Carbapenem and colistin resistance patterns, mechanism and molecular epidemiology of *Acinetobacter* species in Korea

Yangsoon Lee

Department of Medicine
The Graduate School, Yonsei University

Carbapenem and colistin resistance patterns, mechanism and molecular epidemiology of *Acinetobacter* species in Korea

Directed by Professor Seok Hoon Jeong

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Doctor of Philosophy

Yangsoon Lee

June 2012

This certifies that the Doctoral Dissertation of
Yangsoon Lee is approved.

Thesis Supervisor : Seok Hoon Jeong

Thesis Committee Member#1 : Kyungwon Lee

Thesis Committee Member#2 : Young Goo Song

Thesis Committee Member#3: Sang Sun Yoon

Thesis Committee Member#4: Wonkeun Song

The Graduate School
Yonsei University

June 2012

ACKNOWLEDGEMENTS

I am honored to thank many grateful benefactors who contributed this thesis. In particular, I deeply appreciate supervisor, Prof. Seok Hoon Jeong for his sincere principles, thorough guidance and honest enthusiasm. With great appreciation, I acknowledge Prof. Kyungwon Lee for endless encouragement, valuable advice and support. In addition, I am grateful to be guided by Prof. Young Goo Song, Prof. Sang Sun Yoon and Prof. Wonkeun Song who gave me expert counsel and encouragement to complete this thesis.

I deeply thank my family and coworkers for patience, sacrifice, faith, love and pray for me. Especially, I thank my husband, Joseph Cho, for his love and patience. Thanks God to give me health and wisdom to complete my thesis. I love my Lord, Jesus Christ and I will follow him.

Jesus answered, “I am the way and the truth and the life.”

John 14:6

June 2012

Yangsoon Lee

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Carbapenem and colistin resistance patterns, mechanism and molecular epidemiology of *Acinetobacter* species in Korea

Yangsoon Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Seok Hoon Jeong)

Recent increasing carbapenem resistance in *Acinetobacter* species is very worrisome, resulting in a very limited antimicrobial treatment option. Colistin is the only potent therapeutic options available for treatment of nosocomial infections caused by multi-drug resistant *Acinetobacter* species. However, colistin-resistant *Acinetobacter* species of clinical isolates were reported. In present study, it was investigated that resistance patterns and mechanisms to carbapenems and colistin in *Acinetobacter* species isolated from clinical

specimens from 19 hospitals in six provinces in Korea in 2008, and the epidemiological traits of carbapenem-non-susceptible *Acinetobacter baumannii* (CNSAB), as well as the usefulness of phylogenic grouping based on partial *rpoB* gene sequencing in defining the epidemiological traits of CNSAB.

A total of 547 non-duplicate clinical isolates of *Acinetobacter* species were collected from 19 hospitals. Reduced susceptibility rates to imipenem or meropenem were 70% (272/388) for *A. baumannii*, 5% (4/82) for *A. nosocomialis*, 13% (8/62) for *A. pitti*, and 0% (0/13) for *A. bereziniae*. Colistin-resistant clinical isolates were one *A. baumannii*, one *A. bereziniae* and two *Acinetobacter* genomospecies 14 TU. Molecular characterization of the 272 CNSAB isolates using multilocus sequencing typing identified five sequence types (STs): ST92, ST75, ST137, ST138 and ST69. Those STs were clustered into clonal complex 92, sharing alleles at five to six of seven housekeeping gene loci. ST92 was the predominant ST and was associated with European clone II disseminated worldwide. CNSAB of CC92 carried the $bla_{\text{OXA-23}}$ gene (n = 169), the $bla_{\text{OXA-51}}$-like gene preceded by IS*Aba1* (n = 89) or both (n = 14). Sequence -based analysis of the *rpoB* gene showed single-nucleotide substitutions in zone 2 of the *rpoB* gene, resulting in division into five groups (A to E groups). Notably, all CNSAB isolates belonged to subgroup B-0 with a G428T substitution in zone 2. All of the other isolates belonging to group C, D and E were susceptible to carbapenems.

In conclusion, carbapenem resistance rates of *A. baumannii* are very high, over 70%, whereas colistin resistance rate of *Acinetobacter* species is very low until now. CNSAB isolates of CC92 with the G428T substitution in zone 2 of the *rpoB* gene are disseminated nationwide in our country. *A. baumannii* with the G428T substitution in zone 2 may be more likely to acquire carbapenem resistance than are other isolates.

Key words : *Acinetobacter baumannii*, clonal complex 92, European clone II, multilocus sequence typing, carbapenem, colistin

Carbapenem and colistin resistance patterns, mechanism and molecular epidemiology of *Acinetobacter* species in Korea

Yangsoon Lee

Department of Medicine
The Graduate School, Yonsei University

(Directed by Professor Seok Hoon Jeong)

I. INTRODUCTION

The genus *Acinetobacter* consists of strictly aerobic, gram-negative coccobacillary rods that are oxidase negative, non-motile, usually nitrate negative, and nonfermentative.[1, 2] The genus *Acinetobacter* consists of 22 nomenspecies and at least 11 additional putative species.[3] *Acinetobacter* species are widely distributed in nature and in the hospital environment. *Acinetobacter* species are generally considered to be nonpathogenic to healthy individuals but may cause infections such as pneumonia, bacteremia, and meningitis in debilitated individuals.[2, 4] *Acinetobacter baumannii* is an opportunistic pathogen

and is the most common cause of serious nosocomial infections within the genus *Acinetobacter*, especially those found in intensive care units (ICUs).[3, 5, 6] Carbapenems are β-lactam antibiotics that are highly active even against Gram-negative bacilli producing extended spectrum β-lactamases or hyper-producing AmpC β-lactamases. Therefore, carbapenems are recommended as first-line therapy in the treatment of serious infections caused by *A. baumannii* resistant to oxymino-cephalosporins such as ceftriaxone and cefotaxime. The recent dissemination of carbapenem-non-susceptible *A. baumannii* (CNSAB) in many parts of the world is therefore very worrisome, resulting in limited antimicrobial treatment options.[4, 7, 8]. Carbapenem resistance in *Acinetobacter* species has mainly been ascribed to enzymatic degradation by metallo-β-lactamases (MBLs) including IMPs, VIMs and SIM, or OXA carbapenemases including OXA-23, OXA-24, OXA-51, OXA-58 and their sequence variants.[9-13] Nonenzymatic mechanisms including over expression of efflux pumps and changes in outer membrane proteins or penicillin-binding proteins may also play a role in acquiring carbapenem resistance.[14-16] The chromosome-borne *bla*$_{\text{OXA-51}}$-like genes are ubiquitous to *A. baumannii*. The *bla*$_{\text{OXA-51}}$-like genes play a role in carbapenem resistance when an IS*Aba1* presents upstream of the gene, while they have little effect on carbapenem susceptibility in the absence of this insertion sequence.[17, 18] Colistin and polymixin B are the only potent options available for treatment of nosocomial

infections by multi-drug resistant *A. baumannii* isolates. However, Ko *et al*.[19] reported high rates of resistance to colistin and polymixin B in subgroups of *A. baumannii* isolates.

Worldwide dissemination of carbapenem-resistant European clones I and II (EU-I and EU-II) has been reported.[20] Mugnier *et al*.[21] identified eight EU-I isolates of sequence type (ST) 25, ST44, or two novel STs, as well as ten EU-II isolates of ST92 (ST22 was moved to ST92 after an update of allelic profiles at PubMLST website, http://pubmlst.org) or ST118 (moved from ST53) in 20 *A. baumannii* isolates carrying the $bla_{\text{OXA-23}}$ gene, originating from 15 countries. Wide dissemination of CNSAB isolates of ST92 and its single-locus variants have also been described in China and Korea.[22, 23] Nemec *et al*.[24] found that 20 isolates carrying $bla_{\text{OXA-58}}$-like, $bla_{\text{OXA-24}}$-like, or $bla_{\text{OXA-51}}$-like genes preceded by IS*Aba1* from 23 *A. baumannii* isolates with carbapenem MICs of ≥ 8 μg/mL isolated from Czech Republic belonged to EU-II. Dissemination of EU-II isolates carrying the $bla_{\text{OXA-58}}$ gene in ICUs in Rome has also been reported.[25] In contrast, Kulah *et al*.[26] reported that multiple clones of *A. baumannii* carrying the $bla_{\text{OXA-58}}$ gene were responsible for a sustained outbreak that occurred in a hospital in Turkey; however, those isolates were not associated with the *A. baumannii* EU-I, EU-II, or EU-III strains.

