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Analysis of the Class 1 Integrons, Carbapenemase Genes and Biofilm Formation Genes Occurrence in *Acinetobacter baumannii* Clinical Isolates

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Abstract

Acinetobacter baumannii is a non-fermentative Gram-negative bacterium that can cause nosocomial infections in critically ill patients. Carbapenem-resistant *A. baumannii* (CRAB) has spread rapidly in clinical settings and has become a key concern. The main objective of this study was to identify the distribution of integrons and biofilm-formation-related virulence genes in CRAB isolates. A total of 269 *A. baumannii* isolates (219 isolates of CRAB and 50 isolates of carbapenem-sensitive *A. baumannii* (CSAB)) were collected. Carbapenemase genes (bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $bla_{OXA\text{-}23\text{-}like}$) and biofilm-formation-related virulence genes (*abal*, *bfms*, *bap*, and *cusE*) were screened with PCR. Class 1 integron was screened with PCR, and common promoters and gene cassette arrays were determined with restriction pattern analysis combined with primer walking sequencing. Whole-genome sequencing was conducted, and data were analyzed for a $bla_{OXA\text{-}23\text{-}like}$-negative isolate. All 219 CRAB isolates were negative for bla_{KPC}, bla_{VIM}, bla_{IMP}, and bla_{NDM}, while $bla_{OXA\text{-}23\text{-}like}$ was detected in 218 isolates. The detection rates for *abal*, *bfms*, *bap*, and *cusE* in 219 CRAB were 93.15%, 63.93%, 88.13%, and 77.63%, respectively. Class 1 integron was detected in 75 CRAB (34.25%) and in 3 CSAB. The single gene cassette array *aacA4-catB8-aadA1* with relatively strong PcH2 promoter was detected in class 1 integrons. The $bla_{OXA\text{-}23\text{-}like}$-negative CRAB isolate was revealed to be a new sequence type (Oxford 3272, Pasteur 2520) carrying $bla_{OXA\text{-}72}$, $bla_{OXA\text{-}259}$, and $bla_{ADC\text{-}26}$. In conclusion, $bla_{OXA\text{-}23\text{-}like}$ was the main reason for CRAB's resistance to carbapenems. A new (Oxford 3272, Pasteur 2520) CRAB sequence type carrying the $bla_{OXA\text{-}72}$, $bla_{OXA\text{-}259}$, and $bla_{ADC\text{-}26}$ was reported.

K e y w o r d s: integron, virulence gene, carbapenem, antimicrobial resistance, *Acinetobacter baumannii*

Introduction

Acinetobacter species are known as the primary causes of many severe infections and illnesses in recent years (Migliaccio et al. 2023). *Acinetobacter baumannii* has a high capacity for survival and causes outbreaks and long-lasting epidemics in hospitals. The difficulty with clinical treating these infections is increased by the growing number of strains resistant to many drugs because of their pan-drug and all-drug resistance (Lee et al. 2017). Human health is at risk because *A. baumannii* strains resistant to carbapenem are widespread (Harding et al. 2018). *A. baumannii* can build biofilms on non-living and biologic surfaces (Greene et al. 2016). Biofilm formation can promote the emergence of new and more antimicrobial-resistant phenotypes (Khoshnood et al. 2023). In a multicenter cohort study, all catheter-related urinary or bloodstream infections due to *A. baumannii* were caused by biofilm-forming strains, highlighting the unique role that biofilm formation plays in the development of infections related to medical devices (Rodríguez-Baño et al. 2008).

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A. baumannii cells in biofilms have the ability to go dormant and become metabolically inactive in unfavorable environmental conditions, allowing them to withstand environmental stress (Greene et al. 2016). Therefore, forming biofilms makes it easier for *A. baumannii* to adhere to medical implants and biological surfaces, facilitating colonization and infection. In addition, integrons also play a crucial role in the spread of bacterial drug resistance. Site-specific recombination allows integrons to express and capture external drug-resistance gene cassettes, a vital process in developing and disseminating drug resistance in bacteria. By identifying the presence of integrons and drug-resistance gene cassettes in multi-drug resistant strains, we can comprehend the associated drug-resistance mechanisms and their patterns of spread, as well as offer recommendations for clinical anti-infection treatments and nosocomial infection prevention (Gillings 2014). Understanding carbapenem-resistant *A. baumannii* (CRAB), including its epidemiology, distribution of integron, virulence factors, and carbapenem resistance mechanisms, is essential to preventing the continuing development of resistance to antibiotics in this species. Therefore, the primary purpose of this study was to identify the distribution of biofilm-formation-related virulence genes and integrons in CRAB isolates and their relationship with drug resistance.

Experimental

Materials and Methods

Bacterial isolates and susceptibility testing. Two hundred and sixty-nine non-repetitive clinical *A. baumannii* isolates, including 219 CRAB and 50 carbapenem-sensitive *A. baumannii* (CSAB), were collected at Anhui University of Science and Technology Affiliated Fengxian Hospital, Shanghai, from January 2022 to December 2022. MALDI-TOF Mass Spectrometry Microflex® LT/SH (Bruker Daltonics GmbH & Co., Germany) was used to identify each isolate. The VITEK® 2 Compact Automatic Microbial Analyzer (bioMérieux, France) and VITEK® 2 AST-N335 were used to test antibiotic susceptibilities (including imipenem, meropenem, ticacillin/clavulanate, ceftazidime, ciprofloxacin, tobramycin, doxycycline, trimethoprim/sulfamethoxazole, levofloxacin, amikacin, cefepime, minocycline, tigecycline, and colistin). The susceptibility data were interpreted in accordance with the 2021 standards of the Clinical and Laboratory Standard Institute (CLSI) (CLSI 2021). *Escherichia coli* ATCC® 25922™ and *Pseudomonas aeruginosa* ATCC® 27853™ were used as quality control strains. *Proteus mirabilis* 47437 (positive control in screening for class 1 integron integrase gene) and *E. coli* DH5α were preserved in our laboratory (Wei et al. 2014).

