Resistance Traits and Molecular Characterization of Multidrug-resistant *Acinetobacter baumannii* Isolates from an Intensive Care Unit of a Tertiary Hospital in Guangdong, Southern China

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Research Article

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Abstract

Purpose:

This study aims to characterize antimicrobial resistance (AMR), with particular reference to carbapenems and aminoglycosides, in MDR *A. baumannii* isolates recovered from an intensive care unit in a tertiary hospital.

Methods:

A. baumannii (*n* = 95 strains) isolated from patients were subjected to antimicrobial susceptibility test (AST) by Vitek 2 Compact system to determine minimum inhibitory concentrations, followed by genotyping by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR). Resistance genes of interest were PCR-amplified and sequenced.

Results:

All isolates were qualified as MDR, with a resistance rate of >80% to 8 antimicrobials tested. In terms of beta-lactamase detection, the bla_{OXA23} and bla_{TEM-1} genes were detected frequently at 92.63% and 91.58%, respectively. The metallo-β-lactamase genes bla_{IMP}, bla_{VIM}, and bla_{NDM} were undetected. *Aph (3')-I* was detected in 82 isolates (86.32%), making it the most prevalent aminoglycoside-modifying enzyme (AMEs) encoding gene. In addition, *ant (3")-I* was detected at 30.53%, while 26.32% of the strains harboured an *aac (6')-Ib* gene. ERIC-PCR typing suggested moderate genetic diversity among the isolates, which might be organized into 10 distinct clusters, with Cluster A (*n* = 86 isolates or 90.53%) being a dominant cluster of epidemic clones.

Conclusions:

Substantial fractions of the *A. baumannii* strains prevailing in the ICU were MDR clones exhibiting extremely high resistance to carbapenems and aminoglycosides as monitored throughout the study period. They principally belonged to a single cluster of isolates carrying bla_{OXA23} and *armA* co-producing different AMEs genes.

Declarations

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Authors' contributions Yuan-Chun Huang designed the experiments, carried out the study, interpreted the data and reviewed the manuscript. Zhuo-Ran Chen, Hui-Wu Guo designed, performed the experiments and wrote the paper. Jun Liu, Mao-Zhang Fu, Ying-Kun Qiu, Qing Pan collected and analyzed the data. All authors read and approved the final manuscript.

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Introduction

Acinetobacter baumannii (*A. baumannii*) is an opportunistic pathogen adept at colonizing and thriving in the hospital environment. In the recent decade, carbapenemase-producing multidrug resistant (MDR) *A. baumannii* has emerged as a prominent cause of healthcare-associated infections (HAIs) notably at intensive care units (ICUs), whose incidence seems to be ascending alarmingly in parts of China (He C et al. 2011; Li Y et al. 2018; Mariana Bitrian et al. 2012; Behdad R et al. 2020). Patients undergoing invasive procedures, immunosuppressive therapy or treatment with broad-spectrum antibiotics are vulnerable to HAIs cause by *A. baumannii*, particularly in the contexts of ventilator-associated pneumonia, bacteremia, septicaemia, urinary tract and wound infections (Mariana Bitrian et al. 2012; del Mar Tomas M et al. 2005; Freire MP et al. 2016; Carmen Gomez-Arrebola et al. 2021).

By virtue of its extraordinary aptitude to survive in the hospital environment and to develop extremely high resistance to an array of common antibiotics including aminoglycoside and carbapenem classes of antibiotics, *A. baumannii* has become a major challenge to medical care at the ICU (Shimose LA et al. 2016; Molter G et al. 2016; Shamsizadeh Z et al. 2017) . One of the most prevalent sequence types (ST) of epidemic clones in China is ST208, which has gained notoriety for causing outbreaks in local ICUs (Bahador A et al. 2015). Analysis on genomic relatedness among clinical isolates is necessary for determining an epidemic strain, as a first step toward informed diagnosis and anti-infective countermeasures.

