계명의대학술지 제31권 1호
Keimyung Med J
Vol. 31, No. 1, June, 2012

High Prevalence of OXA-23 group Carbapenemase and Widespread of IS*Aba1* in Carbapenem-Resistant *Acinetobacter baumannii*

Seong Il Suh, M.D., Won Ki Baek, M.D., Min Ho Suh, M.D.

Department of Microbiology, Institute for Medical Science,
Keimyung University School of Medicine, Daegu, Korea

Abstract

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) cause infections that are difficult to treat and have high mortality rates due to their acquisition of multidrug-resistant (MDR) phenotypes. A total of 172 strains of CRAB isolated from various clinical specimens in Daegu area were tested to provide an information on the current status of the molecular epidemiology of carbapenemase genes in CRAB and the genetic platforms participating in their expression. All the CRAB isolates harboured OXA (oxacillinase)-51-like carbapnenemase gene and 90.7% of them contained OXA-23-like gene in addition to OXA-51-like gene. We did not detect any genes encoding the carbapenemases OXA-24-like and OXA-58-like, and none of the genes encoding metallo-ß-lactamases (IMP: imipenemase, VIM: Verona-imipenemase, GIM: German-imipenemase, SPM: Sao-Paulo-imipenemase, SIM: Seoul-imipenemase) were detected in this study. IS*Aba1* gene was upstream of *bla*$_{OXA}$-23-like in 90.1% of CRAB isolates and 8.7% of isolates contained an IS*Aba1*-*bla*$_{OXA}$-51-like structure. The presence of IS*Aba1* upstream of *bla*$_{OXA}$-23 gene in CRAB was highly correlated with resistance to imipenem and meropenem. High level (minimal inhibitory concentration; MIC=64 mg/L) of resistance to imipenem and meropenem was observed in isolates containing an IS*Aba1*-*bla*$_{OXA}$-23-like structure. One isolate with *bla*$_{OXA}$-23-like gene lacking IS*Aba1* upstream also showed high level (MIC=64 mg/L) of resistance to carbapenems. Some unidentified IS elements may contribute to the carbapeneme resistances of CRAB isolates lacking IS*Aba1* upstream. Repetitive extragenic palindromic sequence-based PCR (REP-PCR) was chosen as the molecular epidemiologic typing method to monitor and control the spread of CRAB in the hospital environment. More than 98% of isolates containing IS*Aba1*-*bla*$_{OXA}$-23-like structure were clustered into one molecular type group 1a, suggesting that they originated from common bacteria and clonally spread in the study hospital. And 86.7% of isolates containing IS*Aba1*-*bla*$_{OXA}$-51-like structure were clustered into group 2a, also suggesting that they originated from another common bacteria and clonally spread. Given that clonal spread is likely responsible for the majority of CRABs presented in this study, CRAB infections may indicate a serious infection control problem and represents a serious challenge to public health. In conclusion, continuous monitoring of CRAB and discovery of mechanisms of the acquisition of resistances by concerted

Corresponding Author: Min Ho Suh, M.D., Department of Microbiology, Institute for Medical Science, Keimyung University School of Medicine, 1095 Dalgubeol-daero, Dalseo-gu, Daegu 704-701, Korea
Tel: +82-53-580-3841 E-mail: minho@dsmc.or.kr

multidisciplinary efforts and rigorous infection control measures are essential to help develop effective therapy regimens and to prevent the further spread of CRAB infections.

Key Words : *Acinetobacter baumannii*, *bla*$_{OXA}$-23, *bla*$_{OXA}$-51, Carbapenem resistance, IS*Aba1*, OXA-carbapenemase genes, REP-PCR.

Introduction

Acinetobacter baumannii are major opportunistic bacteria frequently involved in outbreaks of infections, occurring mostly intensive care units [1,2]. It is a cause of serious infections such as ventilator-associated pneumonia, bloodstream infection, urinary tract infection and wound infection [1-3].

