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ORIGINAL ARTICLE

Antibiotic resistance determinants of a group of multidrug-resistant *Acinetobacter baumannii* in China

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A group of *Acinetobacter baumannii* confers multidrug resistance, but the molecular epidemiology and multidrug resistance mechanisms are poorly understood. Nineteen isolates were identified, and the antimicrobial susceptibility profile was determined using the disc diffusion method. Then, PCR of 78 kinds of resistance-associated genes were performed. A novel variant of bla_{ADC} gene: $bla_{ADC\text{-}67}$ gene (Genbank accession No. JX169789) was prevalent in all 19 isolates. Moreover, IS*Aba*1 could also provide strong promoter to upregulate the expression of bla_{ADC67} to confer resistance to beta-lactam. This is the first report of emergence of $bla_{ADC\text{-}67}$ in *A. baumannii* worldwide, which might confer resistance to beta-lactam.
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Keywords: *Acinetobacter baumannii*; bla_{ADC}; multidrug resistance; novel variant; resistance determinant

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is important opportunistic pathogen responsible for a variety of nosocomial infections, including ventilator-associated pneumonia, bacteremia, surgical-site infections, secondary meningitis and urinary tract infections. Treatment of *A. baumannii* infections is often complicated by multidrug-resistant phenotypes, including resistance to broad-spectrum beta-lactams, aminoglycosides and quinolones.

From February 2011 to October 2011, multidrug-resistant (MDR) *A. baumannii* were continuously isolated from Ningbo No. 2 Hospital in China. Hence, we chose 19 isolates (no. 5: from throat swab, no. 6: from catheter, no. 12: from urine, no. 17 and no. 18: from wound, others: from sputums) and analyzed the antimicrobial susceptibility profile and genetic determinants of antimicrobial resistance in this group of MDR *A. baumannii*. These isolates were multidrug resistant to beta-lactams, aminoglycosides, quinolones; hence, we attempted to detect 15 kinds of class A beta-lactamase genes, 10 kinds of class B beta-lactamase genes, 3 kinds of class C beta-lactamase genes, 10 kinds of class D beta-lactamase genes, 2 kinds of genes for outer membrane porin protein, 9 kinds of aminoglycoside-modifying enzyme genes, 6 kinds of 16SrRNA methylase genes, 7 kinds of quinolone resistance genes. Furthermore, taking into account of roles of mobile genetic elements, drug efflux genes and resistant island genes in resistance, we also detected two kinds of genetic markers of plasmids, three kinds of genetic markers of transposons, five kinds of genetic markers of insertion sequences, four kinds of genetic markers of integrons, two kinds of drug efflux genes and resistant island genes. The objectives of the present study were to investigate the molecular epidemiology and multidrug resistance mechanisms of this group of isolates. Understanding the genetic background of antimicrobial resistance is an important step in defining the impact of MDR *A. baumannii*.

MATERIALS AND METHODS

The isolates were identified by Vitek Gram-negative identification cards (bioMérieux-Vitek, Inc., Hazelwood, MO, USA), and the antimicrobial susceptibility profile was determined using the disc diffusion method according to the CLSI and interpreted with criteria published in 2011.[1] Whole-cell DNA from the isolates of *A. baumannii*, prepared by proteinase K digestion, was used as a template in PCR assays. PCR was performed using the primers (Wuxi Clone Gen-Tech Institute, Wuxi, Jiangsu, China) for 78 kinds of resistance-associated genes mentioned above, which were designed based on genotypes published in GenBank (Table 1). PCR conditions were 2 min at 93 °C; 35 cycles of 30 s at 93 °C, 30 s at 55 °C and 1 min at 72 °C; and, finally, 5 min at 72 °C. Positive and negative controls were performed in every PCR assays. The amplicons were purified with PCR Kits (Wuxi Clone Gen-Tech Institute, Wuxi, Jiangsu, China) and were subsequently sequenced on an ABI PRISM377 sequencer analyzer (Applied Biosystems, FosterCity, CA, USA). Sequences of primers and length of aimed product were shown in Table 2.

