Molecular typing, and integron and associated gene cassette analyses in *Acinetobacter baumannii* strains isolated from clinical samples

LINGQING XU[1], SHUFEI DENG[1], WEIHONG WEN[1], YINGXIAN TANG[1], LINJUAN CHEN[1], YUZHEN LI[1], GUOQUAN ZHONG[1], JIEHUA LI[1], WEI-JEN TING[1] and BISHI FU[1,2]

[1]Department of Clinical Laboratory, The Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, Qingyuan, Guangdong 511518; [2]School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou, Guangdong 511436, P.R. China

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Abstract. The present study aimed to investigate the association between drug resistance and class I, II and III integrons in *Acinetobacter baumannii* (ABA). Multilocus sequence typing (MLST) is a tool used to analyze the homology among house-keeping gene clusters in ABA and ABA prevalence and further provides a theoretical basis for hospitals to control ABA infections. A total of 96 clinical isolates of non-repeating ABA were harvested, including 74 carbapenem-resistant ABA (CRABA) and 22 non-CRABA strains, and used for bacterial identification and drug susceptibility analysis. Variable regions were sequenced and analyzed. Then, 7 pairs of housekeeping genes were amplified and sequenced via MLST and sequence alignment was performed against the Pub MLST database to determine sequence types (STs) strains and construct different genotypic evolutionary diagrams. The detection rate of CRABA class I integrons was 13.51% (10/74); no class II and III integrons were detected. However, class I, II and III integrons were not detected in non-CRABA strains. The variable regions of 9 of 10 class I integrons were amplified and 10 gene cassettes including aacC1, aac1, aadDA1, aadA1a, aacA4, dfrA17, aadA5, aadA1, aadA22 and aadA23 were associated with drug resistance. The 96 ABA strains were divided into 21 STs: 74 CRABA strains containing 9 STs, primarily ST208 and ST1145 and 22 non-CRABA strains containing 18 STs, primarily ST1145. Class I integrons are a critical factor underlying drug resistance in ABA. CRABA and non-CRABA strains differ significantly; the former primarily contained ST208 and ST1145, and the latter contained ST1145. Most STs were concentrated in intensive care units (ICUs) and the department of Neurology, with the patients from the ICUs being the most susceptible to bacterial infection. In the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, ABA is potentially horizontally transmitted and MLST can be used for clinical ABA genotyping.

Correspondence to: Dr Bishi Fu, School of Basic Medical Sciences, Guangzhou Medical University, 1 Guangyi Road, Panyu, Guangzhou, Guangdong 511436, P.R. China
E-mail: fubishi@gzhmu.edu.cn

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Introduction

Acinetobacter baumannii (ABA) is an important conditional pathogen in hospitals, and often causes infection in critically ill patients (1,2). Carbapenems have always been considered as an important class of antibiotics to treat various infections. However, the proportion of carbapenem-resistant *A. baumannii* (CRABA) has gradually increased in recent years (3). Data from the China Antimicrobial Surveillance Network (2016) indicate that ABA ranked third among all the isolates (4). From 2005 to 2016, the resistance rate of ABA to imipenem rose from 31.0-68.6% and that to meropenem rose from 39.0-71.4% (5).

Integrons are closely associated with the multidrug resistance of ABA (1). Integron cassettes are mobile genetic materials in the bacterial genome that carry components of site-specific recombination systems that can integrate a number of drug resistance gene cassettes together to form multiple drug resistance (6). There are currently 10 types of integrons that have been discovered and identified in humans, but only 5 integrons are associated with drug resistance gene cassettes (7). Class I integrons are the commonest type and their structures include 5' and 3' conserved ends and an internal variable region encoding antibiotic resistance genes (2). The gene sequence of class II integrons is similar to that of class I integrons; the encoded integrin has 46% homology with class I. It is located on transposon Tn7 and its derivatives and is a defective DNA integrase Intl gene (6), Class III integrons have been identified on a resistant plasmid of *Serratia marcescens* that is resistant to imipenem. Its integrase gene encodes 320 amino acids and has 51% homology with integrase I (8). Class IV integrons are a special type of integrons: They were discovered by Cambray *et al* (9) from *Vibrio cholerae*. These

integrons are 126 kb in length and contain ≥179 genes. The primary function of these integrons is to encode adaptability genes. Class V integrons are located on a complex transposon carried by *Vibrio salmonicidum* (10).

At present, studies are mainly focusing on type I, II and III integrons, as they serve a vital role in drug resistance transmission. The product encoded by them can resist most clinical antibiotics (10). Those antibiotics can be divided into nine categories, of which class A are carbocylase dilutes: Imipenem (IPM) and meropenem (MEM); class B are aminoglycosides: Gentamicin (GM) and amikacin, (AN); class C are quinolones: Ciprofloxacin (CIP), levofloxacin (LVX), and ciprofloxacin (MXF); class D are cephalosporins: Cefazoxime (CZ), ceftazidime (CAZ), cefotaxime (CTX) and cefepime (FEP); class E are broad-spectrum penicillins: Ampicillin/sulbactam (SAM), piperacillin/tazobactam (TZP), via ampicillin (AMC) and piperacillin (PIP); class F are β-lactams: Aztreonam (ATM) and amoxicillin/clavulanic acid (AM); class G are sulfonamides: Compound neomin (SXT); class H are tetracyclinea: Tetracycline (TE); and class I are amides and alcohols: Chloramphenicol (C). The resistance rate of integron-positive strains to β-lactams, aminoglycosides and quinolone antibiotics is increased compared with integron-negative strains (7). In China, integrons with resistance gene cassettes are considered as the main drug resistance factor, as the detection rate of integrons is between 52.3-69.6% (11).

