Observational Study

Medicine®

OPEN

$bla_{OXA-23-like}$ and bla_{TEM} rather than $bla_{OXA-51-like}$ contributed to a high level of carbapenem resistance in *Acinetobacter baumannii* strains from a teaching hospital in Xi'an, China

Lei Han, PhD[a,b], Jine Lei, MMed[a,b,c], Jiru Xu, PhD[a,b], Shaoshan Han, MD, PhD[d,*]

Abstract

Acinetobacter baumannii is one of the major threats in clinical infections due to its antibiotic resistance ability. It shows increasing resistance to carbapenems, mainly due to β-lactamase mediated mechanisms. The aim of this study was to investigate carbapenem resistance (CR) profiles and analyze β-lactamases genes composition of clinical *A. baumannii* strains from a teaching hospital in Xi'an. The resistance patterns to imipenem and meropenem were checked for 51 clinical *A. baumannii* strains. The existence of 15 β-lactamases genes was detected by polymerase chain reaction (PCR), and the positive genes were sequenced. The correlation between PCR-positive genes and CR phenotype was analyzed using Chi-square test and contingency coefficient. The expressions of PCR-positive genes were investigated. Forty-five out of 51 strains were resistant to imipenem and meropenem. bla_{TEM}, $bla_{OXA-23-like}$, and $bla_{OXA-51-like}$ were positive among 15 β-lactamases genes, and TEM-1, OXA-23, and OXA-66/69 were their subtypes. TEM and OXA-23-like only showed up in CR isolates, with the occurrence rate of 91.1% and 97.8%, respectively, whereas OXA-51-like appeared in all strains. IS*Aba1* was present in the upstream of OXA-23-like, but absent from that of OXA-51-like in our strains. OXA-23-like had highest relationship with CR, followed by TEM, but OXA-51-like had no correlation. This was verified by RT-qPCR that the expression was positive for OXA-23 and TEM-1, but negative for OXA-66/-69. A high rate of CR *A. baumannii* was detected in this study. Coexistence of TEM, OXA-23-like, and OXA-51-like was the primary resistance profile. The expressions of OXA-23-like and TEM genes were closely related with CR, while OXA-51-like had no contribution to the CR phenotype.

Abbreviations: CR = carbapenem resistance, CS = carbapenem susceptible, MH = Muller-Hinton, MICs = minimal inhibitory concentrations, PCR = polymerase chain reaction.

Keywords: β-lactamases genes, *Acinetobacter baumannii*, $bla_{OXA-23-like}$, $bla_{OXA-51-like}$, bla_{TEM}, carbapenem resistance

1. Introduction

Acinetobacter baumannii is one of the leading causes of nosocomial infections, and is responsible for increasing number of infections in intensive care units (ICUs) worldwide.[1] It is resistant to a number of commonly used antimicrobial agents intrinsically, and has a notable capacity to develop antibiotic resistance via diverse mechanisms.[2,3]

As atypical β-lactam antibiotics, carbapenems also show their bactericidal effects by inhibiting penicillin-binding proteins (PBPs), which are involved in cell-wall synthesis.[4] Carbapenems are widely used in the treatment of multidrug-resistant *A. baumannii* infections. However, resistance to these antibiotics has remarkably increased due to β-lactamase mediated and non-β-lactamase mediated mechanisms.[5] The production of Ambler class A, class B, and class D β-lactamases is the main reason for β-lactamase mediated resistance.[6]

In this study, the resistance patterns to imipenem and meropenem were investigated for 51 nonduplicate *A. baumannii* clinical strains collected from the First Affiliated Hospital of Xi'an Jiaotong University in Xi'an, China. The molecular determinants responsible for carbapenem resistance (CR), and their relationship with CR phenotype were further analyzed. Moreover, the expressions of positive CR genes were determined to reveal the particular genes playing a key role in CR.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Fifty-one *A. baumannii* clinical strains were collected from the First Affiliated Hospital of Xi'an Jiaotong University in 2013. The isolates were identified by VITEK 2 system (bioMérieux, Marcy l'Etoile, France), and further confirmed using specific PCR

Editor: Fateh Rahimi.

Funding/support: This work was supported by the Natural Science Foundation of Shaanxi Province (2016JQ8027 and 2016JQ8029), and the Fundamental Research Funds for the Central Universities (xjj2017140).

There is no conflict of interest.