Detection of carbapenemase and biofilm formation genes. Following the supplier's instructions, total genomic DNA was isolated using the Ezup Column Bacterial Genomic DNA Extraction Kit (Sangon Biotech (Shanghai) Co., Ltd., China). Common carbapenemase genes, including bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $bla_{OXA\text{-}23\text{-}like}$, and biofilm formation genes *abal*, *bfms*, *bap*, and *cusE* were screened with PCR. Primers used in this study were listed in Table I, and PCR conditions were the same as those described in corresponding references.

Detection of class 1 integrons. Primers intF and P2R (Table I) were used in PCR screening for the class 1 integron integrase gene (*intI1*). Each round included a blank control (distilled water), a negative control (*E. coli* DH5α), and a positive control (*P. mirabilis* 47437).

Identification of the variable regions of class 1 integrons. Using TaKaRa LA Taq® DNA Polymerase (Takara Bio Inc., Japan) and primers intF or 5CS and 3CS (Table I), variable regions of class 1 integrons in *intI1*-positive isolates were amplified. Same-sized amplicons were compared by restriction analysis with Hinf I (Takara Bio Inc., Japan). Primer walking was used to sequence at least one representative of each type of amplicon, starting with the primers intF, 3CS, and 5CS. Sequence comparisons were carried out using BLAST programs (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Gene cassette arrays in variable regions, and common promoters Pc and P2, were identified as previously described (Wei et al. 2013).

Whole-genome sequencing of the selected isolate. The whole-genome sequencing of the $bla_{OXA\text{-}23\text{-}like}$-negative isolate CRAB-134 was conducted by Novogene Bioinformatics Technology Co., Ltd. (China). A total of 0.2 μg DNA per sample was used as input material for the DNA library preparations. Briefly, each genomic DNA sample was fragmented by sonication to a size of about 350 bp. After that, DNA fragments were amplified by PCR once more after being end-polished, A-tailed, and ligated with the full-length adaptor for Illumina® sequencing. Following PCR product purification using the AMPure® XP system (Beckman Coulter, Inc., USA), library quality was assessed on the Agilent 5400 system (Agilent Technologies, Inc., USA) and quantified by qPCR (1.5 nM). The qualified libraries were sequenced on Illumina® platforms with the NovaSeq™ 6000 sequencer (Illumina, Inc., USA) by Novogene Co., Ltd. (UK), according to effective library concentration and data amount required. *De novo* assembly was completed using the Unicycler v0.5.0 (https://github.com/rrwick/Unicycler) assembler (Wick et al. 2017). Sequence analysis was performed using several bioin-

Table I
Primers used in this study.

Primer	Primer sequence (5'–3')	Target gene	References
intF	CCAAGCTCTCGGGTAACATC	*intI1*	Wang et al. 2023
P2R	CCCGAGGCATAGACTGTA		
5CS	GGCATCCAAGCAGCAAG	variable region	Wang et al. 2023
3CS	AAGCAGACTTGACCTGA		
AACF	ATCTCATATCGTCGAGTGG	*aac(6')-Ib*	Wang et al. 2023
AACR	TGCGTGTTCGCTCGAATGC		
AADAF	GCAGCGCAATGACATTCTTG	*aadA1*	Wang et al. 2023
AADAR	ATCCTTCGGCGCGATTTTG		
CATF	TTACTCTGGCTACTATCAC	*catB8*	Wang et al. 2023
CATR	TGATGGCATAAGGCTCTAC		
KPC-F	CGTCTAGTTCTGCTGTCTTG	bla_{KPC}	Wang et al. 2023
KPC-R	CTTGTCATCCTTGTTAGGCG		
VIM-F	GATGGTGTTTGGTCGCATA	bla_{VIM}	Wang et al.2023
VIM-R	CGAATGCGCAGCACCAG		
NDM-F	GGTTTGGCGATCTGGTTTTC	bla_{NDM}	Wang et al. 2023
NDM-R	CGGAATGGCTCATCACGATC		
OXA-23-like-F	CCGTCGTTTACGACATTCA	$bla_{OXA-23-like}$	Wang et al.2023
OXA-23-like-R	AAAGAGCGCATTGCTTTGAT		
IMP-F	GGAATAGAGTGGCTTAAYTCTC	bla_{IMP}	Wang et al.2023
IMP-R	GGTTTAAYAAAACAACCACC		
bfmS-F	ATATATGCGGGGCTGGTAATTC	*bfms*	Cai et al. 2017
bfmS-R	ATGCAGGTGCTTTTTTATTGGT		
cusE-F	ATGCATGTTCTCTGGACTGATGTTGAC	*cusE*	Cai et al. 2017
cusE-R	CGACTTGTACCGTGACCGTATCTTGATAAG		
bap-F	CGTTTCCTGGGTCTGATGTATT	*bap*	Cai et al. 2017
bap-R	GTTATTGAAGGCTTCTTTAGTG		
abal-F	GTGGCTCAAGACAGAGAATC	*abal*	Wang and Ye 2021
abal-R	TCAATCATCATTGGTGGACC		This study

formatics tools. The isolate AB-134 was reconfirmed via in silico methods using SpeciesFinder v2.0 (https://cge.cbs.dtu.dk/services/SpeciesFinder) in the Center for Genomic Epidemiology (CGE) (Larsen et al. 2014). Drug resistance genes were screened using ResFinder v4.4.2 (https://cge.cbs.dtu.dk/services/ResFinder) with default software settings (thresholds for ID% were 90%, minimum length of 60%). The flanking sequences of $bla_{ADC-259}$ were analyzed using BLAST programs (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).

Multilocus sequence typing (MLST). The whole genome of the isolate was submitted to PubMLST (http://pubmlst.org/abaumannii), and the housekeeping genes *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, *rpo*, and *cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB* of *A. baumannii* were matched and compared via two schemes (Oxford and Pasteur, respectively) to obtain the sequence type (ST) of the isolate.