The present study collected CRABA and non-CRABA clinical samples from different clinical departments in the Sixth Affiliated Hospital of Guangzhou Medical University, investigated the association between integron distribution and drug resistance in ABA, and analyzed the homology among multilocus sequence types (MLSTs) in ABA. ABA integrase genes, variable regions and 7 housekeeping genes from 96 strains were amplified and sequenced.

Materials and methods

Strain conservation. A total of 96 ABA strains were harvested and separated from clinical samples between August 2014 and October 2018, and duplicates were eliminated. Samples were obtained from the Intensive Care Unit (ICU), and departments of Neurology, Infectious Diseases, Respiratory Medicine, Hematology, neurosurgery, Urology, Neonatal and ENT. All the experiment are performed under the permission of Medical Ethics committee of Qingyuan People's Hospital. Quality control strains were *Escherichia coli* ATCC25922, *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* ATCC27853 (American Type Culture Collection). Among the 96 ABA strains, 74 were CRABA strains and 22 were non-CRABA strains (Fig. 1). Written informed consent was obtained from all patients.

Instruments and reagents. Instruments included a Phoenix M50 automatic bacterial detector (Becton, Dickinson and Company), a T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) and a Gel Dox XR+gel imaging system (Bio-Rad Laboratories, Inc.). Reagents included Colombia blood Agar plate (Guangzhou Dijing Microbiology Co., Ltd), bacterial group DNA extraction kit, 2X Taq PCR MasterMix, Gel Red nucleic acid dye and Marker I, II, III DNA Ladder (all from Tiangen Biotech (Beijing) Co., Ltd.) and primers from Shanghai Shenggong Bioengineering Technology Service Co., Ltd.

Total DNA extraction from bacteria. The strains were stored in sterile milk at 4˚C and resuscitated prior to use. Following separation and culturing on blood-containing media, a sufficient number of bacterial colonies was selected with aseptic cotton and inoculated in a 1.5-ml sterile saline Eppendorf (EP) tube to prepare a bacterial suspension and DNA was extracted in accordance with the protocol of the DNA extraction kit, and transferred to aseptic 1.5 ml EP tube and stored at -80˚C.

Primer design. Primers were designed as described previously (12,13) and are listed in Tables I and II.

PCR for integrase genes. The PCR mix for class I, II and III integrons comprised: 12.5 μl 2X Taq PCR MasterMix, including Taq DNA polymerase, dNTPs, reaction buffer and $MgCl_2$, 0.5 μl each of the forward and reverse primers, 0.5 μl DNA template and 25 μl ddH_2O. The reaction conditions were: Initial denaturation at 95˚C for 5 min, followed by 26 cycles of denaturation at 94˚C for 30 sec, annealing at 62˚C for 30 sec and extension at 72˚C for 1 min, and then a final extension at 72˚C for 5 min. The PCR products were visualized electrophoretically on a 2% agarose gel for 45 min at 80 V, and visualized using a gel imaging system (Bio-Rad Laboratories, Inc.).

PCR for the variable region. The PCR mix for variable regions of class I, II and III integrons comprised: 12.5 μl 2X Taq PCR MasterMix, 0.5 μl each of the forward and reverse primer, 1 μl DNA template and 25 μl ddH_2O. The reaction conditions were as follows: Initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 30 sec, annealing at 62˚C for 30 sec and extension at 72˚C for 2 min, and a final extension at 72˚C for 7 min. The PCR products were visualized electrophoretically on a 1.2% agarose gel for 35 min at 80 V and visualized using a gel imaging system (Bio-Rad Laboratories, Inc.).

PCR for 7 housekeeping genes in MLSTs. The PCR mix for 7 housekeeping genes was: 12.5 μl 2X Taq PCR MasterMix, 0.5 μl each of the forward and reverse primer, 1 μl DNA template and 25 μl ddH_2O. The reaction conditions were: Initial denaturation at 94˚C for 5 min; 30 cycles of denaturation at 94˚C for 1 min, annealing at 56˚C for 1 min and extension at 72˚C for 2 min; final extension at 72˚C for 7 min. The PCR products were visualized electrophoretically on a 1% agarose gel (35 min, 65 V) and visualized using a gel imaging system (Bio-Rad Laboratories, Inc.).

Sequence analysis of the variable regions and 7 housekeeping genes. The PCR products (cut-off value >2) were sent to Shanghai Bioengineering Company for sequencing, and reads were corrected and spliced using Chromas software (version 2.6.6; http://technelysium.com.au/wp/chromas/) and compared and analyzed using BLAST (BLAST+ version 2.10.0; https://blast.ncbi.nlm.