Scola *et al*.[27] demonstrated the usefulness of RNA polymerase β-subunit (*rpoB*) gene sequencing in the differentiation and identification of *Acinetobacter* spp.

Ko *et al*.[19] reported that *A. baumannii* isolates could be divided into three subgroups (I, II, or III) based on phylogenic grouping inferred from the sequence of a variable region, zone 2, in the *rpoB* gene. Furthermore, they reported that the grouping correlated with antimicrobial resistance profiles. While most isolates of subgroup I were susceptible to colistin and polymyxin B, many isolates of subgroups II and III exhibited resistance to these drugs. However, they did not focus on correlations between the grouping and carbapenem resistance profiles in *A. baumannii*.

The aims of this study were to investigate: (1) carbapenem and colistin resistance patterns and mechanisms according to *Acinetobacter* species isolates from clinical specimens of patients hospitalized at 19 different hospitals in Korea, (2) the epidemiological traits of CNSAB isolates, and (3) the usefulness of phylogenic grouping based on partial *rpoB* gene sequencing in defining the epidemiological traits of CNSAB.

II. MATERIALS AND METHODS

1. Bacterial isolates and reference strains

A total of 547 clinical isolates of *Acinetobacter* spp. were collected from 19 different hospitals in six provinces of Korea in 2008. During those periods, the isolates were consecutively collected at each hospital and recovered from one isolate per patient. The strains were isolated from blood, sputum, wound and urine specimens. The isolates were identified by either conventional methods or the ATB 32 GN system (bioMérieux, Marcy l'Etoile, France) and 16S-23S rRNA intergenic spacer region sequencing.[2, 28]

2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the CLSI disk diffusion methods. The antimicrobial disks used were imipenem (Becton Dickinson, Sparks, MD, USA) and meropenem (Becton Dickinson). MICs of colistin (Sigma-Aldrich, St.Louis, Mo, USA) were determined using the agar dilution method according to CLSI guidelines.[29] *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *A. baumannii* ATCC 19606 and *Acinetobacter* genomospecies 14TU ATCC 17905 were used as the control strains.

3. Investigation of carbapenem resistance mechanisms

A. Screening for carbapenemase production

The modified Hodge test was performed on MacConkey agar plates as described previously for the screening of carbapenemases.[30] Briefly, the indicator organism, *E. coli* ATCC 25922, at a turbidity of 0.5 McFarland standard, was used to swab inoculate the surface of a MacConkey agar plate (Becton Dickinson), and the test strain was heavily streaked from the center to the plate periphery. After the plate was allowed to stand for 15 min at room temperature, a 10-μg imipenem disk (Becton Dickinson) was placed at the center, and the plate was incubated overnight. The presence of a distorted inhibition zone was interpreted as a positive result for carbapenem hydrolysis screening.

The imipenem and EDTA-sodium mercaptoacetic acid (SMA) double-disk synergy (IEDDS) test were performed on Mueller-Hinton agar plates as described previously for the screening of and MBLs.[30] Briefly, a 10-μg imipenem disk (Becton Dickinson) was placed on Mueller-Hinton agar plate, and a blank filter paper disk added 10 μL of 0.5 M EDTA and SMA solution was placed at a distance of 10 mm a blank filter paper disk. After overnight incubation, the presence of even a small synergistic inhibition zone was interpreted as positive.

B. Detection of OXA carbapenemase and MBL genes

A few isolated colonies of test organism were suspended in 100 μL of distilled water and then boiled for 10 min to extract DNA. After centrifugation for 2 min at 13,000 rpm, the supernatant was used as a template. PCR premix (Accupower® PCR Premix, Bioneer, Daejeon, Korea) was used for PCR reaction. Multiplex PCR was performed to detect *bla*$_{OXA}$ genes as described by Woodford *et al*.[31] Multiplex PCR was done using PCR primer sets listed in Table 1. PCR condition was as follows; 94°C for 5 min, 94°C for 25 sec, 52°C for 40 sec, 72°C for 50 sec for 30 cycles and final extension at 72°C for 6 min. PCR product was identified in 1.5% agarose gel electrophoresis stained with ethidium bromide. Product sizes expected were 501 bp (OXA-23-like gene), 246 bp (OXA-24-like gene), 353 bp (OXA-51-like gene) and 599 bp (OXA-58-like gene). To detect any upstream presence of IS*Aba1*, primer ISAba1-F in combination with reverse primers for the relevant *bla*$_{OXA-51}$-like gene.

PCR experiments were carried out to detect the genes encoding MBLs (IMP-1 variants, VIM-2 variants, and SIM-1) as described previously[32]. PCR condition was as follows; 94°C for 5 min, 94°C for 30 sec, 52°C for 40 sec, 72°C for 50 sec for 35 cycles and final extension at 72°C for 5 min. The nucleotide sequences were analyzed using PCR-generated amplicons at a commercial laboratory (Macrogen, Seoul, Korea).

Table 1. Primers used for detection and sequencing of OXA carbapenemases and metallo-β-lactamases

Targets	Primers	Sequence (`5 to 3`)
OXA-23-like	OXA-23-F	GATCGGATTGGAGAACCAGA
	OXA-23-R	ATTTCTGACCGCATTTCCAT
OXA-24-like	OXA-24-F	GGTTAGTTGGCCCCCTTAAA
	OXA-24-R	AGTTGAGCGAAAAGGGGATT
OXA-51-like	OXA-51-F	TAATGCTTTGATCGGCCTTG
	OXA-51-R	TGGATTGCACTTCATCTTGG
OXA-58-like	OXA-58-F	AAGTATTGGGGCTTGTGCTG
	OXA-58-R	CCCCTCTGCGCTCTACATAC
bla$_{VIM}$	VIM-F	GATGGTGTTTGGTCGCATA
	VIM-R	CGAATGCGCAGCACCAG
bla$_{IMP}$	IMP-F	GGAATAGAGTGGCTTAAYTCTC
	IMP-R	CCAAACYACTASGTTATCT
bla$_{SIM}$	SIM-F	TACAAGGGATTCGGCATCG
	SIM-R	TAATGGCCTGTTCCCATGTG
IS*Aba1*	ISAba1-F	CACGAATGCAGAAGTTG
	ISAba1-R	CGACGAATACTATGACAC

C. *bla*$_{\text{OXA-23}}$ gene location

(A) Pulsed-field gel electrophoresis and Southern blotting

Strains were cultured onto a Muller-Hinton agar media for 18-20 h. Harvested cells suspended into 1 mL of saline-EDTA solution (0.15 M NaCl, 10 mM EDTA, pH 8.0) were centrifuged at 12,000 rpm for 5 min. Sediment cells were added 130 μL of cold Pett IV buffer (1 M NaCl, 10 mM EDTA, pH 8.0) and 2 μL of lysozyme (10 mg/mL). After that, 165 μL of 2% InCert agarose (Cambrex Bio Science Rockland, Inc. Rockland, ME, USA) was added and transferred into the plug mold, and harden at 4°C for 10 min. A plug was divided into 3 pieces and lysed with 1 mL of lysozyme and 1 mL of proteinase K 10 μL plus ES solution (1% N-lauroyl-sarcosine, 0.25M EDTA, pH 8.0) 1 mL at 50°C overnight. Samples added 1.5 mL of 1 mM phenylmethylsulfonyl fluride in TE buffer (10 mL Tris-HCl, 1 mM EDTA, pH 8.0) solution were incubated for 4 h and washed three times, each for 10 min, in 1 mL of TE buffer. Plug was washed once for 50 min, in 1 mL of 0.1 mM EDTA-TE buffer. Plugs containing whole genomic DNA of the isolates were digested with S1 nuclease or I-*Ceu*I restriction enzyme. DNA fragments were separated using PFGE with a CHEF-DRII device (Bio-Rad, Hercules, CA, USA). PFGE was performed at 6 V/cm for 20 h with pulse times ranging from 9 to 90 s at a temperature of 14°C. PFGE was performed in 1% agarose gel electrophoresis and stained with ethidium bromide for 30 min. The gels with S1 nuclease-treated linearized

plasmids and I-*Ceu*I-digested chromosomal DNA were blotted onto nylon membranes (Bio-Rad) and hybridized with probes specific for the $bla_{\text{OXA-23}}$ gene or 16S rRNA gene. The probes were obtained via PCR experiments as described above. Probe labeling, hybridization, and detection were performed with the DIG DNA Labeling and Detection kit (Roche Diagnostics, Indianapolis, IN, USA).