[a] Department of Microbiology and Immunology, School of Basic Medical Science, Xi'an Jiaotong University Health Science Center, [b] Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education, [c] Department of Laboratory Medicine, [d] Department of Hepatobiliary Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China.

[*] Correspondence: Shaoshan Han, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, 277 West Yanta Road, Xi'an 710061, China (e-mail: han.shaoshan@foxmail.com).

Copyright © 2017 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

Medicine (2017) 96:48(e8965)

Received: 12 July 2017 / Received in final form: 5 November 2017 / Accepted: 8 November 2017

http://dx.doi.org/10.1097/MD.0000000000008965

with primers sp2F, sp4F, and sp4R.[7] *A. baumannii* standard stain ATCC 19606 was kept in our laboratory and used as a positive control. The bacteria were grown on Mueller–Hinton agar (MHA; OXOID, Hampshire, UK) plates and stored in Muller–Hinton broth (MHB; OXOID, Hampshire, UK) containing 20% glycerol at −80°C.

2.2. Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using VITEK 2 system. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The minimal inhibitory concentrations (MICs) of imipenem and meropenem were determined by Etest strips (BIO-KONT, Wenzhou, China), which had antibiotic concentrations of 0.008, 0.016, 0.032, 0.064, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 μg/mL. The susceptibility results were interpreted according to the M100-S27 guidelines of Clinical and Laboratory Standards Institute (CLSI).[8]

2.3. DNA extraction and detection of carbapenem resistance genes

Genomic DNAs of 51 *A. baumannii* strains were extracted using TIANamp Bacteria DNA Kit (TIANGEN, Beijing, China) according to the manufacturer's instruction. A total of 15 β-lactamase encoding genes were screened, in which bla_{KPC}, bla_{GES}, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}1,3,10–12,15}$ were class A β-lactamases genes, $bla_{IMP\text{-}1,4–6,9,10,18}$, $bla_{IMP\text{-}2,8,13,19,20}$, $bla_{VIM\text{-}1,2,4,5}$, $bla_{VIM\text{-}2,6,8–11}$, $bla_{SIM\text{-}1}$, $bla_{NDM\text{-}1}$ were class B β-lactamases genes, and $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$ were class D β-lactamases genes.[9] The primer sequences are summarized in Table 1. PCR was performed using Golden Easy PCR System (TIANGEN, China) in a total volume of 25 μL containing 12.5 μL of 2 × Reaction Mix, 0.5 μmol/L of each primer, 0.5 U of Golden DNA Polymerase, and 1 μL of DNA template. The amplification reaction consisted of a predenaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 35 seconds, and extension at 72°C for 1 minute, followed by a final elongation at 72°C for 10 minutes. Positive PCR products were purified and sequenced by Sangon Biotech (Shanghai, China). In addition, the existence of IS*Aba1* in the upstream of $bla_{OXA\text{-}23\text{-}like}$ and $bla_{OXA\text{-}51\text{-}like}$ genes was also investigated using primer pairs IS*Aba1*F/OXA-23-likeR and IS*Aba1*F/OXA-51-likeR respectively according to Turton et al.[10]

2.4. Correlation analysis between CR genes and CR phenotype

The correlation of CR genes with CR phenotype was statistically analyzed by Chi-square test using SPSS 22.0 (SPSS Inc, Chicago, IL.). *P* value and contingency coefficient were obtained for each gene. The gene was considered correlated with CR phenotype if *P* value < .01. A higher contingency coefficient suggested stronger correlation.

2.5. RNA extraction and quantitative real-time PCR (RT-qPCR)

RNA templates were extracted with RNAprotect Bacteria Reagent (QIAGEN, Hilden, Germany) and RNeasy Mini Kit (QIAGEN) following the manufacturer's protocol. Residual DNA was removed by DNase I (Thermo Scientific, Vilnius, Lithuania). One microgram of DNA-free total RNA from each sample was subsequently reverse transcribed to cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) with random hexamer primer. The expression levels of PCR-positive genes TEM (*TEM-1*), OXA-23-like (*OXA-23*), and OXA-51-like (*OXA-66* and *OXA-69*) were analyzed by RT-qPCR using the Agilent Mx3005P QPCR System (Agilent Technologies, Santa Clara) and SYBR Select Master Mix (Applied Biosystems, Austin) in a final volume of 20 μL. The primers used for analysis are listed in Table 1. Cycling condition was carried out at 50°C for 2 minutes and 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds, 55°C for 15 seconds, and 72°C for 1 minute. Triplicate samples were analyzed and standardized against 16S rRNA gene expression.[11]

Table 1

Primers used in this study.