Infections with nosocomial *A. baumannii* have created a challenge for concordant therapy due to their acquisition of multidrug-resistant (MDR) phenotypes, such as resistance to carbapenems, extended-spectrum cephalosporins, aminoglycosides and fluoroquinolones [4]. Antimicrobial resistance increases the morbidity, mortality and costs of treating infectious diseases [5]. This bacterium is also able to survive for prolonged periods throughout the hospital environment, potentiating its ability for nosocomial outbreaks [6].

Until recently, carbapenems remained active against MDR *A. baumannii,* however, resistances to carbapenems are now common occurrences in many countries [7]. Increasing reports of carbapenem resistance have raised serious concerns about the loss of this critical treatment option [8].

Carbapenem resistance in *A. baumannii* is mediated most often by OXA-type carbapenemases and less frequently by metallo-ß-lactamases (MBLs) [9]. *A. baumannii* is intrinsically resistant to many antimicrobials and transposable elements play an important role in the expression of resistance genes in *A. baumannii* [10]. In some carbapenem-resistant isolates, an insertion element, such as IS*Aba1*, may be seen between downstream of the intergenic spacer sequence and upstream of the *bla*$_{OXA}$ gene, and this usually provides a strong promoter to drive the expression of the *bla*$_{OXA}$ gene [11,12].

Carbapenem-resistant *A. baumannii* (CRAB) isolates have been associated with infection outbreaks and have contributed to patient mortality [7]. Sentinel surveillance is necessary to monitor and control the spread of CRAB in the hospital environment [8,13]. This study aims to provide an information on the current status of the molecular epidemiology of carbapenemase genes in CRAB and the cognate genetic platforms participating in their expression.

Materials and Methods

1. Bacterial strains

A total of 172 *Acinetobacter baumannii* isolates were obtained between July and December 2011 from Keimyung University Dongsan Medical Center, Daegu, Korea. Identification to species level was undertaken using a conventional method, 16S rRNA gene sequencing and intrinsic chromosomal *bla*$_{OXA}$-51-like gene detection [14-17]. DNA sequencing was undertaken using an ABI PRISM BigDye Terminator v3.1 cycle sequencing method (Applied Biosystems, Foster City, California, USA).

2. Antimicrobial susceptibility test

The minimal inhibitory concentrations (MICs) to antimicrobial agents were determined by the agar dilution method according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [18]. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used as reference strains in susceptibility testing. The breakpoints used were defined by the CLSI for *Acinetobacter baumannii* [18]. All CRAB in the study were stored at -70℃ in tryptic soy broth (Becton, Dickinson and Co., Sparks, MD, USA), supplemented with 20% glycerol until they were tested.

3. DNA preparation

Bacteria were grown on MacConkey's agar (Becton, Dickinson and Co., Sparks, MD, USA) overnight. A colony of each isolate was suspended in 50 μl of distilled sterile H_2O, and boiled for 10 min. After a 10 min of centrifugation, aliquots of each sample were subjected to PCR amplification.

4. Multiplex PCR assay for detection of OXA-carbapenemase genes

Detection of the four groups of OXA-carbapenemase genes (*bla*$_{OXA}$-23-like, *bla*$_{OXA}$-24-like, *bla*$_{OXA}$-51-like and *bla*$_{OXA}$-58-like) was carried out using a multiplex assay [19]. Primers used are given in Table 1. The amplification conditions were, initial denaturation at 94℃ for 5 min, 30 cycles of 94℃ for 25 sec, 52℃ for 40 sec and 72℃ for 50 sec, and a final elongation at 72℃ for 6 min.

5. PCR assay for detection and mapping of IS*Aba1*

IS*Aba1* was sought as described by Segal, *et al*. [20]. Primers used are given in Table 1. The amplification conditions were, initial denaturation at 95℃ for 5 min, 35 cycles of 95℃ for 45 sec, 56℃ for 45 sec and 72℃ for 3 min, and a final elongation at 72℃ for 5 min. PCR mapping experiments using combinations of the IS*Aba1* primers and the OXA-23-like and OXA-51-like reverse primers were carried out exactly as the IS*Aba1* PCR, except that an annealing temperature of 58℃ was used for the IS*Aba1*/OXA-51-likeR PCR [11].

6. Multiplex PCR assay for detection of MBLs

Detection of the five groups of MBL genes (IMP family, VIM family, GIM-1, SPM-1 and SIM-1) was carried out using a multiplex assay [21]. Primers used are given in Table 1. The amplification conditions were, initial denaturation at 94℃ for 5 min, 36 cycles of 94℃ for 30 sec, 52℃ for 40 sec and 72℃ for 50 sec, and a final elongation at 72℃ for 5 min.

7. Repetitive extragenic palindromic sequence-based PCR (REP-PCR)

Epidemiological typing of CRAB isolates was performed by REP-PCR [22-24]. The primers used had the following sequences: REP1 5'-III GCG CCG ICA TCA GGC-3' and REP2 5'-ACG TCT TAT CAG GCC TAC-3' to amplify putative REP-like elements in the genomic bacterial DNA [22]. These primers have the nucleotide inosine at ambiguous positions in the REP consensus sequence. Inosine contains the purine base hypoxanthine and is able to base pair with A, C, G or T [25]. Final volume of 50 μl PCR amplificaton was performed with an initial denaturation at 94℃ for 10 min, followed by 35 cycles of 94℃ for 1 min, 40℃ for 1 min, 65℃ for 1 min, and a final extension 65℃ for 16 min. Aliquots of each sample were subjected to electrophoresis in

1.2% agarose gels at 100 volts for 30 min.

Results

The CRAB isolates were investigated for the presence of OXA-type and MBL genes (Table 2). All isolates harboured OXA-51-like carbapenemase gene. One hundred and fifty-six (90.7%) isolates had, in addition, OXA-23-like gene. None of the genes encoding the carbapenemases OXA-24-like and OXA-58-like were detected. We did not detect any MBLs (IMP, VIM, GIM, SPM and SIM).

IS*Aba1*-was upstream of *bla*$_{OXA}$-23-like in 155 (90.1%) CRAB isolates and 15 (8.7%) isolates contained an IS*Aba1-bla*$_{OXA}$-51-like structure (Table 3). Among the remaining isolates without IS*A*ba1 upstream of *bla*$_{OXA}$ genes, one isolate contained both *bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like structure, and one contained only a *bla*$_{OXA}$-51-like structure. No isolate had both IS*Aba1-bla*$_{OXA}$-23-like and IS*Aba1-bla*$_{OXA}$-51-like structure in this study.

Results of the molecular epidemiologic groups

Table 1. Oligonucleotide primers used in this study

Primer name	Nucleotide sequence (5'-3')	Amplicon size (bp)
OXA-23-likeF	AT CGG ATT GGA GAA CCA GA	501
OXA-23-likeR	ATT TCT GAC CGC ATT TCC AT	
OXA-24-likeF	GGT TAG TTG GCC CCC TTA AA	246
OXA-24-likeR	AGT TGA GCG AAA AGG GGA TT	
OXA-51-likeF	TAA TGC TTT GAT CGG CCT TG	353
OXA-51-likeR	TGG ATT GCA CTT CAT CTT GG	
OXA-58-likeF	AAG TAT TGG GGC TTG TGC TG	599
OXA-58-likeR	CCC CTC TGC GCT CTA CAT AC	
IS*Aba1*-F	CAC GAA TGC AGA AGT TG	549
IS*Aba1*-R	CGA CGA ATA CTA TGA CAC	
IMP-F	GGA ATA GAG TGG CTT AAC TCT C	188
IMP-R	CCA AAC CAC TAG GTT ATC T	
VIM-F	GAT GGT GTT TGG TCG CAT A	390
VIM-R	CGA ATG CGC AGC ACC AG	
GIM-F	TCG ACA CAC CTT GGT CTG AA	477
GIM-R	AAC TTC CAA CTT TGC CAT GC	
SPM-F	AAA ATC TGG GTA CGC AAA CG	271
SPM-R	ACA TTA TCC GCT GGA ACA GG	
SIM1-F	TAC AAG GGA TTC GGC ATC G	571
SIM1-R	TAA TGG CCT GTT CCC ATG TG	

