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Molecular tracking of carbapenem-resistant *Acinetobacter baumannii* clinical isolates: a multicentre study over a 4-year period across eastern China

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Abstract

Introduction. Colonization by carbapenem-resistant *Acinetobacter baumannii* (CRAB) causes therapeutic and economic problems for critically ill patients.

Gap Statement. The analysis of CRAB in China was limited to certain regions.

Aims. To investigate the antibiotic susceptibility, molecular characterization and clonal relationship among CRAB isolates from multiple hospitals of eastern China.

Methodology. Isolates from 29 tertiary hospitals from September 2015 to September 2018 were recovered. All strains were analysed using antimicrobial susceptibility testing to detect their tolerance. PCR was also used to detect multiple β-lactamase genes. After multilocus sequence typing (MLST) of seven house-keeping genes. eBURST was used to assess clonal complexes and explore evolutionary relationships.

Results. All isolates showed resistance to carbapenems, while remaining susceptible to colistin and tigecycline. All isolates were detected with $bla_{\text{OXA-51}}$ gene by PCR, and 80.1% harboured the $bla_{\text{OXA-23}}$ gene. The prevalence of $bla_{\text{OXA-23}}$ gene was remarkably increased from 50.7% in 2015 to 90.5% in 2018. Other genes such as $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$, $bla_{\text{IMP-2/4}}$, $bla_{\text{VIM-2}}$, bla_{SHV}, bla_{AmpC} and bla_{TEM} were also obtained. While bla_{KPC}, $bla_{\text{NDM-1}}$, $bla_{\text{IMP-4}}$ and $bla_{\text{SIM-1}}$ were not found in these strains. MLST showed all isolates could be divided into 26 known sequence types (STs) and ten novel STs and 47.2% isolates belong to ST195 and ST208. eBURST revealed clonal complex 92 as the major clonal complex (98.4%), which includes 88.5% (23/26) of known STs and 80% (8/10) of unknown STs. Phylogenetic analysis also found that almost all CRAB isolates could cluster into one lineage, suggesting an epidemic of this CRAB lineage. This indicated severe nosocomial infections of CRAB in multiple hospitals of eastern China.

Conclusion. An outbreak of ST195 and ST208 CRAB-resistant clones with $bla_{\text{OXA-23}}$ gene might be happening in multiple hospitals in eastern China.

INTRODUCTION

Acinetobater baumannii is recognized as a significant opportunistic Gram-negative pathogen, responsible for a broad series of nosocomial infections, including ventilator-associated pneumonia, meningitis, and urinary tract and central nervous system infection [1]. Multidrug-resistant (MDR) *A. baumannii* are common in these infected patients, who often require intensive care and experience higher morbidity and mortality rates [2]. Carbapenems are taken as the frontline treatment for MDR *A. baumannii* infections, due to their excellent antibacterial activities and limited side effects. However, the appearance of carbapenem-resistant

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Keywords: *Acinetobacter baumannii*; Carbapenem-resistant *Acinetobacter baumannii*; clonal complex (CC); multilocus sequence typing.

Abbreviations: CHINET, China Antimicrobial Surveillance Network; CLSI, Clinical and Laboratory Standards Institute; ESBLs, extended-spectrum β-lactamases; ICUs, intensive care units; MBLs, metallo-β-lactamases; MDR, multidrug-resistant; MLST, multilocus sequence typing; PCR, polymerase chain reaction.

A supplementary table is available with the online version of this article.

A. Baumannii (CRAB) currently has become a global concern [3, 4]. For instance, the SENTRY Antimicrobial Surveillance Program, involving seven countries in Latin America, revealed that the number of CRAB infections among hospitalized patients increased from 22.8% in 1997 to 85.6% in 2016 [5]. In 2017, CRAB was listed as one of the most critical pathogens with the highest priority in new antibiotic development by the World Health Organization [6].

CRAB is mediated by several coexisting mechanisms, among which, β-lactamase enzymes remain the most important resistance mechanism [7]. According to sequence motifs and differences in hydrolytic mechanism, there are four different types of β-lactamase enzymes, including the Ambler class A β-lactamase (bla_{GES}, bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER}, bla_{VEB} and bla_{KPC}), Ambler class B or metallo-beta-lactamases (bla_{IMP}, bla_{VIM}, bla_{SIM} and bla_{NDM}), Ambler class C chromosomal AmpC-type β-lactamases (bla_{ADC}), and Ambler class D beta-lactamases or oxicillinases (bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, $bla_{OXA-143}$ and $bla_{OXA-235}$) [8, 9]. Although the population structure of *A. baumannii* strains is quite diverse, there seems to be a clonal spread of a few epidemic lineages that predominate over the rest [10]. Particularly, the clonal complex 92 (CC92, Oxford scheme) and clonal complex 109 (CC109, Oxford scheme), which occupy the largest *A. baumannii* infections worldwide and are frequently accompanied by various kinds of β-lactamase-encoding genes [11].

Nosocomial outbreaks of CRAB have been reported worldwide. Molecular epidemiological research of *A. baumannii* revealed that CC92 plays a pivotal role in nosocomial infection outbreaks [12]. In China, previous studies also reported CC92 as the main clonal complex in many hospitals, which usually carries endemic carbapenemase genes and insertion sequences, such as OXA-23 and Tn2008 [13]. However, the samples of these studies were limited to certain regions. A more detailed analysis using more samples of multiple centres in China is needed. Large-array phylogenomic and phenotypic analysis were currently applied to investigate the genome of *A. baumannii*, providing valuable insights into the adaptation and evolution of *A. Baumannii* [14, 15]. In this study, a total of 443 clinical strains of CRAB from 29 hospitals of eastern China were collected and analysed using large-array phylogenomic and phenotypic methods. We aimed to analyse the phenotypic resistance patterns of CRAB and gain insights into the mechanism of carbapenem resistance. The epidemic spectrum of CRAB in eastern China was drawn.

METHODS

Identification of bacterial isolates and strains

A total of 443 clinical strains of CRAB from 29 tertiary care hospitals of eastern China between September 2015 and September 2018 were collected and analysed in this study. Each of the strains were isolated from different patients, and were identified by API 20NE (BioMérieux, Marcy-l'Étoile, France), and *gyrB* multiplex PCR of bla_{OXA-51} genes [16, 17].