RESULTS AND DISCUSSION

According to antimicrobial susceptibility testing by the disc diffusion method, all isolates of *A. baumannii* were defined as MDR strains as they were resistant to three or more unique antimicrobial classes: including aminoglycosides, quinolones, extended-spectrum cephalosporins, beta-lactam/beta-lactamase inhibitor combination (Table 3).

By sequencing and BLASTn analysis, $bla_{ADC\text{-}67}$(Genbank accession No. JX169789) was identified as a novel variant of the ADC

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Table 1 Resistance-associated genes detected in 19 isolates of MDR *A. baumannii*

Beta-lactam	Class A beta-lactamase gene	TEM, SHV, CTX-M-1 cluster, CTX-M-2 cluster, CTX-M-8 cluster, CTX-M-9 cluster, CTX-M-25 cluster, PER, GES, VEB, CARB, KPC, RTG, SCO, promoter-TEM
	Class B beta-lactamase gene	IMP, VIM, SIM, GIM, NDM, AIM, SPM, KHM, DIM, TMB
	Class C beta-lactamase gene	DHA, ADC, IS*aba*1-ADC
	Class D beta-lactamase gene	OXA-1 cluster,OXA-2 cluster, OXA-10 cluster, OXA-20 cluster, OXA-23 cluster, OXA-24 cluster, OXA-51 cluster, OXA-58 cluster, IS*aba*1-OXA23, IS*aba*1-OXA66
	Gene for outer membrane porin protein	*car*O, *opr*D
Aminoglycoside	Aminoglycoside-modifying enzyme gene	*aac*(3)-I, *aac*(3)-II, *aac*(6′)-Iad, *aac*(6′)-Ib, *aac*(6′)-II, *ant*(2″)-I, *ant*(3″)-I, *ant*(4′)-I, *aph*(3′)-I
	16SrRNA methylase gene	*arm*A, *rmt*A, *rmt*B, *rmt*C, *rmt*D, *npm*A
Quinolone	Quinolone resistance gene	*gyr*A, *par*C, *qnr*A, *qnr*B, *qnr*S, *aac*(6′)-Ib-Cr, *qep*A
Mobile genetic element	Genetic marker of plasmid	*tra*A, *trb*C
	Genetic marker of transposon	*mer*A, *tnp*U, *tnp*513
	Genetic marker of insertion sequence	IS*aba*1, IS*aba*4, IS*aba*9, IS26, IS903
	Genetic marker of integron	*int*I1, *int*I2,*int*I3, *qac*EΔ1
Drug efflux pump	Drug efflux gene	*ade*B, *qac*EΔ1
Resistance island		Aba-RI

