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Molecular epidemiological study and detection of multi-drug resistant *Acinetobacter baumannii* -related resistance genes

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This study aimed to investigate the existence and molecular epidemiological characteristics of multi-drug resistant *Acinetobacter baumannii* (MDRAB)-related resistance genes isolated from our hospital. Microdilution and disk diffusion methods were used to detect the antibiotic susceptibility of 46 MDRAB isolates that carried OXA-51 gene. Pulsed-field gel electrophoresis (PFGE) was used to analyze the homologies among the strains. Resistance genes were tested through polymerase chain reaction. Among the 46 MDRABA, a total of nine, isolates were categorized as strain A (19.6%), eight as strain B (17.4%), and four as strain O (8.7%). A total of 41 isolates (89.1%) carried the OXA23 gene, 17 (37.0%) carried the PER gene, and six (13%) carried the IMP gene. Six strains (13%) did not carry the membrane pore protein gene carO. The numbers of isolates that carried a particular gene were as follows: 40 (87.0%), armA; 41 (89.1%), ant(3")-I; 33 (71.7%), aac(3)-I; 2 (4.3%), aac(3)-II; 1 (2.2%), aac(6')-II; and 1 (2.2%) and aph(3')-VI. Most of the isolates (93.5%) carried the qacE△1 gene. These hospital MDRAB isolates were proven to simultaneously carry a variety of drug resistance genes. Strains A and B were the major epidemic strains of nosocomial MDRAB.

Key words: *Acinetobacter baumannii*, resistance gene, pulsed-field gel electrophoresis, multi-drug resistant.

INTRODUCTION

Acinetobacter baumannii has recently emerged as a significant pathogen, with a surprisingly rapid acquisition of antibiotic resistance and fast proliferation within hospitals (Jiang et al., 2012). Several studies have demonstrated that Multidrug-resistant *A. baumannii* (MDRAB) and extensive drug-resistant *A. baumannii* have caused significant challenges in clinical treatment (Munoz and Weinstein, 2008; Peleg et al., 2008; Doi et al., 2009). Carbapenems, fluoroquinolones, aminoglycosides, sulbactam, and sulbatacm combinations are often used against *A. baumannii*. However, the number and incidence of MDRAB strains had increased considerably during the past decade. The current study examined the β-lactam, aminoglycoside, fluoroquinolones, and disinfectant-related resistance genes (qacE△1 gene) of MDRAB strains isolated from our hospital. In addition, the statistical distribution of the resistant strains was also investigated to analyze the resistance mechanisms and relationships among MDRAB strains from our hospital.

MATERIALS AND METHODS

Source of strain

Clinical isolates were isolated from the Microbiology Laboratory in our hospital using standard procedures. A total of 46 MDRAB isolates were collected from June 2010 to June 2011, of which 44 isolates were from the sputum, one from puncture fluid, and one

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from secretions.

Bacterial identification and antibiotic susceptibility determination

MDRAB is *A. baumannii* resistant to multiple antibiotics, often defined as three or more antimicrobials (aminoglycoside, ampicillin-sulbactam, antipseudomonal carbapenem, antipseudomonal cephalosporin, fluoroquinolone). The bacteria were identified using WalkAway 96 PLUS NC31 composite panels. Antimicrobial susceptibility test was performed using the Kirby-Bauer disk diffusion method. Susceptibility testing was performed in accordance with Clinical and Laboratory Standards Institute Guidelines of 2011. Susceptibility was tested for the following antimicrobial agents: amikacin, ceftazidime, ceftriaxone, ciprofloxacin, cefepime, gentamicin, imipenem, levofloxacin, meropenem, piperacilin, cotrimoxazole, piperacilkin/tazobactam, tobramycin, cefoperazone / sulbactam, minocycline, and tigecycline. Susceptibility to polymyxin E was examined using ATB PSE strips. MH agar and susceptibility paper were both purchased from British Oxoid.

Resistance gene detection

Bacterial DNA was extracted using the boil extraction method and resistance gene detection was performed via polymerase chain reaction (PCR). Table 1 lists the pairs of oligonucleotide primers designed to target β-lactam resistance genes. The primers were designed according to previously published procedure (Shen et al., 2008; Hu et al., 2008). Sixteen (16) pairs of oligonucleotide primers (Table 2) were designed to target aminoglycosides, plasmid-mediated fluoroquinolones, and disinfectant-related resistance genes. The primers were designed according to previously published procedure (Zhi et al., 2005; Wang et al., 2009; Yang et al., 2011; Chi et al., 2006; Turton et al., 2006).

A number of positive gene PCR products were analyzed for nucleotide sequence by Shanghai Sunny Biotechnology Co., Ltd. The resulting sequence data were compared with the data from the GenBank database using BLAST at the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov/).

Homology analysis

Pulsed-field gel electrophoresis (PFGE) was performed following the protocol of Gautom with some modifications. The PFGE patterns were analyzed by computer-assisted program (Bio-Numerics software. Version 5.1, Appliedmaths, Inc.) and by manual or visual comparison of each band patterns. Band patterns were compared and classified as nondistinguishable and unrelated with 90% as the homology threshold.

RESULT

Antibiotic Resistance

The sensitivities of the 46 strains to 17 antimicrobials are presented in Table 3.

PFGE

The PFGE patterns of the 46 MDRAB isolates were analyzed using computer-assisted program (BioNumerics software. Version 5.1, Appliedmaths, Inc.) and by manual or visual comparison of each band patterns. A 90% interlinkage homology level between patterns was considered as the cutoff for defining close genetic relationship isolates. Isolates were categorized into 20 major groups and designated as Strains A to T based on the generated dendrogram (Figure 1). A total of nine isolates were categorized as, strain A (19.6%), eight as strain B (17.4%), and four as strain O (8.7%). The other types of strains only contained 1 or 2 isolates. All strains were found in the intensive care wards. The distribution of which is shown in Table 4.

Detection of β-lactam resistance gene and sequencing results

Approximately 100% of the 46 MDRAB isolates carried β-lactam resistance genes. The numbers of isolates that carried a particular gene were as follows: 41 (89.1%) OXA23, 17 (37%) PER, 6 (13%) IMP, and 4 (8.7%) of both OXA23 and IMP-4 genes. Sequence analysis of PCR amplification products of the three OXA23 gene-positive isolates showed 100% homology with JN207493. Four of the amplification products of the six IMPgene positive isolate showed that four isolates were 100% homologous to JN106667 and the rest only showed 99%. The sequence analysis of the PCR amplification products of 4 PER gene-positive isolates showed 99% homology with FE535600.

Detections of aminoglycoside and plasmid-mediated quinolone resistance genes as well as qacEΔ1 gene

The numbers of isolates among the 46 MDRAB isolates that carried a particular gene were as follows: 40 (87.0%), armA; 41 (89.1%), ant(3")-; 33 (71.7%), aac(3)-I; 2 (4.3%), aac(3)-II; 1 (2.2%), aac(6')-II; 1 (2.2%), aph(3')-VI; 41 (89.1%), AMES; 40 (87%), 16SrRNA methylase; and 43 (93.5%), qacEΔ1. The aac(3)-III, aac(3)-IV, aac(6')-I, and ant(2")-I AMES genes were not detected. No rmtB16SrRNA methylase gene was detected. Plasmid-mediated fluoroquinolone resistance genes, namely, qnrA, qnrB, or qnrC, were not detected. Three MDRAB isolates that only carried armA16SrRNA methylase genes were resistant to amikacin, tobramycin, and gentamicin. Two isolates that did not carry AMES or 16SrRNA methylase were sensitive to amikacin, tobramycin, and gentamicin.

Analysis of the clinical data

A total of 44 isolates collected from sputum samples of patients carried MDRAB. These patients had symptoms of respiratory tract infection. MDRAB were detected in both sputum samples and thoracentesis fluid from 1 patient. MDRAB was detected in the fluid drained from one patient' after surgery. All patients with MDRAB

Table 1. PCR ampification primer sequences.