4. Evaluation of molecular epidemiologic traits

A. Repetitive sequence-based PCR

Repetitive sequence-based PCR (rep-PCR) experiments were performed using the DiversiLab system (bioMérieux Inc., Grenoble, France) according to the manufacturer's instructions to investigate the clonal relatedness of *A. baumannii* isolates. Briefly, the whole microbial DNA using beads were extracted, and rep-PCR amplification was performed in a thermal cycler. PCR product was loaded in the DNA chip and run in the Agilent® 2100 Bioanalyzer. Results were analyzed with DiversiLab software using the Kullback-Leibler method to determine distance matrices and the unweighted pair group method with arithmetic averages to create a dendrogram. Isolates showing >95% similarity were considered to be related.[33]

B. Multilocus sequence typing

Multilocus sequence typing (MLST) was performed using the method of Bartual *et al*.[34] Fragments of seven housekeeping genes (*gltA, gyrB, gdhB, recA, cpn60, gpi,* and *rpoD*) were amplified by PCR and sequenced. Two primer sets were redesigned to detect *gpi* and *rpoD* genes (Table 2). Multiplex PCR condition was as follows; 94°C for 5 min, 94°C for 1 min, 55°C for 1 min, 72°C for 2 min for 30 cycles and final extension at 72°C for 2 min. PCR products loaded in 1.5% agarose gel were extracted using QIAquick gel extraction kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The nucleotide sequences were analyzed using PCR-generated amplicons at a commercial laboratory (Macrogen). Allele numbers were assigned a sequence type after the distinct allele sequences were submitted to a dedicated database (http://pubmlst.org). A clonal complex (CC) was used to assess the genetic relatedness of the STs; the most stringent definition - sharing alleles at six of seven loci - was used.[35] MLST described by Diancourt *et al.*[36] was performed for representative strains allocated to the STs associated with CNSAB. Allelic profiles of seven housekeeping genes (*cpn60, fusA, gltA, pyrG, recA, rplB* and *rpoB*) were analyzed using *Acinetobacter* MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/).

Table 2. Primers used for detection and sequencing of seven housekeeping genes using MLST analysis

Targets	Primers	Sequence (`5 to 3`)
gltA	gltA-F	AATTTACAGTGGCACATTAGGTCCC
	gltA-R	GCAGAGATACCAGCAGAGATACACG
gyrB	gyrB-F	TGTAAAACGACGGCCAGTGCNGGRTCYTTYTCYTGRCA
	gyrB-R	CAGGAAACAGCTATGACCAYGSNGGNGGNAARTTYRA
	gyrB-sF	TGTAAAACGACGGCCAGT
	gyrB-sR	CAGGAAACAGCTATGACC
gdhB	gdhB-F	GCTACTTTTATGCAACAGAGCC
	gdhB-R	GTTGAGTTGGCGTATGTTGTGC
	gdhB-sF	ACCACATGCTTTGTTATG
	gdhB-sR	GTTGGCGTATGTTGTGC
recA	recA-F	CCTGAATCTTCYGGTAAAAC
	recA-R	GTTTCTGGGCTGCCAAACATTAC
cpn60	cpn60-F	ACTGTACTTGCTCAAGC
	cpn60-R	TTCAGCGATGATAAGAAGTGG
gpi	gpi-F	AATACCGTGGTGCTACG
	gpi-R	TTCAGGAGCAATCCCCCACT
rpoD	rpoD-F	CGAATYGCATTGCAAAACG
	rpoD-R	CNGCAATYTTTTGYTGGAA

5. Phylogenic grouping based on partial *rpoB* gene sequencing.

The 450-bp sequence (zone 2) of the *rpoB* gene was amplified using the primers Ac1005F (5'-GTGATAARATGGCBGGTCGT-3') and Ac1598R (5'-CGBGCR TGCATYTTGTCRT-3') as previously described.[27] The phylogenic relationship based on the *rpoB* zone 2 sequence was estimated using the neighbor-joining method in molecular evolutionary genetic analysis software.

6. Statistical analysis

Statistical analysis was done by SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Differences between categorical variables were analyzed using the chi-square test (Fisher's exact test). *P* values less than 0.05 were considered significant.

III. RESULTS

1. Collection and identification of *Acinetobacter* species

Clinical isolates of *Acinetobacter* species were collected from 19 hospitals in six provinces in Korea in 2008 (Fig. 1). The 547 clinical isolates of *Acinetobacter* species were identified as *A. baumannii* (n = 388), *Acinetobacter nosocomialis* (n = 82), *Acinetobacter pittii* (n = 62), *Acinetobacter bereziniae* (n = 13), and *Acinetobacter* genomospecies 14TU (n = 2) (Table 3).

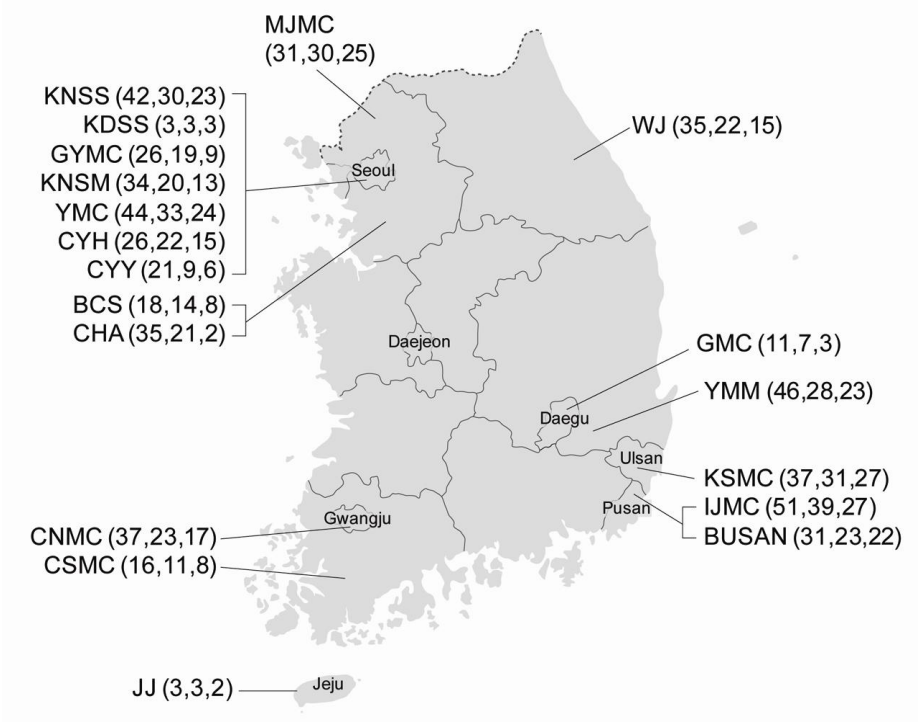


Fig. 1. The geographic location of 19 hospitals and numbers of clinical isolates (*Acinetobacter* spp., *A. baumannii*, and carbapenem-non-susceptible *A. baumannii*) collected in South Korea.

Table 3. Collection of *Acinetobacter* species clinical isolates

Hospitals	*A. baumannii*	*A. nosocomialis*	*A. pitti*	*A. bereziniae*	*A.* genomosp. 14TU	Total
KNSM	20	5	9	0	0	34
KNSS	30	8	4	0	0	42
KDSS	3	0	0	0	0	3
GYMC	19	4	2	1	0	26
KSMC	31	5	1	0	0	37
GMC	7	1	3	0	0	11
MJMC	30	0	1	0	0	31
BUSAN	23	6	1	0	1	31
BCS	14	1	3	0	0	18
CHA	21	3	4	7	0	35
YMC	33	9	1	0	1	44
YMM	28	7	11	0	0	46
WJ	22	2	10	1	0	35
IJMC	39	7	4	1	0	51
CNMC	23	13	1	0	0	37
JJ	3	0	0	0	0	3
CSMC	11	3	2	0	0	16
CYY	9	8	1	3	0	21
CYH	22	0	4	0	0	26
Total	388	82	62	13	2	547

2. Carbapenem and colistin resistance patterns of *Acinetobacter* species

Reduced susceptibility rates to imipenem or meropenem were 70% (272/388) for *A. baumannii*, 5% (4/82) for *A. nosocomialis*, 13% (8/62) for *A. pitti*, and 0% (0/13) for *A. bereziniae*. Over 60% of resistance rates to carbapenem in *A. baumannii* were showed in 15 of 19 hospitals (Table 4). All but four *Acinetobacter* species isolates exhibited susceptibility to colistin. Only one of 403 *A. baumannii* isolates (MIC, 8 μg/mL) and one of 13 *A. bereziniae* isolates (MIC, 8 μg/mL) showed low-level resistance to colistin. None of the 82 *A. nosocomialis* isolates and the 62 *A. pitti* isolates showed resistance to colistin (Table 5). However, both *Acinetobacter* genomospecies 14TU isolates showed high-level resistance to colistin (MIC, 32 μg/mL). Interestingly, the *Acinetobacter* genomospecies 14TU ATCC17905 reference strain also showed high-level resistance to the drug (MIC, 64 μg/mL).