Primer	Direction	Primer sequence (5'-3')	Reference
bla_{KPC}	Forward	TGTCACTGTATCGCCGTC	[9]
	Reverse	CTCAGTGCTCTACAGAAAACC	
bla_{GES}	Forward	GTTTTTGCAATGTGCTCAACG	[9]
	Reverse	TGCCATAGCAATAGGCGTAG	
bla_{TEM}	Forward	ATAAAATTCTTGAAGACGAAA	[9]
	Reverse	GACAGTTAGCAATGCTTAATCA	
bla_{SHV}	Forward	GCCTTTATCGGCCCTCACTCAAG	[9]
	Reverse	TTAGCGTTGCCAGTGCTCGATCA	
$bla_{CTX\text{-}M\text{-}1,3,10–12,15}$	Forward	CGTCACGCTGTTGTTAGGAA	[9]
	Reverse	ACCGTCGGTGACGATTTTAG	
$bla_{IMP\text{-}1,4–6,9,10,18}$	Forward	ACCGCAGCAGAGTCTTTGCC	[9]
	Reverse	ACAACCAGTTTTGCCTTACC	
$bla_{IMP\text{-}2,8,13,19,20}$	Forward	GTTTTATGTGTATGCTTCC	[9]
	Reverse	AGCCTGTTCCCATGTAC	
$bla_{VIM\text{-}1,2,4,5}$	Forward	AGTGGTGAGTATCCGACAG	[9]
	Reverse	ATGAAAGTGCGTGGAGAC	
$bla_{VIM\text{-}2,6,8\text{-}11}$	Forward	ATGTTCAAACTTTTGAGTAAG	[9]
	Reverse	CTACTCAACGACTGAGCG	
$bla_{SIM\text{-}1}$	Forward	TACAAGGGATTCGGCATCG	[9]
	Reverse	TAATGGCCTGTTCCCATGTG	
$bla_{NDM\text{-}1}$	Forward	ATGGAATTGCCCAATATTATGCACCCGG	[9]
	Reverse	TCAGCGCAGCTTGTCGGCCATG	
$bla_{OXA\text{-}23\text{-}like}$ seq	Forward	GATGTGTCATAGTATTCGTCG	[9]
	Reverse	TCACAACAACTAAAAGCACTG	
$bla_{OXA\text{-}24\text{-}like}$ seq	Forward	CAAGAGCTTGCAAGACGGACT	[9]
	Reverse	TCCAAGATTTTCTAGCTACTTATA	
$bla_{OXA\text{-}51\text{-}like}$ seq	Forward	ATGAACATTAAAGCACTC	[9]
	Reverse	CTATAAAATACCTAATTGTTC	
$bla_{OXA\text{-}58\text{-}like}$ seq	Forward	TTATCAAAATCCAATCGGC	[9]
	Reverse	TAACCTCAAACTTCTAATTC	
IS*Aba1*F	Forward	CACGAATGCAGAAGTTG	[10]
OXA-23-likeR	Reverse	ATTTCTGACCGCATTTCCAT	
OXA-51-likeR	Reverse	TGGATTGCACTTCATCTTGG	
TEM-1 qRT	Forward	ACCAACACGCTTCACTTCCT	This study
	Reverse	CTGCAACTTTATCCGCCTCC	
OXA-23 qRT	Forward	TAATGCTCTAAGCCGCGCAA	This study
	Reverse	TGACCTTTTCTCGCCCTTCC	
OXA-66+69 qRT	Forward	TCGGCCTTGAGCACCATAAG	This study
	Reverse	ACCAACACGCTTCACTTCCT	
16S rRNA	Forward	CAGCTCGTGTCGTGAGATGT	[11]
	Reverse	CGTAAGGGCCATGATGACTT	

2.6. Ethical review

This study was approved by the Ethics Committee of Xi'an Jiaotong University Health Science Center.

Table 2

Antibiotic resistance patterns of *A. baumannii* strains.