Primer	Sequence of primers (5'→3')	Size (bp)
OXA23gp-F	CCCCGAGTCAGATTGTTC	291
OXA23gp-R	GCTTCATGGCTTCTCCTAG	
OXA24gp-F	ACGAGCAAATAAAGAATATGTCCC	496
OXA24gp-R	CACCCAACCAGTCAACCAAC	
OXA48gp-F	GGGATGGACAGACMCGSGATA	300
OXA48gp-R	TGGCTTGRTTGACYATACGC	
OXA55gp-F	GCTGAGGGTTGGCAAGAGGT	179
OXA55gp-R	AACGCAATAAGGCTGGAGGG	
OXA58gp-F	TGGCACGCATTTAGACCG	507
OXA58gp-R	AAACCCACATACCAACCC	
OXA60gp-F	TCACCGCCGACCGTACCTAT	177
OXA60gp-R	CGTGCTCCCACTGCTCGTAA	
OXA64gp-F	TCAGCCTGCTCACCTTAT	406
OXA64gp-R	CACGCTTCACTTCKTTAGAC	
OXA66qc-F	ATGAACATTAAAGCACTC	825
OXA66qc-R	CTATAAAATACCTAATTG	
VIM-2gp	TCCGACAGTCAYCGAAAT	435
VIM-2gp	GCAGCACCYGGATAGAAGAG	
VIM-7	TACACCTCACCCTTGACACGC	246
VIM-7	ATTGGCATCGGCAACATTAC	
IMP-1gp-F	TCTCATTTTCATAGRGACAG	353
IMP-1gp-R	ACCAGTTTTGCCTTACCATA	
IMP-2gp-F	CTTGTAAACACWGACGCCTAT	134
IMP-2gp-R	GTGCTGTCGCTATGGAAAT	
IMP-11gp-F	GKGTCTTTGCCTGATTTA	256
IMP-11gp-R	CTATTCCACCCGWGCTGT	
IMP-14gp-F	RGACAGTACGGCTGGAATAG	239
IMP-14gp-R	CAAAGCAACCACCGAATAAA	
IMP-12gp-F	TTRCATAGCGACAGRACG	277
IMP-12gp-R	CARCCAAATTACCWAGACC	
GIM1-F	ATTACTTGTAGCGTTGCC	418
GIM1-R	CTCTATAAGCCCATTTCC	
SPM1-F	GCCATCAATACGCACTTTCA	526
SPM1-R	ACAGTCTCATTTCGCCAACG	
KPC-gp-F	GCGGAACCATTCGCTAAACTC	340
KPC-gp-R	CGCCCAACTCCTTCAGCAACA	
TEM-F	AGGAAGAGTATGATTCAACA	535
TEM-R	CTCGTCGTTTGGTATGGC	
SHV-F	GGTTATGCGTTATATTCGCC	867
SHV-R	TTAGCGTTGCCAGTGCTC	
PER-F	AGTCAGCGGCTTAGATA	978
PER-R	CGTATGAAAAGGACAATC	
EBC-MF	TCGGTAAAGCCGATGTTGCGG	302
EBC-MR	CTTCCACTGCGGCTGCCAGTT	
VEB-F	GCGGTAATTTAACCAGA	961
VEB-R	GCCTATGAGCCAGTGTT	
MOX-F	GCTGCTCAAGGAGCACAGGAT	520
MOX-F	CACATTGACATAGGTGTGGTGC	
CTT-MF	TGGCCAGAACTGACAGGCAAA	462
CTT-MR	TTTCTCCTGAACGTGGCTGGC	
ACC- MF	AACAGCCTCAGCAGCCGGTTA	346

Table 1. Contd.

ACC- MR	TTCGCCGCAATCATCCCTAGC	
FOX- MF	AACATGGGGTATCAGGGAGATG	190
FOX- MR	CAAAGCGCGTAACCGGATTGG	

Table 2. PCR ampification primer sequences.

Target gene	Sequence of primers(5'→3')	Size (bp)
aac(3)-Ⅰ	P1:ACCTACTCCCAACATCAGCC P2:ATATAGATCTCACTACGCGC	169
aac(3)-Ⅱ	P1:ACTGTGATGGGATACGCGTC P2:CTCCGTCAGCGTTTCAGCTA	237
aac(3)-Ⅲ	P1: CACAAGAACGTGGTCCGCTA P2:AACAGGTAAGCATCCGCATC	185
aac(3)-Ⅳ	P1:CTTCAGGATGGCAAGTTGGT P2:TCATCTCGTTCTCCGCTCAT	286
aac(6′)-Ⅰ	P1:TATGAGTGGCTAAATCGA P2:CCCGCTTTCTCGTAGCA	394
aac(6′)-Ⅱ	P1: TTCATGTCCGCGAGCACCCC P2:GACTCTTCCGCCATCGCTCT	178
aph(3′)-Ⅵ	P1:ATACAGAGACCACCATACAGT P2:GGACAATCAATAATAGCAAT	234
ant(3″)-Ⅰ	P1:TGATTTGCTGGTTACGGTGAC P2:CGCTATGTTCTCTTGCTTTTG	284
ant(2″)-Ⅰ	P1:GAGCGAAATCTGCCGCTCTGG P2:CTGTTACAACGGACTGGCCGC	320
armA	P1:AGGTTGTTTCCATTTCTGAG P2:TCTCTTCCATTCCCTTCTCC	591
rmtB	P1:ATGAACATCAACGATGCCC P2:CCTTCTGATTGGCTTATCCA	769
qnrA	P1:ATTTCTCACGCCAGGATTTG P2:GATCGGCAAAGGTTAGGTCA	516
qnrB	P1:GATCGTGAAAGCCAGAAAGG P2: ACGATGCCTGGTAGTTGTCC	469
qnrS	P1:ACGACATTCGTCAACTGCAA P2: TAAATTGGCACCCTGTAGGC	417
aac（6’）-Ib	P1:TTGCGATGCTCTATGAGTGGCTA P2: CTCGAATGCCTGGCGTGTTT	482
qacE△1	TAGCGAGGGCTTTACCTAAGC ATTCAGAATGCCGAACACCG	300

Table 3. Susceptibility results of 46 isolates of MDRAB.

Antimicrobial drugs	Sensitive (number (%) of isolates)	Intermediary resistance (number (%) of isolates)	Resistance (number (%) of isolates)
Amikacin	3(6.5)	0(0.0)	43(93.5)
Ceftazidime	0(0.0)	0(0.0)	46(100)
Ceftriaxone	0(0.0)	0(0.0)	46(100)
Ciprofloxacin	0(0.0)	0 (0.0)	46(100)
Cefepime	0(0.0)	0(0.0)	46(100)

Table 3. Contd

Gentamicin	2(4.3)	1(2.2)	43(93.5)
Imipenem	0(0.0)	0 (0.0)	46(100)
Levofloxacin	0(0.0)	2(4.3)	44(95.7)
Meropenem	0(0.0)	0(0.0)	46(100)
Piperacilin	0(0.0)	0(0.0)	46(100)
Cotrimoxazole	1(2.2)	0(0.0)	45(97.8)
Piperacillin/tazobactam	0(0.0)	0(0.0)	46(100)
Tobramycin	3(6.5)	0(0.0)	43(93.5)
Cefoperazone/sulbactam	6(14)	15(32)	25(54)
Minocycline	25(54.3)	20(43.5)	1(2.2)
Tigecycline	28(60.9)	0(0.0)	
Polymyxin E	46(100)		

Table 4. Distribution of 20 strains in the intensive care wards.

ICU	Number of strain																			
	A	B	C	D	E	F	G	H	Y	Z	K	L	M	N	O	P	Q	R	S	T
ICU1	5	0	0	0	1	0	1	0	2	1	1	0	0	0	1	2	1	1	0	0
ICU2	2	1	1	1	0	0	0	2	0	1	0	0	1	0	2	0	0	0	0	0
ICU3	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1
ICU4	0	7	0	1	0	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0
ICU5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ICU6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

(46/46) used broad-spectrum antimicrobial drugs and suffered from a variety of acute and (or) chronic diseases.

DISCUSSION

MDRAB has emerged as the most severe nosocomial pathogens . A previous study has reported that suction devicesand ventilator, as well as air conditioning, andtransfusion systems can be contaminated with this pathogen, spreading, MDRAB to patients (Shen and Ou, 2008). *A. baumannii* is the most common opportunistic pathogen that causes respiratory tract infection. Among the 46 MDRAB isolates from our hospital, 44 isolates were collected from sputum samples of patients carrying MSRAB. All of the 44 patients exhibited symptoms of respiratory tract infection.

Carbapenemases are β-lactamases with various hydrolytic capacities. Carbapenemases are members of molecular classes A, B, and D β-lactamases. KPC carbapenemases are the most prevalent in the class A carapenemase group. Class B enzymes include metallo-β-lactamases which contain zinc in the active site and belong to the IMP, VIM, SPM, CIM, and SIM families. These enzymes have been detected primarily in *Pseudomonas aeruginosa. The class D carbapenemases consist of* OXA-type β-lactamases. OXA-23, which can hydrolyze carbopenems antibiotics, is one of main factors that caused the multi-drug resistance of *A. baumannii* (Zou et al., 2010; Yu et al., 2011). The OXA enzyme is the major product of *A. baumannii*. Several strains of *A. baumannii* also produce extended-spectrum β-lactamase, cephalosporinase, and metal enzyme, which can result in resistance to β-lactam antimicrobial agents. For example, IMP-4 mental enzymecan hydrolyze β-lactam antibiotics, including carbopenem and cephalosporin antibiotics. In addition, PRE-1 Extended-spectrum β-lactam enzyme can hydrolyze cephalosporin antibiotics. Our study shows that 41 isolates carried the OXA23gene (89.1%), 17 (37%)) the PER gene, and 6 (13%) carried the IMP gene. The sequence analysis of the PCR amplification products of three OXA23 gene positive isolates showed that they were all OXA-23 gene. The sequence analysis of the PCR amplification products of four PER gene positive isolates showed that they were all PER-1 gene. The results showed that OXA-23 is the most common carbapenemases gene among the MDRAB isolates collected from our hospital. This phenomenon is similar to the results of Ma et al. (2011) in which the OXA-type enzyme carbapenemase was found to have an important function in imipenem resistance of *A. baumannii* and the OXA-23 type enzyme is common in China. In addition, PER-1 and

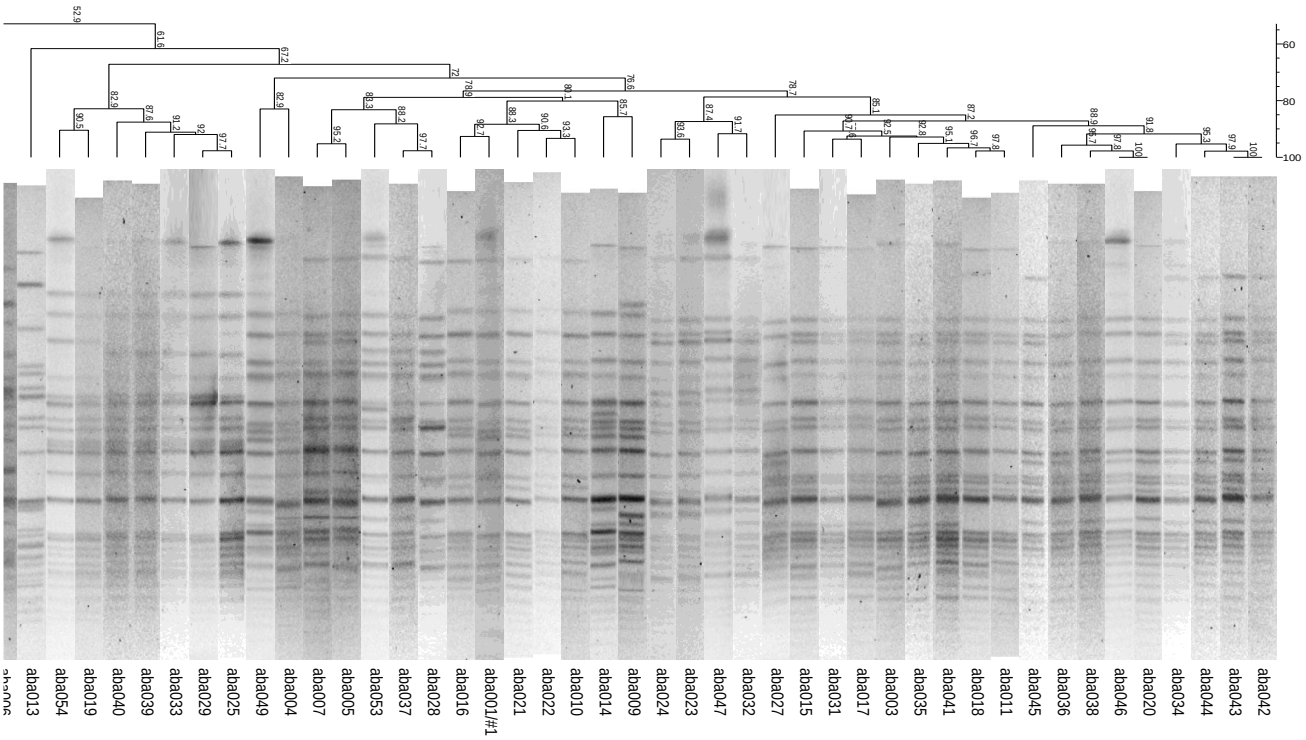

Figure 1. PFGE results.

IMP-4 mental enzymes may be related to the β-lactam antibiotic resistance of MDRAB in our hospital.

Galimand et al. (2000) reported that resistance to aminoglycoside antibiotics was caused by actions of aminoglycoside-modifying enzyme (AME) and mutations in the 16SrRNA genes of aminoglycoside antibiotics'. AMEs can be divided into acetyltransferase, phosphotransferase, and nucleoside transferase according to the different functions of the enzymes. More than 30 AME enzymes had been found (Chang et al., 2004). A total of 46 MDRAB isolates were found in our study, of which 41 isolates carried ant (of which enaac(3)-I gene, 2 carried aac(3)-II gene, 1 carried aac(6c(6I gene, 1 carried aac (6aph(3h(3I gene, 1 carried aac(6c(6 gene, 1 was 89.1%, indicating that the resistance of MDRAB in our hospital to animoglycoside drug is related with AMEs. The positive rate of armA 16SrRNA was 87% (40/46), which is higher than the positive rate of 69.77% in 43 isolates of *A. baumannii* resistant to amilacin reported by Miao et al. (2011). Feng et al. (2008) have reported that the 16S rRNA methylase *armA* and *rmtB* genes were not detected in 20 MDRAB isolated. 16S rRNA methylase may have an important role in the resistance of MDRAB to aminoglycoside antibiotics in our hospital. In the current study, three MDRAB isolates that only carry the armA 16SrRNA methylase gene were resistant to amikacin, tobramycin, and gentamicin. Thus, armA 16SrRNA methylase gene may lead to resistance to aminoglycoside antibiotics alone. One isolate of MDRAB that only carry AMEs was sensitive to amikacin and tobramycin and was intermediary resistant to gentamicin. Two MDRAB isolates without AMEs or 16SrRNA methylase gene were all sensitive to amikacin, tobramycin, and gentamicin. These results indicate that AMEs and 16SrRNA methylase gene were related with the resistance of MDRAB' to aminoglycoside antibiotics. The positive rate of qacEΔ1 in our hospital was 93.5% (43/46). The high resistance to disinfectants may be an important factor of nosocomial infection, which should be addressed by the disinfection department in our hospital.