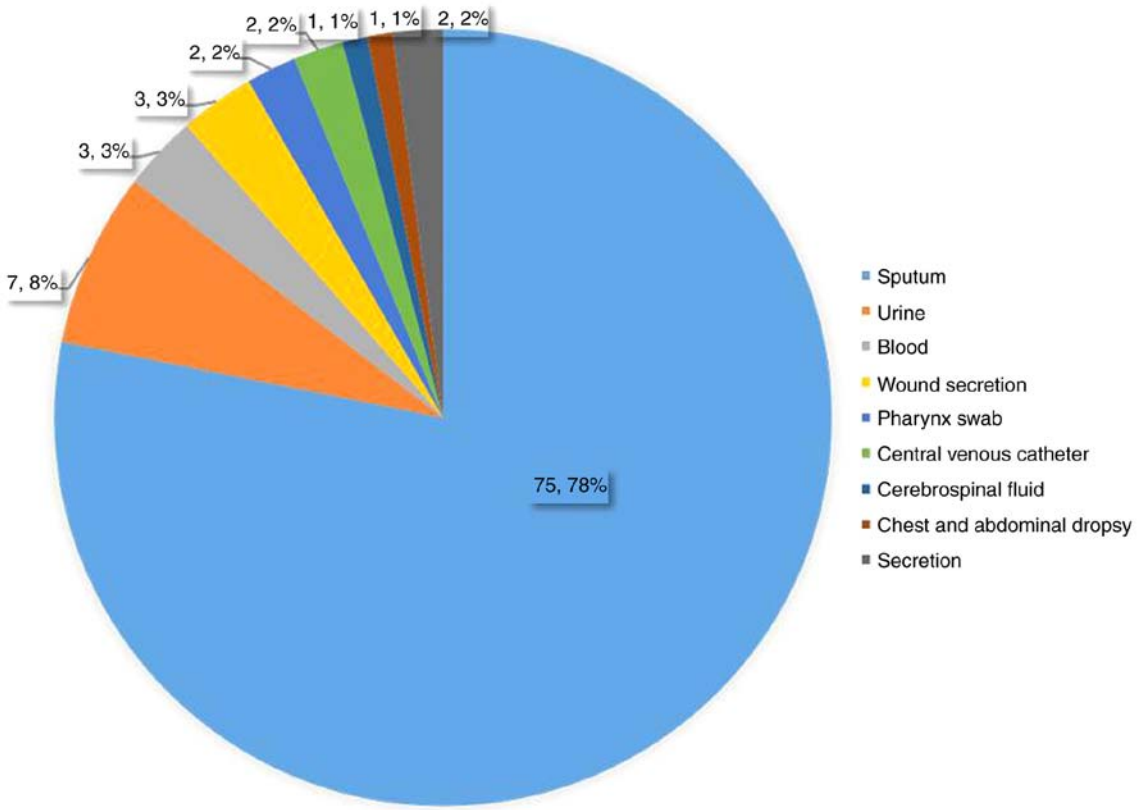

Figure 1. Distribution of 96 *Acinetobacter baumannii* strains in sample types.

nih.gov/Blast.cgi) and reads with the highest degree of coincidence were selected.

Genotyping analysis of ABA MLSTs. From the ABA MLST database (https://pubmlst.org/abaumannii/), the sequencing reads were subjected to BLAST analysis and different allele values were obtained. All strains were ranked, thus yielding an allelic spectrum in the order of *gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi* and *rpoD*.

Whole-genome sequencing. Genomic DNA was extracted from the culture with a QIAamp DNA kit according to the manufacturer's instructions (Qiagen). Purified DNA was fragmented by sonication with a Covaris S2 instrument (Covaris, Inc.). Indexed shotgun sequencing libraries were prepared using the Kapa Library Preparation kit [Kapa Biosystems (Pty) Ltd.], following the manufacturer's instructions. All libraries were sequenced on HiSeq 2000 instruments (Illumina, Inc.) using paired-end 101 bp reads with an index read of 9 bp. SPAdes (version 3.13.1; with the following parameters, k 21,33,55,77,99 and 127) was used for assembly of reads (14,15). The phylogenetic tree were constructed using core genome single nucleotide polymorphism (CG-SNP) analysis with Parsnp (version 1.2; with the following parameters, -x -c) (16), followed by displaying with iTOL (http://itol.embl.de) (17). The scale represents relative genetic distance.

Pulsed-field gel electrophoresis (PFGE). All the bacterial cells were suspended into a cell suspension buffer (100 mM Tris-HCl, 100 mM EDTA, pH 8.0). Proteinase K (20 μl) was added to 400 μl of the suspension along with 400 μl 1% SeaKem Gold Agar. These were mixed quickly, and approximately 300 μl was dispensed into prepared plug molds. Once solidified, the plugs were placed into 1.5 ml cell lysis buffer (50 mM Tris-HCl, 50 mM EDTA, pH 8.0, 1% Sarcosyl) and 40 μl of Proteinase K and incubated for 1.5 h at 54˚C in a shaking water bath. The plugs were washed twice in ultrapure water for 15 min in a 50˚C water bath followed by four washings in Tris-ETDA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The washed plugs were stored in TE buffer at 4˚C. For PFGE, the plugs were cut into 3-mm by 9-mm pieces and then digested in 173 μl of sterile water, 2 μl of bovine serum albumin, 20 l of 10XReAct II buffer, and 5 μl of XbaI (10 U/l) at 37˚C in a shaking water bath for 1.5 h. The plugs were run in a 1% agarose gel using a CHEF III Pulsed-Field System (Bio-Rad) in 0.5% Tris-borate-EDTA buffer (Sigma) at 10˚C. The parameters were set with the initial switch time at 2.2 sec, the final switch time at 64 sec, a voltage of 6 V/cm, and a duration of 21 h. The gel was stained with ethidium bromide and recorded on a Gel Doc system (Bio-Rad Laboratories, Inc., Hercules, CA). The file images were processed by BioNumerics software (Applied Maths BVBA, Kortrijk, Belgium).