Table 4. Carbapenem resistance rates of clinical isolates of *Acinetobacter* species collected from 19 hospitals

	A. baumannii		*A. nosocomialis*		*A. pitti*	
Hospitals	no. of isolates	NS (%)	no. of isolates	NS (%)	no. of isolates	NS (%)
KNSM	20	13 (65)	5	0	9	1
KNSS	30	23 (77)	8	1	4	1
KDSS	3	3 (100)	0	0	0	0
GYMC	19	9 (47)	4	0	2	0
KSMC	31	27 (87)	5	0	1	0
GMC	7	3 (43)	1	0	3	0
MJMC	30	25 (83)	0	0	1	1
BUSAN	23	22 (96)	6	0	1	1
BCS	14	8 (57)	1	0	3	0
CHA	21	2 (10)	3	0	4	1
YMC	33	24 (73)	9	1	1	1
YMM	28	23 (82)	7	0	11	1
WJ	22	15 (68)	2	1	10	0
IJMC	39	27 (69)	7	0	4	0
CNMC	23	17 (74)	13	0	1	0
JJ	3	2 (67)	0	0	0	0
CSMC	11	8 (73)	3	1	2	0
CYY	9	6 (67)	8	0	1	0
CYH	22	15 (68)	0	0	4	0
Total	388	272 (70)	82	4 (5)	62	8 (13)

Table 5. Antimicrobial susceptibilities to carbapenem and colistin in *Acinetobacter* species

Species (no. of isolates)	IPM (breakpoints, mm)			MEM (breakpoints, mm)			CST (MIC, μg/mL)	
	R (≤ 13)	I (14-15)	S (≥16)	R (≤ 13)	I (14-15)	S (≥16)	R (≥4)	S (≤ 2)
A. baumannii (388)	221	23	144	270	2	116	1	387
A. nosocomialis (82)	2	2	78	4	0	78	0	82
A. pitti (62)	8	0	54	8	0	54	0	62
A. bereziniae (13)	0	0	13	0	0	13	1	12
A. genomospecies 14TU (2)	0	0	2	0	0	2	2	0

Abbreviations: IPM, imipenem; MEM, meropenem; CST, colistin; R, resistant; I, intermediate; S, susceptible

3. Carbapenem resistance mechanisms of *Acinetobacter* species

While 70% (272/388) of *A. baumannii* isolates exhibited intermediate or resistance to imipenem and/or meropenem, the majority of non-*baumannii* *Acinetobacter* isolates was susceptible to these drugs (Table 5). Most CNSAB (266/272) showed positive results on the modified Hodge test; however, none had positive results on the IEDDS test. The $bla_{\text{OXA-23}}$ gene was detected in 169/272 CNSAB isolates, while the IS*Aba1* element associated with the $bla_{\text{OXA-51}}$-like gene was detected in 89/272 isolates. Fourteen isolates, all of which were collected from the same hospital, carried both genes (Table 6). Genes encoding OXA-24-like and OXA-58-like carbapenemases or MBLs were not detected in the *A. baumannii* isolates. Most (10/12) of the carbapenem-non-susceptible non-*baumannii* *Acinetobacter* (NBA) isolates showed positive results on the IEDDS test, suggesting the production of MBLs. The $bla_{\text{IMP-1}}$ and the $bla_{\text{VIM-2}}$ genes were detected in six and four of these NBA isolates, respectively. With the exception of an *A. pitti* isolate carrying the $bla_{\text{OXA-23}}$ gene, no genes encoding OXA carbapenemases were detected in any of the NBA isolates.

Table 6. Carbapenem resistance mechanisms of *Acinetobacter* species

Species (no. of isolates)	Carbapenem resistance determinants							
	bla$_{\text{OXA-23}}$ only	IS*Aba1*-*bla*$_{\text{OXA-51}}$ only	*bla*$_{\text{OXA-23}}$, IS*Aba1*-*bla*$_{\text{OXA-51}}$	*bla*$_{\text{OXA-24}}$ -like	*bla*$_{\text{OXA-58}}$ -like	*bla*$_{\text{IMP-1}}$	*bla*$_{\text{VIM-2}}$	*bla*$_{\text{SIM-1}}$
A. baumannii (272)	169	89	14	0	0	0	0	0
A. nosocomialis (4)	0	0	0	0	0	1	2	0
A. pitti (8)	1	0	0	0	0	5	2	0

4. Location of the *bla*$_{\text{OXA-23}}$ gene

A probe specific for the *bla*$_{\text{OXA-23}}$ gene hybridized with 100-kb to 150-kb I-*Ceu*I chromosomal fragments from *A. baumannii* and *A. pitti* isolates, respectively, but not with S1 nuclease-treated linearized plasmids. The 16S rDNA probe also hybridized with the same chromosomal fragments, suggesting the presence of the *bla*$_{\text{OXA-23}}$ gene on the chromosome rather than on a plasmid.

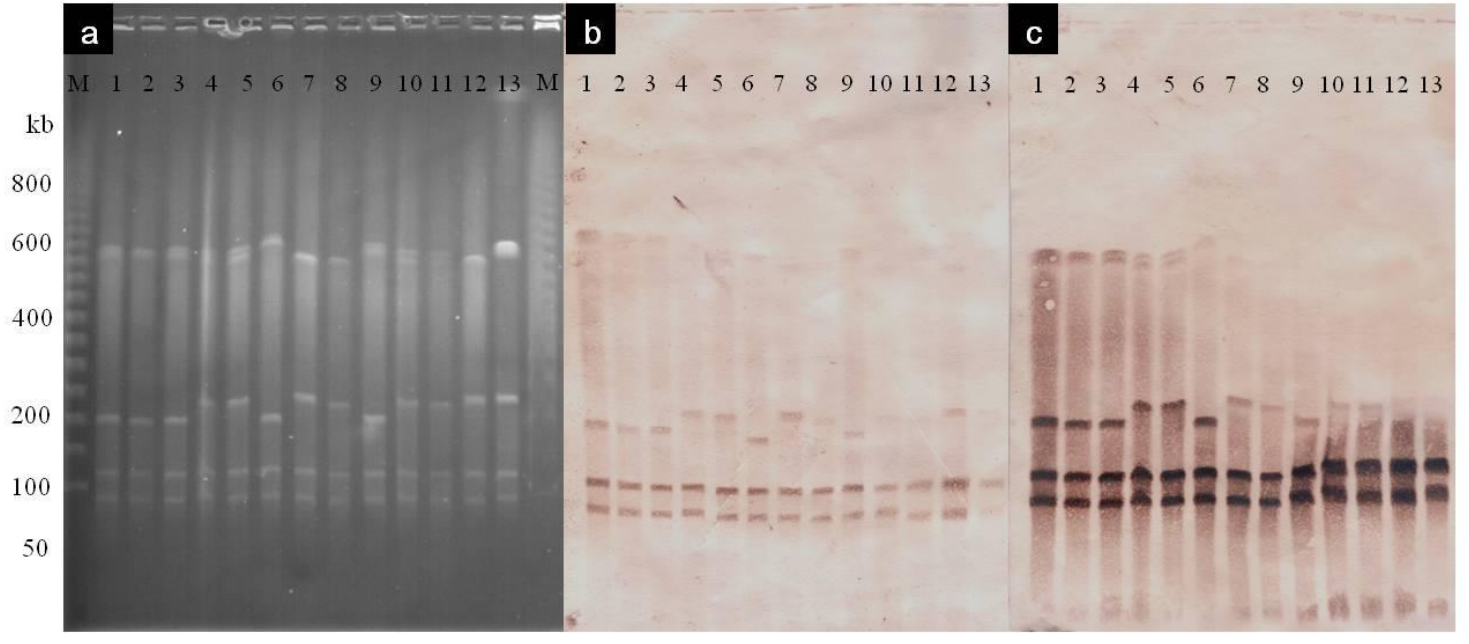


Fig. 2. PFGE pattern of genomic DNA of *Acinetobacter* species digested by I-*Ceu*I enzyme (a), Southern blot hybridization with $bla_{\text{OXA-23}}$ probe (b) and 16S rRNA gene probe (c). Lane M, lambda ladder (Bio-Rad) as a DNA size marker; lanes 1 to 12, *A. baumannii* isolates; lane 13, *A. pitti* isolate.