Although substantial efforts have been made over the years in monitoring the epidemicity and AMR trends of *A. baumannii* in China, the scope of previous studies tends to be limited to highly populous urban centers in northern and eastern China (Ning NZ et al. 2017; Zhou K et al. 2018). In southern China including Guangdong province (population 108.5 million), where the humid subtropical climate indeed favors microbial growth, epidemiological surveys on *A. baumannii* in HAIs were only with moderate frequencies and again covered only very large urban centers such as Guangzhou (population 14.9 million)(Zhou Y et al. 2015; Li Y et al. 2013). In contrast, studies on HAIs by *A. baumannii* and underlying

mechanisms of AMR are otherwise scant in other Chinese regions overlooked in epidemiological survey. In this regard, we undertook the current study to examine the AMR traits, molecular determinants of AMR and clonal relationship of *A. baumannii* strains isolated from an ICU of a teaching tertiary hospital in the Chaoshan metropolitan area (13.93 million residents) in Guangdong province, southern China. We found that the isolates (*n* = 95) generally exhibited very high resistance to most of the commonly used clinical antibiotics including aminoglycosides and carbapenems, carried blaOXA23, blaTEM-1 and *aph (3')-I* as major resistance genes and consisted mostly of strains belonging to a single dominant cluster (Cluster A in ERIC-PCR analysis).

Materials And Methods

Research settings and bacterial isolates

This study was conducted at the ICU of a tertiary-level teaching hospital affiliated to the Shantou University Medical College (SUMC) in Shantou city in Guangdong, a populous province in southern China. The hospital (1816 inpatient beds) serves the Chaoshan metropolitan area in eastern Guangdong. A total of 95 non-duplicated *A. baumannii* isolates were systematically collected from patients' samples during the period of January 1st to December 31st, 2015. This study had been reviewed and approved by the Research Ethics Committee of the First Affiliated Hospital of Shantou University Medical College. The study was given a waiver of informed consent on the ground that it focuses only on characterizing bacterial isolates and involves no patient's information.

Antimicrobial susceptibility

All isolates were first identified by using Vitek 2 Compact system (bioMérieux, France) and their antimicrobial susceptibility profiles obtained by using the Gram negative susceptibility cards (GN16 cards), according to the manufacturer's instructions. Antimicrobial susceptibility test (AST) results for MICs (minimum inhibitory concentrations) were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute (CLSI 2015). Additional validation for *A. baumannii* was done by PCR to test for the intrinsic bla_{OXA51} gene. Confirmed *A. baumannii* isolates were stored at -80°C for subsequent experiments.

Detection of antimicrobial resistance genes

Whole genomic DNA was extracted by using TIANamp Bacteria DNA kit (Tiangen Biotech, China), according to the manufacturer's instructions. Detection of antimicrobial resistance (AMR) genes by PCR amplification was carried out with specific primers (See details in Table 1) to screen for the following genes of interest: extended-spectrum β-lactamases (ESBLs) encoding gene (bla_{TEM-1}, bla_{shv}), metallo-β-lactamases encoding genes (bla_{IMP}, bla_{VIM-2}, bla_{NDM-1}), cephalosporinase encoding gene (bla_{ADC}), OXA carbapenemases encoding genes (bla_{OXA51}, bla_{OXA23}, bla_{OXA24}, bla_{OXA58}), aminoglycoside-modifying enzyme (AME) encoding genes (*aac(6')-Ib*, *ant(3")-I*, *aph(3')-I*) and 16s rRNA methylase encoding gene

(*armA*) were detected. For PCR amplification, the following thermal cycling conditions were adopted: initial denaturation at 94°C for 3 min, followed by 30 cycles (94°C for 1 min, 58-62°C for 1 min and 72°C for 1 min), and a final extension step of 8 min at 72°C. PCR products were separated by electrophoresis (at 100 V through a 1% agarose gel in 0.5×TBE running buffer), stained with ethidium bromide and observed under ultraviolet light. Identity of all PCR products was confirmed by DNA sequencing (Beijing Genomics Institute, BGI).

Genotyping of isolates

For determination of genetic relatedness of the isolates, enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) was performed with primer ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Bahador A et al. 2015) to amplify the conserved sequences of bacterial strains, by using the following thermal cycling conditions: initial denaturation at 94°C for 5 min, 4 cycles (94°C for 1 min, 26°C for 1 min, 72°C for 1 min), then 40 cycles (94°C for 30 sec, 40°C for 30 sec and 72°C for 1 min), and extension at 72°C for 5 min. To resolve the PCR products, each PCR product was analyzed by electrophoresis in a 1.5% agarose gel stained with ethidium bromide. Results for ERIC-PCR banding patterns were appraised by the software Quantity One (version 4.6.2) and scored as absent (0) or present (1) to construct a dendrogram according to the the unweighted pair group (UPGMA) method, using the software NTSYS-pc (version 2.10e). Isolates with more than 90% similarity were considered as belonging to the same cluster.

Statistics analysis

Statistical analysis on the correlation between different resistance genes was performed by rank-order correlation with SPSS 19.0 software. The difference in AMR rates was analyzed by *Chi*-squared test. Statistical significance was determined at p value <0.05.