Strain	Imipenem MIC, μg/mL	Meropenem MIC, μg/mL	β-lactamase encoding genes content*
1	≥32	≥32	TEM, OXA-23-like, OXA-51-like
2	≥32	≥32	TEM, OXA-23-like, OXA-51-like
3	≥32	≥32	TEM, OXA-23-like, OXA-51-like
4	≥32	≥32	TEM, OXA-23-like, OXA-51-like
5	≥32	≥32	TEM, OXA-23-like, OXA-51-like
6	≥32	≥32	TEM, OXA-23-like, OXA-51-like
7	≥32	≥32	TEM, OXA-23-like, OXA-51-like
8	≥32	≥32	TEM, OXA-23-like, OXA-51-like
9	≥32	≥32	TEM, OXA-23-like, OXA-51-like
10	≥32	≥32	TEM, OXA-23-like, OXA-51-like
11	≥32	≥32	TEM, OXA-23-like, OXA-51-like
12	≥32	≥32	TEM, OXA-23-like, OXA-51-like
13	≥32	≥32	TEM, OXA-23-like, OXA-51-like
14	≥32	≥32	TEM, OXA-23-like, OXA-51-like
15	≥32	≥32	TEM, OXA-23-like, OXA-51-like
16	≥32	≥32	TEM, OXA-23-like, OXA-51-like
17	≥32	≥32	TEM, OXA-23-like, OXA-51-like
18	≥32	≥32	TEM, OXA-23-like, OXA-51-like
19	≥32	≥32	TEM, OXA-51-like
20	≥32	≥32	TEM, OXA-23-like, OXA-51-like
21	≥32	≥32	TEM, OXA-23-like, OXA-51-like
22	≥32	≥32	TEM, OXA-23-like, OXA-51-like
23	≥32	≥32	TEM, OXA-23-like, OXA-51-like
24	≥32	≥32	TEM, OXA-23-like, OXA-51-like
25	≥32	≥32	TEM, OXA-23-like, OXA-51-like
26	≥32	≥32	TEM, OXA-23-like, OXA-51-like
27	≥32	≥32	TEM, OXA-23-like, OXA-51-like
28	≥32	≥32	TEM, OXA-23-like, OXA-51-like
29	≥32	≥32	TEM, OXA-23-like, OXA-51-like
30	≥32	≥32	TEM, OXA-23-like, OXA-51-like
31	≥32	≥32	TEM, OXA-23-like, OXA-51-like
32	≥32	≥32	TEM, OXA-23-like, OXA-51-like
33	≥32	≥32	OXA-23-like, OXA-51-like
34	≥32	≥32	TEM, OXA-23-like, OXA-51-like
35	≥32	≥32	TEM, OXA-23-like, OXA-51-like
36	≥32	≥32	TEM, OXA-23-like, OXA-51-like
37	≥32	≥32	TEM, OXA-23-like, OXA-51-like
38	≥32	≥32	TEM, OXA-23-like, OXA-51-like
39	≥32	≥32	OXA-23-like, OXA-51-like
40	≥32	≥32	TEM, OXA-23-like, OXA-51-like
41	≥32	≥32	TEM, OXA-23-like, OXA-51-like
42	≥32	≥32	TEM, OXA-23-like, OXA-51-like
43	≥32	≥32	OXA-23-like, OXA-51-like
44	≥32	≥32	OXA-23-like, OXA-51-like
45	≥32	≥32	TEM, OXA-23-like, OXA-51-like
S1	0.125	0.125	OXA-51-like
S2	0.125	0.125	OXA-51-like
S3	0.25	0.25	OXA-51-like
S4	0.125	0.125	OXA-51-like
S5	0.25	0.25	OXA-51-like
S6	0.125	0.125	OXA-51-like

* TEM = bla_{TEM}, OXA-23-like = $bla_{OXA\text{-}23\text{-}like}$, OXA-51-like = $bla_{OXA\text{-}51\text{-}like}$.

3. Results

3.1. Bacterial strains and antibiotic susceptibility testing

All 51 bacterial strains were confirmed as *A. baumannii* using the methods of VITEK 2 and specific PCR amplification. Antibiotic susceptibility testing showed that strains 1 to 45 were resistant to both imipenem and meropenem, and strains S1 to S6 were sensitive to these 2 antibiotics (Table 2). The resistance ratio reached to 88.2%.

Table 3

Carbapenem resistance gene profile of 51 *A. baumannii* strains.

Carbapenem resistance gene profile	Type of isolates	Positive rate, % (n = 51)
TEM + OXA-23-like + OXA-51-like	Carbapenem resistance	78.43 (40)
TEM + OXA-51-like	Carbapenem resistance	1.96 (1)
OXA-23-like + OXA-51-like	Carbapenem resistance	7.84 (4)
OXA-51-like	Carbapenem susceptible	11.76 (6)

3.2. Carbapenem resistance gene analysis

Among 15 resistance-related genes, 3 were positive in bacterial genomic DNAs checked by PCR (Table 2). In which, bla_{TEM} and $bla_{OXA\text{-}23\text{-}like}$ genes only showed up in CR strains, with the appearance ratio of 91.1% (41/45) and 97.8% (44/45), respectively. It was noteworthy that $bla_{OXA\text{-}51\text{-}like}$ gene was positive in all 51 *A. baumannii* strains including both CR and carbapenem-sensitive isolates. Moreover, IS*Aba1* was found upstream to $bla_{OXA\text{-}23\text{-}like}$ rather than $bla_{OXA\text{-}51\text{-}like}$. Thus, the resistance gene profile in this study was divided into 4 types: TEM + OXA-23-like + OXA-51-like, TEM + OXA-51-like, OXA-23-like + OXA-51-like, and only OXA-51-like (Table 3). Sequencing of positive PCR products revealed that the resistance genes were subtype TEM-1 for TEM, OXA-23 for OXA-23-like, and OXA-66 and OXA-69 for OXA-51-like.

3.3. Correlation analysis between CR genes and CR phenotype

bla_{TEM} and $bla_{OXA\text{-}23\text{-}like}$ genes (both *P* values were .001) rather than $bla_{OXA\text{-}51\text{-}like}$ gene were statistically associated with CR phenotype analyzed by Chi-square test. The contingency coefficient of 0.675 for OXA-23-like and 0.595 for TEM showed that they were highly correlated with CR. There was no statistic relationship between OXA-51-like and CR phenotype, as both resistant and sensitive strains harbored this gene.

3.4. Expression of resistance-related genes

In order to ascertain the genes that played a key role in leading our *A. baumannii* strains to the CR phenotype, the expressions of 3 PCR-positive genes were evaluated by RT-qPCR. TEM-1 and OXA-23 showed positive results, but no expression was detected for OXA-66/69 in either susceptible or resistant strains (Fig. 1).

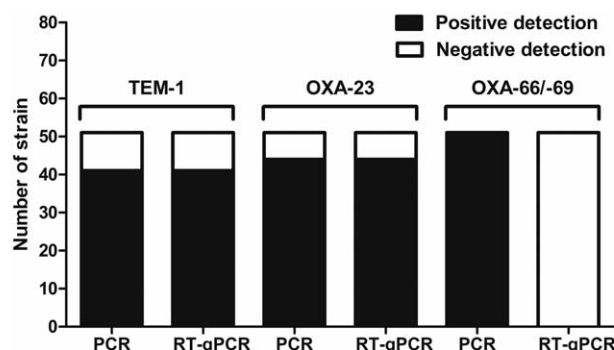

Figure 1. Expression of TEM-1, OXA-23, and OXA-66/-69 as assessed by PCR and RT-qPCR in clinical isolates.

4. Discussion

Since the 1990s, strains from *A. baumannii* show a dramatic escalation in antimicrobial resistance, even with the appearance of isolates resistant to all antimicrobials except colistin.[2] Surveillances carried out in China annually demonstrate an ascending tendency of imipenem and meropenem resistance in *A. baumannii* from 2005 to 2014. The resistance rate ranges from 31.0% to 62.4% for imipenem, and 39.0% to 66.7% for meropenem.[12]*A. baumannii* strains in our observation had a higher resistance level at 88.2% (45 out of 51 isolates) for both imipenem and meropenem. This difference might be caused by the survey spectrum. The surveillances of China were carried out in 17 teaching hospitals including various departments, whereas our strains were mostly obtained in ICUs, for example, central ICU, respiratory ICU, and surgical ICU. In addition, an outbreak caused by extensively drug-resistant *A. baumannii* was covered during the period of sample collection.[13] Third, as our hospital is a tertiary care teaching hospital in Xi'an, many patients with severe diseases have been infected by drug-resistant *A. baumannii* before admitted in. Similar and even higher ratios of CR in *A. baumannii* have been reported.[14,15] In those studies, except of medical and surgical wards, samples are mainly collected from the ICUs.

Bacteria can overcome β-lactam antibiotics by 4 primary mechanisms: changes in the active site of PBPs, modification of porin proteins in the outer membrane of Gram-negative bacterial cell walls, overexpression of multicomponent drug efflux pump systems, and production of β-lactamases. Among which, the last one is the most common and important mechanism of resistance in Gram-negative bacteria.[4] In this study, β-lactamase encoding genes from class A, B, and D, which are responsible for CR, were investigated from both CR strains and carbapenem-susceptible strains.

Three (bla_{TEM}, $bla_{\text{OXA-23-like}}$, and $bla_{\text{OXA-51-like}}$) out of 15 genes were found positive in the samples collected from our hospital. And coexistence of resistance genes was detected in most isolates, especially in CR strains. From these 3 genes, OXA-23-like was closely related to CR phenotype according to the correlation analysis, followed by TEM. OXA-23, the subtype of OXA-23-like in this study, belongs to class D β-lactamase, which is also named oxacillinase. It is the first reported acquired oxacillinase with appreciable carbapenem-hydrolyzing activity isolated from *A. baumannii*,[16] and subsequently showing expression globally through both plasmid and chromosomal carriage.[17] The positive expression results confirmed its relationship with CR phenotype. The high possession rate (97.8%) of IS*Aba1*-OXA-23 indicated its leading role in causing CR in this study. TEM-1 encodes a class A β-lactamase, and has been found in many CR *A. baumannii* strains in different regions.[18–21] It also contributed to CR occurrence in our study, as all the CR isolates carrying this gene showed positive expression.

It was worth noting that OXA-51-like gene existed in all 51 clinical isolates, no matter they were CR or susceptible. OXA-51-like genes have been reported to be present in *A. baumannii* chromosomes,[22,23] and control the production of so-called intrinsic carbapenemases.[24] Nonetheless, they are normally expressed at a low level, and show weak hydrolytic activity toward carbapenems, unless an upstream insertion of the IS*Aba1* sequence.[10,25] IS*Aba1* has been shown to be associated with $bla_{\text{OXA-23}}$-like- and $bla_{\text{OXA-51}}$-like-related CR of *A. baumannii*.[26,27] Its absence in the upstream of OXA-51-like but presence in that of OXA-23-like revealed that OXA-51-like was not as important as OXA-23-like in contributing to the CR phenotype in our study. This was proofed by the null expression of OXA-66/-69 in the strains.

In conclusion, a high rate of CR in *A. baumannii* from our hospital was noticed. A coexistence of β-lactamases encoding genes bla_{TEM}, $bla_{\text{OXA-23-like}}$, and $bla_{\text{OXA-51-like}}$ was the primary resistance profile. And the drug resistance was closely associated with the expression of $bla_{\text{OXA-23-like}}$ and bla_{TEM}. $bla_{\text{OXA-51-like}}$ was found in the chromosome of all clinical strains, but neither IS*Aba1* in its upstream nor the expression of this gene was found, thus $bla_{\text{OXA-51-like}}$ did not contribute to the CR phenotype.

Acknowledgment

We thank senior statistician Guangmin Xu for the help in statistical consultation.