5. Grouping of *A. baumannii* isolates based on partial *rpoB* gene sequencing

A phylogenic tree clustered the 388 *A. baumannii* isolates into five groups according to the *rpoB* zone 2 sequence using the neighbor-joining method (Fig. 3). The group A isolate was defined as that carrying the same nucleotide sequence in zone 2 of the *rpoB* gene that is carried by the *A. baumannii* ATCC19606 reference strain (Table 7). The group B isolate was defined as that with a G-to-T substitution at the 428th nucleotide in zone 2 compared to the *A. baumannii* ATCC19606 reference strain sequence. Isolates belonging to subgroup B-0 had only one G428T substitution. Group C, D and E isolates were defined as those with a C449T, A407G and G116A substitution, respectively, in zone 2 compared to the *A. baumannii* ATCC19606 reference strain sequence (Table 7). Interestingly, all 272 CNSAB isolates belonged to subgroup B-0. However, some (37/309) isolates belonging to subgroup B-0 were susceptible to carbapenems. All of isolates belonging to group C, D and E were susceptible to carbapenems.

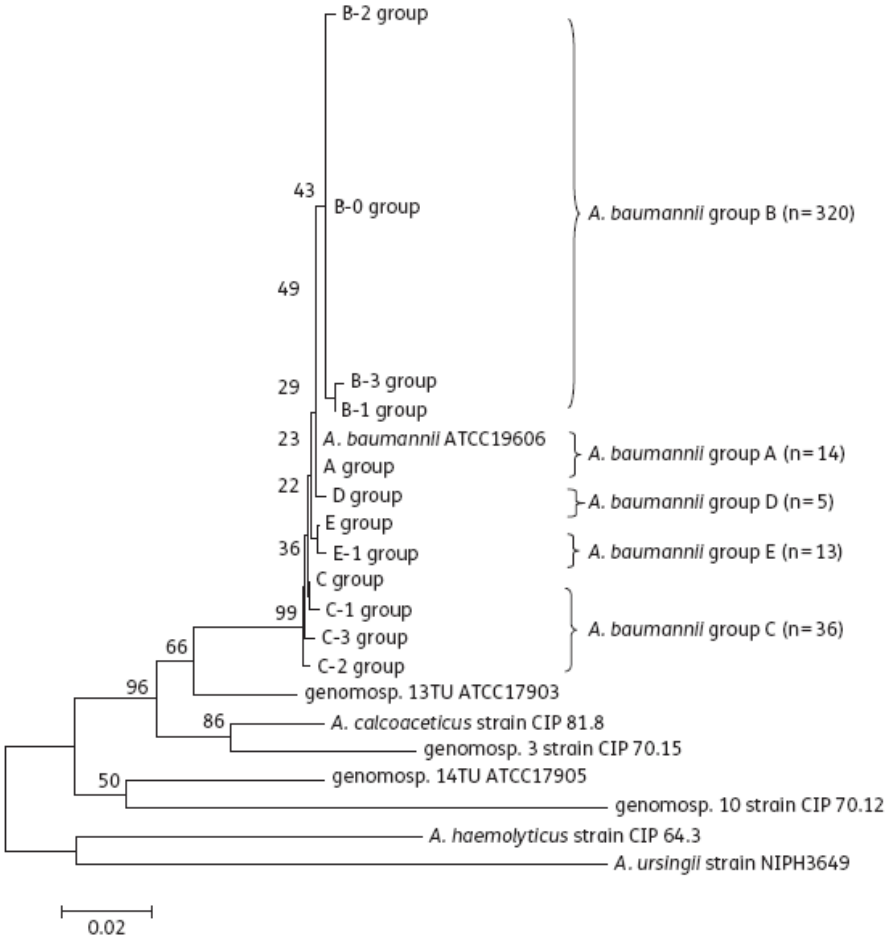


Fig. 3. Phylogenic tree of 388 isolates of *A. baumannii* inferred from zone 2 sequencing of the *rpoB* gene. This tree was grouped using the neighbor-joining method in molecular evolutionary genetic analysis software. Values on the branches of the clusters represented the results of bootstrapping analysis.

Table 7. Antimicrobial susceptibilities and *A. baumannii* groups according to substitution in zone 2 of the *rpoB* gene sequence

Species (no. of isolates)			IPM (Breakpoints, mm)*			MEM (Breakpoints, mm)			CST (MIC, μg/mL)	
A. baumannii (388)	Group	Substitution†	R (≤ 13)	I (14-15)	S (≥16)	R (≤ 13)	I (14-15)	S (≥16)	R (≥4)	S (≤ 2)
	B (320)									
	B-0‡	G428T	221	23	65	270	2	37	0	309
	B-1	G428T, C292T	0	0	4	0	0	4	0	4
	B-2	G428T, C292T, G89C	0	0	5	0	0	5	0	5
	B-3	G428T, T431A	0	0	2	0	0	2	0	2
	C (36)									
	C-0	C449T	0	0	8	0	0	8	0	8
	C-1	C449T, C269A	0	0	23	0	0	23	0	23
	C-2	C449T, C239T	0	0	3	0	0	3	0	3
	C-3	C449T, C114T	0	0	2	0	0	2	0	2
	E (13)									
	E-0	G116A	0	0	12	0	0	12	0	12
	E-1	G116A, C449T	0	0	1	0	0	1	0	1
	D (5)	A407G	0	0	5	0	0	5	0	5
	A (14)		0	0	14	0	0	14	1	13
A. nosocomialis (82)			2	2	78	4	0	78	0	82
A. pitti (62)			8	0	54	8	0	54	0	62
A. bereziniae (13)			0	0	13	0	0	13	1	12
Acinetobacter genomospecies 14TU (2)			0	0	2	0	0	2	2	0

Abbreviations: IPM, imipenem; MEM, meropenem; CST, colistin; R, resistant; I, intermediate; S, susceptible

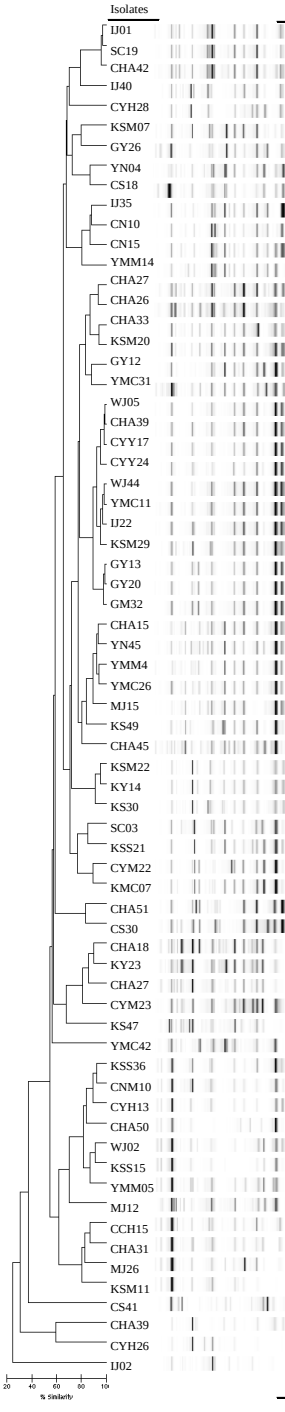
* The CLSI breakpoints were applied for the interpretation of the results.

† The reference sequence of *A. baumannii* ATCC19606 was defined as group A.