Antimicrobial susceptibility and resistant genes

Antimicrobial susceptibility was tested by the agar dilution method in accordance to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The tested antimicrobials included imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, gatifloxacin, minocycline [18]. Susceptibility of cefoperazone/sulbactam, tigecycline and colistin were also assessed by the broth microdilution method. Because of the absence of breakpoint for sulbactam, this analysis is based on ampicillin/sulbactam breakpoint (defined sulbactam susceptibility $\leq$4 mg l^{-1}, intermediate susceptibility=8 mg l^{-1} and resistance $\geq$16 mg l^{-1}) for *Acinetobacter* spp., then the breakpoints for cefoperazone/sulbactam would be defined as (susceptibility $\leq$16/4 mg l^{-1}, intermediate susceptibility=32/8 mg l^{-1} and resistance $\geq$64/16 mg l^{-1}) [19–21]. Tigecycline non-susceptibility was defined as at least 4 mg l^{-1}, according to the recommendations of the US Food and Drug Administration (FDA) for tigecycline susceptibility breakpoints of Enterobacteriaceae criteria (susceptible $\leq$2 mg l^{-1}, resistant $\geq$ 8 mg l^{-1}) [22, 23]. Colistin resistance was as recommended by CLSI breakpoints [18]. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as controls. All experiments were repeated three times.

Resistant genes were amplified by PCR, including 10 β-lactamases encoding genes (bla_{TEM}, bla_{SHV}, bla_{KPC}, bla_{NDM-1}, bla_{SIM-1}, $bla_{IMP-2,4}$, bla_{VIM-2}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51} and bla_{OXA-58}) and AmpC β-lactamases encoding gene bla_{ADC} [24–29]. Agarose gel electrophoresis was applied to detect the PCR products. The primers of PCR were shown in Table 1.

Molecular typing by MLST and eBURST analysis

House-keeping genes, including *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD* (https://pubmlst.org/primers-used-mlst-acinetobacter-baumannii-complex-oxford-scheme) were also sequenced after PCR. MLST was used to characterize the population structure of *A. baumannii* using the sequenced house-keeping genes [30]. All sequencing data were submitted to the PubMLST database (https://pubmlst.org/abaumannii/).

eBURST (version 3.0) was used to analyse clusters of related STs [31]. A clonal complex includes a founding ST as a common ancestor and several other closely related STs descended from the predicted founding genotype. The default calculation of the bootstrap value (1000 resamples) is used to estimate the confidence of the predicted founder of a CC.

Table 1. The PCR primers of each gene used in this study

Primers	Sequences (5'–3')	Length
bla_{TEM}	F-ATGAGTATTCAACATTTCCGTG	47
	R-TTACCAATGCTTAATCAGTGAG	
bla_{SHV}	F-AAGATCCACTATCGCCAGCAG	47
	R-ATTCAGTTCCGTTTCCCAGCGG	
bla_{KPC}	F-CGTCTAGTTCTGCTGTCTTG	26
	R-CTTGTCATCCTTGTTAGGCG	
AmpC	F-ACAGAGGAGCTAATCATGCG	47
	R-GTTCTTTTAAACCATATACC	
bla_{OXA-23}	F-GAT CGGATTGGAGAACCAGA	27
	R-ATTTCTGACCGCATT TCCAT	
bla_{OXA-24}	F-GGTTAGTTGGCC CCCTTAAA	27
	R-AGTTGAGCGAAAAGGGGATT	
bla_{OXA-51}	F-TAATGC TTTGATCGGCCTTG	27
	R-TGGATTGCACTTCATCTTGG	
bla_{OXA-58}	F-AAGTATTGGGGCTTGTGCTG	27
	R-CCCCTCTGCGCTCTACATAC	
bla_{IMP-4}	F-ATC CAAGCAGCAAGC GCGTTA	45
	R-AGGCGTGCTGCTGCAACGACTTGT	
bla_{IMP-2}	F-CATGGTTTGGTGGTTCTTGT	46
	R-ATAATTTGGCGGACTTTGGC	
bla_{VIM-2}	F-ATGTTCAAACTTTTGAGTAAG	46
	R-CTACTCAACGACTGAGCG	
bla_{SIM-1}	F-TACAAGGGATTCGGCATCG	46
	R-TAATGGCCTGTTCCCATGTG	
bla_{NDM-1}	F-GGTTTGGCGATCTGGTTTTC	48
	R-CGGAATGGCTCATCACGATC	

Phylogenetic analysis

SeqKit v0.80 and DNAstar v7.1.0 were used to analyse the sequence data. The seven house-keeping gene sequences were aligned using MAFFT (version 7) [32]. The phylogenetic tree was inferred using the maximum-likelihood method implemented in iqtree with parameters'-mp –bb 1000' to determine the best-fit model of nucleotide substitution and to bootstrap 1000 times [33]. The output newick file was visualized in iTOL [34].

RESULTS

Bacterial isolates

A total of 443 CRAB isolates were recovered from patients from 29 tertiary referral hospitals, of whom 289 (65.2%) were male and 154 (34.8%) were female. Most patients were elderly, including 220 patients aged from 61 to 97 and 160 aged from 51 to 60. These isolates were collected from samples, including sputum (349), wound secretion (42), blood (15), cerebrospinal fluid (14), urine (12) and drainage fluid (12). The majority of the isolates were from intensive care unit (ICU) ward (213) and surgical ward (115), followed by medical ward (76), cancer ward (32), and paediatric ward (7). The baseline of the patients shown in Table 2.

Table 2. Clinical characteristics of 443 patients with Carbapenem-resistant *Acinetobacter baumannii* (CRAB)

Gender	Total no.	Source	Total no.	Department	Total no.
Male	286 (65.2%)	Sputum	348 (78.5%)	Intensive care unit	213 (48.1%)
Female	154 (34.8%)	Wound secretion	42 (9.5%)	Surgical ward	115 (25.9%)
Age (years)	**Total no.**	Blood	15 (3.4%)	Medical ward	76 (17.2%)
0–20	20 (4.5%)	Cerebrospinal fluid	14 (3.2%)	Cancer ward	32 (7.2%)
21–50	43 (9.7%)	Urine	12 (2.7%)	Paediatric ward	7 (1.6%)
51–60	160 (36.1%)	Drainage fluid	12 (2.7%)		
61–97	220 (49.7%)				

Antimicrobial susceptibility

Antimicrobial susceptibility tests revealed a high rate of antimicrobial resistance to the most antibiotics tested in this study (Table 3). CRAB isolates were then selected for further analysis, showing the resistance rates of imipenem and meropenem as high as 100%. Meanwhile, the rates of susceptibility to minocycline and levofloxacin were 45 and 29.46%, respectively. Tigecycline retained excellent activity against isolates, with a susceptibility rate of 90.47%. More than 99% of the CRAB isolates were highly susceptible to colistin.

Analysis of Carbapenem-resistant genes

In this study, all isolates were detected with $bla_{\text{OXA-51}}$ gene by PCR, 80.1% isolates were found carrying the $bla_{\text{OXA-23}}$ gene. The prevalence of the $bla_{\text{OXA-23}}$ gene in the isolates was remarkably increased from 50.7% in 2015 to 90.5% in 2018. In addition, there were four (0.9%) strains with bla_{OXA24}, six (1.4%) with bla_{OXA58}, nine (2.1%) strains with $bla_{\text{IMP-2}}$, five (1.2%) strains with $bla_{\text{VIM-2}}$, 48 (10.9%) with bla_{SHV} and 410 (92.5%) with bla_{ADC}. Among them, 204 (46.1%) CRAB strains harboured more than four different resistant genes. The detailed detected results of each isolate were shown in Table S1, available with the online version of this article.

MLST

All the isolates fell into 36 STs based on PubMLST database (https://pubmlst.org/abaumannii/), including 26 existing STs and 10 novel STs (Fig. 1). ST195 was the most dominant ST type (31.6%) in this study, followed by ST208 (69), ST540 (59), ST369 (59), ST368 (25), ST191 (18), ST547 (11), ST469 (9), ST1791 (9), ST1451 (7), ST381 (4), ST705 (3), ST136 (2), ST801 (2), ST784 (2), ST1967 (2), ST436 (1), ST620 (1), ST1295 (1), ST75 (1), ST193 (1), ST138 (1), ST218 (1), ST373 (1), ST1779 (1) and ST1472 (1). The novel STs comprised 12 isolates, which belonged to unreported STs: three isolates belonged to N-ST4. All other N-ST types contained only one isolate. The sequence data of this study were submitted to the GenBank database.

Table 3. Susceptibility analyses of 443 *A. baumannii* in this research

Antimicrobial agents	Antibiotic susceptibility (%)			MIC (μg/ml)		
	S	I	R	S	I	R
imipenem	0	0	100	≤2	4	≥8
meropenem	0	0	100	≤2	4	≥8
amikacin	11.24	2.26	86.5	≤16	32	≥64
gentamicin	1.62	2.52	95.86	≤4	8	≥16
ciprofloxacin	2.26	1.13	96.61	≤1	2	≥4
levofloxacin	10.81	18.65	70.54	≤2	4	≥8
gatifloxacin	10.42	3.58	86	≤2	4	≥8
minocycline	39.62	5.38	55	≤4	8	≥16
Cefoperazone/sulbactam	30.16	13.52	56.32	≤16/4	32/8	≥64/16
tigecycline	90.47	–	9.53	≤2	–	≥8
colistin	99.22	–	0.78	≤2	–	≥4