Table 2. OXA-type and metallo-carbapenemase genes detected among 172 carbapenem-resistant *A. baumannii*

	OXA-type carbapenemase genes				metallo-carbapenemase genes				
	bla$_{OXA}$-23	*bla*$_{OXA}$-24	*bla*$_{OXA}$-51	*bla*$_{OXA}$-58	IMP	VIM	GIM	SPM	SIM
No. of isolates (%)	156 (90.7)	0 (0)	172 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

detected among 172 CRAB isolate using REP-PCR are shown in Table 3. All the *A. baumannii* isolates clustered into six distinct groups (Table 3 and Fig. 1). CRAB isolates containing IS*Aba1*-*bla*$_{OXA}$-23-like structure clustered in one largest group (group 1). Group 1 was further divided into four subgroups (group 1a, 1b, 1c and 1d) with minor band pattern differences, most (152/155) of isolates belonged to group 1a. Isolates containing IS*Aba1*-*bla*$_{OXA}$-51-like structure clustered in the second largest group (group 2). Group 2 was further divided into two subgroups (group 2a and 2b), most (13/15) of them belonged to group 2a. One isolate without IS*Aba1* upstream of *bla*$_{OXA}$-23-like gene belonged to group 1a, and another isolate containing *bla*$_{OXA}$-51-like structure lacking IS*Aba1* upstream belonged to group 2a. Carbapenem-susceptible *A. baumannii* control strains clustered into four distinct groups (group 3, 4, 5 and 6).

Relationship between OXA-carbapenemase gene structure and MICs of CRAB isolates to carbapenems are shown in Table 4. High level (MIC≥64 mg/L) of

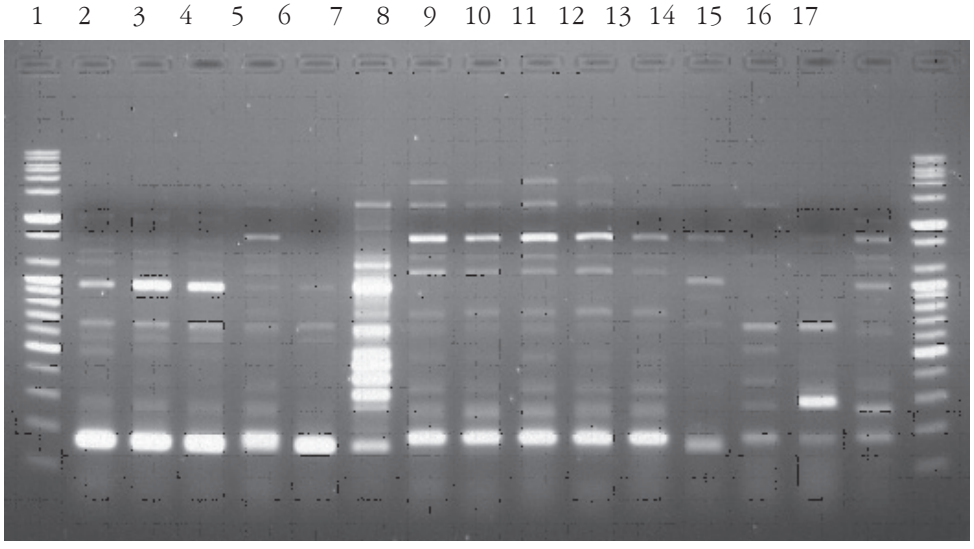

Fig. 1. DNA patterns obtained by repetitive extragenic palindromic sequence-based PCR (REP-PCR). Lanes: 1 and 17, 100 bp DNA size markers; 2 to 4, Carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates belonged to group 1a; 5, CRAB isolate belonged to group 1b, 6, CRAB isolate belonged to group 1c; 7, CRAB isolate belonged to group 1d; 8 to 11, CRAB isolates belonged to group 2a, 12, CRAB isolate belonged to group 2b; 13 to 16, carbapenem-susceptible *A. baumannii* isolates belonged to group 3, 4, 5 and 6, respectively.

Table 3. Arrangement of *bla*$_{OXA}$ genes with upstream IS*Aba1* and their molecular epidemiologic types detected among 172 carbapenem-resistant *A. baumannii*

Arrangement of *bla*$_{OXA}$ genes with upstream IS*Aba1*	No. (%) of isolates	Molecular types by REP-PCR	No. (%) of isolates
Both IS*Aba1*-*bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like	155 (90.1)	1a	152 (88.4)
		1b	1 (0.6)
		1c	1 (0.6)
		1d	1 (0.6)
IS*Aba1*-*bla*$_{OXA}$-51-like only	15 (8.7)	2a	13 (7.6)
		2b	2 (1.2)
Both *bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like	1 (0.6)	1a	1 (0.6)
bla$_{OXA}$-51-like only	1 (0.6)	2a	1 (0.6)
Negative control[a]	4	3	1 (0.6)
		4	1 (0.6)
		5	1 (0.6)
		6	1 (0.6)

a; Carbapenem-susceptable *A. baumannii*.

resistance to imipenem and meropenem was observed in CRAB isolates containing an IS*Aba1*-*bla*$_{OXA}$-23-like structure. One isolate with *bla*$_{OXA}$-23-like gene lacking IS*Aba1* upstream also showed high level (MIC≥64 mg/L) of resistance to carbapenems. Isolates containing an IS*Aba1*-*bla*$_{OXA}$-51-like structure showed moderate level (MIC 16-32 mg/L) resistance to carbapenems.

Discussion

A. baumannii has emerged globally as an increasingly important nosocomial pathogen [1,2]. Carbapenems have been the antibiotics of choice for treatment of infections caused by this organism, but resistance to carbapenems is becoming common, and very few therapeutic options remain [7,8,26]. With the increase in resistance, resistance surveillance has become increasingly important [5,27].

Presence of OXA-type carbapenemase and IS*Aba1* upstream of *bla*$_{OXA}$ genes has emerged as a mechanism of high-level resistance to carbapenems among CRAB [9-11]. In the present study, all 172 CRAB isolates harboured OXA-51-like carbapnenemase gene. Most (90.7%) of them contained OXA-23-like gene in addition to OXA-51-like gene. We did not detect any genes encoding the carbapenemases OXA-24-like and OXA-58-like. Lee *et al*. [28] and Kim, *et al*. [29] also reported that most resistance in *A. baumannii* was due to OXA-23-like or upregulated OXA-51-like genes. The OXA-23 group has been reported worldwide and is particularly prominent in

Table 4. Relationship between OXA-carbapenemase gene structure and MICs of carbapenem-resistant *A. baumannii* isolates

OXA-carbapenemase gene structure	No. of isolates in							
	MIC[a]s (mg/L) of imipenem				MICs (mg/L) of meropenem			
	>64	64	32	16	>64	64	32	16
Both IS*Aba1*-*bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like	91	62	2		17	87	51	
IS*Aba1*-*bla*$_{OXA}$-51-like only			12	5			14	1
Both *bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like	1					1		
bla$_{OXA}$-51-like only			1				1	

a; minimal inhibitory concentration.

certain geographical regions, including China [30,31]. Poirel *et al*. [32] reported that *bla*$_{OXA}$-23 in CRAB originated from *A. radioresistens* and *bla*$_{OXA}$-23 has been found in environmental *A. baumannii*, this suggests possible niches of gene transfer [33].

MBLs have received most attention as a source of carbapenem resistance in Korea [28]. But none of the genes encoding MBLs (IMP, VIM, GIM, SPM and SIM) were detected in this study. Other reports also showed that they did not detect any of the MBL genes in *A. baumannii* and some MBL-producing isolates belonged to non-*baumannii* species [28,29,34,35].