‡ There was a significant difference (P <0.001) in carbapenem susceptibility between B-0 group (88.0%) and non-B-0 groups (0%) by the Chi-square test (Fisher's exact test) using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA).

6. Clonal relationship

Results of rep-PCR experiments on the 388 *A. baumannii* isolates are shown in Figure 4, illustrating that the *A. baumannii* isolates were clustered into 57 distinct band patterns, using a clonal relationship index of >95% as a threshold. All 272 CNSAB isolates belonged to one of three band patterns (17, 18, or 19). These band patterns were similar to those of the EU-II control strain described by Higgins *et al.*[20] A total of 57 different STs were identified in 388 *A. baumannii* isolates using MLST experiments by the method of Bartual *et al.*[34], and the 272 CNSAB isolates were classified into five STs (ST92, ST75, ST138, ST137, and ST69; Fig. 4). However, the isolates of aforementioned five STs were identified as an identical ST, ST2 (2-2-2-2-2-2-2), by the MLST method described by Diancourt *et al.*[36] Many (159/272) CNSAB isolates were identified as ST92 including 76 isolates carrying the $bla_{\text{OXA-23}}$ gene, 69 isolates carrying the IS*Aba1* element associated with the $bla_{\text{OXA-51}}$-like gene, and 14 isolates carrying both. One hundred five isolates carrying the $bla_{\text{OXA-23}}$ gene (n = 93) or the IS*Aba1* element associated with the $bla_{\text{OXA-51}}$-like gene (n = 12) were identified as ST75 (n = 64), ST137 (n = 3), or ST138 (n = 38), single-locus variants of ST92. The remaining eight isolates carrying the IS*Aba1* element associated with the $bla_{\text{OXA-51}}$-like gene were identified as ST69, a double-locus variant of ST92.



Rep-PCR	*gltA*	*gyrB*	*gdhB*	*recA*	*cpn60*	*gpi*	*rpoD*	ST	*rpoB* type	no. of isolates	no. (%) of isolates with carbapenem susceptibility			
											Susceptible	Carbapenem-resistance determinant		
												bla $_{OXA-23}$	IS*Aba1*-*bla* $_{OXA-51}$-like	*bla* $_{OXA-23}$ + IS*Aba1*-*bla* $_{OXA-51}$-like
1	1	3	65	45	4	4	26	148*	B-1	1	1	0	0	0
1	1	48	65	45	4	90	26	171*	B-0	1	1	0	0	0
1	1	48	65	45	4	90	26	171*	E-0	1	1	0	0	0
2	21	12	2	28	1	86	4	161*	C-1	1	1	0	0	0
3	21	48	58	42	36	83	4	176*	B-1	1	1	0	0	0
4	35	60	77	11	26	11	48	182*	B-1	1	1	0	0	0
5	36	31	70	28	4	54	4	184*	B-0	1	1	0	0	0
6	2	38	42	1	1	4	49	150*	B-1	1	1	0	0	0
7	2	38	42	1	1	4	49	150*	E-0	1	1	0	0	0
8	1	15	13	12	4	14	2	146*	C-0	1	1	0	0	0
9	1	15	13	12	4	14	2	146*	C-0	2	2	0	0	0
10	1	15	13	12	4	14	2	146*	C-0	2	2	0	0	0
11	1	15	13	12	4	12	2	20	C-0	1	1	0	0	0
12	1	31	79	43	4	25	50	157*	C-3	1	1	0	0	0
13	1	31	79	43	4	25	50	157*	C-3	1	1	0	0	0
14a	21	35	2	28	1	85	4	158*	C-1	1	1	0	0	0
14a	21	35	2	28	1	52	4	145*	C-1	1	1	0	0	0
15	1	15	12	6	28	59	32	140*	A	2	2	0	0	0
16	21	35	2	28	1	52	4	145*	C-1	1	1	0	0	0
17a	1	3	3	2	2	7	3	92	B-0	183	24 (6)	76 (20)	69 (18)	14 (4)
17a	1	47	67	28	1	10	6	143*	B-3	1	1	0	0	0
17a	1	3	3	2	2	12	3	137	B-0	3	0	0	3	0
17a	1	46	3	2	2	58	3	69	B-0	8	0	0	8 (2)	0
17b	1	3	3	2	2	50	3	138	B-0	3	1	1	1	0
17b	1	3	3	2	2	11	3	75	B-0, E-1	60	1○	55 (14)	4 (1)	0
17b	1	47	3	2	2	7	30	141*	A	1	1	0	0	0
17b	1	15	81	1	1	72	45	152*	C-1	1	1	0	0	0
18	1	3	3	2	2	11	3	75	B-0	5	0	2	3	0
18	1	12	12	11	4	10	3	17	B-2	5	5	0	0	0
18	1	3	3	2	2	50	3	138	B-0, E-0	37	1○	35 (9)	1	0
19a	1	15	2	28	1	25	32	156*	C-1	6	6	0	0	0
19b	1	56	2	44	1	52	6	160*	C-1	1	1	0	0	0
19c	1	47	67	28	1	82	6	149*	B-3	1	1	0	0	0
19d	1	15	2	28	1	52	32	110	C-1	4	4	0	0	0
19e	1	15	2	28	1	52	32	110	C-1	2	2	0	0	0
20	33	21	12	49	1	54	7	177*	C-0	1	1	0	0	0
21	1	15	2	28	1	52	32	110	C-1	1	1	0	0	0
22a	21	12	2	28	32	83	4	151*	C-1	2	2	0	0	0
22a	21	35	2	28	1	84	4	153*	C-1	1	1	0	0	0
22b	21	55	13	28	1	84	2	154*	C-0	1	1	0	0	0
23	37	53	13	47	32	64	52	185*	B-0	1	1	0	0	0
24	1	15	12	6	28	59	3	139*	A	1	1	0	0	0
25	1	57	78	12	1	10	51	159*	C-2	1	1	0	0	0
26	1	57	78	12	1	25	51	155*	C-2	1	1	0	0	0
27	1	57	78	12	1	10	51	159*	C-2	1	1	0	0	0
28	33	12	40	26	32	83	5	167*	D	1	1	0	0	0
29	33	12	40	26	32	84	5	164*	D	1	1	0	0	0
30	33	12	40	26	32	91	5	163*	D	1	1	0	0	0
31	33	12	40	26	25	84	5	165*	D	1	1	0	0	0
32	33	12	40	26	32	87	5	166*	D	1	1	0	0	0
33	35	62	75	28	35	7	30	179*	A	2	2	0	0	0
34	1	17	82	39	1	92	7	175*	A	3	3	0	0	0
35a	1	17	81	1	1	91	26	183*	B-0	1	1	0	0	0
35b	1	54	62	31	4	55	45	147*	E-0	3	3	0	0	0
35c	1	3	15	2	2	7	3	106	B-0	1	1	0	0	0
36	1	58	29	2	4	9	53	173*	B-0	1	1	0	0	0
37a	38	59	80	48	18	58	43	186*	B-0	1	1	0	0	0
37b	40	1	83	6	4	7	3	162*	B-0	3	3	0	0	0
38	31	61	70	46	16	58	6	178*	E-0	1	1	0	0	0
39	1	63	12	6	28	59	30	180*	A	1	1	0	0	0
40	1	34	80	28	1	72	45	172*	B-0	1	1	0	0	0
41	1	12	80	28	35	89	30	170*	E-0, E-1	3	3	0	0	0
42	1	34	70	28	4	90	45	169*	E-0	1	1	0	0	0
43	1	12	65	45	4	88	26	168*	E-0	1	1	0	0	0
44	11	27	14	21	15	22	15	174*	B-0	1	1	0	0	0
45	21	35	2	28	22	52	4	144*	C-1	1	1	0	0	0
46	1	64	76	1	23	81	32	181*	A	1	1	0	0	0
47	10	12	4	11	1	9	30	142*	A	3	3	0	0	0
									Total	388	116 (30)	169 (44)	89 (23)	14 (4)

Fig. 4. Rep-PCR banding patterns and STs of 388 isolates of *A. baumannii*. Isolates that showed >95% similarity were assigned the same banding pattern number, and a small letter (a to e) was given to isolates with >90% similarity. The asterisks indicate novel STs found in this study. Open circles indicate the number of isolates belonged to E group.