Results

Isolate characteristics and resistance rates

As shown in Figure 1, resistance rates of total *A. baumannii* against commonly used antibiotics (notably imipenem, gentamycin and tobramycin) had been persistently high(>70%) at the ICU in recent years, especially in a spiral upward trend to the antibiotics such as cefepime (FEP), imipenem (IPM), piperacillin/tazobactam (TZP), gentamycin (GEN) and ciprofloxacin (CIP). LVX was the only suvival with a resistance rate under 70.00%. In this study, a total of 95 non-duplicative *A. baumannii* strains were isolated from ICU patients. Strains from male patients evidently outnumbered those from females at a ratio of 65 (68.42%) to 30 (31.58%). Affected patients had a mean age of 61.93±1.87 years (range of 7 to 89 years old). The major isolation sites were sputum (n = 91), drain (n = 2) and stool (n = 2). As shown in Table 2, AST results suggested that all isolates could be qualified as multidrug resistant (MDR) *A. baumannii,* which showed high rates of extensive resistance to 8 antibiotics tested including cefepime (FEP), ceftriaxone (CRO), imipenem (IPM), gentamycin (GEN), tobramycin (TOB), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT), piperacillin/tazobactam (TZP) at 93.68%, 92.63%, 93.68%,

89.47%, 86.32%, 94.74%, 81.05% and 83.16%, respectively. Importantly, despite a full resistance rate of below 55.00%, levofloxacin (LVX) might not be deemed any more effective than the abovementioned agents against *A. baumannii*, as it had a notable rate of intermediate-level resistance (37.90%). The susceptibility rates of the isolates toward all tested antibiotics were below 20.00%.

Genotypic patterns in ERIC-PCR analysis

ERIC-PCR was used to compare the genetic relatedness among the *A. baumannii* isolates. All PCR banding patterns ranging from 550 bp to 2000 bp were analyzed by the NTSYS software to construct a dendrogram, as shown in Figure 2. In general, the analyzed *A. baumannii* strains could be organized into 10 distinct clusters, where 86 (or 90.53%) of the isolates belonged to the major Cluster A, while the remaining 9 isolates exhibited substantially different banding patterns, additionally designated as Clusters B, C, D, F, G, H, J and K. In longitudinal analysis, strains belonging to Cluster A were detectable throughout the study period in 2015, indicating that members of this cluster correspond to major epidemic clones.

Determination of antimicrobial resistance genes

Analysis on AMR genes suggests that the *A. baumannii* isolates included in this study had high carriage rates for some specific AMR genes. Among the 95 strains, 87 (91.58%) were tested positive for the ESBL encoding gene bla_{TEM-1}, while all strains contained the cephalosporinase gene bla_{ADC}. In terms of detection of carbapenemase genes, 88 strains (92.63%) were found to harbour the bla_{OXA23} gene, while the bla_{OXA51} gene was universally detected in all isolates, consistent with the premise that it is an intrinsic resistance determinant in *A. baumannii.* Gene *armA*, a member of 16S rRNA methylases, was detected in 84 isolates (88.42%), while the most prevalent AMEs encoding gene *aph (3')-I* was found in 82 isolates (86.32%). In comparison, 29 (30.53%) and 25 (26.32%) of the isolates harboured the *ant (3")-I* and *aac (6')-Ib* genes, respectively. In further analysis, the genes bla_{SHV}, bla_{IMP}, bla_{VIM-2}, bla_{NDM-1}, bla_{OXA24} and bla_{OXA58} were undetected in any of the *A. baumannii* strains.

Through genotyping and detection of resistance genes, we classified the 95 isolates of *A. baumannii* in this study, as shown in Table 3. Cluster A could be categorized into 9 subtypes on the basis of different AMR gene combinations. The most prevalent subtype in Cluster A was subtype Ai, comprising 55 (63.95%) isolates expressing the genes bla_{OXA23}, bla_{OXA51}, bla_{TEM-1}, bla_{ADC}, *armA* and *aph (3')-I*. Among subtype Ai isolates, 38 (44.19%) were insensitive to all of the antibiotics tested. Twenty two (25.58%) isolates in group Aii harboured 8 different genes, namely, bla_{OXA23}, bla_{OXA51}, bla_{TEM-1}, bla_{ADC}, *armA, aph (3')-I, aac (6')-Ib,* and *ant (3")-I.* The 20 isolates in subtype Aii were also insensitive to all antibiotics tested. Gene bla_{TEM-1} was absent in group Aix which, however, was also found to be insensitive to cefepime and ceftriaxone. The aminoglycoside resistance genes *aph (3')-I* and *ant (3")-I* were only detected in subtypes Aviii and Aix, but surprisingly these were susceptible to gentamycin and tobramycin. The rest of the cluster showed various combinations of AMR genes, presumably giving rise to different resistance patterns. Members of Clusters C, E, J and K also expressed the genes bla_{OXA51} and bla_{ADC} and showed an

appreciably higher level of susceptibility than isolates in other clusters. Notably, despite the absence of carbapenemase gene *bla*$_{OXA23}$ in Cluster F, its members showed insensitivity to imipenem, in comparison with other clusters.