Statistical analysis. All statistical analysis was performed using the SPSS software (Version 18.0; SPSS, Inc.). The antibiotic resistance data between the integron-positive isolates and integron-negative isolates was compared and the χ^2 test was used to calculate the P-value in terms of the resistant and susceptible numbers. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. The primer sequence of three integrase gene and variable area.

Target gene	Oligonucleotide	Sequence (5'→3')	Length, base pairs
Int 1	int 1-F	GGTCAAGGATCTGGATTTCG	493
	int 1-R	ACATGCGTGTAAATCATCGTC	
Int 2	int 2-F	CACGGATATGCGACAAAAAGGT	789
	int 2-R	GTAGCAAACGAGTGACGAAATG	
Int 3	int 3-F	AGTGGGTGGCGAATGAGTG	922
	int 3-R	TGTTCTTGTATCGGCAGGTG	
Int-variable area	5'-CS	GGCATCCAAGCAGCAAG	Variable
	3'-CS	AAGCAGACTTGACCTGA	

F, forward; R, reverse; CS, conserved segment; int, integrase gene.

Table II. The primer sequence of multilocus sequence typing.

Gene	Oligonucleotide and sequence	Length/bp
gltA	gltA(F):5'-AATTTACAGTGGCACATTAGGTCCC-3'	722
	gltA(R):5'-GCAGAGATACCAGCAGAGATACACG-3'	
gyrB	gyrB(F):5'-TGAAGGCGGCTTATCTGAGT-3'	594
	gyrB(R):5'-GCTGGGTCTTTTTCCTGACA-3'	
gdhB	gdhB(F)):5'-GCTACTTTTATGCAACAGAGCC-3'	774
	gdhB(R):5'-GTTGAGTTGGCGTATGTTGTGC-3'	
recA	recA(F):5'-CCTGAATCTTCYGGTAAAAC-3'	425
	recA(R):5'-GTTTCTGGGCTGCCAAACATTAC-3'	
cpn60	cpn60(F):5'-GGTGCTCAACTTGTTCGTGA-3'	640
	cpn60(R):5'-CACCGAAACCAGGAGCTTTA-3'	
Gpi	gpi(F):5'-GAAATTTCCGGAGCTCACAA-3'	456
	gpi(R):5'-TCAGGAGCAATACCCCACTC-3'	
rpoD	rpoD(F):5'-ACCCGTGAAGGTGAAATCAG-3'	672
	rpoD(R):5'-TTCAGCTGGAGCTTTAGCAAT-3'	

F, forward; R, reverse; gltA, citrate synthase gene; gyrB, DNA gyrase subunit B gene; gdhB, NAD-specific glutamate dehydrogenase; recA, recombinase A gene; cpn60, chaperonin protein 60 gene; gpi, glucose-6-phosphate isomerase gene; rpoD, RNA polymerase sigma factor gene.

Results

Departmental distribution of ABA in the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital. A total of 54 from 74 CRABA strains were obtained from the ICUs; the remaining 9 strains were obtained from the department of Neurology, 3 strains were obtained from the department of Infectious Diseases and the remaining strains were distributed among other departments. Of the 22 non-CRABA strains, 10 were obtained from the ICUs and the departments of Neurology and Urology each contributed 3 strains, while the remaining strains were distributed among other departments. These observations indicated that the ICUs have the highest number of contaminated with ABA and are most prone to bacterial infections.

Antimicrobial resistance profile. Among the 96 ABA strains, the resistance rate of strains harboring class I integrons towards tetracycline, piperacillin, cefepime, ampicillin/sulbactam, meropenem, gentamycin, levofloxacin and piperacillin/tazobactam was almost 100%, while that against ciprofloxacin, ceftazidime, cefotaxime and imipenem was >90%. With the exception of trimethoprim/sulfamethoxazole and amikacin, the resistance rate of class I integron-positive strains to all antibiotics was increased compared with of class I integron-negative strains. The results are summarized in Table III.

PFGE analysis and whole-genome sequencing of 10 CRABA strains. In this study, Ten Integron-positive strains could be divided into four subtypes (A, B, C, and D) by PFGE (Fig. 2). Those strains harbored class I integrons, of which 13.51% (10 of 74) were CRABA strains, and none of them was non-CRABA strains. None of the 96 strains of ABA strains harbored class II and III integrons. A total of 10 *A. baumannii* isolates were clustered into 2 main clades (Clade 1 and Clade 2), although the isolates had highly similar SNPs

Table III. Comparison of drug resistance phenotypes between 96 ABA profile I integron positive strains and profile I integron negative strains.

		Profile I integron positive strains (10 strains)			Profile I integron negative strains (86 strains)			
Antibacterials	D. R. R (%)	D. R. R (%)	I.R. (%)	S.R. (%)	D. R. R (%)	I.R. (%)	S.R. (%)	P-value
Ciprofloxacin	85.4	90.0	10.0	0.0	84.9	0.0	15.1	<0.05
Tetracycline	86.5	100.0	0.0	0.0	84.9	0.0	15.1	<0.05
Piperacillin	89.6	100.0	0.0	0.0	88.4	0.0	11.6	<0.05
Ceftazidime	85.4	90.0	0.0	10.0	84.9	1.2	14.0	<0.05
Cefotaxime	83.3	90.0	0.0	10.0	82.6	8.1	9.3	<0.05
Cefepime	87.5	100.0	0.0	0.0	86.1	1.2	12.8	<0.05
Ampicillin/Sulbactam	87.5	100.0	0.0	0.0	86.1	0.0	14.0	<0.05
Meropenem	85.4	100.0	0.0	0.0	82.6	0.0	17.4	<0.05
Gentamycin	86.5	100.0	0.0	0.0	84.9	0.0	15.1	<0.05
Imipenem	83.3	90.0	0.0	10.0	82.6	0.0	17.4	<0.05
Levofloxacin	86.5	100.0	0.0	0.0	84.9	0.0	15.1	<0.05
Bactrim	71.9	30.0	0.0	70.0	76.7	0.0	23.3	<0.05
Colistin	9.4	10.0	0.0	90.0	9.3	0.0	90.7	<0.05
Amikacin	77.1	20.0	0.0	80.0	83.7	0.0	16.3	<0.05
Piperacillin/tazobactam	87.5	100.0	0.0	0.0	86.1	1.2	12.8	<0.05

D.R.R., drug resistance rate; I.R., intermediary rate; S.R., sensitivity rate.

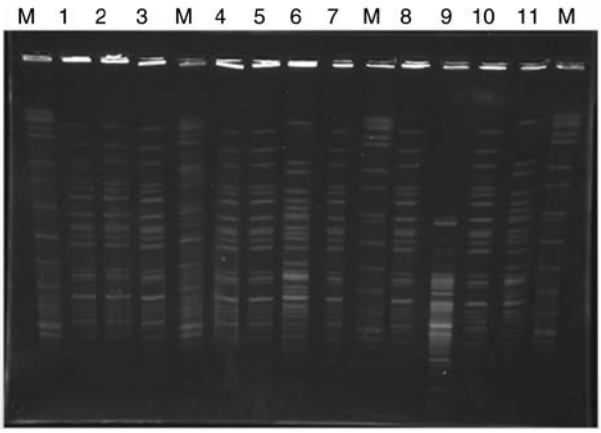

Figure 2. PFGE of 10 Integron-positive strains. ALL 10 sample genomes were treat with XbaI Lane 1, 2, 3, 4, 5 are the same clone as type A, Lane 6, 11 are the same clone as type B. Lane 7, 8, 10 are the same clone as type C, Lane 9 as type D. Lane M: Salmonella H9812. Lane 11 is an integron-negative strain.

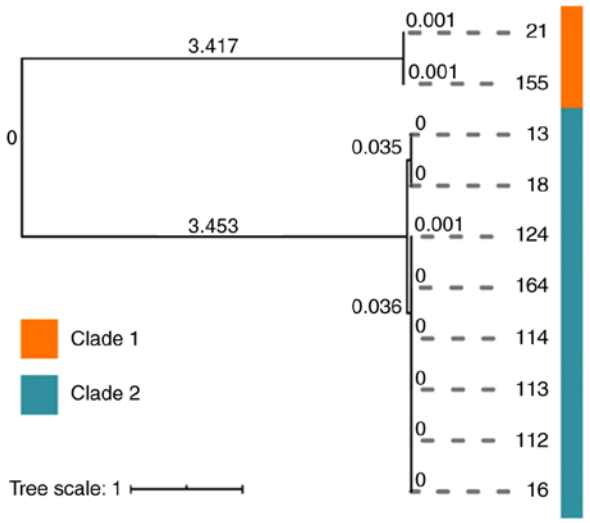

Figure 3. *Acinetobacter baumannii* isolates were clustered into two main clades via core genome SNP analysis. All the genome data were sequenced with a HiSeq (Illumina, Inc.) platform and assembly was performed with SPAdes (version 3.13.1, with the following parameters: k 21,33,55,77,99 and 127). The phylogenetic tree was constructed by core genome single nucleotide polymorphisms (CG-SNP) analysis with Parsnp (version 1.2, with the following parameters -x -c), followed by displaying with iTOL (http://itol.embl.de). The scale represents relative genetic distance.

in each clade, the genetic distance between the two clades was comparatively high (Fig. 3).

Association between class I integrons and antibacterial resistance. A total of 8 class I integron-positive strains and 1 class I integron-negative strain (n=9 strains) were sent for genome sequencing and 10 drug resistance gene cassettes were detected (Table IV). The integrated substructure is presented in Fig. 4. The actual drug resistance genes of 9 successfully sequenced strains and the variable regions in the gene cassette are summarized in Table IV. The drug resistance rates of ABA class I integron-positive strains against meropenem, imipenem, ceftazidime, cefotaxime, cefixime, tetracycline, ciprofloxacin, cefazolin, piperacillin, ampicillin/sulbactam, gentamicin, levofloxacin and piperacillin/tazobactam were significantly increased compared with the negative strains (P<0.05), indicating that the mechanism underlying the acquisition of drug resistance in ABA strains was closely

Table IV. The comparison between drug resistance phenotype and variable region gene box.

Sample number	Resistance phenotype	The size of variable Area (base pairs)	GM	AN	Bactrim	The combination of variable region gene box
8	a, b, c, d, e, g, h	3000	+	-	+	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a
12	a, b, c, d, e, g, h	3000	+	-	+	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a
24	a, b, c, d, e, h	3000	+	-	-	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a
55	a, b, c, d, e, g, h	3000	+	+	+	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a
60	a, b, c, d, e, g, h	3000	+	-	-	aacC1, aadA1, aadDA1, aac1aacA4, aadA1a
64	a, b, c, d, e, g, h	3000	+	+	-	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a
67	a, b, c, d, e, g, h	3000	+	+	-	aadA22, aadA23
75	a, b, c, d, e, g, h	3000	+	-	-	dfrA17, aadA5
130	a, b, c, d, e, g, h	3000	+	-	-	aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a

Drug-resistant is shown with '+' and drug sensitive is shown with '-'.

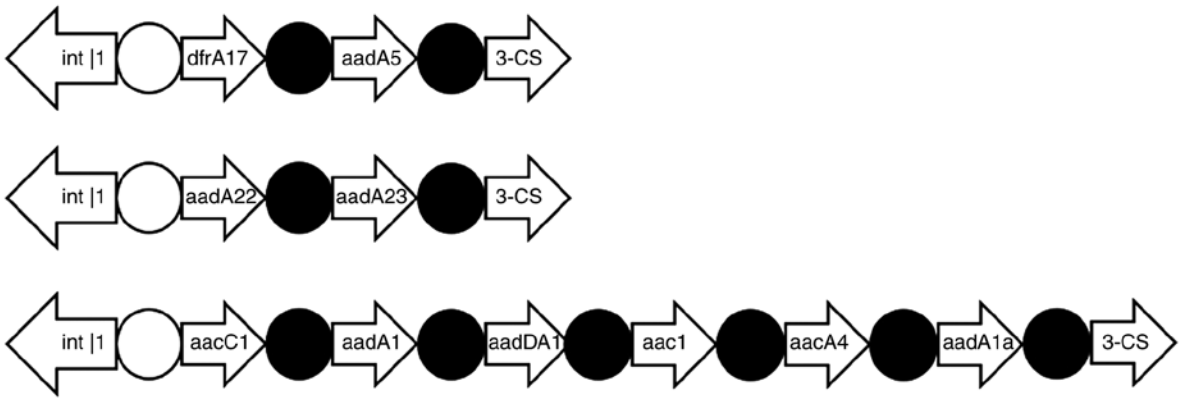

Figure 4. Integrated substructures of integrons.

associated with the presence of class I integrons. However, the resistance rate of class I integron-negative ABA strains to a number of antibiotics was also relatively high, indicating that multidrug resistance among ABA strains is not only associated with class I integrons but also with other drug resistance mechanisms, which warrants further investigation.

MLST data. A total of 21 sequence types (STs) were obtained from 96 ABA strains, including 22 strains with ST208, 22 strains with ST1145, 12 strains with ST1417 and 11 strains with ST195. The MLSTs of CRABA and non-CRABA strains were compared, among which ST208 and ST1145 were the most prominent STs of the CRABA strain and ST1145 was the most prominent ST of the non-CRABA strain, while others exhibited a scattered distribution (Table V). The genotypic distribution of each branch is presented in Fig. 5.

Discussion

Integrons contain specific recombinant sites, which can propagate antibiotic resistance, enable transmission of different drug resistance gene cassettes from bacterial drug resistance genes among different strains and lead to multidrug-resistance among bacteria (18). The most common integron type of ABA are the class I integrons, which contains various drug resistance genes and is closely associated with the acquisition of drug resistance (19).

In the present study, class I, II and III integrons and variable regions were sequenced in 96 ABA strains. Of these, 10 strains harbored class I integrons, with a positive rate of 10.42%, and all of them were CRABA strains; none of them harbored class II and III integrons. The results of the present study are in contrast to those of a previous study, in which Goudarzi and Azimi (20) reported that the prevalence of class I and II integrons was 66.7 and 20%, respectively, and that, for the first time, class III integrons were reported in 3 *A. baumannii* strains (2.9%). These results differ from the present study, considering the difference of regional distribution and the sample resources of *A. baumannii* strains. Although the positive rate of class I integrons was relatively low, the variable region was amplified and the drug resistance gene cassettes were detected in 9 of 10 class I integron-positive strains, all of which were CRABA strains. These results show that the distribution of class I integrons in CRABA was markedly different compared with the non-CRABA strains and that class I integron-positive strains serve an important role in the acquisition of drug resistance, thus providing essential and novel clinical insights.

Table V. Non-CRABA and CRABA strain multilocus sequence typing results and distribution rate (%).