The existence of plasmid-mediated fluoroquinolone resistance gene in MDRAB has been reported in several cities in China. The qnr gene positive rate was 43.9% in 57 isolates of *Klebsiella pneumoniae* producing extended - spectrum β-lactamase. Although the qnr gene was mainly detected from Enterobacter sp, Yang et al. (2009) reported that 2 of the 115 *A. baumannii* isolates were qnrB gene-positive. No plasmid-mediated quinolone fluoroquinolone resistance gene was detected from the 46 MDRAB isolates in our hospital, so MDRAB resistance to fluoroquinolones was not related to the plasmid-mediated fluoroquinolone resistance gene.

Among the 46 patients with MDRAB, 45 patients had symptoms of respiratory tract infection. The 46 patients used broad-spectrum antimicrobial drugs and were suffering from a variety of acute and (or) chronic diseases. Among the nine patients infected by MDRAB strain A, four patients were cured, three patients improved, and

two patients died. Analytical results show that several infected patients were related to nosocomial infections. However, the clinical effect was related to the patients' physical condition and the proper use of antibacterial drugs. A total of 44 isolates were collected from the sputum samples of patients with MDRAB. Although they were all qualified specimens, distinguishing infectious bacteria from bacterial colonization was difficult. Therefore, clinicians should improve the submission rate of blood culture.

Table 4 shows that nine isolates of MDRAB strain A distributed in four intensive care units in our hospital were mainly derived from ICU 1. Eight isolates of MDRAB strain B were distributed in two intensive care units, seven came from ICU 2, and four isolates of MDRAB strain O were distributed in three intensive care units. Therefore, the common MDRAB stains in our hospital were mainly strains A and B, followed by strain O. Some of the patients infected by MDRAB strain A or B were transferred from other hospitals, so strains A and B may be the major epidemic MDRAB strains in this area.

In our study, the resistance rate of MDRAB to gentamicin, tobramycin, and amilacin were 93%. Two isolates exhibited intermediary resistance to levofloxacin. All the isolates were resistant to ciprofloxacin, ceftazidime, ceftriaxone, ciprofloxacin, cefepime, imipene, meropenem, piperacillin, and piperacillin/tazobactam. They also exhibited high sensitivity to tigecycline (60.9%) and minocycline (54.3%). The resistance rate to cefoperazone/sulbactam was 54%, and the intermediary resistance rates to cefoperazone/sulbactam were 32%. One of the isolates was sensitive to cotrimoxazole. None of the isolates was resistant to polymyxin E. Therefore, treatments of choice for MDRAB infection in our hospital are polymyxin + cefoperazone/sulbactam; and cefoperazone/sulbactam + minocycline or cefoperazone / sulbactam+ tigecycline.

Attention should be paid when clinicians select treatments for MDRAB infections because optional antibiotics for MDRAB are very limited. Clinicians should be aware of the characteristics of MDRAB in the area and strengthen the awareness of drug combination. Moreover, rational use of drug and prevention of the spread of resistant bacterium are important factors for controlling MDRAB in our hospital.

REFERENCES

Chang LL, Chen HF, Chang CY (2004). Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of Stenotrophomonas maltophili. J. Antimicrob. Chemother. 53:518-521.

Chi Hye Park, Ari Robicsek, George A. Jacoby, Deniel Sahm, David C. Hooper (2006). Prevalence in the United States of aac(6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents Chemother. 50:3953-3955.

Doi Y, Husain S, Potoski BA, McCurry KR, Paterson DL (2009). Extensively drug resistant Acinetobacter *baumannii.* Emerg. Infect. Dis. 15: 980-982. PMID: 19523312

Feng Gan-zhu, Zhang Yang, Yao Kun, Zhao Shui-di (2008). Study on the genes of 16SrRNA methylase and minoglycoside modifying enzyme in multi-resistant Acinetobacter baumannii. Chin. J. Infect. Chemother. 8:303-306.