IV. DISCUSSION

A. baumannii is an opportunistic pathogen and the most common cause of serious nosocomial infections, especially in intensive care units[3, 5]. The aims of present study were to investigate: resistance patterns and mechanisms to carbapenems and colistin in *Acinetobacter* species isolates from clinical specimens of patients hospitalized at 19 different hospitals in Korea, the epidemiological traits of CNSAB isolates, and the usefulness of phylogenic grouping based on partial *rpoB* gene sequencing in defining the epidemiological traits of CNSAB.

The increasing prevalence of carbapenem-resistant *A. baumannii* clinical isolates has become a serious worldwide problem. The prevalence of antibiotic-resistant bacteria varies greatly from country to country. Carbapenem resistance rates of *A. baumannii* isolates were high over 50% in Greece, Spain, Portugal and Turkey.[37] The Korean Nationwide Surveillance of Antimicrobial Resistance (KONSAR) study group reported that imipenem-resistance rate of *Acinetobacter* species steadily increased from 1% in 1997 to 22% in 2007, and after that the increase was drastic, reaching 51% in 2009.[38, 39] In the present study, imipenem or meropenem non-susceptible *A. baumannii* isolates was approximately 70 % (range 10-87%). High resistance rates to imipenem or meropenem in *A. baumannii* were revealed in almost hospitals. The increasing carbapenem resistance in *A. baumannii* is very

worrisome considering the fact those isolates show multi-drug resistance to the other class antibiotics such as aminoglycosides and fluoroquinolones[40]. Colistin and polymixin B are the only potent therapeutic options available for treatment of severe hospital acquired infections caused by multi-drug resistant *A. baumannii*. Results of our study correspond well with those of Ko *et al.*' study which reported subgroups of *A. baumannii* using partial *rpoB* gene sequences. However, colistin resistance rates differ from the result of this study. Although Ko *et al.*[19] reported high rates of resistance to colistin and polymixin B in *A. baumannii*, colistin-resistant *A. baumannii* isolate was rare until now as shown in this study. Their study is different from this study in that small number of hospitals and antimicrobial susceptibility testing method. Molecular characterization of the 272 CNSAB isolates using multilocus sequencing typing identified five sequence types: ST92, ST75, ST137, ST138, and ST69. The CNSAB of ST92, one of its single-locus variants (ST75, ST138, and ST137), or its double-locus variant (ST69). ST92 and its single-locus variants can be clustered into a single clonal complex 92 because they share five to six of seven housekeeping gene loci. The results of Institute Pasteur MLST scheme also supported the clonally relatedness of the isolates in a broader sense, which they share an identical ST, ST 2. CC92 also includes other single-locus variants of ST92, including ST118 (1-3-3-2-2-3-3) identified from an isolate from France, ST88 (1-3-3-2-2-10-3) and ST90

(1-3-3-2-2-62-3) from China, and ST4 (1-12-3-2-2-7-3) from the UK. Therefore, clonal expansion of *A. baumannii* CC92 may be the main cause of the recent worldwide dissemination of CNSAB.

A. baumannii isolates clustered into five groups according to the sequence of zone 2 of the *rpoB* gene. Grouping correlated well with the carbapenem resistance profiles of the *A. baumannii* isolates. All 272 CNSAB isolates belonged to subgroup B-0, while all of the isolates belonging to the other groups were susceptible to carbapenems. Subgroup B-0 was comprised of 37 carbapenem-susceptible isolates and 272 CNSAB isolates, including 169 isolates carrying the $bla_{\text{OXA-23}}$ gene, 89 isolates carrying the IS*Aba1* element upstream of the $bla_{\text{OXA-51}}$-like gene, and 14 isolates carrying both (Fig. 4). It was interesting that only *A. baumannii* isolates with the single G428T substitution in zone 2 of the *rpoB* gene acquired carbapenem-resistance determinants. Further studies are needed to investigate the reasons for these phenomena. CNSAB isolates of CC92 with a G428T substitution in zone 2 of the *rpoB* gene were widely disseminated in Korea, as has been reported in European countries and China.

While the $bla_{\text{OXA-23}}$ gene has mainly been detected in *A. baumannii*, reports of NBA isolates harboring the OXA carbapenemase are rare. Recently, two *A. pitti* isolates carrying the $bla_{\text{OXA-23}}$ gene were reported in the Irish Republic.[41] One *A. pitti* isolate harboring chromosome-borne OXA-23 was identified in

this study. To our knowledge, this is the second report of an OXA-23-produc -ing NBA isolate.

In this study, only one of 403 *A. baumannii* isolates was identified as colistin-resistant, in contrast to results reported by Ko *et al*.[9] Interestingly, both *Acinetobacter* genomospecies 14TU showed resistance to colistin. Furthermore, the *Acinetobacter* genomospecies 14TU ATCC 17905 reference strain also showed resistance to colistin. These results suggest that the *Acinetobacter* genomospecies 14TU may be intrinsically resistant to colistin, as described by Nemec *et al*.[42]

V. CONCLUSION

A total of 547 non-duplicate clinical isolates of *Acinetobacter* species were collected from 19 hospitals. Reduced susceptibility rates to imipenem or meropenem were 70% (272/388) for *A. baumannii*, 5% (4/82) for *A. nosocomialis*, 13% (8/62) for *A. pitti*, and 0% (0/13) for *A. bereziniae*. Over 60% of resistance rates to carbapenems in *A. baumannii* were showed in 15 of 19 hospitals. Colistin-resistant *A. baumannii* isolates was rare, 0.25% (1/388). However, all of isolates of *Acinetobacter* genomospecies 14 TU as well as *Acinetobacter* genomospecies 14TU ATCC 17905 were resistant to colistin, resulting in possibility of natural resistance to colistin in *Acinetobacter* genomospecies 14TU.

The 272 CNSAB isolates were clustered into CC92, sharing alleles at five or six of seven housekeeping gene loci. ST92 was the predominant ST and was associated with European clone II. CNSAB of CC92 carried the $bla_{\text{OXA-23}}$ gene, the $bla_{\text{OXA-51}}$-like gene preceded by IS*Aba1* or both. Sequence-based analysis of the *rpoB* gene showed single-nucleotide substitutions in zone 2 of the *rpoB* gene, resulting in division into five groups (A to E groups). All CNSAB isolates belonged to subgroup B-0 with a G428T substitution in zone 2. CNSAB isolates of CC92 with the G428T substitution in zone 2 of the *rpoB* gene are disseminated nationwide in Korea. *A. baumannii* with the G428T substitution in zone 2 may be more likely to acquire carbapenem resistance than are other isolates.

REFERENCES

1. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev 1996;9:148-65.
2. C.Schreckenberger P, Daneshvar MI, Hollis DG. *Acinetobacter*, *achromobacter*, *Chryseobacterium*, *Moraxella*, and other nonfermentative Gram-negative rods. Manual of clinical microbiology: ASM press; 2007. p.770-802.
3. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin Microbiol Rev 2008;21:538-82.
4. Munoz-Price LS, Weinstein RA. *Acinetobacter* infection. N Engl J Med 2008;358:1271-81.
5. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51:3471-84.
6. Villegas MV, Hartstein AI. *Acinetobacter* outbreaks, 1977-2000. Infect Control Hosp Epidemiol 2003;24:284-95.
7. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-36.
8. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol

2007;5:939-51.

9. Peleg AY, Franklin C, Walters LJ, Bell JM, Spelman DW. OXA-58 and IMP-4 carbapenem-hydrolyzing beta-lactamases in an *Acinetobacter junii* blood culture isolate from Australia. Antimicrob Agents Chemother 2006;50:399-400.

10. Bou G, Oliver A, Martinez-Beltran J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob Agents Chemother 2000;44:1556-61.

11. Fournier PE, Richet H. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin Infect Dis 2006;42:692-9.

12. Villegas MV, Kattan JN, Correa A, Lolans K, Guzman AM, Woodford N, et al. Dissemination of *Acinetobacter baumannii* clones with OXA-23 Carbapenemase in Colombian hospitals. Antimicrob Agents Chemother 2007;51:2001-4.

13. Donald HM, Scaife W, Amyes SG, Young HK. Sequence analysis of ARI-1, a novel OXA beta-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob Agents Chemother 2000;44:196-9.

14. Limansky AS, Mussi MA, Viale AM. Loss of a 29-kilodalton outer membrane protein in *Acinetobacter baumannii* is associated with imipenem resistance. J Clin Microbiol 2002;40:4776-8.

15. Lee Y, Yum JH, Kim CK, Yong D, Jeon EH, Jeong SH, et al. Role of OXA-23 and AdeABC efflux pump for acquiring carbapenem resistance in an *Acinetobacter baumannii* strain carrying the blaOXA-66 gene. Ann Clin Lab Sci 2010;40:43-8.

16. Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. Antimicrob Agents Chemother 2005;49:1432-40.

17. Heritier C, Poirel L, Lambert T, Nordmann P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49:3198-202.

18. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 2006;258:72-7.

19. Ko KS, Suh JY, Kwon KT, Jung SI, Park KH, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. J Antimicrob Chemother 2007;60:1163-7.

20. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2010;65:233-8.

21. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of *Acinetobacter baumannii*. Emerg Infect Dis 2010;16:35-40.

22. Park YK, Lee GH, Baek JY, Chung DR, Peck KR, Song JH, et al. A single clone of *Acinetobacter baumannii*, ST22, is responsible for high antimicrobial resistance rates of *Acinetobacter* spp. isolates that cause bacteremia and urinary tract infections in Korea. Microb Drug Resist 2010;16:143-9.

23. Fu Y, Zhou J, Zhou H, Yang Q, Wei Z, Yu Y, et al. Wide dissemination of OXA-23-producing carbapenem-resistant *Acinetobacter baumannii* clonal complex 22 in multiple cities of China. J Antimicrob Chemother 2010;65:644-50.

24. Nemec A, Krizova L, Maixnerova M, Diancourt L, van der Reijden TJ, Brisse S, et al. Emergence of carbapenem resistance in *Acinetobacter baumannii* in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. J Antimicrob Chemother 2008;62:484-9.

25. D'Arezzo S, Capone A, Petrosillo N, Visca P, Ballardini M, Bartolini S, et al. Epidemic multidrug-resistant *Acinetobacter baumannii* related to European clonal types I and II in Rome (Italy). Clin Microbiol Infect 2009;15:347-57.

26. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N, et al. Characterisation of carbapenem-resistant *Acinetobacter baumannii*

outbreak strains producing OXA-58 in Turkey. Int J Antimicrob Agents 2010;36:114-8.

27. La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the rpoB gene and flanking spacers for molecular identification of *Acinetobacter* species. J Clin Microbiol 2006;44:827-32.

28. Zarrilli R, Giannouli M, Di Popolo A, Tomasone F, Chu YW, Vaneechoutte M, et al. Identification of *Acinetobacter* genomic species 13TU by sequence analysis of the 16S-23S rRNA gene spacer region. J Clin Microbiol 2009;47:1281-2.

29. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. M100-S20, CLSI, Wayne, PA, USA, 2010.

30. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of Pseudomonas spp. and *Acinetobacter* spp. J Clin Microbiol 2003;41:4623-9.

31. 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.

32. Lee K, Yum JH, Yong D, Lee HM, Kim HD, Docquier JD, et al. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea.

Antimicrob Agents Chemother 2005;49:4485-91.

33. 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.

34. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodriguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005;43:4382-90.

35. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J Bacteriol 2004;186:1518-30.

36. Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 2010;5:e10034.

37. Bae Ik, Jeong SH, Lee K. Carbapenem-resistant *Acinetobacter baumannii*. Korean J Clin Micobiol 2012;15:1-7.

38. Lee K, Kim MN, Kim JS, Hong HL, Kang JO, Shin JH, et al. Further increases in carbapenem-, amikacin-, and fluoroquinolone-resistant

isolates of *Acinetobacter* spp. and *P. aeruginosa* in Korea: KONSAR study 2009. Yonsei Med J 2011;52:793-802.

39. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y. VIM- and IMP-type metallo-beta-lactamase-producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean hospitals. Emerg Infect Dis 2003;9:868-71.

40. Mera RM, Miller LA, Amrine-Madsen H, Sahm DF. *Acinetobacter baumannii* 2002-2008: increase of carbapenem-associated multiclass resistance in the United States. Microb Drug Resist 2010;16:209-15.

41. Boo TW, Walsh F, Crowley B. First report of OXA-23 carbapenemase in clinical isolates of *Acinetobacter* species in the Irish Republic. J Antimicrob Chemother 2006;58:1101-2.

42. Nemec A, Dijkshoorn L. Variations in colistin susceptibility among different species of the genus *Acinetobacter*. J Antimicrob Chemother 2010;65:367-9.

ABSTRACT (IN KOREAN)

국내 분리 *Acinetobacter* 균종의 carbapenem과 colistin 내성 양상, 기전 및 분자역학적 특성

<지도교수 정석훈>

연세대학교 대학원 의학과

이 양 순

최근 carbapenem 내성인 *Acinetobacter* 균종의 증가는 이 세균에 의한 감염증 환자의 치료에 있어서 항균제 선택을 크게 제한함으로 많은 우려가 되고 있다. Colistin이 다약제 내성 *A. baumannii*에 의한 병원내 감염 환자의 유일한 치료제이나, 이에 대한 내성 균주도 출현하였다. 본 연구에서는 우리나라 전국 병원에서 수집된 *Acinetobacter* 균종별 carbapenem과 colistin의 내성양상 및 기전을 분석하였다. 또한 *A. baumannii* 균주를 대상으로 multilocus sequencing typing (MLST) 방법과 *rpoB* 유전자 염기서열 분석을 통하여, carbapenem 내성인 *A. baumannii*의 분자역학적인 특성을 규명하고자

하였다.

2008년 전국 6개 지역 19개 주요 병원에서 분리된 *Acinetobacter* 균종 547주를 수집하였다. Imipenem 혹은 meropenem에 감수성이 저하된 균주는 각각 *A. baumannii* 65% (272/388), *A. nosocomialis* 5% (4/82), *A. pitti* 13% (8/62) 이었고, *A. bereziniae* 은 모두 감수성이었다. Colistin 내성인 균주는 *A. baumannii*와 *A. bereziniae* 각각 1주 였고 *Acinetobacter* genomospecies 14 TU 2주가 colistin 에 모두 내성이었다. 분리된 *A. baumannii* 388주에 대해서 MLST 및 *rpoB* 유전자의 다중중합효소반응 및 염기서열 분석을 시행하였다. MLST 분석 결과, carbapenem에 감수성이 저하된 *A. baumannii*은 모두 5개의 sequence type (ST), ST92, ST75, ST137, ST138, ST69에 속하였고, 이들은 7개의 유전자중 5-6개 유전자가 동일한 clonal complex 92 를 이루고 있었다. 이 중에서 ST92는 세계적으로 분포하고 있는 European clone II 에 속하는 ST으로 본 연구에서 가장 많이 분리되었다. CC92에 속하는 carbapenem 비감수성 *A. baumannii*의 carbapenem 내성기전은 OXA-23형 유전자를 가진 경우가 169주, OXA-51형를 과발현한 균주는 89주, OXA-23형과 OXA-51형을 과발현한 균주는 14주였다. 한편, *rpoB* 유전자의 zone2 부분을 PCR 및 염기서열 분석하였을 때, 특정 위치의 치환기에 따라 5개군(A-E군)으로 분류됨을 알 수 있었다. 특히, carbapenem에 비감수성인 *A. baumannii* 272주는 모두 *rpoB* 유전자 zone 2의 428번째 염기가 G에서 T로 치환된 B-0군에

속하였다. B-0군 이외의 군에서는 carbapenem 내성인 균주가 없었다.

결론적으로, 우리나라 환자에서 분리된 *Acinetobacter* 균종 중 carbapenem 내성률은 *A. baumannii*가 가장 높아 70%에 이르렀다. 반면, colistin 내성률은 현재까지는 매우 낮음을 알 수 있었다. 분자역학적인 연구결과, *rpoB* 유전자 zone2의 G428T 치환을 가진 carbapenem 내성 *A. baumannii* clonal complex 92 균주들이 우리나라에 널리 확산되어 있음을 확인할 수 있었다. 또한, *rpoB* 유전자 zone2의 G428T 치환기를 가진 *A. baumannii*는 다른 균주들보다 carbapenem 내성을 잘 획득하여 확산된 것으로 판단되었다.

핵심되는 말 : *Acinetobacter baumannii*, clonal complex 92, European clone II, multilocus sequence typing, carbapenem, colistin

PUBLICATION LIST

Lee Y, Lee J, Jeong SH, Bae IK, