguanides was mainly observed in Gram-positive bacteria, especially in Methicillin-resistant Staphylococcus aureus(MRSA) [31,32]. Sim-

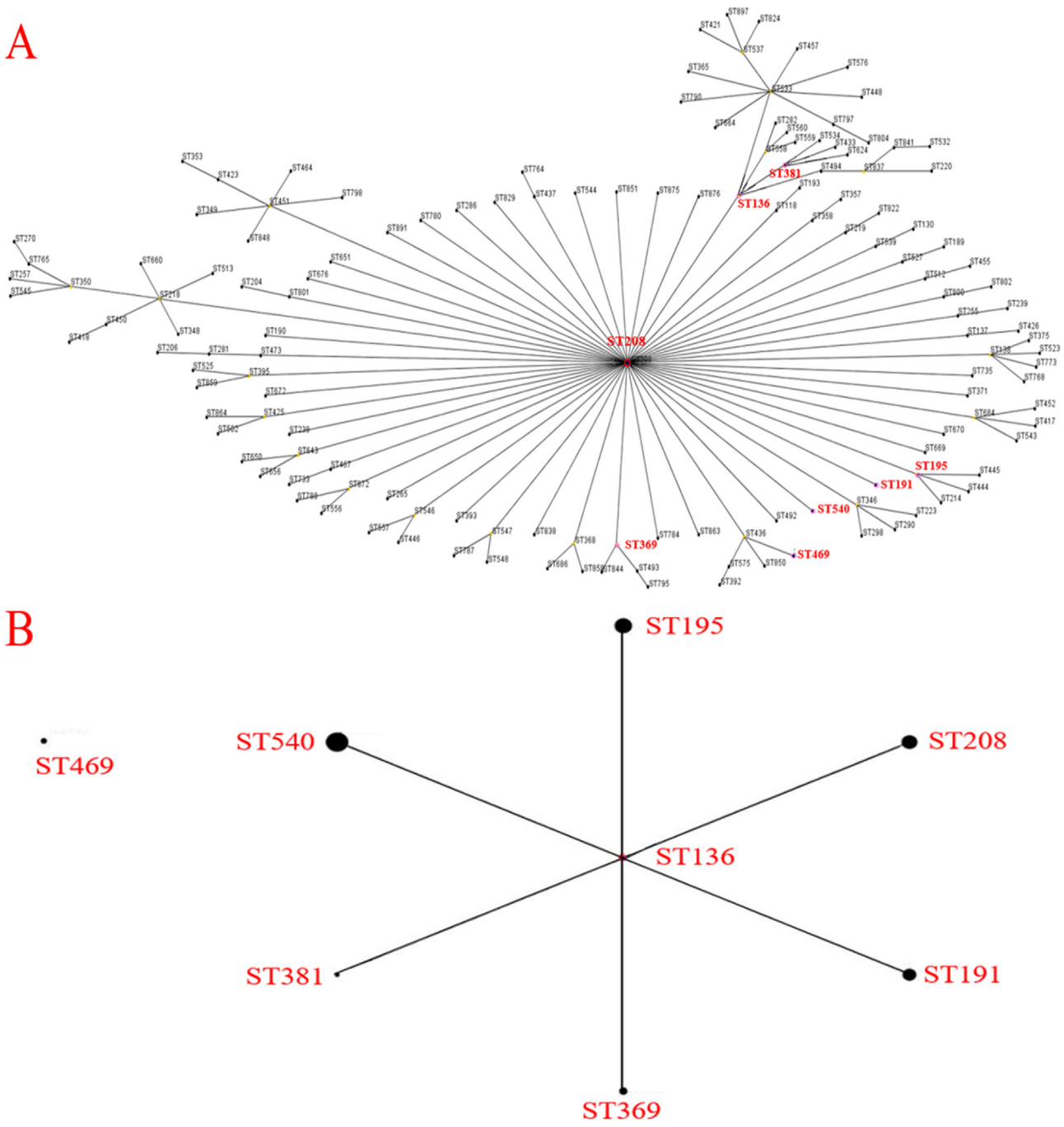

Fig. 1. Analysis of the 82 CRAB isolates using eBURST. (A) Minimum spanning tree analysis based on MLST data. Each dot indicates a specific sequence type (ST), and dot size is proportional to the number of isolates falling within that ST. Relationships among STs are indicated with solid lines. The eight STs shown in red were identified in the present study. (B) Relationships among the eight STs identified in the present study. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ilarly, a study of intensive care unit patients in India found 40% prevalence for *qacE* and 80% prevalence for *qac△E1* among CRAB isolates [33]. Furi's article claimed that the isolates carrying *qacA/B* exhibited a 4-fold increase in MIC to benzalkonium chloride(BC); [34] Wen's study found that there exists a higher MIC to BC for those isolates harboring *qacE* [19]. In our study, the significant difference ($p < 0.05$) was also observed in MIC for *qacE* positive and negative isolates; However, the presence or absence of *qac△E1* genes has no significant impact ($p > 0.05$) on MICs of disinfectants, which was consistent with a study in Malaysia [12].