Table 2 Primer sequences of target genes and lengths of aimed products

Gene	*Primer sequences of target genes (5′→3′)and lengths of aimed products*
Beta-lactam	
Gene for outer membrane porin protein	
carO	P1:ATGAAAGTATTACGTGTTTTAGTGACAAC; P2:TTACCAGTAGAATTCNACACCAACT(729 bp)
oprD	P1:ATGCTAAAAGCACAAAAACTTACATTAGCA; P2:TTAGAATAATTTCACAGGAATATCTAAGAA(1320 bp)
Class A beta-lactamase gene	
TEM	P1:AGGAAGAGTATGATTCAACA; P2:CTCGTCGTTTGGTATGGC(535 bp)
SHV	P1:TGCGCAAGCTGCTGACCAGC; P2:TTAGCGYTGCCAGTGCTCGA(305 bp)
CTX-M-1 cluster	P1:ATGGTTAAAAAATCACTGCGYCAGTTC; P2:TCACAAACCGTYGGTGACGATTTTAGCCGC(876 bp)
CTX-M-2 cluster	P1:ATGATGACGCAGAGCATTCGCCGCTCA; P2:TCAGAAACCGTGGGTTACGATTTTCGC(876 bp)
CTX-M-8 cluster	P1:ATGATGAGACATCGCGTTAAGCGG; P2:TTAATAACCGTCGGTGACGATTTTCGCG(876 bp)
CTX-M-9 cluster	P1:ATGGTGACAAAGAGAGTGCAACGG; P2:TTACAGCCCTTCGGCGATGATTCTCGC(876 bp)
CTX-M-25 cluster	P1:ATGATGAGAAAAAGCGTAAGGCGGGCG; P2:TTAATAACCGTCGGTGACAATTCTGGC(876 bp)
PER	P1:AGTCAGCGGCTTAGATA; P2:CGTATGAAAAGGACAATC(978 bp))
GES	P1:ATGCGCTTCATTCACGCAC; P2:CTATTTGTCCGTGCTCAGG(846 bp))
VEB	P1:GCGGTAATTTAACCAGA; P2:GCCTATGAGCCAGTGTT(961 bp))
CARB	P1:AAAGCAGATCTTGTGACCTATTC; P2:TCAGCGCGACTGTGATGTATAAAC(588 bp))
RTG	P1:TATGTCTCACGCTATCATTAAATGC; P2:ATAATGTGGCCTGACACAGCTCT(338 bp))
SCO	P1: ATGACAAGATCTGCCCTTTTGAT; P2:TTATTCCAGAACTTCGGCAGCA(867 bp)
promoter-TEM	P1:ATAAAATTCTTGAAGACGAAA; P2:CTCGTCGTTTGGTATGGC(735 bp)
KPC	P1:ATGTCACTGTATCGCCGTCTA; P2:TTACTGCCCGTTGACGCCCAA(882 bp)
Class B beta-lactamase gene	
IMP	P1:CGGCCKCAGGAGMGKCTTT; P2:AACCAGTTTTGCYTTACYAT(587 bp)
VIM	P1:ATTCCGGTCGGMGAGGTCCG; P2:GAGCAAGTCTAGACCGCCCG(633 bp)
SPM	P1:CTGCTTGGATTCATGGGCGCG; P2:CCTTTTCCGCGACCTTGATCG(786 bp)
GIM	P1:CCTGTAGCGTTGCCAGCTTTA; P2:CAGCCCAAGAGCTAATTGAGG(562 bp)
SIM	P1:ACAAGGGATTCGGCATCGTT; P2:TTATCTTGAGTGTGTCCTGG(355 bp)
AIM	P1:CGTCGCTTCACCCTGCTGGGCAGC; P2:AGGCGAGGCGACCGCCGTCAGGCC(535 bp)
NDM	P1:ATGGAATTGCCCAATATTATGCACCCG; P2:TCAGCGCAGCTTGTCGGCCATGCG(813 bp)
KHM	P1:ATGAAAATAGCTCTTGTTATATCG; P2:TCACTTTTTAGCTGCAAGCGCTTC(726 bp)
DIM	P1:ATGAGAACACATTTTACAGCGTTA; P2:TCAATCAGCCGACGCGTTAGCGTT(756 bp)
TMB	P1:TATGCCTCAGCGCTGACTAAT; P2:TCAGCGGTCGCCGTGATTGGC(400 bp)
Class C beta-lactamase gene	
DHA cluster	P1:AACTTTCACAGGTGTGCTGGGT; P2:CCGTACGCATACTGGCTTTGC(405 bp)
ADC	P1:GGTATGGCYGTGGGBGTYATTC; P2:CTAAGASTTGGTCRAARGGT(739 bp)
ISaba1-ADC	P1:GATGTGTCATAGTATTCGTCG; P2:CTAAGASTTGGTCRAARGGT(variable length)
Class D beta-lactamase gene	
OXA-1 cluster	P1:CTGTTGTTTGGGTTTCGCAAG; P2:CTTGGCTTTTATGCTTGATG(440 bp)
OXA-2 cluster	P1:CAGGCGCYGTTCGYGATGAGTT; P2:GCCYTCTATCCAGTAATCGCC(233 bp)
OXA-10 cluster	P1:GTCTTTCRAGTACGGCATTA; P2:GATTTTCTTAGCGGCAACTTA(822 bp)

Table 2 (Continued)