References

[1] Abbott I, Cerqueira GM, Bhuiyan S, et al. Carbapenem resistance in Acinetobacter baumannii: laboratory challenges, mechanistic insights and therapeutic strategies. Expert Rev Anti Infect Ther 2013;11:395–409.
[2] Clark NM, Zhanel GG, Lynch JP3rd. Emergence of antimicrobial resistance among Acinetobacter species: a global threat. Curr Opin Crit Care 2016;22:491–9.
[3] Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant Acinetobacter baumannii. Nat Rev Microbiol 2007;5:939–51.
[4] Bonomo RA. beta-lactamases: a focus on current challenges. Cold Spring Harb Perspect Med 2017;7:pii: a025239.
[5] Vijayakumar S, Gopi R, Gunasekaran P, et al. Molecular characterization of invasive carbapenem-resistant Acinetobacter baumannii from a Tertiary Care Hospital in South India. Infect Dis Ther 2016;5:379–87.
[6] Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826–36.
[7] Higgins PG, Wisplinghoff H, Krut O, et al. A PCR-based method to differentiate between Acinetobacter baumannii and Acinetobacter genomic species 13TU. Clin Microbiol Infect 2007;13:1199–201.
[8] CLSIPerformance Standards for Antimicrobial Susceptibility Testing. 27th edition.Clinical and Laboratory Standards Institute, Wayne, PA:2017.
[9] Nie L. Molecular Characteristics of Aminoglycoside and Carbapenem Resistance in Acinetobacter baumannii. Institute of Medicinal Biotechnology, Peking Union Medical College, Beijng:2013.
[10] Turton JF, Ward ME, Woodford N, et al. The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. FEMS Microbiol Lett 2006;258:72–7.
[11] Fernando D, Kumar A. Growth phase-dependent expression of RND efflux pump- and outer membrane porin-encoding genes in Acinetobacter baumannii ATCC 19606. J Antimicrob Chemother 2012;67:569–72.
[12] Hu FP, Guo Y, Zhu DM, et al. Resistance trends among clinical isolates in China reported from CHINET surveillance of bacterial resistance, 2005–2014. Clin Microbiol Infect 2016;22(Suppl 1):S9–14.
[13] Lei J, Han S, Wu W, et al. Extensively drug-resistant Acinetobacter baumannii outbreak cross-transmitted in an intensive care unit and respiratory intensive care unit. Am J Infect Control 2016;44:1280–4.
[14] Maraki S, Mantadakis E, Mavromanolaki VE, et al. A 5-year surveillance study on antimicrobial resistance of Acinetobacter baumannii clinical isolates from a tertiary Greek hospital. Infect Chemother 2016;48:190–8.
[15] Gong YL, Yang ZC, Yin SP, et al. [Analysis of the pathogenic characteristics of 162 severely burned patients with bloodstream infection]. Zhonghua Shao Shang Za Zhi 2016;32:529–35.
[16] Paton R, Miles RS, Hood J, et al. ARI 1: beta-lactamase-mediated imipenem resistance in Acinetobacter baumannii. Int J Antimicrob Agents 1993;2:81–7.
[17] Da Silva GJ, Domingues S. Insights on the horizontal gene transfer of carbapenemase determinants in the opportunistic pathogen Acinetobacter baumannii. Microorganisms 2016;4:pii: E29.

[18] Zhou Y, Teng SJ, Yang L, et al. A novel variant of the beta-lactamase ADC-61 gene in multi-drug resistant Acinetobacter baumannii. Genet Mol Res 2015;14:7092–100.
[19] Vranic-Ladavac M, Bedenic B, Minandri F, et al. Carbapenem resistance and acquired class D beta-lactamases in Acinetobacter baumannii from Croatia 2009–2010. Eur J Clin Microbiol Infect Dis 2014;33:471–8.
[20] Cicek AC, Duzgun AO, Saral A, et al. Detection of class 1 integron in Acinetobacter baumannii isolates collected from nine hospitals in Turkey. Asian Pac J Trop Biomed 2013;3:743–7.
[21] Manageiro V, Jones-Dias D, Ferreira E, et al. Genetic diversity and clonal evolution of carbapenem-resistant Acinetobacter baumannii isolates from Portugal and the dissemination of ST118. Int J Antimicrob Agents 2012;40:398–403.
[22] Asai S, Umezawa K, Iwashita H, et al. An outbreak of blaOXA-51-like- and blaOXA-66-positive Acinetobacter baumannii ST208 in the emergency intensive care unit. J Med Microbiol 2014;63:1517–23.
[23] Shoja S, Moosavian M, Rostami S, et al. Characterization of oxacillinase and metallo-beta-lactamas genes and molecular typing of clinical isolates of Acinetobacter baumannii in Ahvaz, South-West of Iran. Jundishapur J Microbiol 2016;9:e32388.
[24] Patel G, Bonomo RA. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol 2013;4:48.
[25] Chen TL, Lee YT, Kuo SC, et al. Emergence and distribution of plasmids bearing the blaOXA-51-like gene with an upstream ISAba1 in carbapenem-resistant Acinetobacter baumannii isolates in Taiwan. Antimicrob Agents Chemother 2010;54:4575–81.
[26] Khurshid M, Rasool MH, Ashfaq UA, et al. Emergence of ISAba1 harboring carbapenem-resistant Acinetobacter baumannii isolates in Pakistan. Future Microbiol 2017;12:1261–9.
[27] Mirshekar M, Shahcheraghi F, Azizi O, et al. Diversity of class 1 integrons, and disruption of carO and dacD by insertion sequences among Acinetobacter baumannii isolates in Tehran, Iran. Microb Drug Resist 2017;Doi 10.1089/mdr.2017.0152. Epub ahead of print.