Discussion

ERIC-PCR, a genotyping method premised on amplification of conserved regions of genomic DNA, has the advantage of facile instrumentation and reliability comparable to Pulsed Field Gel Electrophoresis (PFGE). It has been proven useful for determining genomic relationship across strains with heterogeneous backgrounds (Cartelle Gestal M et al. 2016; Ece G et al. 2015). In the present study, a dendrogram based on ERIC-PCR results identifies 10 distinct clusters (A, B, C, D, E, F, G, H, J, K). Notably, 90.52% (86/95) of the strains were classified into Cluster A, the principal cluster. Indeed, throughout the study period, isolates belonging to Cluster A were detected each month. In terms of resistance phenotype, strains in Cluster A were consistently more insensitive to all tested antibiotics than were strains in other clusters. This suggested that a single dominant clone of MDR-*A. baumannii* prevailed in the ICU in 2015 (Jan. to Dec.). By using ERIC-PCR as a genotyping method, Ning and coworkers reported carbapenem-resistant clones of *A. baumannii* spreading at an ICU in western China (Ning NZ et al. 2017). Chen and coworkers also described a major epidemic strain spreading at different hospital units in Hunan province of southern China (Chen D et al.2016). In our study, the spreading of *A. baumannii* strains in the ICU lasted for a substantial period and their resistance rates to antibiotics were extremely high. We found that among the 9 subtypes of strains within Cluster A, the most frequent type of AMR gene combination was *bla*$_{OXA23}$*-bla*$_{OXA51}$*-bla*$_{TEM-1}$*-bla*$_{ADC}$*-aph (3')-I-armA,* which accounted for 63.95% (55/86) of the cases. Strains harbouring this gene combination could be routinely isolated throughout the study period, suggesting the existence of entrenched extrinsic factors favoring their spread. Cross-transmission and contamination within the ward environment might underpin this process, which calls for greater awareness for monitoring and timely disinfection of the ward environment.

In our study, we found that multidrug-resistant *Acinetobacter baumannii (*MDRAB) strains simultaneously carrying the *bla*$_{OXA23}$ gene and multiple aminoglycoside resistance genes are apparently spreading in southern China. The carriage of *bla*$_{OXA23}$ carbapenemases in *A. baumannii* has been documented worldwide and *bla*$_{OXA23}$ was one of the most prevalent carbapenemase genes detected in Chinese hospitals (Ruan Z et al. 2013; Shoja S et al. 2017). While the prevalence of *A. baumannii* co-expressing aminoglycoside resistance genes and carbapenemases genes have been reported in eastern China (Wang Y et al. 2016), to the best of our knowledge, there have been no studies on the epidemicity of *A. baumannii* co-carrying AMR genes against aminoglycosides and carbapenems in southern China.

A high percentage (95.80%; *n* = 91) of the total strains in our study originated from sputum, which is similar to findings in a recent epidemiological study covering 10 tertiary-care teaching hospitals in northern China (Jiang M et al. 2016).It has been suggested that most of the HAIs took the form of

ventilator-associated pneumonia caused by mechanical ventilation, which occasionally even gave rise to outbreaks in ICUs by cross-infections (Munoz-Price LS and Weinstein RA. 2008; Peng H et al. 2015).

A. baumannii resistance rates to antibiotics seem to be increasing across the years. That high resistance to almost all antibiotics tested in this study indicated doctors were facing embarrassed situations on selecting antibiotic for infections caused by MDRAB in ICU. Since current treatment options for MDRAB were extremely limited, colistin is often considered as the last line of therapy for it (Gounden et al. 2009), although colistin-resistant *A. baumannii* had been reported (Al-Agamy MH et al. 2014).