Statistical analysis. SPSS statistical software 26.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Student's test and Fisher's exact tests were carried out for data analysis. $p < 0.05$ in all experiments was considered statistically significant.

Results

Clinical distribution and drug resistance of CRAB and CSAB isolates. Two hundred and nineteen isolates of CRAB were mainly collected from the ICU (Intensive Care Unit, 72/219), NICU (Neurosurgery Intensive Care Unit, 54/219), and EICU (Emergency Intensive Care Unit, 50/219). A total of 176 (80.37%) CRAB isolates were collected from these three wards. While only 44.00% (22/50) of CSAB isolates were from ICU (8/50), EICU (8/50), and NICU (6/50) (Fig. 1). Both CRAB and CSAB were mainly isolated from sputum or throat swabs (CRAB 86.76%, CSAB 88.00%) (Fig. 2).

In addition to resistance to the carbapenem antibiotics imipenem and meropenem, 219 isolates of CRAB

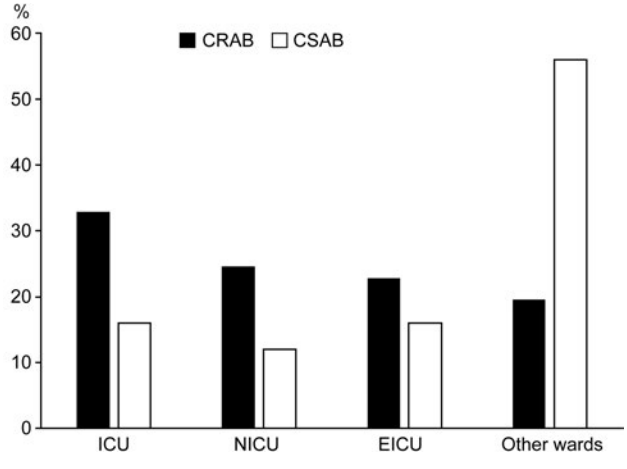


Fig. 1. Clinical ward distribution of CRAB and CSAB isolates.

also had a drug resistance rate of 100% to ticacillin/clavulanate. The drug resistance rates to ceftazidime (99.54%), ciprofloxacin (99.09%), doxycycline (98.63%), and tobramycin (94.06%) were over 90%. CRAB isolates resistant to tigacycline (5.02%) and colistin (1.37%) also appeared in this study. The resistance rates of 50 CSAB isolates to these commonly used antibiotics were only 10% to 16%, and the resistance rate to minocycline was 4%. All these CSAB isolates were sensitive to tigacycline and colistin (Table II).

Presence of carbapenem resistance and biofilm formation genes. The *abal*, *bfms*, *bap*, and *cusE* detection rates were 93.15%, 63.93%, 88.13%, and 77.63%, respectively, in the 219 CRAB isolates. The $bla_{\text{OXA-23-like}}$ gene was detected in 218 of 219 CRAB isolates (99.54%). All the 219 CRAB isolates were negative for bla_{KPC}, bla_{VIM}, bla_{IMP}, and bla_{NDM}. No carbapenem resistance genes were detected in 50 CSAB isolates, and the detection rates of *abal*, *bfms*, *bap*, and *cusE* were 38%, 62%, 10%, and 48%, respectively. Among them, the rates of *abal*-, *bap*-, *cusE*-, and $bla_{\text{OXA-23-like}}$-positivity in CRAB were higher than those in CSAB, with statistical differences ($p < 0.05$) (Table III).

Table II
Resistance rates of CRAB and CSAB isolates to commonly used antibiotics.

Antibiotics	CRAB (n = 219)		CSAB (n = 50)		*p*
	No.	Rate (%)	No.	Rate (%)	
CAZ	218	99.54	6	12.00	0.000*
CIP	217	99.09	6	12.00	0.000*
TOB	206	94.06	6	12.00	0.000*
DOX	216	98.63	6	12.00	0.000*
SXT	166	75.80	8	16.00	0.000*
LVX	175	79.91	5	10.00	0.000*
AMK	131	59.82	6	12.00	1.000
FEP	169	77.17	6	12.00	0.384
MNO	78	35.62	2	4.00	0.000*
TGC	11	5.02	0	0.00	0.226
COL	3	1.37	0	0.00	1.000

CAZ – ceftazidime, CIP – ciprofloxacin, TOB – tobramycin, DOX – doxycycline, SXT – trimethoprim/sulfamethoxazole, LVX – levofloxacin, AMK – amikacin, FEP – cefepime, MNO minocycline, TGC – tigecycline, COL – colistin, CRAB – carbapenem-resistant *Acinetobacter baumannii*, CSAB – carbapenem-sensitive *A. baumannii*
n – the number of strains in the group
* – $p < 0.05$

In CRAB, the drug resistance rate of *bfmS*-negative isolates to trimethoprim/sulfamethoxazole was higher than that of *bfmS*-positive isolates ($p < 0.05$). In contrast, the drug resistance rates of *cusE*-positive isolates to levofloxacin and cefepime were higher than those of negative isolates ($p < 0.05$) (Table IV). The resistance rates of *bap*-positive isolates to tobramycin were higher than those of *bap*-negative isolates ($p < 0.05$) (Table IV).