Transposable elements play an important role in the expression of resistance genes in *A. baumannii* [10]. High-level carbapenem resistance due to the expression of genes encoding OXA-carbapenemases requires a strong promoter like that provided by the IS*Aba1* [11,12,36]. In the present study, IS*Aba1*-was upstream of *bla*$_{OXA}$-23-like in most (90.1%) CRAB isolates and 15 (8.7%) isolates contained an ISA*ba1*-*bla*$_{OXA}$-51-like structure. Other reports also showed that IS*Aba1* was inserted upstream of *bla*$_{OXA}$-23 and *bla*$_{OXA}$-51 in most CRAB isolates [28-30,36,37]. The presence of IS*Aba1* upstream of *bla*$_{OXA}$-23 gene in CRAB was highly correlated with resistance to imipenem and meropenem. High level (MIC≥64 mg/L) of resistance to imipenem and meropenem was observed in isolates containing an IS*Aba1*-*bla*$_{OXA}$-23-like structure. Isolates containing an IS*Aba1*-*bla*$_{OXA}$-51-like structure showed moderate level (MIC 16-32 mg/L) resistance to carbapenems. *bla*$_{OXA}$-51-like gene is chromosomally located intrinsic ß-lactamase gene and is normally quiescent [9,38]. Its expression is mediated by a promoter of IS*Aba1* located upstream of the structural gene, but the yield may not be as much as that of *bla*$_{OXA}$-23-like [9,38]. It is likely that differences in transcriptional level regulation of *bla*$_{OXA}$ genes could affect carbapenem resistance levels [37].

Among the remaining CRAB isolates without IS*Aba1* upstream of *bla*$_{OXA}$ genes, one isolate contained both *bla*$_{OXA}$-23-like and *bla*$_{OXA}$-51-like structure, and one contained only a *bla*$_{OXA}$-51-like structure in this study. One isolate with *bla*$_{OXA}$-23-like gene lacking IS*Aba1* upstream also showed high level (MIC≥64 mg/L) of resistance to carbapenems. It has been suggested that alternative mechanisms, such as overexpression of an efflux

pump and loss of outer membrane protein, may be responsible for resistance to carbapenems [9,37]. But nonenzymatic mechanisms were considered not to be major factors contributing to carbapenem resistance [9,37]. Poirel *et al.* [12] reported that non-IS*Aba1* IS elements such as IS*Aba2*, IS*Aba3* or IS18 provide promoter sequences for the expressions of some OXA-carbapenemase genes, such as *bla*$_{OXA}$-58. Taken together, some unidentified IS elements may contribute to the carbapenem resistances of CRAB isolates lacking IS*Aba1* upstream [9,12].

CRAB isolates have been associated with infection outbreaks and information on the current status of the molecular epidemiology is important to monitor and control the spread of them in the hospital environment [7,8,13]. In this study, REP-PCR was chosen as the molecular epidemiologic typing method for its easy of use, high throughput and discriminatory power that has been shown to be comparable to that of PFGE and fluorescent amplified fragment-length polymorphism [23,24,34]. Most (152/155) of isolates containing IS*Aba1-bla*$_{OXA}$-23-like structure were clustered into one molecular type group 1a, suggesting that they originated from common bacteria and clonally spread in the study hospital [29,34]. And majority (13/15) of isolates containing IS*Aba1-bla*$_{OXA}$-51-like structure were clustered into group 2a, also suggesting that they originated from another common bacteria and clonally spread. Given that clonal spread is likely responsible for the majority of CRABs presented in this study, CRAB infections may indicate a serious infection control problem and represents a serious challenge to public health [34]. The carbapenems are the drugs of choice for treating infections caused by extended-spectrum ß-lactamase (ESBL)-producing bacteria [7]. The spread may have been facilitated by the extensive use of carbapenems for treating ESBL-producing MDR Gram-negative bacteria that are frequently isolated in ICUs [6,35,39]. The persistence of *A. baumannii* in the environment and the effect of antibiotic usage suggest that restriction of antibiotic prescribing and early discharge of patients from hospital are important measures in combating outbreaks of this pathogen [35].

In conclusion, continuous monitoring of CRAB and discovery of mechanisms of the acquisition of resistances by concerted multidisciplinary efforts and rigorous infection control measures are essential to help develop effective therapy regimens and to prevent the further spread of CRAB infections [37,40].

Summary

A total of 172 strains of CRAB isolated from clinical specimens were tested to provide an information on the current status of the molecular epidemiology of carbapenemase genes in CRAB and the genetic structures. All the CRAB isolates harboured OXA-51-like carbapnenemase gene and most (90.7%) of them contained OXA-23-like gene in addition to OXA-51-like gene. We did not detect any genes encoding the carbapenemases OXA-24-like and OXA-58-like, and none of the genes encoding metallo-ß-lactamases (IMP, VIM, GIM, SPM and SIM) were detected in this study. IS*Aba1*-was upstream of *bla*$_{OXA}$-23-like in most (90.1%) CRAB isolates and 15 (8.7%) isolates contained an IS*Aba1-bla*$_{OXA}$-51-like structure. The presence of IS*Aba1* upstream of *bla*$_{OXA}$-23 gene in CRAB was highly correlated with resistance to imipenem and meropenem. In REP-PCR epidemiologic typing, most (152/155) of isolates containing IS*Aba1-bla*$_{OXA}$-23-like structure were clustered into one molecular type group 1a, suggesting that they originated from common bacteria and clonally spread in the study hospital. In conclusion, continuous monitoring of CRAB and discovery of mechanisms of the

resistances by concerted efforts and infection control measures are essential to help develop effective therapy regimens and to prevent the further spread of CRAB infections.

Acknowledgements

We thank Sung Duck Paik, Hye Kyung Shin and Eun Young Park for expert technical assistances.

References

1. Bergogne-Bérézin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin Microbiol Rev* 1996;**9**:148–65.
2. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008;**21**:538–82.
3. Gaynes R, Edwards JR. Overview of nosocomial infections caused by Gram-negative bacilli. *Clin Infect Dis* 2005;**41**:848-54.
4. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nature Rev Microbiol* 2007;**5**:939-51.
5. Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009;**64**(Suppl 1):i3-10.
6. Kempf M, Rolain M. Emergence of resistance to carbaphenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. *Int J Antimicrob agents* 2012;**39**:105-14.
7. Brown S, Amyes S. OXA ß-lactamases in *Acinetobacter*: the story so far. *J Antimicrob Chemother* 2006;**57**:1-3.
8. Coelho J, Woodford N, Turton J, Livermore DM. Multiresistant *Acinetobacter* in the UK: how big a threat? *J Hosp Infect* 2004;**58**:167-9.
9. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 2006;**12**:826-36.
10. Mugnier PD, Poirel L, Nordmann P. Functional analysis of insertion sequence IS*Aba1*, responsible for genomic plasticity of *Acinetobacter baumannii*. *J Bacteriol* 2009;**191**:2414-8.
11. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R. The role of IS*Aba1* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006;**258**:72-7.
12. Poirel L, Nordmann P. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*$_{OXA}$-58 in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006;**50**:1442-8.
13. Lu PL, Huang LY, Lian ST, Chang K, Lin CL, Hwang IJ, *et al*. How carbapenem-resistant *Acinetobacter* spp. established in a newly constructed hospital. *Int J Antimicrob Agents* 2008;**31**:463-6.
14. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. *Manual of Clinical Microbiology*. 9th ed. Washington, DC, ASM Press; 2007, p.770-802.
15. Loffler FE, Sun Q, Li J, Tiedje JM. 16S rRNA gene-based detection of tetrachloroethene-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. *Appl Environ Microbiol* 2000;**66**:1369-74.
16. Dortet L, Legrand P, Soussy CJ, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol* 2006;**44**:4471-8.
17. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the *bla*$_{OXA}$-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006;**44**:2974-6.
18. Clinical and Laboratory Standard Institute. *Performance standards for antimicrobial susceptibility*