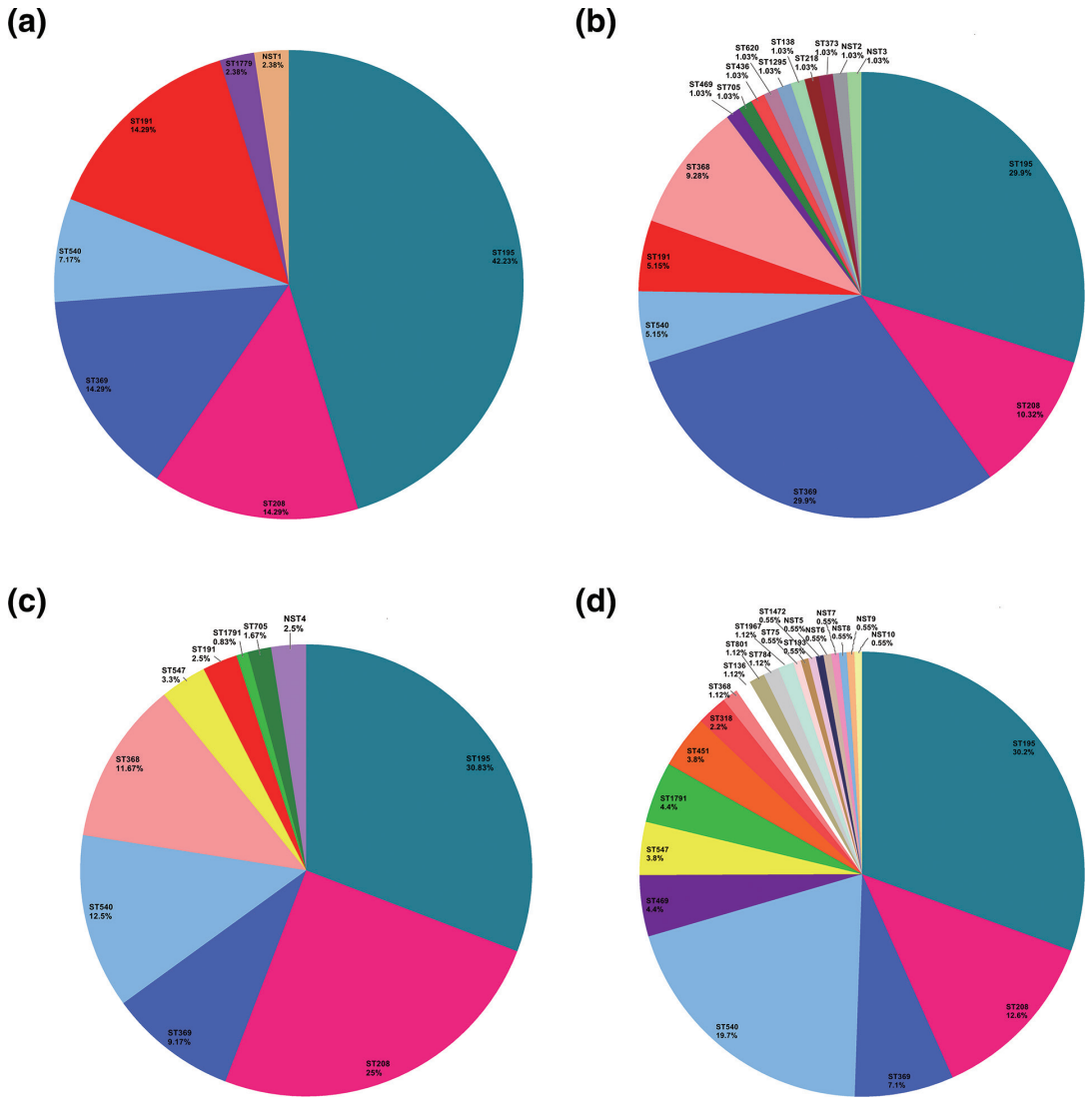

Fig. 1. Distribution of STs in different years: (a) distribution of STs in 2015; (b) distribution of STs in 2016; (c) distribution of STs in 2017; (d) distribution of STs in 2018.

eBURST analysis

The dominance of ST195 and ST208 remained unchanged over the 4-year study period (Fig. 1). However, the proportion dropped from 56.52% in 2015 to 40.22% in 2016, and then increased to 42.8% in 2018. ST369 was isolated from six strains (14.29%) in 2015, while the number increased to 29 strains (29.9%) in 2016, and then fell to 13 strains (7.1%) in 2018. In addition, only three strains (7.14%) of ST540s were isolated in 2015, and the number gradually increased to 36 strains (19.7%) by 2018. The ST populations ranged every year, with some subtypes decreasing or transforming into other subtypes. For example, the ST368 require only a single change to transform into ST1791. The appearance of ST1791 and the disappearance of ST368 in 2018 might indicate a ST transforming between the two STs (Fig. 1a-d).

eBURST was used to divide the MLST data in this study into multiple groups. The evolutionary patterns among the descendants of different STs shown in Fig. 2. In this study, 88.5% (23/26) of known STs and 80% (8/10) of unknown STs were clustered in CC92, which is one of the most common clonal complexes of CRAB in the world.

Phylogenetic analysis

Compared with eBURST, phylogenetic analysis can reveal evolutionary relationships among groups or singletons that eBURST could not find. Phylogenetic analysis of *A. baumannii* showed that all the strains could be classified to seven clusters (Fig. 3 and Table S1). A total of 150 strains (33.9%) were clustered into cluster 1. The STs of this cluster included ST195, ST136, ST381,