Integron and variable region analysis. There were 75 *intI1*-positive isolates among the 219 CRAB isolates, while only three *intI1*-positive isolates were detected among the 50 CSAB isolates. Seventy-five variable regions were successfully amplified from 78 *intI1*-positive isolates, while we failed to amplify the variable

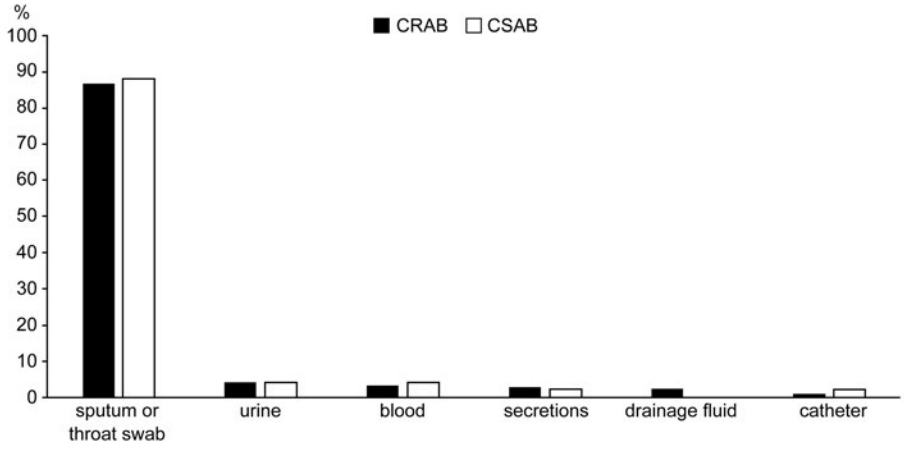


Fig. 2. Clinical specimen type distribution of CRAB and CSAB isolates.

Table III
Virulence- and resistance-gene-carrying status of CRAB and CSAB.

Genes		CRAB (n = 219)	CSAB (n = 50)	*p*
abal	+	204	19	0*
	–	15	31	
bfmS	+	140	31	0.798
	–	79	19	
bap	+	193	5	0*
	–	26	45	
cusE	+	170	24	0*
	–	49	26	
$bla_{OXA\text{-}23\text{-}like}$	+	218	0	0*
	–	1	50	

CRAB – carbapenem-resistant *Acinetobacter baumannii*, CSAB – carbapenem-sensitive *A. baumannii*
n – the number of strains in the group
* – $p < 0.05$

region in 3 *intI1*-positive CRAB isolates. All amplified fragments of the variable region had the same restriction enzyme digestion pattern. The gene cassette array of the variable region was *aacA4-catB8-aadA1*, which had more than 99% homology to the sequences of fragment corresponding to GenBank accession number CP050904. This gene cassette array can confer resistance to chloramphenicol and aminoglycoside. The Pc promoters of variable regions were all relatively strong (PcH2).

Correlation between class 1 integron and antibiotic resistant rates in CRAB. The resistance rates of the 219 CRAB isolates to commonly used antibiotic agents are shown in Table V. Compared with the *intI1*-negative CRAB isolates, the *intI1*-positive CRAB isolates had significantly increased tobramycin-, trimethoprim/sulfamethoxazole-, amikacin-, and minocycline-resistant rates; however, the resistance rate of the *intI1*-positive isolates to cefepime was lower than that of the *intI1*-negative isolates, and the difference was statistically significant ($p < 0.05$), as shown in Table V. As to the correlation between class 1 integron and biofilm formation genes, the positive rates of *intI1*-positive CRAB isolates for *abal* (100%) and *bap* (96%) were significantly higher than those of *intI1*-negative CRAB isolates ($p < 0.05$) (Table VI).

Analysis of whole-genome sequencing of AB-134. The isolate AB-134 was reconfirmed by in silico methods using SpeciesFinder v2.0 in CGE. The drug-resistance genes $bla_{OXA\text{-}72}$, $bla_{OXA\text{-}259,}$ and $bla_{ADC\text{-}26}$ were also found in this isolate through ResFinder v4.4.2. In the MLST, we searched for the ST of AB-134 in the public database of PubMLST, and the results showed unknown sequence types. The data were uploaded to PubMLST and new STs ST3272 (Oxford) and ST2520 (Pasteur) were obtained. The types of different housekeeping genes of AB-134 are shown in Table VII. The whole-genome sequence of AB-134 was submitted to GenBank with accession No. JAZGUW000000000. Using BLAST programs, the flanking sequences (5 kbp upstream and 5 kbp downstream) of $bla_{OXA\text{-}259}$ were found to be the same as those flanking $bla_{OXA\text{-}98}$ in *A. baumannii* ATCC19606 (GenBank accession No. AP025740), with the FxsA family protein coding gene and GNAT family N-acetyltransferase coding gene flanking $bla_{OXA\text{-}259}$. The $bla_{OXA\text{-}72}$-containing assembly

Table IV
Correlations between virulence genes and antibiotic resistance in 219 CRAB isolates.

Antibiotics	*abal*			*bfmS*			*cusE*			*bap*		
	+ (n = 204)	– (n = 15)	*p*	+ (n = 140)	– (n = 79)	*p*	+ (n = 170)	– (n = 49)	*p*	+ (n = 193)	– (n = 26)	*p*
MNO	72	6	0.782	47	31	0.4	59	19	0.6	68	10	0.747
DOX	201	15	1	139	77	0.296	168	48	0.534	191	25	0.317
SXT	154	12	1	98	68	0.008*	124	42	0.066	145	21	0.528
CIP	202	15	1	139	78	1	168	49	1	192	25	0.224
LVX	163	12	1	109	66	0.313	141	34	0.037*	152	23	0.246
TOB	191	15	0.607	129	77	0.142	161	45	0.494	185	21	0.011*
AMK	125	6	0.105	79	52	0.173	96	35	0.06	119	12	0.13
TGC	11	0	1	9	2	0.335	9	2	1	11	0	0.369
CAZ	203	15	1	139	79	1	169	49	1	192	26	1
FEP	156	13	0.74	112	57	0.184	141	28	0*	148	21	0.641
COL	3	0	1	2	1	1	2	1	0.534	2	1	0.317

CAZ – ceftazidime, CIP – ciprofloxacin, TOB – tobramycin, DOX – doxycycline, SXT – trimethoprim/sulfamethoxazole, LVX – levofloxacin, AMK – amikacin, FEP – cefepime, MNO minocycline, TGC – tigecycline, COL – colistin, CRAB – carbapenem-resistant *Acinetobacter baumannii*
n – the number of strains in the group
* – $p < 0.05$

Table V
Correlation between class 1 integron and antibiotic resistant rates in 219 CRAB isolates.

Antibiotics	*intI1*(+) (n = 75)		*intI1*(–) (n = 144)		*p*
	No.	Rate (%)	No.	Rate (%)	
CAZ	75	100	143	99.31	1.000
CIP	75	100	142	98.61	0.548
TOB	75	100	132	91.67	0.009*
DOX	74	98.67	142	98.61	1.000
SXT	73	97.33	93	64.58	0.000*
LVX	62	82.67	113	78.47	0.462
AMK	58	77.33	78	54.17	0.001*
FEP	44	58.67	125	86.81	0.000*
MNO	36	48	42	29.17	0.006*
TGC	1	1.33	10	6.94	0.103
COL	1	1.33	2	1.39	1.000

CAZ – ceftazidime, CIP – ciprofloxacin, TOB – tobramycin, DOX – doxycycline, SXT – trimethoprim/sulfamethoxazole, LVX – levofloxacin, AMK – amikacin, FEP – cefepime, MNO minocycline, TGC – tigecycline, COL – colistin
CRAB – carbapenem-resistant *Acinetobacter baumannii*
n – the number of strains in the group
* – $p < 0.05$

Table VI
Correlation between class 1 integron and virulence and resistance genes in 219 CRAB isolates.

Genes		*intI1*(+) (n = 75)	*intI1*(–) (n = 144)	*p*
abal	+	75	129	0.003*
	–	0	15	
bfmS	+	53	87	0.134
	–	22	57	
bap	+	72	121	0.009*
	–	3	23	
cusE	+	53	116	0.088
	–	22	28	
$bla_{\text{OXA-23-like}}$	+	75	143	1.000
	–	0	1	

CRAB – carbapenem-resistant *Acinetobacter baumannii*
n – the number of strains in the group
* – $p < 0.05$

contig was very short and did not have any meaningful information, and no further analysis was conducted on the flanking sequences of $bla_{\text{OXA-72}}$.

Discussion

CRAB is a significant cause of nosocomial infections that are associated with high mortality rates. It is commonly spread in ICUs (Chukamnerd et al. 2022). This is consistent with the report in this study. CRAB was mostly isolated from intensive care wards, while CSAB was widely distributed in different wards. The reason for this may be that most patients in intensive care wards have primary diseases, severe diseases with many complications, long hospitalization times, long-term indwelling catheters, and have been subjected to invasive operations for examination or treatment. In addition, the long-term use of broad-spectrum antibiotics increases the chance of infection. Sputum or throat swabs were the primary sources of the isolates, which is consistent with previous reports in our hospital (Xiao et al. 2019). It may be because *A. baumannii* is mainly related to respiratory infections, especially ventilator-associated pneumonia (Huang et al. 2019).

The mechanisms of drug resistance in *A. baumannii* include β-lactamases, aminoglycoside-modifying enzymes, permeability defects, the alteration and replacement of antibiotic target sites, enzymatic inactivation, and multidrug efflux pumps (Ibrahim et al. 2021). In this study, the resistance rates of 219 CRAB isolates to levofloxacin, cefepime, trimethoprim/sulfamethoxazole, amikacin, and minocycline were 79.91%, 77.17%, 75.80%, 59.82%, and 35.62%, respectively, while the resistance rates to other commonly used antibiotics were over 90%. Some isolates were resistant to tigecycline (5.02%) and colistin (1.37%). However, 50 CSAB isolates were sensitive to tigecycline and colistin, and the resistance rates to other commonly used antibiotics were only between 4% and 16%, indicating that the CRAB isolated in our hospital had severe drug resistance.

Bacteria can also spread drug-resistance genes between different isolates through horizontal gene transfer, spreading bacterial resistance. This gene transfer occurs through gene-moving elements such as plasmids or transposons. A large-scale survey in Colombia (Villegas et al. 2007) found that 33.6% of *A. baumannii* was resistant to carbapenems, and 98.5% of resistant isolates carried the $bla_{\text{OXA-23}}$ group. IMP and VIM enzymes have specific detection rates in the Far East and Europe and are related to the carbapenem resistance of *A. baumannii* (El-Ageery and Al-Hazmi 2014;

Table VII
Results of multilocus sequence typing (MLST).

Oxford	ST	*gltA*	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*
	3272	1	12	56	1	1	385	26
Pasteur	ST	*cpn60*	*fusA*	*gltA*	*pyrG*	*recA*	*rplB*	*rpoB*
	2520	3	2	2	2	3	4	95

Ibrahim 2019; Nikibakhsh et al. 2021). In 2013, 13 (38.2%) of 34 CRAB isolates from a hospital in Huaibei (a city in Anhui province, China) produced the KPC-2 carbapenem enzyme (Zhang et al. 2015). The detection rate for bla_{NDM-1} in CRAB from Algeria was 10.6% (Khorsi et al. 2015). It is worth noting that bla_{IMP}, bla_{VIM}, $bla_{KPC,}$ and bla_{NDM} were not detected in this study. Most of the 219 CRAB isolates in this study carried $bla_{OXA-23-like}$ (99.54%), which is consistent with previous reports, and carbapenem-resistant *A. baumannii* in our country mainly carry $bla_{OXA-23-like}$ (Xiao et al. 2019).

In this study, whole-genome sequencing analysis of a $bla_{OXA-23-like}$-negative isolate indicated that this isolate carried bla_{OXA-72}, $bla_{OXA-259}$, and bla_{ADC-26} at the same time, which could confer β-lactam resistance. bla_{OXA-72} is one of the most important allelic variants of $bla_{OXA-24/40-like}$ (Lasarte-Monterrubio et al. 2022). However, $bla_{OXA-259}$ is a member of the bla_{OXA-51} family and has only been reported in one hospital in Hangzhou, China (Jia et al. 2019). *Acinetobacter*-derived cephalosporinases (ADC) are cephalosporinases produced by *Acinetobacter*; bla_{ADC-26} is a variant of ADC and was first discovered in France (Rodríguez-Martínez et al. 2010). In this study, the resistance phenotype of AB-134 provided resistance not only to carbapenems but also to ceftazidime, and the detection of bla_{ADC-26} indicated that this gene might mediate ceftazidime resistance. In addition, MLST typing of this isolate resulted in the discovery of new STs, ST3272 (Oxford) and ST2526 (Pasteur).