The ESBL-producing genes bla_{TEM-1} and bla_{ADC} identified in this study belong to Ambler class A and class C on β-lactamase coding genes, respectively. The gene bla_{ADC} along with the chromosomally located gene bla_{OXA51} were consistently detected in *A. baumannii*, suggesting that they may be useful as signature genes for species confirmation. With the absence of bla_{TEM-1} gene, strains in Clusters C, D, E, H, J and K were susceptible to FEP compared to those in Cluster F and G, but were still insensitive to CRO. In view of the above observations, we speculated that strains with bla_{TEM-1} gene may gain advantage in FEP resistance. It is noteworthy that bla_{TEM-1} gene was the most prevalent ESBL gene in the present study, which differs from a previous study, where bla_{CTX-M} was reported to be the predominant ESBL gene (Mahamat A et al. 2016).

Conventionally, aminoglycosides are used in combination with β-lactams in anti-infective regimens to treat *A. baumannii* infections (Wang Y et al. 2016). However, aminoglycoside resistance *A. baumannii* has been reported with increasing frequency in China in recent years (Gao L et al. 2017; Jiang M et al. 2014; Lin T et al. 2015). The resistance rates for GEN and TOB in this study were 89.47% and 86.32%, respectively (Table 2). A study reported by Wen *et al.* (2014) showed high resistance rates in Jiangsu province, eastern China. Aminoglycoside modify enzymes (AMEs) and 16S rRNA methylase have been attributed as a molecular basis for aminoglycoside resistance. In the present study, 3 different AMEs and one 16S rRNA methylase genes were detected. The most prevalent of the AMEs was *aph(3')-I* (86.32%), followed by *ant(3")-I* (30.53%), with 84 (88.42%) of the strains carrying *armA*. In a study on *A. baumannii* from Jiangsu province, China the most prevalent AMEs were identified as *aac(3')-I* and *aac(6')-Ib* (Wen JT et al. 2014). We noted that *A. baumannii* from different parts of China feature different aminoglycoside resistance genes (Wang H et al. 2017). For example, the most representative aminoglycoside resistance gene combination in the present study was *armA-aph(3')-I* (58.95%). It is known that AMEs genes could be detected in both aminoglycoside-resistant and susceptible strains (as in Cluster H). Gene products of AMEs are reportedly responsible for moderate-level resistance, whereas 16S rRNA methylase has been attributed to high-level aminoglycosides resistance (Wang Y et al. 2016). Interestingly, strains in Cluster B exhibited susceptibility to both GEN and TOB with only *armA* gene being detected. Some possible reasons include that 16S rRNA methylase may not be acting alone and that some uncharacterized mechanisms modulate *armA* activity.

In addition, high levels of aminoglycoside resistance co-occurring with carbapenems resistance have been reported in epidemic clones of *A. baumannii* from western China (Lin T et al. 2015). The imipenem

resistance rates of *A. baumannii* were extremely high in China and numerous studies have raised concerns over the emergence and spread of imipenem-resistant *A. baumannii* in hospitals (Neves FC et al. 2016). Resistance rates for imipenem reported in different Chinese ranged from 58% to 100% (Jiang M et al. 2016; Zong Z et al. 2008; Ji S et al. 2014; Wu W et al. 2015)and this alarming trend seems to continue to rise unabated each year. For *A. baumannii* strains included in this study, the resistance rate for imipenem was 93.68%. Our current results suggested that efficacy of carbapenems as treatment for MDR-AB infections seemed to be fast diminishing, especially in ICU contexts. Ruan and coworkers found that a carbapenem-resistant status of *A. baumannii* isolates was predictive of high resistance rates to commonly used antibiotics. In terms of mechanisms, carriage of metallo-β-lactamases (MBLs) and carbapenem-hydrolyzing class D β-lactamases (CHDLs) has been attributed to carbapenem resistance (Ruan Z et al. 2013). A growing body of literature documents bla_{OXA23} as a predominant carbapenemase genotype among epidemic clones in China (Chen Y et al. 2017; Rapee Thummeepak et al. 2016) and outbreaks caused by bla_{OXA23} producing *A. baumannii* paralleled those occurring worldwide (Neves FC et al. 2016; Hammoudi D et al. 2015;Novovic K et al. 2015;Koh TH et al. 2007;Martins AF et al. 2009). In this present study, we found that 88 of the *A. baumannii* strains (92.63%) harboured a bla_{OXA23} gene, suggestive of a level of prevalence seen in other parts of China (Ana Kovacic et al. 2017). In contrast, alternative AMR genes supporting the developing of MDR phenotypes such as bla_{OXA24}, bla_{OXA58}, bla_{IMP}, bla_{VIM-2}, bla_{NDM-1} genes were detected. Collectively, we proposed that the presence of bla_{OXA23} gene could be a cardinal molecular determinant of carbapenem resistance in our study.

Conclusion

In this study, we described the resistance traits and genetic relatedness of MDR *A. baumannii* strains with high resistance that prevailed at the ICU of a teaching tertiary hospital in the Chaoshan area of Guangdong province, a populous yet epidemiologically overlooked region in southern China. Extreme resistance to carbapenem and aminoglycoside classes of antibiotics including imipenem and gentamycin/tobramycin may be associated with the carriage of bla_{OXA23} and AMEs genes as determined in PCR assays. A single Cluster A of epidemic clones seemed to dominate the spread of MDR A. baummannii at the ICU. Surveillance work in this study represents a first step towards a better understanding of MDR *A. baumannii* as a causative agent in ICUs, which calls for greater attention to continued monitoring and rational use of antibiotics.

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Tables

Table 1. Primer sequences used in this study for detecting resistance genes.

Target gene	Sequence 5'→3'	Annealing temp. (°C)	Amplicon size (bp)
bla_{TEM-1}	ACCCAGAAACGCTGGTGAAA	57	724
	TGACTCCCCGTCGTGTAGAT		
bla_{shv}	TTATCTCCCTGTTAGCCACC	55	795
	GATTTGCTGATTTCGCTCGG		
bla_{IMP}	AATTGAGAAGCTTGAAGAAGGCG	56	621
	TTAACAGCCTGCTCCCATGT		
bla_{VIM-2}	AGTCTCCACGCACTTTCAT	57	505
	CACAACCACCATAGAGCACA		
bla_{NDM-1}	GGTTTGGCGATCTGGTTTTC	55	621
	CGGAATGGCTCATCACGATC		
bla_{ADC}	TAAACACCACATATGTTCCG	58	663
	ACTTACTTCAACTCGCGACG		
bla_{OXA51}	TCCAAATCACAGCGCTTCAA	57	703
	TCGAACAGAGCTAGGTATTCCTTT		
bla_{OXA23}	TTTCTGGTTGTACGGTTCAGCA	57	646
	AACCAGCCCACTTGTGGTTTT		
bla_{OXA24}	GTTTCTCTCAGTGCATGTTCATCT	55	664
	CCCAACCAGTCAACCAACCT		
bla_{OXA58}	CCAATCGGCTTTTTCTTCAGCA	56	837
	TCATCACCAGCTTTCATTTGCAT		
aac(6')-Ib	TTGCGATGCTCTATGAGTGGCTA	57	482
	CTCGAATGCCTGGCGTGTTT		
ant(3")-I	GCCATACAGCGATATTGATTTG	58	306
	AAGGCAACGCTATGTTCTCTTG		
aph(3')-I	CGTTGCCAATGATGTTACAGAT	58	333
	TTACGCTCGTCATCAAAATCAC		
armA	TGAAAAGGTTGTTTCCATTTCTGA	57	669

	TCATTCCCTATAACCTTCGAATCA

Table 2 Antimicrobial susceptibility profiles of *A. baumannii* isolates.

Antibiotics	Resistance		Intermediate		Susceptible		MIC range	MIC_{50}	MIC_{90}
	n	Rate (%)	*n*	Rate (%)	*n*	Rate (%)			
FEP	89	93.68	0	0.00	6	6.32	1-64	64	64
CRO	88	92.63	7	7.37	0	0.00	1-64	64	64
IPM	89	93.68	0	0.00	6	6.32	1-16	16	16
GEN	85	89.47	2	2.11	8	8.42	1-16	16	16
TOB	82	86.32	0	0.00	13	13.68	1-16	16	16
LVX	51	53.68	36	37.90	8	8.42	0.25-8	4	8
CIP	90	94.74	0	0.00	5	5.26	0.25-4	4	4
SXT	77	81.05	0	0.00	18	18.95	1-16	16	16
TZP	79	83.16	4	4.21	12	12.63	4-28	128	128

Table 3. Classification of MDR *A. baumannii* isolates based upon ERIC-PCR and genotypic profiles.

Cluster	*n* (%)	Subtype (*n*)	Resistance genes[a]	Resistance patterns (R+I)[b]
A	86 (90.35)	Ai (55)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *aph(3')-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT (40)
				FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT (6)
				FEP, CRO, IPM, GEN, TOB, LVX, CIP (5)
				FEP, CRO, IPM, GEN, TOB, LVX, CIP (4)
				FEP, CRO, IPM, GEN, TOB, CIP (1)
		Aii (22)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *aph(3')-I*, *aac(6')-Ib*, *ant(3")-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT (20)
				FEP, CRO, IPM, GEN, TOB, LVX, CIP (1)
				FEP, CRO, IPM, GEN, TOB, LVX, CIP (1)
		Aiii (2)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *aac(6')-Ib*, *ant(3")-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT
		Aiv (1)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *ant(3")-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP
		Av (1)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *aph(3')-I*, *ant(3")-I*	FEP, CRO, IPM, GEN, LVX, CIP, SXT
		Avi (1)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *aac(6')-Ib*, *ant(3")-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT
		Avii (2)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT
		Aviii (1)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *bla*$_{TEM-1}$, *aph(3')-I*	FEP, CRO, IPM, LVX, CIP, SXT
		Aix (1)	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *ant(3")-I*	FEP, CRO, IPM, LVX, CIP
B	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{OXA23}$, *armA*	FEP, CRO, IPM, SXT
C	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$	CRO
D	1	–	*bla*$_{OXA51}$, *bla*$_{ADC}$, *aph(3')-I*, *ant(3")-I*	CRO, GEN, LVX, CIP, SXT

	(1.05)			
E	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$	CRO, CIP
F	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$, *bla*$_{TEM-1}$	FEP, CRO, IPM, GEN, TOB, LVX, CIP, SXT
G	1 (1.05)	–	*bla*$_{OXA23}$, *bla*$_{TEM-1}$, *armA*, *aph(3')-I*	FEP, CRO, IPM, GEN, TOB, LVX, CIP
H	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$, *aph(3')-I*	CRO
I	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$	CRO
J	1 (1.05)	–	*bla*$_{OXA51}$, *bla*$_{ADC}$	CRO, CIP, SXT

[a] All isolates were tested negative for *bla*$_{SHV}$, *bla*$_{VIM-2}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{OXA58,}$ *bla*$_{OXA24}$;

[b] R+I: Resistant and intermediate;

Figures

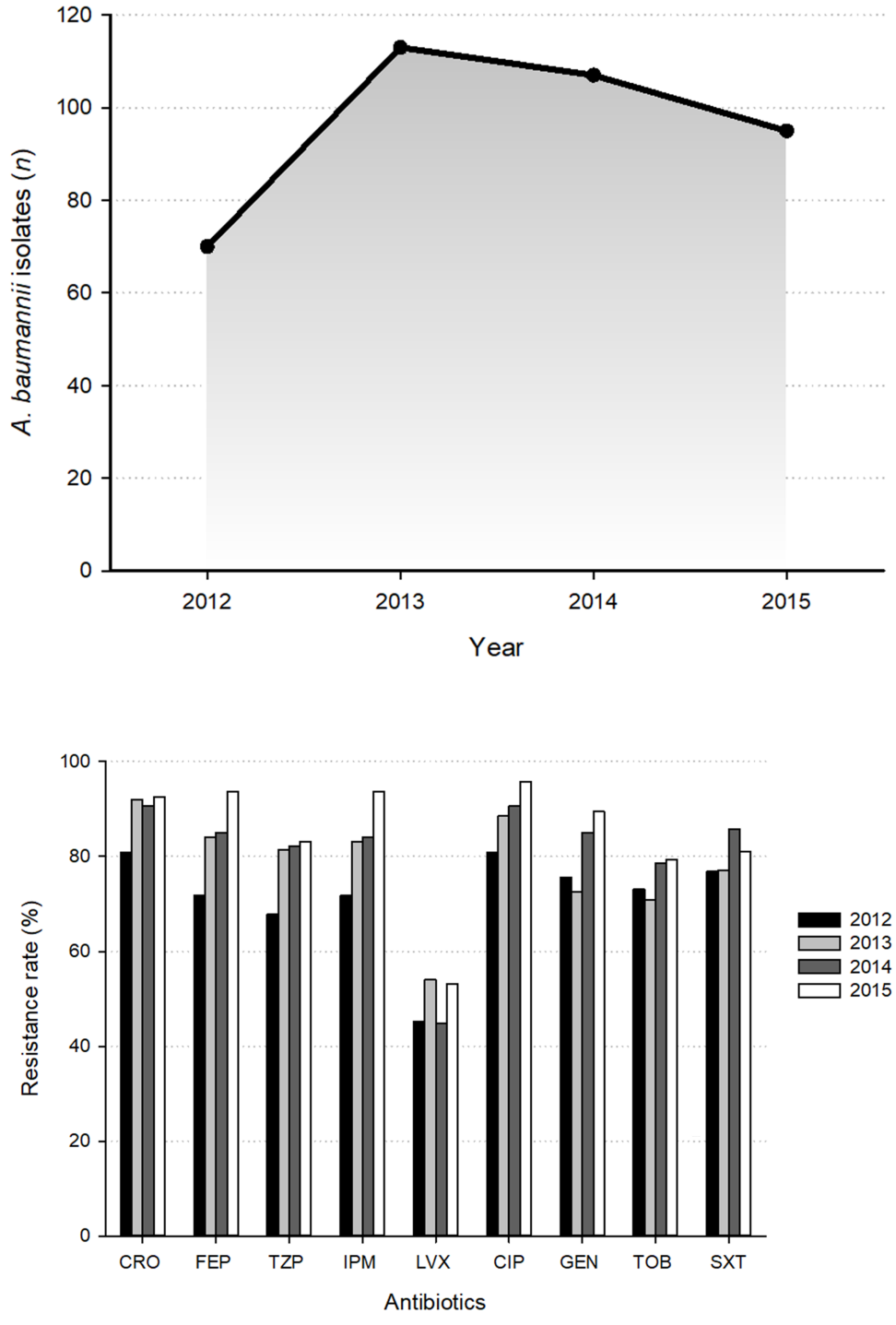

Figure 1

Distribution of A. baumannii isolates recovered at the ICU (upper panel) and their resistance rates for individual antibiotics (lower panel) over the period of 2012 to 2015.

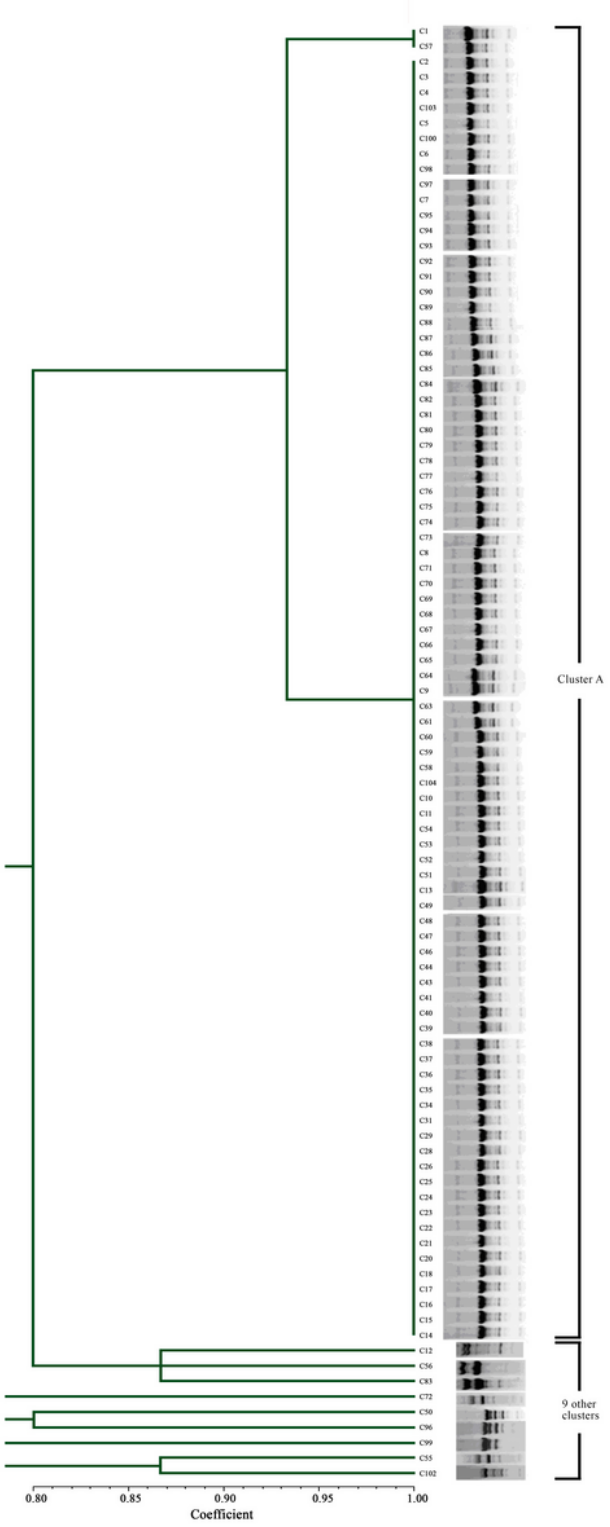

Figure 2

Dendrogram depicting genetic relationships of A. baumannii isolates.