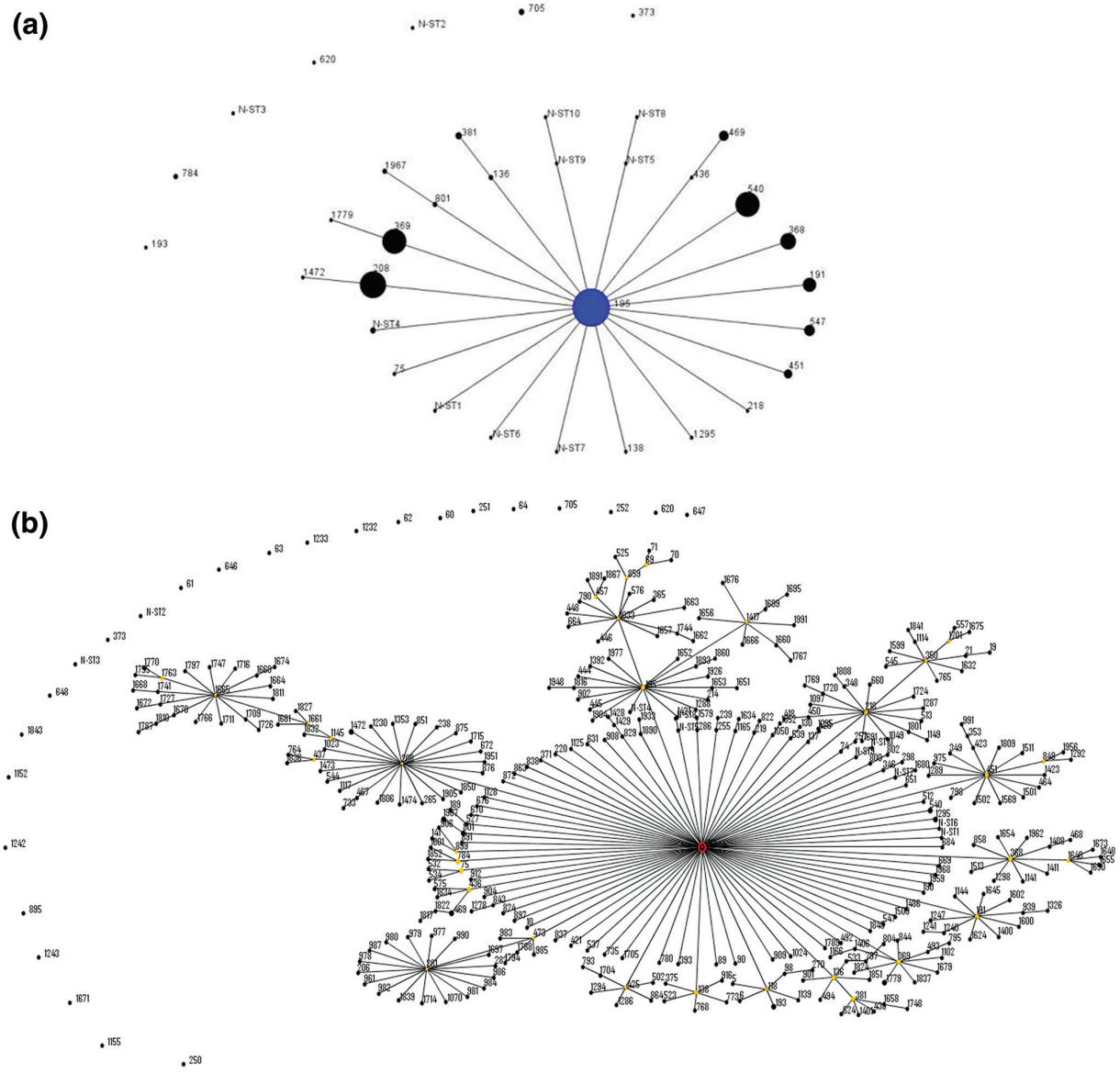

Fig. 2. *A. baumannii* STs analysed by eBURST. (a) Relationships among the 36 STs in this study, each node represents an ST, and the size of a node represents different numbers of isolates. (b) All of the clonal complexes were found in this study. The red circle indicates the original primary founder, and the yellow node indicated the founder of the subgroups extended by the original primary founder. Relationships among STs are indicated with solid lines.

ST138 and N-ST4. A total of 129 strains (29.2%) were clustered into cluster 3. The isolates belonged to six known STs (ST208, ST191, ST368, ST547, ST784 and ST1295) and two novel STs (N-ST1 and N-ST7). There were also 62, 59, 25, 13 and 5 isolates clustered into cluster 2, cluster 7, cluster 5, cluster 6 and cluster 4, respectively. Interesting, the dominant STs (ST195 and ST208) were divided into two different clusters, which were also the main clusters in this study.

DISCUSSION

CRAB is a hazardous nosocomial pathogen causing infection with relatively high morbidity and mortality, which gives rise to a sizeable health threat and economic burden [1, 35–37]. Hospital settings play a vital role as storage points for the transmission of CRAB and may become the propagation source for emerging epidemics under certain circumstances [38]. This study assessed the genetic characterization of hospital-acquired CRAB and the dissemination of CC92 in multiple hospitals across eastern China over a 4-year period.

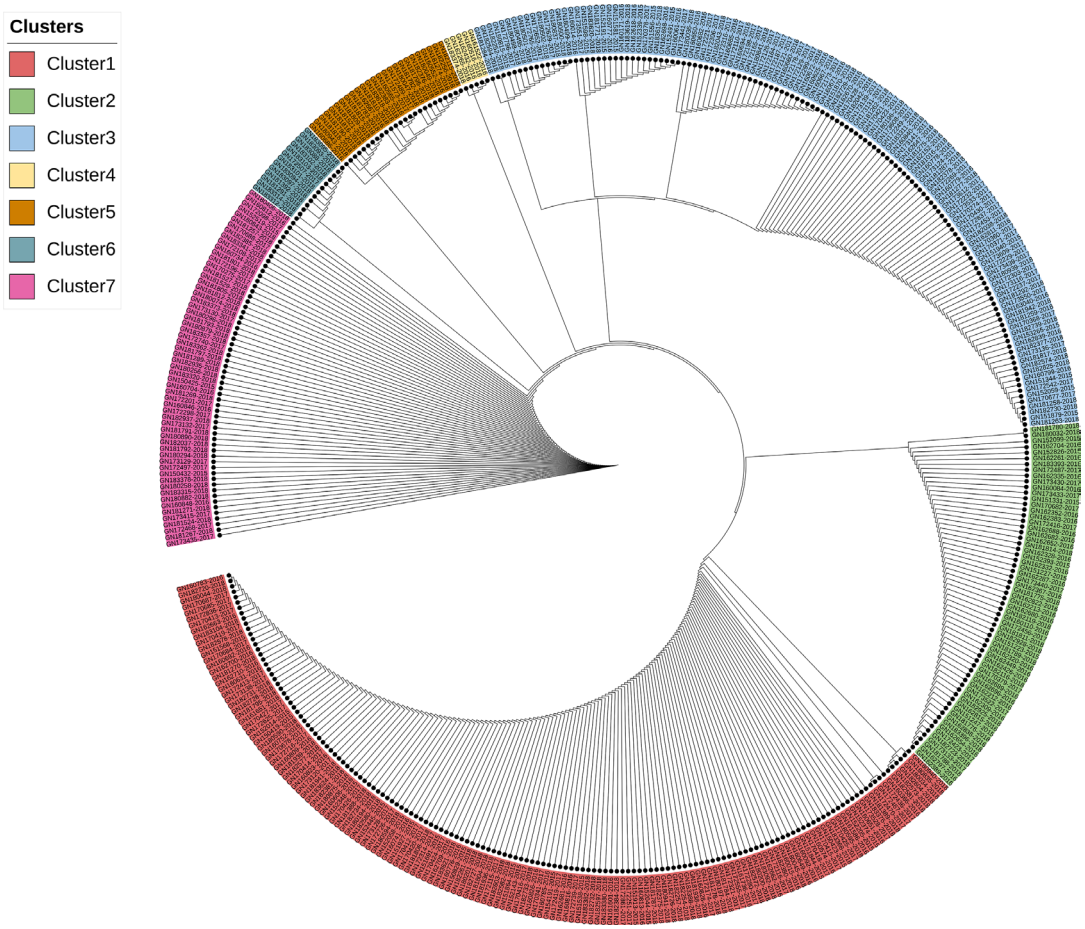

Fig. 3. Phylogenetic tree of *A. baumannii* isolated from clinical strains. All the strains were classified into seven clusters. A total of 150 strains classified into ST195, ST136, ST381, ST138 and N-ST4 were clustered into cluster 1. A total of 129 strains (29.2%) were clustered into cluster 3. The isolates belonged to six known STs (ST208, ST191, ST368, ST547, ST784 and ST1295) and two novel STs (n-st1 and n-st7). There were also 62, 59, 25, 13 and 5 isolates clustered into cluster 2, cluster 7, cluster 5, cluster 6 and cluster 4, respectively.