One of the most important virulence factors of *A. baumannii* is its formation of biofilms, which facilitate the bacterial adherence to medical equipment and colonization of the surface of human skin for long-term survival (Dolma et al. 2022). Studies have confirmed that *abal*, *cusE*, *bap,* and *bfmS* genes are all related to the formation of biofilms. *CsuE* is a key gene for the fimbriae synthesis in *A. baumannii* (Pakharukova et al. 2018). The expression of fimbrial synthesis genes is regulated by the *bfmRS* two-component regulatory system (Roy et al. 2022). In addition, the quorum sensing system also governs the formation of bacterial biofilms, and the quorum sensing signaling-molecule-encoding gene *abal* plays a role in forming bacterial biofilms on abiotic surfaces (Sun et al. 2021). Studies have shown that mutations of the *bap* gene mainly affect the volume and thickness of the biofilms formed by *A. baumannii* (Azizi et al. 2016). In this study, the detection rates of the virulence genes *abal*, *bap,* and *cusE* in CRAB were higher than those of CSAB, with a statistical difference ($p < 0.05$). In CRAB, the resistance rates of *cusE*-positive isolates to levofloxacin and cefepime were higher than those of *cusE*-negative CRAB isolates ($p < 0.05$). The resistance rates of *bap*-positive isolates to tobramycin were higher than those of *bap*-negative isolates ($p < 0.05$). When bacteria exist in the form of a biofilm, drug resistance is significantly enhanced (10–1,000 times); antibiotics cannot effectively remove the bacterial biofilm and can also induce drug resistance (Aliramezani et al. 2016), thus aggravating clinical infection and increasing the difficulty of treatment. While the resistance rates of *bfmS*-negative isolates to trimethoprim/sulfamethoxazole were higher than those of *bfmS*-positive isolates ($p < 0.05$), this may have been due to *bfmS* negatively regulating antimicrobial resistance in *A. baumannii* (Kim et al. 2019).

Integrons can capture and express resistance gene cassettes through site-specific recombination and play important roles in the production and spread of drug resistance genes (Lee et al. 2017). In this study, we screened for *intI1* in 269 *A. baumannii* isolates. Among them, 75 isolates of CRAB and three isolates of CSAB were positive for *intI1*, consistent with our previous reports (Xiao et al. 2019). The amplified 75 variable regions in 78 *intI1*-positive isolates were all *aacA4-catB8-aadA1*, which can confer resistance to chloramphenicol and aminoglycoside. It can explain, in part, how the drug resistance rates of the *intI1*-positive isolates to tobramycin and amikacin were higher than those of the *intI1*-negative isolates ($p < 0.05$). Apart from tobramycin and amikacin, the resistance rates of *intI1*-positive CRAB isolates to trimethoprim/sulfamethoxazole and minocycline were also higher than those of *intI1*-negative CRAB. The *sul1-qacEΔ1* regions in the 3' conserved segment of the class 1 integron may partly explain the higher resistance rates of *intI1*-positive CRAB isolates to trimethoprim/sulfamethoxazole. In addition, the class 1 integron was often embedded in transposons or located in drug-resistance plasmids, in which many other drug-resistance genes were also carried. This can partly explain the relatively higher resistance rates of *intI1*-positive isolates to other antibiotics that cannot be conferred by antibiotic-resistance gene cassettes in variable regions of class 1 integrons.

In this study, the Pc promoters of the variable regions in 78 *intI1*-positive isolates, such as PcH2, were all relatively strong, and the expression levels of downstream gene cassettes were relatively high, which can confer a higher level of drug resistance to bacteria (Wei et al. 2011; Barraud and Ploy 2015). Meanwhile, the efficiency of exogenous gene cassette integration into the *attI1* site downstream of strong Pc promoters was also lower (Wei et al. 2011). The integrase protein corresponding to a stronger Pc (PcS or PcH2) has a weaker ability to capture exogenous gene cassettes to maintain the relative stability of integrons (Barraud and Ploy 2015). This may be one of the reasons for the identity of gene cassette array *aacA4-catB8-aadA1* found in this study.

Because the Pc of class 1 integrons is opposite to the promoter of integrase, there is mutual interference, and studies have shown that the stronger Pc of class 1

integrons can affect the expression of integrase (Lacotte et al. 2017). The SOS stress response system regulates the expression of intI1, and binding the LexA protein to the adjacent sites of the −10 region of the *intI1* promoter P_{int} can inhibit the transcription of *intI1*. Studies have shown that *A. baumannii* lacks LexA-driven integrase repression but restricts the expression of *intI1* through the selective retention of the strong Pc to prevent the toxic effects caused by the massive expression of integrase protein (Couvé-Deacon et al. 2019). This may explain why the Pc of the class 1 integron in this study's clinical isolates of *A. baumannii* was relatively strong.

Conclusions

In summary, $bla_{OXA-23-like}$ may be the main reason for CRAB's resistance to carbapenem. A new (Oxford 3272, Pasteur 2520) CRAB isolate ST carrying the bla_{OXA-72}, $bla_{OXA-259}$, and bla_{ADC-26} was discovered.

Availability of data and material

The data used to support this study are available from the corresponding author upon request.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Aliramezani A, Douraghi M, Hajihasani A, Mohammadzadeh M, Rahbar M. Clonal relatedness and biofilm formation of OXA-23--producing carbapenem resistant *Acinetobacter baumannii* isolates from hospital environment. Microb Pathog. 2016 Oct;99:204–208. https://doi.org/10.1016/j.micpath.2016.08.034

Azizi O, Shahcheraghi F, Salimizand H, Modarresi F, Shakibaie MR, Mansouri Sh, Ramazanzadeh R, Badmasti F, Nikbin V. Molecular analysis and expression of *bap* gene in biofilm-forming multi-drug-resistant *Acinetobacter baumannii*. Rep Biochem Mol Biol. 2016 Oct; 5(1):62–72.