Galimand M, Gerbaub G, Courvalin P (2000). Spectinomycin resistance in Neisseria spp due to mutat ions in 16S rRNA. Antimicrob. Agents Chemother. 44:1365-1366.

Hu Fu-pin, Zhu De-mei, Ye Xin-yu, Guo Yan, Wu Pei-cheng (2008). The molecular characteristics of E.coli and Klebsiella spp. with CLSI ESBL-screening test positive but cefepime- susceptible. Chin. J. Lab. Med. 31: 1128-1123

Jiang Mei-jie, Sun QI-ying, Li Yu-chen (2012). Drug resistance of *Acinetobacter baumanii* from 2066-2010. Chin J Nosocomiol. 22: 1469-1450.

Ma Xu-zhu, Lv Yuan, Zhang Jia, Li Yun, Yang Wei-wei, Zheng Bo (2011). Prevalence of antibiotics resistance and OXA carbapenemases genes in imipenem-resistant Acinetobacter baumannii isolates in China. Chin. J. Clin. Pharmacol. 27:268-271.

Miao Xin-jun, Chen Zhi-li, Chen Yu-xi, Chen Hong-hui, Li Yong (2011). Study on 16S rRNA methylase genes in 134 acinetobacter baumannii strains. Chin. J. Nosocomiol. 6:1075-1077.

Munoz-Price LS, Weinstein RA (2008). Acinetobacter Infection. N. Engl. J. Med. 358: 1271-1281. PMID:18354105

Peleg AY, Seifert H, Paterson DL (2008). Acinetobacter baumannii: Emergence of a successful pathogen. Clin. Microbiol. Rev. 21: 538-582. PMID:18625687

Shen Cai-hong, Ou Miao (2008). Clinical distribution and drug resistance of 122 strains of Acinetobacter baumannii. Lab. Med. Clin. 5:853-854.

Shen Ji-lu, Zhu De-mei, Wu Wei-hong, Xu Xiao-gang, Wang Ming-gui (2008). The relationship between acquired carbapenemases and resistance of gram-negative bacill. Chin. J. Lab. Med. 31: 408-414

Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL (2006). Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 44(8):2974-6

Wang Wei, MAO Jian-feng, XU Wei-zhen (2009). Drug-reisitance and Disinfectant-resistant Genes of Chlorhexidine-sulfadiazine-resistant Escherichia coli Isolates. Chin. J. Nosocomiol. 19:247-249.

Yang Ren-guo, Yu Ru-jia, Gao Yan-yu, Lv Xiao-ju (2009). Detection of qnr gene and drug resistance in clinical isolates of Acinetobacter baumannii. Chin. J. Antibio. 34:747-749.

Yang Yinmei, Ye Huifen, Zhang Weihong, Chen, Huiling, Zhou Xiaomian (2011). Detection of New Dilhi metallo-β-lactamase 1 gene in Klebsiella ezaenae and Acinetobacter baumannii. Int. J. Lab. Med. 32:1407-1409.

Yu LIn, Jiang Feng-ru, Lu Jian-cai, Su Dan-hong (2011). Study on the genotypes of OXA-23 and qacE△1 in mutidrug-resistant Acinetobacter baumannii. Lab. Med. Clin. 8:1793-1795.

Zhi Zhi-quan, He Zhi-heng, Jiang Peng (2005). The study on genotyping of β-lactamases and aminoglycoside-modifying enzyme in multi-resistant Pseudomonas aeruginosa. Chin. J. Lab. Med. 28: 1121-1124.

Zou Jiu-ming, Zhang Ai-ping, Li Zhi-shan, Yang Yan, Deng San-ji (2010). AN infection outbreak of OXA-23 carbapenemase-producing imipenem-resistant Acinetobacter baumannii. Chin. J. Infect Control 9:235-238.