Inpatients with invasive procedures and long hospital stays are most vulnerable to CRAB infections [39]. This study showed that most of the patients were elderly male patients with low immunity and long hospital stays. In addition, CRAB isolates can cause a wide range of infections, including pulmonary infection, wound infection, bloodstream infection, urinary tract infection and so on. What caught our attention was that almost all patients had suffered multiple diseases at the same time. The most common infections in this study were pulmonary infections, including pneumonia and respiratory failure. This is consistent with previous studies showing that CRAB infection is often associated with pneumonia [40, 41]. With regard to our resistance profiles, all of the isolates were characterized with carbapenem resistance, of which 250 (56.32%) were resistant to cefoperazone/sulbactam, and 244 (55%) were resistant to minocycline (Table 3). Though 90.47% of the research isolates remained susceptible to tigecycline and colistin. The presence of tigecycline and colistin-resistant isolates is of great concern. They are not sensitive to known commercially available drugs, and infections caused by these isolates are tough to treat. These CRAB may further cause considerable infection control issues [42].

Oxacillinases are kind of β-lactamases with potent carbapenemase activity and are frequent in *A. baumannii* isolates [7]. In our study, 80.1% of the strains harboured $bla_{\text{OXA-23}}$, and 100% harboured the $bla_{\textit{OXA-51}}$ gene. This is analogous to previous findings that $bla_{\text{OXA-23}}$ was the most widely distributed gene, while $bla_{\text{OXA-51}}$ was a natural resistant gene [43]. It is worth noting that the $bla_{\text{OXA-23}}$ gene can either be located on the chromosome or on plasmids. Different isolates can get carbapenem resistance through horizontal gene transfer [10]. $bla_{\text{OXA24/58}}$ β-lactamase was first identified on plasmids of *A. baumannii* in the early 2000s, from an outbreak in Europe [44, 45]. Here, we also detected the $bla_{\text{OXA24/58}}$ gene in CRAB from eastern China, indicating its wide spread in the world. Though only 2.3% of our isolates contained $bla_{\text{OXA24/58}}$ genes, the distribution of these genes should be monitored immediately [7, 10]. Compared with class D β-lactamases (CHDLs), metallo-β-lactamases (MBLs) can catalyse the hydrolysis of virtually all β-lactamases (including carbapenems), conferring high levels of carbapenem resistance in *A. baumannii*. Four

groups of these enzymes have been described in *A. baumannii*, including $bla_{\text{IMP-like}}$, $bla_{\text{SIM-1}}$, $bla_{\text{NDM-like}}$ and $bla_{\text{VIM-like}}$ carbapenemase [7, 40]. In this study, we found that only 2.1% of the isolates carried $bla_{\text{VIM-2}}$, and 1.2% of the isolates carried $bla_{\text{IMP-2}}$. No bla_{KPC}, $bla_{\text{IMP-4}}$, $bla_{\text{SIM-1}}$ or $bla_{\text{NDM-1}}$ encoding genes were detected.

bla_{TEM} is an extended-spectrum β-lactamase (ESBL) gene of *A. baumannii* [46]. In this study, bla_{TEM} was detected in 63.6% of CRAB isolates. Of these bla_{TEM} strains, 56.3% were resistant to sulbactam. This was consistent with previous reports that $bla_{\text{TEM-1}}$ as a common narrow-spectrum β-lactamases gene had the ability to resist sulbactam in CRAB strains [47]. We also found 10.9% CRAB isolates of this study carried bla_{SHV}, which is also an extended-spectrum β-lactamase gene, which confirmed with the previous findings. In addition, 92.5% of the isolates also had chromosomally encoded *AmpC*. Previous studies also detected most CRAB isolates (84.0 and 90.2%) carried *AmpC*. It is worth noting that a high proportion of CRAB isolates were identified with more than one ESBL gene in China in this study as well as previous articles, indicating ESBLs, *AmpC,* and some other resistance genes (such as MBLs) might co-mediate the multi-drug resistance of CRAB isolates in China.

There were also some isolates with carbapenem resistance but without related genes detected by PCR. This might be due to the simultaneous presence of other resistance mechanisms. For example, it is possible that they may carry class A/C β-lactamase resistance genes and other resistance mechanism factors, such as outer-membrane protein mutations, increased expression of efflux pumps, and penicillin-binding proteins' modification [7, 10, 41].

There have been numerous reports of CRAB clonal outbreaks, mainly associated with international clonal lineages I–III [48]. In the Pasteur/Oxford MLST scheme, most outbreak strains belong to CC1/CC109 and CC2/CC92, corresponding to international clonal lineages I and II, respectively. CC92 is a widely disseminated variant with advantages in acquiring resistance determinants and survival in the nosocomial environment [49]. So far, more than 398 STs have been found in CC92. In this study, we also found that 98.4% of the 443 isolates from 88.5% (23/26) of known STs and 80% (8/10) of unknown STs were clustered in CC92. As the ancestry and the most common ST of CC92, ST92 has been reported in many countries, including China. However, we did not detect any ST92 in this study. Instead, ST195 and ST208 were the most prevalent STs from multiple hospitals in eastern China, indicating an outbreak of ST195 and ST208 instead of ST92 in eastern China. Of note, our result showed that 80.1% of CRAB carried the $bla_{\text{OXA-23}}$ gene. Particularly ST368, ST208 and ST369 isolates, the content of the bla_{OXA23} gene could reach 100%. We speculate that CC92 strains carrying the OXA-23 gene are the most common strains in eastern China.

Notably, four isolates of ST195 and ST208 were detected carrying the $bla_{\text{OXA-24}}$ gene from ICU patients of three nearby tertiary-care hospitals in 2016, 2017 and 2018. Similarly, we also found the OXA-58 gene in six N-ST and ST195 isolates from ICU patients of one tertiary hospital. OXA-58 gene has been isolated in Greece, Italy, Lebanon, Turkey, Brazil and Vietnam [48]. The appearance of these isolates with high resistance to meropenem and imipenem in eastern China reminded us to prevent their further outbreaks. An instant monitoring is needed.