Barraud O, Ploy MC. Diversity of class 1 integron gene cassette rearrangements selected under antibiotic pressure. J Bacteriol. 2015 Jul;197(13):2171–2178. https://doi.org/10.1128/JB.02455-14

Cai X, Hu Z, Li J, Li Y, Tian B. [Clinical isolates of *Acinetobacter baumannii* from bloodstream infections: PFGE characterization and virulence-associated factors] (in Chinese). Chin J Lab Med. 2017 Mar;40(3):201–205.

Chukamnerd A, Singkhamanan K, Chongsuvivatwong V, Palittapongarnpim P, Doi Y, Pomwised R, Sakunrang C, Jeenkeawpiam K, Yingkajorn M, Chusri S, et al. Whole-genome analysis of carbapenem-resistant *Acinetobacter baumannii* from clinical isolates in Southern Thailand. Comput Struct Biotechnol J. 2022 Jan;20:545–558. https://doi.org/10.1016/j.csbj.2021.12.038

CLSI. Performance standards for antimicrobial susceptibility testing. 31st ed. CLSI supplement M100. Wayne (USA): Clinical and Laboratory Standards Institute; 2021.

Couvé-Deacon E, Jové T, Afouda P, Barraud O, Tilloy V, Scaon E, Hervé B, Burucoa C, Kempf M, Marcos JY, et al. Class 1 integrons in *Acinetobacter baumannii*: A weak expression of gene cassettes to counterbalance the lack of LexA-driven integrase repression. Int J Antimicrob Agents. 2019 Apr;53(4):491–499. https://doi.org/10.1016/j.ijantimicag.2018.11.012

Dolma KG, Khati R, Paul AK, Rahmatullah M, de Lourdes Pereira M, Wilairatana P, Khandelwal B, Gupta C, Gautam D, et al. Virulence characteristics and emerging therapies for biofilm-forming *Acinetobacter baumannii*: A review. Biology. 2022 Sep; 11(9):1343. https://doi.org/10.3390/biology11091343

El-Ageery SM, Al-Hazmi SS. Microbiological and molecular detection of VIM-1 metallo beta lactamase-producing *Acinetobacter baumannii*. Eur Rev Med Pharmacol Sci. 2014;18(7):965–970.

Gillings MR. Integrons: Past, present, and future. Microbiol Mol Biol Rev. 2014 Jun;78(2):257–277. https://doi.org/10.1128/MMBR.00056-13

Greene C, Vadlamudi G, Newton D, Foxman B, Xi C. The influence of biofilm formation and multidrug resistance on environmental survival of clinical and environmental isolates of *Acinetobacter baumannii*. Am J Infect Control. 2016 May;44(5):e65–e71. https://doi.org/10.1016/j.ajic.2015.12.012

Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. Nat Rev Microbiol. 2018 Feb;16(2):91–102. https://doi.org/10.1038/nrmicro.2017.148

Huang Y, Zhou Q, Wang W, Huang Q, Liao J, Li J, Long L, Ju T, Zhang Q, Wang H, et al. *Acinetobacter baumannii* ventilator-associated pneumonia: Clinical efficacy of combined antimicrobial therapy and *in vitro* drug sensitivity test results. Front Pharmacol. 2019 Feb; 10:92. https://doi.org/10.3389/fphar.2019.00092

Ibrahim ME. Prevalence of *Acinetobacter baumannii* in Saudi Arabia: Risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance. Ann Clin Microbiol Antimicrob. 2019 Jan; 18(1):1. http://doi.org/10.1186/s12941-018-0301-x

Ibrahim S, Al-Saryi N, Al-Kadmy IMS, Aziz SN. Multidrug-resistant *Acinetobacter baumannii* as an emerging concern in hospitals. Mol Biol Rep. 2021 Oct;48(10):6987–6998. https://doi.org/10.1007/s11033-021-06690-6

Jia H, Sun Q, Ruan Z, Xie X. Characterization of a small plasmid carrying the carbapenem resistance gene bla_{OXA-72} from community-acquired *Acinetobacter baumannii* sequence type 880 in China. Infect Drug Resist. 2019 Jun;12:1545–1553. https://doi.org/10.2147/IDR.S202803

Khorsi K, Messai Y, Hamidi M, Ammari H, Bakour R. High prevalence of multidrug-resistance in *Acinetobacter baumannii* and dissemination of carbapenemase-encoding genes $bla_{OXA-23-like}$, $bla_{OXA-24-like}$ and bla_{NDM-1} in Algiers hospitals. Asian Pac J Trop Med. 2015 Jun;8(6):438–446. https://doi.org/10.1016/j.apjtm.2015.05.011

Khoshnood S, Sadeghifard N, Mahdian N, Heidary M, Mahdian S, Mohammadi M, Maleki A, Haddadi MH. Antimicrobial resistance and biofilm formation capacity among *Acinetobacter baumannii*

strains isolated from patients with burns and ventilator-associated pneumonia. J Clin Lab Anal. 2023 Jan;37(1):e24814. https://doi.org/10.1002/jcla.24814

Kim SY, Kim MH, Kim SI, Son JH, Kim S, Lee YC, Shin M, Oh MH, Lee JC. The sensor kinase BfmS controls production of outer membrane vesicles in *Acinetobacter baumannii*. BMC Microbiol. 2019 Dec;19(1):301. https://doi.org/10.1186/s12866-019-1679-0

Lacotte Y, Ploy MC, Raherison S. Class 1 integrons are low-cost structures in *Escherichia coli*. ISME J. 2017 Jul;11(7):1535–1544. https://doi.org/10.1038/ismej.2017.38