Gene	*Primer sequences of target genes (5′→3′)and lengths of aimed products*
OXA-20 cluster	P1:TTGATAATCCGATTTCTAGCAC; P2:CTAGTTGGGTGGCAAAGCAT(801 bp)
OXA-23 cluster	P1:ATGAATAAATATTTTACTTGCTATGTG; P2:TTAAATAATATTCAGCTGTTTTAATGA(822 bp)
OXA-24 cluster	P1:CAAGAGCTTGCAAGACGGACT; P2:TCCAAGATTTTCTAGCRACTTATA(420 bp)
OXA-51 cluster	P1:ATGAACATTAAAGCACTCTTACTT; P2:CTATAAAATACCTAATTGTTCTAA(825 bp)
OXA-58 cluster	P1:TCGATCAGAATGTTCAAGCGC; P2:ACGATTCTCCCCTCTGCGC(530 bp)
ISaba1-OXA23	P1:GATGTGTCATAGTATTCGTCG; P2:TCACAACAACTAAAAGCACTG(variable length)
ISaba1-OXA66	P1:GATGTGTCATAGTATTCGTCG; P2:CTATAAAATACCTAATTGTTCTAA(variable length)
Aminoglycoside	
Aminoglycoside-modifying enzyme gene	
aac(3)-I	P1:ACCTACTCCCAACATCAGCC; P2:ATATAGATCTCACTACGCGC(169 bp)
aac(3)-II	P1:ACTGTGATGGGATACGCGTC; P2:CTCCGTCAGCGTTTCAGCTA(237 bp)
aac(6′)-Iad	P1:ATGATTAGAAAAGCAACTGTCCAAG; P2:TTAAAGTTGCTTTGTAAAACAAATC(435 bp)
aac(6′)-Ib	P1:ATGACTGAGCATGACCTTGC; P2:TTAGGCATCACTGCGTGTTC(519 bp)
aac(6″)-II	P1: TTCATGTCCGCGAGCACCCC; P2:GACTCTTCCGCCATCGCTCT(178 bp)
ant(2″)-I	P1:GAGCGAAATCTGCCGCTCTGG; P2:CTGTTACAACGGACTGGCCGC(320 bp)
ant(3″)-I	P1:TGATTTGCTGGTTACGGTGAC; P2:CGCTATGTTCTCTTGCTTTTG(284 bp)
ant(4″)-I	P1:CGTGGAGCGATATCGATTTCG; P2:TCTGGTTCGGCGGCCGGATGC(266 bp)
aph(3″)-I	P1:ATGTGCCATATTCAACGGGAAACG; P2:TCAGAAAAACTCATCGAGCATCAA(816 bp)
16SrRNA methylase gene	
armA	P1:ATGGATAAGAATGATGTTGTTAAG; P2:TTATTTCTGAAATCCACTAGTAATTA(774 bp)
rmtA	P1:CCTAGCGTCCATCCTTTCCTC; P2:AGCGATATCCAACACACGATGG(315 bp)
rmtB	P1:ATGAACATCAACGATGCCCTC; P2:TTATCCATTCTTTTTTATCAAGTATAT(756 bp)
rmtC	P1:ATGAAAACCAACGATAATTATC; P2:TTACAATCTCGATACGATAAAATAC(846 bp)
rmtD	P1:ATGAGCGAACTGAAGGAAAAACTGCT; P2:TCATTTTCGTTTCAGCACGTAAAACAG(744 bp)
npmA	P1:TTGGGTACTGGAGACGGTAG; P2:CAGCTTTGTATTGTTCGCTC(421 bp)
Quinolone	
Quinolone resistance gene	
gyrA	P1:AAATCTGCCCGTGTCGTTGGT; P2:GCCATACCTACGGCGATACC(344 bp)
parC	P1:AAACCTGTTCAGCGCCGCATT; P2:AAAGTTGTCTTGCCATTCACT(327 bp)
qnrA	P1:CAAGAGGATTTCTCACGCCAG; P2:GAACTCTATGCCAAAGCAGTTGG(240 bp)
qnrB	P1:ATGRCTCTGGCAMTMGTTGGCGA; P2:CTARCCAATMAMCGCGATGCCAAG(645 bp)
qnrS	P1:ATGGAAACCTACMRTCAYACATAT; P2:TTAGTCAGGAWAAACAACAATACCC(657 bp)
aac(6′)-Ib-Cr	P1:ATGACTGAGCATGACCTTGC; P2:TTAGGCATCACTGCGTGTTC(519 bp)
qepA	P1:GCCGAACGCCGCTGCCGACAG; P2:TGCTGCTGACGCTGGCGCTC(501 bp)
Mobile genetic element	
Genetic marker of plasmid	
traA	P1:AAGTGTTCAGGGTGCTTCTGCGC; P2:GTCATGTACATGATGACCATTT(272 bp)
trbC	P1:CGGYATWCCGSCSACRCTGCG; P2:GCCACCTGYSBGCAGTCMCC(255 bp)
Genetic marker of transposon	
merA	P1:GACCAGCCGCAGTTCGTCTA; P2:GCAGCASGAAAGCTGCTTCA(462 bp)
tnpU	P1:CCAACTGATGGCGGTGCCTT; P2:CGGTATGGTGGCTTTCGC(403 bp)
tnp513	P1:ATGTCGCTGGCAAGGAACGC; P2:GGGTTCGCTGCGAGGATTGT(240 bp)
Genetic marker of insertion sequence	
ISaba1	P1:AATGATTGGTGACAATGAAG; P2:ATGCAGCGCTTCTTTGCAGG(372 bp)
ISaba4	P1:TTACGGATAAGCCAAAGATTTAATC; P2:AGAGGCTACATTAGCCAACCATTA(303 bp)
ISaba9	P1:GTTGTTACTCAGCCCCTGAGA; P2:ACGCTGAATGAGCTGTGCCAT(240 bp)
IS26	P1:CACATGAACCCATTCAAAGGCC; P2:TCTTTGCCCGTGGCACATGGATGAA(240 bp)
IS903	P1:GCAATACGCACGCTTTCAGGC; P2:ACTGCACGGTTACGGTCTGCA(240 bp)
Genetic marker of integron	
intI1	P1:CCGAGGATGCGAACCACTTC; P2:CCGCCACTGCGCCGTTACCA(373 bp)
intI2	P1:CACGGATATGCGACAAAAAGGT; P2:GTAGCAAACGAGTGACGAAATG(789 bp)
intI3	P1:GCCTCCGGCAGCGACTTTCAG; P2:GATGCTGCCCAGGGCGCTCG(433 bp)
qacEΔ1	P1:TAGCGAGGGCTTTACTAAGC; P2:ATTCAGAATGCCGAACACCG(300 bp)
Drug efflux pump	
Drug efflux gene	
adeB	P1:TACCGGTATTACCTTTGCCGGA; P2:GTCTTTAAGTGTCGTAAAAGCCAC(250 bp)
qacEΔ1	P1:TAGCGAGGGCTTTACTAAGC; P2:ATTCAGAATGCCGAACACCG(300 bp)
Resistance island	
Aba-RI	P1:TCCATTTTACCGCCACTTTC; P2:AATCGATGCGGTCGAGTAAC (variable length)

Table 3 Antimicrobial susceptibility profiles of 19 isolates of multidrug-resistant *A. baumannii*

Antimicrobial agent	*Resistance (R%)*	*Intermediate (I%)*	*Susceptibility (S%)*
Cefazolin	19 (100.0)	0 (0)	0 (0)
Ampicillin	19 (100.0)	0 (0)	0 (0)
Cefuroxime	19 (100.0)	0 (0)	0 (0)
Cefotaxime	19 (100.0)	0 (0)	0 (0)
Ceftazidime	17 (89.5)	2 (10.5)	0 (0.0)
Cefepime	19 (94.7)	0 (0.0)	1 (5.3)
Cefoxitin	19 (100.0)	0 (0.0)	0 (0.0)
Imipenem	18 (94.7)	0 (0.0)	1 (5.3)
Meropenem	19 (94.7)	0 (0.0)	1 (5.3)
Piperacillin/tazobactam	16 (84.2)	3 (15.8)	0 (0.0)
Cefoperazone/sulbactam	4 (21.1)	11 (57.8)	4 (21.1)
Gentamicin	6 (31.6)	0 (0.0)	13 (68.4)
Amikacin	2 (10.5)	0 (0.0)	17 (89.5)
Levofloxacin	17 (89.5)	2 (10.5)	0 (0.0)

chromosomal cephalosporinase from *A. baumannii*. However, its biochemical characteristics, biological activity, new host were still unknown, which need further study.

As shown in Table 4, linkage detection of IS*aba*1-$bla_{\text{OXA-23}}$ was positive, which suggested that $bla_{\text{OXA-23}}$ cluster was confirmed as $bla_{\text{OXA-23}}$. In addition, promoter was found upstream of bla_{TEM} in isolate no.10 and no.13 to result in high-level expression of bla_{TEM}, and IS*Aba*1 could also provide strong promoter to upregulate the expression of $bla_{\text{ADC-67}}$ and $bla_{\text{OXA-23}}$ to confer resistance to beta-lactam in positive isolates.[2] Moreover, the epidemic isolates of *A. baumannii* in Korea,[3] Thailand,[4] Australia[5] all carried $bla_{\text{OXA-23}}$, whereas isolates in Italy carried either $bla_{\text{OXA-58}}$-like (22.8%) or $bla_{\text{OXA-23}}$-like (71.1%). However, *A baumannii* isolates in Australia had higher positive rate of bla_{TEM}(88%), and IS*aba*1 were also found upstream of *amp*C beta-lactamase gene and attributed to increased *amp*C expression.[5]

In Gram-negative bacteria, the outer membrane limits the rate of antimicrobials entering the cell, and variations in porin's structure are survival strategies to escape from antibacterial pressure. Resistance to both imipenem and meropenem in MDR *A. baumannii* is associated with the loss of a 29-kDa outer membrane protein, called CarO,[6] and a 43-kDa protein, which shows significant peptide homology with OprD from *P. aeruginosa*.[7] CarO may function as a carbapenem-unspecific channel, and the OprD-like protein may function as a carbapenem-specific channel. In this investigation, full length of CarO and OprD were sequenced and confirmed as mutations in their structure. Moreover, it's the first report that *opr*D was positive in *A. baumannii* in China.

Four kinds of aminoglycoside-modifying enzyme genes: *aac*(3)-I (10.7%), *aac*(6′)-Ib (17.9%), *ant*(2″)-I(14.3%), *aph*(3′)-I(17.9%), and a kind of 16SrRNA methylase gene: *arm*A(17.9%), were positive in PCR assays and involved in aminoglycoside resistance. However, *A.baumannii* isolates in Algeria had higher positive rate of aminoglycoside-modifying enzyme genes: *aph*(3′)-VI (50.7%), *aad*A (63.4%), *aac*(3)-Ia (91.1%).[8]

Futhermore, mutations in the QRDR of *gyrA*(Ser83Leu), and mutations in the QRDR of *par*C (Ser80Leu), would help explain the clinical observation of quinolones resistance. Moreover, *A.baumannii* isolates in Algeria also had double mutations Ser83Leu and Ser80Leu (or Ser84Leu) in *gyr*A and *par*C (69.0%),[8] while isolates in Australia only had Ser80Leu mutation in *gyr*A.[5]

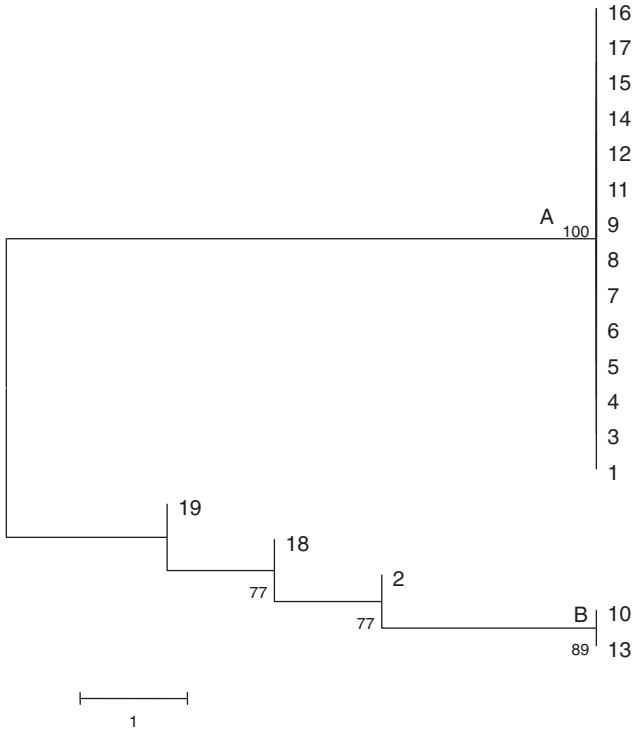

Figure 1 Cluster analysis of 19 isolates of multidrug-resistant *Acinetobacter baumannii* (by MEGA5.0).