Previous studies showed CRAB isolates carrying the $bla_{\text{OXA-23}}$ gene from Pakistan and tertiary-care hospitals were dominated by single lineage [48, 50]. Phylogenetic analysis of our study also showed 63.0% of strains clustered into two clusters, indicating the dominance of these isolates. Interesting, the dominance of ST195 and ST208, which fell into these two clusters, also remained unchanged over the 4-year study period. There seems to be a clonal spread of a few epidemic lineages that predominate over the rest. The rest strains (57.0%) clustered into five other clusters. The percentages of strains in each cluster ranged from 1.1–14.0%, indicating heterogeneity. The majority of CRAB isolates belong to a few lineages, while sporadic strains coexist with epidemic ones. This suggested an outbreak of CRAB from a few lineages happened in multiple hospitals in eastern China. Clinicians should pay more attention to the management of patients, especially for patients with prolonged duration of mechanical ventilation support, longer hospitalization or ICU stay, higher exposure to infected or colonized patients in an adjacent hospital setting, greater number of interventions, receipt of transfusion of blood products, greater disease severity as measured by a relevant scoring system and use of broad-spectrum antimicrobial agents.

Conclusions

In this study, we analysed the antibiotic susceptibility and molecular characterization of CRAB isolates from 29 tertiary hospitals across eastern China over a 4-year period. All strains had high resistance phenotypes, CC92 strains of CRAB carrying the $bla_{\text{OXA-23}}$ gene are popular in eastern China. Phylogenetic analysis also found that almost all CRAB isolates could cluster into one lineage, suggesting an outbreak of CRAB from a single lineage in multiple hospitals in eastern China. Also, we should pay attention to the prevalence of other clonal lineage strains carrying β-lactamase genes such as $bla_{\text{OXA-58}}$, $bla_{\text{OXA-24}}$. Implementation of effective measures is highly essential to prevent further spreading of CRAB.

GenBank accession numbers for the new sequence data of *A. baumannii* isolates were shown in the following table:

STs	gltA	gyrB	gdhB	recA	Cpn60	gpi	rpoD
N-ST1	MW625941	MW625942	MW625943	MW625944	MW625945	MW625946	MW625947
N-ST2	MW656398	MW656399	MW656400	MW656401	MW656402	MW656403	MW656404
N-ST3	MW656405	MW656406	MW656407	MW656408	MW656409	MW656410	MW656411
N-ST4	MW656412	MW656413	MW656414	MW656415	MW656416	MW656417	MW656418
N-ST5	MW656419	MW656420	MW656421	MW656422	MW656423	MW656424	MW656425
N-ST6	MW656426	MW656427	MW656428	MW656429	MW656430	MW656431	MW656432
N-ST7	MW656433	MW656434	MW656435	MW656436	MW656437	MW656438	MW656439
N-ST8	MW656440	MW656441	MW656442	MW656443	MW656444	MW656445	MW656446
N-ST9	MW656447	MW656448	MW656449	MW656450	MW656451	MW656452	MW656453
N-ST10	MW656454	MW656455	MW656456	MW656457	MW656458	MW656459	MW656460

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Author contribution
Professor J.B.L. and Y.Y. contribute substance to ideas and design. Y.Z. conceived and coordinated the study, performed and analysed the experiments, wrote the paper. L.Z. and H.Z. carried out the data collection, data analysis. Y.Z. gave a lot of assistance and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of interest
The authors declare that there is no conflict of interest.

Ethical statement
Ethical approval is not applicable in this study. All of the clinical strains were already sampled before the experiment.

References

1. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 2007;5:939–951.
2. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008;21:538–582.
3. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother* 2011;55:4943–4960.
4. Ayobami O, Willrich N, Harder T, Okeke IN, Eckmanns T, *et al*. The incidence and prevalence of hospital-acquired (carbapenem-resistant) *Acinetobacter baumannii* in Europe, Eastern Mediterranean and Africa: a systematic review and meta-analysis. *Emerg Microbes Infect* 2019;8:1747–1759.
5. Gales AC, Seifert H, Gur D, Castanheira M, Jones RN, *et al*. Antimicrobial susceptibility of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex and *Stenotrophomonas maltophilia* clinical isolates: results from the SENTRY antimicrobial surveillance program (1997-2016). *Open Forum Infect Dis* 2019;6:S34–S46.
6. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, *et al*. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 2018;18:318–327.
7. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens* 2021;10:373.
8. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;39:1211–1233.
9. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, *et al*. β-Lactamases and β-Lactamase inhibitors in the 21st century. *J Mol Biol* 2019;431:3472–3500.
10. Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. *Int J Antimicrob Agents* 2015;45:568–585.
11. Karah N, Sundsfjord A, Towner K, Samuelsen Ø. Insights into the global molecular epidemiology of carbapenem non-susceptible clones of *Acinetobacter baumannii*. *Drug Resist Updat* 2012;15:237–247.
12. Ruan Z, Chen Y, Jiang Y, Zhou H, Zhou Z, *et al*. Wide distribution of CC92 carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* in multiple provinces of China. *Int J Antimicrob Agents* 2013;42:322–328.
13. Chen Y, Gao J, Zhang H, Ying C. Spread of the blaOXA–23-containing Tn2008 in carbapenem-resistant *Acinetobacter baumannii* isolates grouped in CC92 from China. *Front Microbiol* 2017;8.
14. Hwang SM, Cho HW, Kim TY, Park JS, Jung J, *et al*. Whole-genome sequencing for investigating a health care-associated outbreak of carbapenem-resistant *Acinetobacter baumannii*. *Diagnostics* 2021;11:201.
15. Makke G, Bitar I, Salloum T, Panossian B, Alousi S, *et al*. Whole-genome-sequence-based characterization of extensively drug-resistant *Acinetobacter baumannii* hospital outbreak. *mSphere* 2020;5:e00934-19.
16. Lee MJ, Jang SJ, Li XM, Park G, Kook J-K, *et al*. Comparison of rpoB gene sequencing, 16S rRNA gene sequencing, gyrB multiplex PCR,

and the VITEK2 system for identification of Acinetobacter clinical isolates. *Diagn Microbiol Infect Dis* 2014;78:29–34.

17. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, *et al*. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006;44:2974–2976.
18. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. Wayne, PA, 2021.
19. Ku YH, Yu WL. Cefoperazone/sulbactam: new composites against multiresistant gram negative bacteria? *Infect Genet Evol* 2021;88:104707.
20. Jones RN, Barry AL, Packer RR, Gregory WW, Thornsberry C. In vitro antimicrobial spectrum, occurrence of synergy, and recommendations for dilution susceptibility testing concentrations of the cefoperazone-sulbactam combination. *J Clin Microbiol* 1987;25:1725–1729.
21. Yang Q, Xu Y, Jia P, Zhu Y, Zhang J, *et al*. In vitro activity of sulbactam/durlobactam against clinical isolates of *Acinetobacter baumannii* collected in China. *J Antimicrob Chemother* 2020;75:1833–1839.
22. Sader HS, Castanheira M, Farrell DJ, Flamm RK, Mendes RE, *et al*. Tigecycline antimicrobial activity tested against clinical bacteria from Latin American medical centres: results from SENTRY Antimicrobial Surveillance Program (2011-2014). *Int J Antimicrob Agents* 2016;48:144–150.
23. Liu J-W, Ko W-C, Huang C-H, Liao C-H, Lu C-T, *et al*. Agreement assessment of tigecycline susceptibilities determined by the disk diffusion and broth microdilution methods among commonly encountered resistant bacterial isolates: results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) study, 2008 to 2010. *Antimicrob Agents Chemother* 2012;56:1414–1417.
24. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;70:119–123.
25. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, *et al*. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006;27:351–353.
26. Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, *et al*. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. *Antimicrob Agents Chemother* 2001;45:710–714.
27. Lee K, Yum JH, Yong D, Lee HM, Kim HD, *et al*. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob Agents Chemother* 2005;49:4485–4491.
28. Rezaee MA, Pajand O, Nahaei MR, Mahdian R, Aghazadeh M, *et al*. Prevalence of Ambler class A β-lactamases and ampC expression in cephalosporin-resistant isolates of *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis* 2013;76:330–334.
29. Bonnin RA, Nordmann P, Poirel L. Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art. *Expert Rev Anti Infect Ther* 2013;11:571–583.
30. Gaiarsa S, Batisti Biffignandi G, Esposito EP, Castelli M, Jolley KA, *et al*. Comparative analysis of the two *Acinetobacter baumannii* Multilocus Sequence Typing (MLST) schemes. *Front Microbiol* 2019;10:930.
31. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol* 2004;186:1518–1530.
32. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772–780.
33. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 2015;32:268–274.
34. Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* 2016;44:W242–5.
35. Zhen X, Lundborg CS, Sun X, Hu X, Dong H. The clinical and economic impact of antibiotic resistance in China: a systematic review and meta-analysis. *Antibiotics* 2019;8:115.
36. Zhen X, Chen Y, Hu X, Dong P, Gu S, *et al*. The difference in medical costs between carbapenem-resistant *Acinetobacter baumannii* and non-resistant groups: a case study from a hospital in Zhejiang province, China. *Eur J Clin Microbiol Infect Dis* 2017;36:1989–1994.
37. Zhen X, Stålsby Lundborg C, Sun X, Gu S, Dong H. Clinical and economic burden of carbapenem-resistant infection or colonization caused by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*: a multicenter study in China. *Antibiotics* 2020;9:514.
38. Opazo-Capurro A, San Martín I, Quezada-Aguiluz M, Morales-León F, Domínguez-Yévenes M, *et al*. Evolutionary dynamics of carbapenem-resistant *Acinetobacter baumannii* circulating in Chilean hospitals. *Infect Genet Evol* 2019;73:93–97.
39. Vázquez-López R, Solano-Gálvez SG, Juárez Vignon-Whaley JJ, Abello Vaamonde JA, Padró Alonzo LA, *et al*. *Acinetobacter baumannii* resistance: a real challenge for clinicians. *Antibiotics* 2020;9:205.
40. Luna CM, Aruj PK. Nosocomial *Acinetobacter pneumonia*. *Respirology* 2007;12:787–791.
41. Almasaudi SB. *Acinetobacter* spp. as nosocomial pathogens: epidemiology and resistance features. *Saudi J Biol Sci* 2018;25:586–596.
42. Mavroidi A, Likousi S, Palla E, Katsiari M, Roussou Z, *et al*. Molecular identification of tigecycline- and colistin-resistant carbapenemase-producing *Acinetobacter baumannii* from a Greek hospital from 2011 to 2013. *J Med Microbiol* 2015;64:993–997.
43. Zowawi HM, Sartor AL, Sidjabat HE, Balkhy HH, Walsh TR, *et al*. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* isolates in the Gulf Cooperation Council States: dominance of OXA-23-type producers. *J Clin Microbiol* 2015;53:896–903.
44. Bou G, Oliver A, Martínez-Beltrán J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrob Agents Chemother* 2000;44:1556–1561.
45. Di Popolo A, Giannouli M, Triassi M, Brisse S, Zarrilli R. Molecular epidemiological investigation of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries with a multilocus sequence typing scheme. *Clin Microbiol Infect* 2011;17:197–201.
46. Bonnin RA, Nordmann P, Poirel L. Screening and deciphering antibiotic resistance in *Acinetobacter baumannii*: a state of the art. *Expert Rev Anti Infect Ther* 2013;11:571–583.
47. Krizova L, Poirel L, Nordmann P, Nemec A. TEM-1 β-lactamase as a source of resistance to sulbactam in clinical strains of *Acinetobacter baumannii*. *J Antimicrob Chemother* 2013;68:2786–2791.
48. Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. *Microb Genom* 2019;5:e00 .
49. Ruan Z, Chen Y, Jiang Y, Zhou H, Zhou Z, *et al*. Wide distribution of CC92 carbapenem-resistant and OXA-23-producing *Acinetobacter baumannii* in multiple provinces of China. *Int J Antimicrob Agents* 2013;42:322–328.
50. Zarrilli R, Bagattini M, Migliaccio A, Esposito EP, Triassi M. Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in Italy. *Ann Ig* 2021;33:401–409.