Larsen MV, Cosentino S, Lukjancenko O, Saputra D, Rasmussen S, Hasman H, Sicheritz-Pontén T, Aarestrup FM, Ussery DW, Lund O. Benchmarking of methods for genomic taxonomy. J Clin Microbiol. 2014 May;52(5):1529–1539. https://doi.org/10.1128/JCM.02981-13

Lasarte-Monterrubio C, Guijarro-Sánchez P, Bellés A, Vázquez-Ucha JC, Arca-Suárez J, Fernández-Lozano C, Bou G, Beceiro A; Spanish National Study Acinetobacter spp. 2020 Group. Carbapenem resistance in *Acinetobacter nosocomialis* and *Acinetobacter junii* conferred by acquisition of $bla_{OXA-24/40}$ and genetic characterization of the transmission mechanism between *Acinetobacter* genomic species. Microbiol Spectr. 2022 Feb;10(1):e0273421. https://doi.org/10.1128/spectrum.02734-21

Lee CR, Lee JH, Park M, Park KS, Bae IK, Kim YB, Cha CJ, Jeong BC, Lee SH. Biology of *Acinetobacter baumannii*: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol. 2017 Mar;7:55. https://doi.org/10.3389/fcimb.2017.00055

Migliaccio A, Bray J, Intoccia M, Stabile M, Scala G, Jolley KA, Brisse S, Zarrilli R. Phylogenomics of *Acinetobacter* species and analysis of antimicrobial resistance genes. Front Microbiol. 2023 Oct;14:1264030. https://doi.org/10.3389/fmicb.2023.1264030

Nikibakhsh M, Firoozeh F, Badmasti F, Kabir K, Zibaei M. Molecular study of metallo-β-lactamases and integrons in *Acinetobacter baumannii* isolates from burn patients. BMC Infect Dis. 2021 Aug;21(1):782. https://doi.org/10.1186/s12879-021-06513-w

Pakharukova N, Tuittila M, Paavilainen S, Malmi H, Parilova O, Teneberg S, Knight SD, Zavialov AV. Structural basis for *Acinetobacter baumannii* biofilm formation. Proc Natl Acad Sci USA. 2018 May;115(21):5558–5563. https://doi.org/10.1073/pnas.1800961115

Rodríguez-Baño J, Martí S, Soto S, Fernández-Cuenca F, Cisneros JM, Pachón J, Pascual A, Martínez-Martínez L, McQueary C, Actis LA, et al; Spanish Group for the Study of Nosocomial Infections (GEIH). Biofilm formation in *Acinetobacter baumannii*: Associated features and clinical implications. Clin Microbiol Infect. 2008 Mar;14(3):276–278. https://doi.org/10.1111/j.1469-0691.2007.01916.x

Rodríguez-Martínez JM, Poirel L, Nordmann P. Genetic and functional variability of AmpC-type β-lactamases from *Acinetobacter baumannii*. Antimicrob Agents Chemother. 2010 Nov;54(11):930–4933. https://doi.org/10.1128/AAC.00427-10

Roy S, Chowdhury G, Mukhopadhyay AK, Dutta S, Basu S. Convergence of biofilm formation and antibiotic resistance in *Acinetobacter baumannii* infection. Front Med. 2022 Mar;9:793615. https://doi.org/10.3389/fmed.2022.793615

Sun X, Ni Z, Tang J, Ding Y, Wang X, Li F. The *abaI*/*abaR* quorum sensing system effects on pathogenicity in *Acinetobacter baumannii*. Front Microbiol. 2021 Jul;12:679241. https://doi.org/10.3389/fmicb.2021.679241

Villegas MV, Kattan JN, Correa A, Lolans K, Guzman AM, Woodford N, Livermore D, Quinn JP. Dissemination of *Acinetobacter baumannii* clones with OXA-23 carbapenemase in Colombian hospitals. Antimicrob Agents Chemother. 2007 Jun;51(6):2001–2004. https://doi.org/10.1128/AAC.00226-07

Wang T, Zhu Y, Zhu W, Cao M, Wei Q. Molecular characterization of class 1 integrons in carbapenem-resistant *Enterobacterales* isolates. Microb Pathog. 2023 Apr;177:106051. https://doi.org/10.1016/j.micpath.2023.106051

Wang X, Ye Y. [The correlation analysis of the resistance and virulence genes of penicillium carbon alkene resistant *Acinetobacter baumannii*] (in Chinese). Chin J Antibio. 2021 Dec;46(7):699–705.

Wei Q, Hu Q, Li S, Lu H, Chen G, Shen B, Zhang P, Zhou Y. A novel functional class 2 integron in clinical *Proteus mirabili*s isolates. J Antimicrob Chemother. 2014 Apr;69(4):973–976. https://doi.org/10.1093/jac/dkt456

Wei Q, Jiang X, Li M, Chen X, Li G, Li R, Lu Y. Transcription of integron-harboured gene cassette impacts integration efficiency in class 1 integron. Mol Microbiol. 2011 Jun;80(5):1326–1336. https://doi.org/10.1111/j.1365-2958.2011.07648.x

Wei Q, Jiang X, Li M, Li G, Hu Q, Lu H, Chen G, Zhou Y, Lu Y. Diversity of gene cassette promoter variants of class 1 integrons in uropathogenic *Escherichia coli*. Curr Microbiol. 2013 Nov;67(5):543–549. https://doi.org/10.1007/s00284-013-0399-1

Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017 Jun;13(6):e1005595. https://doi.org/10.1371/journal.pcbi.1005595

Xiao C, Cao M, Zhang L, Kong N, Wang X, Xiao L, Wei Q. [Distribution of integrons and antimicrobial resistance in carbapenem-resistant *Acinetobacter baumannii* clinical strains] (in Chinese). Chin J Microbiol Immunol. 2019 Apr;39(4):277–282.

Zhang Q, Cheng X, Shen J. [Detection of carbapenemases and integrons in carbapenem-resistant *Acinetobacter baumannii*] (in Chinese). Chin J Antibiot. 2015 May;40(5):377–381.