testing: Twenty-first informational supplement M100-S21. CLSI, Wayne, PA, USA, 2011.
19. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, *et al*. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006;**27**:351-3.
20. Segal H, Garny S, Elisha BG. Is IS*Aba1* customized for *Acinetobacter*? *FEMS Microbiol Lett* 2005;**243**:425-9.
21. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-ß-lactamase. *J Antimicrob Chemother* 2007;**59**:321-2.
22. Vila J, Marcos MA, Jimenez De Anta MT. A comparative study of different PCR-based DNA fingerprinting techniques for typing of the *Acinetobacter calcoaceticus-A. baumanni* complex. *J Med Microbiol* 1996;**44**:482-9.
23. Saeed S, Fakih MG, Riederer K, Shah AR, Khatib R. Interinstitutional and intrainstitutional transmission of a strain of *Acinetobacter baumannii* detected by molecular analysis: comparison of pulsed-field gel electrophoresis and repetitive sequence-based polymerase chain reaction. *Infect Control Hosp Epidemiol* 2006;**27**:981-3.
24. Fontana C, Favaro M, Minelli S, Bossa MC, Testore GP, Leonardis F, *et al*. *Acinetobacter baumannii* in intensive care unit: a novel system to study clonal relationship among the isolates. *BMC Infect Dis* 2008;**8**:79.
25. Snelling AM, Gerner-Smidt P, Hawkey PM, Heritage J, Parnell P, Porter C, *et al*. Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus-Acinetobacter baumannii complex* and application of the method to the investigation of a hospital outbreak. *J Clin Microbiol* 1996;**34**:1193-202.
26. Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. *Clin Microbiol Infect* 2007;**13**:807-15.
27. Hawkey PM. The growing burden of antimicrobial resistance. *J Antimicrob Chemother* 2008;**62**(Suppl 1):i1-9.
28. Lee K, Kim MN, Choi TY, Cho SE, Lee S, Whang DH, *et al*. Wide dissemination of OXA-type carbapenemases in clinical *Acinetobacter* spp. isolates from South Korea. *Int J Antimicrob Agents* 2009;**33**:520-4.
29. Kim JW, Heo ST, Jin JS, Choi CH, Lee YC, Jeong YG, *et al*. Characterization of *Acinetobacter baumannii* carrying *bla*$_{OXA}$-23, *bla*$_{PER}$-1 and *armA* in a Korean hospital. *Clin Microbiol Infect* 2008;**14**:716–8.
30. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the *bla*$_{OXA}$-23 carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 2010;**16**:35-40.
31. Walsh TR. Emerging carbapenemases: a global perspective. *Int J Antimicrob Agents* 2010;**36**S3:S8-14.
32. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother* 2008;**52**:1252-6.
33. Girlich D, Poirel L, Nordmann P. First isolation of the *bla*$_{OXA}$-23 carbapenemase gene from an environmental *Acinetobacter baumannii* isolate. *Antimicrob Agents Chemother* 2010;**54**:578-9.
34. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010;**65**:233-8.
35. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N, *et al*. Characterization of carbapenem-resistant *Acinetobacter baumannii* outbreak strains producing OXA-58 in Turkey. *Int J Antimicrob Agents* 2010;**36**:114-8.
36. Lin MF, Kuo HY, Yeh HW, Yang CM, Sung CH, Tu CC, *et al*. Emergence and dissemination of *bla*$_{OXA}$-23-

carrying imipenem-resistant *Acinetobacter* sp in a regional hospital in Taiwan. *J Microbiol Immunol Infect* 2011;**44**:39-44.

37. Lin YC, Hsia KC, Chen YC, Sheng WH, Chang SC, Liao MH, *et al*. Genetic basis of multidrug resistance in *Acinetobacter* clinical isolates in Taiwan. *Antimicrob Agents Chemother* 2010;**54**:2078-84.
38. Walsh TR. Clinically significant carbapenemases: an update. *Curr Opin Infect Dis* 2008;**21**:367-71.
39. Cao J, Song W, Gu B, Mei Y, Tang J, Meng L, *et al*. Correlation between carbapenem consumption and antimicrobial resistance rates of *Acinetobacter baumannii* in a university-affiliated hospital in China. *J Clin Pharmacol* 2012.doi:10.1177/009127001143 5988.
40. Queenan AM, Bush K. Carbapenemases: the versatile ß-lactamases. *Clin Microbiol Rev* 2007;**20**:440-58.