	Number of strains	Housekeeping genes							ST types	Distribution rate % (n/74)
		gltA	gyrB	gdhB	recA	cpn60	gpi	rpoD		
CRABA strain	22	1	3	3	2	2	97	3	208	29.73
	22	1	3	3	102	2	97	3	1,145	29.73
	12	1	3	3	102	2	96	3	1,417	16.22
	11	1	3	3	2	2	96	3	195	14.86
	3	1	3	3	2	2	16	3	136	4.05
	1	15	48	58	42	36	54	41	613	1.35
	1	1	81	3	102	2	16	3	1,658	1.35
	1	1	3	3	102	2	169	3	1,142	1.35
	1	61	12	2	28	1	158	5	717	1.35
Non-CRABA strain	2	1	3	3	2	2	97	3	208	9.09
	3	1	3	3	102	2	97	3	1,145	13.64
	2	1	3	3	102	2	96	3	1,417	9.09
	1	1	3	3	2	2	96	3	195	4.55
	1	15	48	58	42	36	54	41	613	4.55
	2	1	47	53	1	1	59	32	73	9.09
	1	33	31	2	28	1	77	5	629	4.55
	1	33	12	40	26	32	91	5	163	4.55
	1	33	50	80	28	1	164	5	702	4.55
	1	31	45	8	6	30	53	6	105	4.55
	1	1	17	56	1	1	91	26	183	4.55
	1	1	34	3	28	1	144	45	1,106	4.55
	1	1	3	3	2	2	157	3	547	4.55
	1	21	35	2	28	1	52	4	145	4.55
	1	21	48	58	42	36	109	4	234	4.55
	1	33	12	59	11	32	11	5	334	4.55
	1	1	54	62	31	4	55	45	147	4.55

CRABA, carbapenem-resistant *Acinetobacter baumannii*; gltA, citrate synthase gene; gyrB, DNA gyrase subunit B gene; gdhB, NAD-specific glutamate dehydrogenase; recA, recombinase A gene; cpn60, chaperonin protein 60 gene; gpi, glucose-6-phosphate isomerase gene; rpoD, RNA polymerase sigma factor gene.

Drug sensitivity analysis indicated that the resistance rate of ABA strains isolated from the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, was as high as 89% to broad-spectrum penicillin, >80% to penicillin, carbapenem and cephalosporins of 3 or 4 generations and >70% to aminoglycosides. These aforementioned drugs can be used in accordance with the patient condition per clinical guidelines. While strains resistant to >3 antimicrobial drugs are considered multi-resistant, class I integron-positive strains are more dangerous. The results from the present study show that ABA, a multidrug-resistant bacterium, can be treated with few drugs and that CRABA or non-CRABA strains can simultaneously cause infections within hospitals over a long time period and spread widely through different modes of infection, thereby resulting in nosocomial infections and leading to the generation of drug-resistant strains; this is of critical concern. In the present study, CRABA carried the resistant cassettes that contained aacC1, aadA1, aadDA1, aac1, aacA4, aadA1a, aadA22, aadA23, dfrA17 and aadA5, and aacC1, aadA1, aadDA1, aac1, aacA4 and aadA1a were the main strains in the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital. This is markedly different from another study in China: Huang *et al* (21) reported that most of the cassettes belong to a class I integron (136/144) encoding arr3, aacA4, dfrA17, aadA5, aadB, cat, blaOXA10, aadA1, aadA2, dfrA and aacC1, and AadA2-HP-dfrA were the prevalent strains in the Second Affiliated Hospital of Chongqing Medical University, China. Currently, numerous class I integron resistance gene cassettes are available. In the present study, 10 types of drug resistance gene cassettes were detected: aacC1, aac1, aadDA1, aadA1a, aacA4, dfrA17, aadA5, aadA1, aadA22 and aadA23. Among these, aadA1, aadA2, aadA5 and aadA22 encoded aminoglycoside nucleotransferase, which imparted spectinycin and streptomycin resistance, aacA4 encoded 6 N-aminoglycan drugs, resulting in tobramycin and amikacin resistance and dfrA12 and dfrA17 encoded sulfonamides, resulting in sulfanilamide resistance. These results demonstrated that the drug resistance rate of integron-positive strains was increased compared with integron-negative strains. Furthermore, other

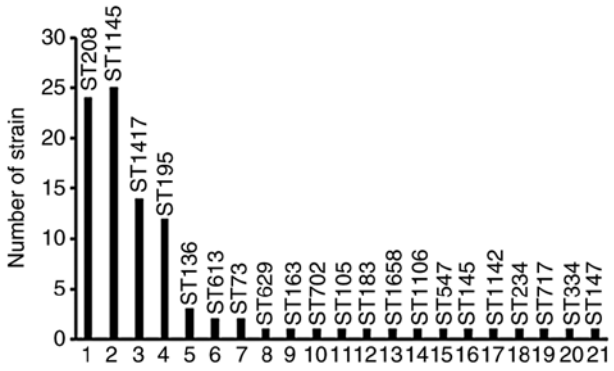

Figure 5. Distribution of various ST strains in the Sixth Affiliated Hospital of Guangzhou Medical University. ST, sequence type. 1. Intensive Care Unit, neurosurgery department, Neurology department, and cardiology department; 2. Intensive Care Unit, Tumor radiology department, Infectious Diseases department, Neonatal pediatrics department, Hematology department, Neurological surgery, and Respiratory Medicine department; 3. Intensive Care Unit, Neurosurgery department, Burns and Plastic Surgery of Department, Respiratory Medicine department, and Neurology department; 4. Intensive Care Unit, Neurology department; 5. Intensive Care Unit, Infectious Diseases department, and Respiratory Medicine department; 6. Intensive Care Unit, Pediatrics department; 7. Intensive Care Unit; 8. Intensive Care Unit; 9. Neurology department; 10. Brain department; 11. Intensive Care Unit; 12. Urological surgery; 13. Infectious diseases department; 14. Neonatal pediatrics department; 15. E.N.T department; 16. Urological surgery department; 17. E.N.T department; 18. Intensive Care Unit; 19. Intensive Care Unit; 20. Intensive Care Unit; 21. Intensive Care Unit.

bacterial drug resistance mechanisms include alterations in bacterial permeability, drug targets, and metabolic pathways, production of enzymes that eliminate antibiotics, active excretion of the antibiotics and inhibition of antibiotic entry into bacteria cells.