The formulation with 70% ethanol and 1% CHG is offen used for hygienic or surgical hand wash in our hospital. As the most widely used QACs, BC is extensively used in mouthwash, hand sanitizers, and eye drops. In order to monitor their sensitivity, our findings were as follows: 78.04% (64/82) and 64.63% (53/82) of isolates showed a 2-fold increase in the MIC of the BB and CHG compared to the susceptible strain; 2.4% (2/82) and 29.26% (24/82) of isolates exhibited 4-fold increase for BB and CHG. Obviously, strains with a higher MIC were less susceptible to BB or CHG. Our laboratory did not collect any *A. baumannii* isolates of skin surface origin, so there may be a bias in the results of the disinfectant sensitiv-

ity study. A research from the UK showed there was a significant association between the CHG exposure and the mean MIC [35]. Specifically, the higher the CHG exposure, the greater the mean MIC of the strain. Therefore, we speculated that isolates derived from skin surface may have higher MIC values for BB and CHG, which will be further studied. Fernandez-Cuenca et al. also concluded that reduced susceptibility to disinfectants was associated with co-resistance to carbapenems, aminoglycosides, tetracycline and ciprofloxacin [36]. The MIC values of all isolates to CLR were 250 mg/L, without relationship to *qac* genes. Several studies have demonstrated that the RND-type efflux pump *AdeABC* and the non-RND efflux pump *AmvA* can discharge disinfectants out of the cell in *A. baumannii* [27,37]. 2000 mg/L chlorine disinfectant solution was usually configured by our nursing staff in nonsterile containers with non-sterile water and the concentration was uniformly applied to the cleaning of hospital settings. According to Casini et al. [38], this will greatly increase the probability of environmental microbial contamination. Perhaps, we should refer to the French norm, which recommends classifying the healthcare zones based on the level of risk of infection from the patient's exposure [39]. For different risk levels zones, we should use different cleaning procedures. As shown by Liu et al. different disinfectant concentrations and action times could produce different cleaning effects [40].

All our CRAB isolates showed multi-drug resistance but retained susceptibility to tigecycline and minocycline. We found the $bla_{OXA\text{-}51\text{-}like}$ gene in all clinical isolates, consistent with its status as an "intrinsic gene" of *A. baumannii* [41]. Similarly, all our isolates contained the $bla_{OXA\text{-}23}$ gene, which is regarded as the primary carbapenem resistance gene [42,43]. Although it has also been reported as a popular carbapenemases in many countries [44,45], we did not identify $bla_{OXA\text{-}24\text{-}like}$ gene in our isolates. $bla_{OXA\text{-}58/143\text{-}like}$ genes are known to cause typically carbapenem resistance but the genes are relatively rare in China. Therefore, $bla_{OXA\text{-}58/143\text{-}like}$ gene did not detected in our study. None of our isolates contained the MBL-encoding genes bla_{NDM}, bla_{IMP} or bla_{VIM}. $bla_{NDM\text{-}1}$ has been reported in only a few *A. baumannii* isolates in China, and these genes are also rare among *A. baumannii* isolates in the US [46–48].

The insertion sequence ISaba1 activates the promoters upstream of the $bla_{OXA\text{-}51}$ [49] and $bla_{OXA\text{-}23}$ genes [50], inducing their overexpression. ISaba1 can move horizontally between strains. All but one isolate in our study contained the ISaba1 sequence upstream of the $bla_{OXA\text{-}51}$ or $bla_{OXA\text{-}23}$ gene. Similarly, a study in Pakistan found ISaba1 upstream of $bla_{OXA\text{-}51}$ in 95.6% of isolates and upstream of $bla_{OXA\text{-}23}$ in 87.6% of isolates [51]. *tpn513,* a newly described transposase gene [52], was found in the multidrug-resistant (MDR) *A. baumannii* isolates from Zhejiang province of China [53], but was not detected in any of our isolates.

The 82 isolates belonged to eight STs, which differed from one another at only one or two housekeeping genes. ST540 and ST195 accounted for 58.53% of all CRAB strains, and isolates in ST195 appear to express resistance genes such as *qacE* and *qac△E1* less frequently (Table 4). Interestingly, ST195 contained the only isolate negative for the ISaba1 sequence. Analysis with eBURST showed that all STs in our study belonged to clonal complex 92, which is widely distributed around the world, especially Asia [54]. An outbreak of clonal complex 92 *A. baumannii* encoding $bla_{OXA\text{-}23}$ was recently reported in Shanghai [55].

Our results suggest that the $bla_{OXA\text{-}23/51}$ gene may be the main factor in CRAB prevalence in Hubei Province of China, and that the ISaba1 plays a key supporting role. At the same time, the high prevalence of *qac* genes and decreased susceptibility to BB or CHG confirm the need for continued vigilance against nosocomial infections. At present, only a few applications of CHG were justified by strong scientific evidence. One application is used in antiseptic rinsing agent for the oral cavity in ventilated patients to prevent ventilator-associated pneumonia [56]. Another application combined with alcohols is applied to the disinfection of puncture sites of central venous catheters, which can effectively reduce the incidence of central line-associated bloodstream infections(CLABSIs) [57,58]. GmbH suggested eliminating those applications without a clear or with a doubtful patient benefit, including CHG soaps, hand rubs with CHG [59]. It seems make sense to establish epidemiological cut-off value, which will contribute to identify acquired resistance to an agent and quantify the dimension.

Competing interests

The authors declare no conflict of interest.

Ethical approval

The article does not contain any studies related to human participants or animals.

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