In addition, previous studies have demonstrated the importance of efflux pumps in removing antimicrobials from the cell and conferring the MDR phenotype. All 19 isolates contained highly conserved *ade*B gene from the AdeABC efflux pump complex, and 5 isolates contained *qac*EΔ1 to confer resistance to antiseptics and disinfectants.

Resistance islands are common in *A. baumannii* as genomic locations for the acquisition and accumulation of genes required for antibiotic resistance.[9] The most well studied of these RIs is AbaR1, but AbaR1 was negative in all 19 isolates.

Futhermore, mobile genetic elements are major forms of horizontal gene transfer. Five kinds of genetic markers of mobile genetic elements were positive to spread resistance among isolates: tnpU, tnp513, *int*I were positive in five isolates, IS*aba*1 and IS26 were positive in all isolates.

From Figure 1, the 19 isolates could be divided into clusters A and B, with three isolates demonstrating unique profiles depending on Cluster analysis[10] by MEGA5.0. Cluster A included 14 isolates: no. 1, 3～9, 11, 12, 14～17, and their positive models were: $bla_{\text{ADC-67}}$+ $bla_{\text{OXA-2}}$ cluster+ $bla_{\text{OXA-23}}$ cluster+ $bla_{\text{OXA-51}}$ cluster+ *ade*B+ IS*aba*1+ IS26+mutation of (*car*O+ *opr*D+ *gyrA* (Ser83Ler) +*par*C (Ser80Ler)). While cluster B included two isolates: no. 10 and 13. Hence, it suggested that clonal spread emerged among these isolates. In fact, patient transfer and hospital staff contact may have enhanced clonal spread among different wards. Early recognition of the presence of carbapenem-resistant *A. baumannii* clones is necessary in order to prevent their spread within the hospital environment.

Sequencing of the whole genome of these isolates is needed to better understand how they have accumulated these resistance-encoding genes.

Table 4 Positive resistance-associated genes in 19 isolates of multidrug-resistant *A. baumannii*

	Beta-lactam										Aminoglycoside					Quinolone				Drug efflux		Mobile genetic element				
	Class A		Class C		Class D				Porin		Aminoglycoside-modifying enzyme genes				16SrRNA methylase gene	gyrA		parC				Transposon		Insertion sequence		Integron
No.	TEM	Promoter-TEM	ADC	ISaba1 ADC	OXA-2 cluster	OXA-23 cluster	OXA-51 cluster	ISaba1 -OXA23	carO	oprD	aac(3)-I	aac(6″)-Ib	ant(2″)-I	aph(3′)-I	armA	83rd TCA	87th GAA	80th TCG	84th GAA	adeB	qacEΔ1	tnpU	tnp513	ISaba1	IS26	intI1
1	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
2	−	−	ADC 67	+	+	+	+	+	M	M	+	+	+	+	+	TTA	GAA	TTG	GAA	+	+	+	+	+	+	+
3	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
4	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
5	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
6	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
7	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
8	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
9	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
10	+	+	ADC 67	+	+	+	+	+	M	M	+	+	+	+	+	TTA	GAA	TTG	GAA	+	+	+	+	+	+	+
11	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
12	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
13	+	+	ADC 67	+	+	+	+	+	M	M	+	+	+	+	+	TTA	GAA	TTG	GAA	+	+	+	+	+	+	+
14	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
15	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
16	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
17	−	−	ADC 67	+	+	+	+	+	M	M	−	−	−	−	−	TTA	GAA	TTG	GAA	+	−	−	−	+	+	−
18	−	−	ADC67	+	+	+	+	+	M	M	−	+	+	+	+	TTA	GAA	TTG	GAA	+	+	+	+	+	+	+
19	−	−	ADC 67	+	+	+	+	+	M	M	−	+	−	+	+	TTA	GAA	TTG	GAA	+	+	+	+	+	+	+
SUM	2	2	19	19	19	19	19	19	19	19	3	5	4	5	5	19	19	19	19	19	5	5	5	19	19	5

Note. M:mutation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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