Pulse-field gel electrophoresis is a standard method for bacterial molecular typing and assessment of homology and is often used for ABA genotyping; however, it is only suitable for short-term epidemiological studies (<3 months), and the method is difficult to standardize and digitize (22); by contrast, MLST has high resolution and can be used to detect housekeeping gene sequences (23). Compared with other typing methods, MLST yields more accurate and realistic results, is superior and reproducible, and enables differential typing of different strains. MLST has been widely used worldwide for molecular epidemiological studies of bacteria, such as *S. aureus* (24), *Vibrio parahaemolyticus* (25), *E. coli* (26) and others. Previous studies have performed MLST to study the molecular epidemiological characteristics of ABA and reported numerous well-known multidrug resistance STs and clone complexes (cc) (27,28). The PubMLST database (https://pubmlst.org/abaumannii/) shows that CC92 is currently the most prominent clone complex and that the majority of ABA multi-drug-resistant strains worldwide are CC92 (29,30). ST92 is the ancestor of the CC92 clone complex, indicating that other ST types have evolved from ST92 (31). Strains with the CC92 clone complex are difficult to eliminate with antibiotics, thereby leading to its increased drug resistance (32,33); however, this requires further verification. In China, a few studies have reported this, although Han *et al* (34) demonstrated that in Xi'an, Shanxi province, 4 ST types (ST195, ST218, ST368 and ST208) were identified among 47 multidrug-resistant *A. baumannii* strains and that ST368 and ST208 have the closest genetic relationship. Lin *et al* (35) reported that in Anhui Province the drug-resistance rates of 87 strains of *A. baumannii* to imipenem and meropenem were 74.7 and 66.7%, respectively; 87 strains were subdivided into 42 ST-types and 37 strains were demonstrated to be dominant types and sub-listed in the ST2 category. The ST2 *A. baumannii* belongs to the clone complex CC1.

Concomitantly, multidrug-resistant ABA shares gene homology in various regions in Europe via amplified fragment length polymorphism analysis. Furthermore, European clones I, II and III of multi-drug-resistant ABA are prevalent, among which European clone II is the most prevalent (36). Higgins *et al* (37) genotyped CRABA strains from 32 countries via repetitive fragment PCR analysis and reported 8 worldwide clones, the European clone II being the most widely distributed. Although the CC92 clone complex is widely prevalent worldwide, different epidemic characteristics persist in different regions. Zhong *et al* (32) reported that ST92 and ST75 are the primary prevalent clones, while another study reported that ST75 is probably the primary epidemic type in Eastern China (38). He *et al* (39) reported a novel STn-1 (polymorphism 1-B1- 3-2- 2- 16- 3, B1 being a novel allele located at the *gyrB* locus) in Chengdu, in addition to ST92. He *et al* (40) hypothesized that ST138 and STn-1 may be the most widespread in Western China; however, some other studies had already reported the primary epidemic types in southern China. For instance, Fu *et al* reported that CC92 was widespread in a number of cities in China from 2004 to 2005, including ST75, ST90 and ST92 (41).

In the present study, the changes in *gyrB* and *gpi* were the most prominent, concurrent with previous studies (42,43) and *gyrB* and *gpi* easily undergo mutation and recombination during evolution. ST208 was widely spread in the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, accounting for 24 strains (25%) of the CC92 clone, concurrent with previous nationwide and international reports (31). The proportions of ST1145 type and ST1417 were 26.04 and 14.58%, respectively, potentially belonging to the new genotype and warranting further investigation. Irrespective of the genotype, a majority of the ABA strains were identified in the ICUs and bacterial infections were most common in the ICUs, thus there is the potential to lead to an outbreak, which warrants immediate attention.

In conclusion, multidrug resistance in ABA strains are closely associated with the presence of class I integrons. The results of the present study highlighted the differences in STs between CRABA and non-CRABA strains; CRABA strains containing primarily ST208 and ST1145, and non-CRABA strains ST1145. The results of the present study indicated that ABA in the Sixth Affiliated Hospital of Guangzhou Medical University, Qingyuan People's Hospital, is capable of horizontal transmission. Furthermore, MLST can be used for clinical ABA genotyping. Owing to multidrug resistance in ABA, further studies are required to investigate the mechanism underlying bacterial drug resistance, strictly monitor susceptible factors in hospitals, control bacterial drug resistance, strengthen disinfection and isolation methods in

hospitals, improve safety awareness among the medical staff and effectively control hospital infections.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LX, SD and WW carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. YT and LC carried out the species identification. YL and GZ participated in the susceptibility tests. JL and WT participated in the PCR. BF conceived the study and participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study was approved by the Medical Ethics committee of Qingyuan People's Hospital. Written informed consent was obtained from each participant's legal guardian.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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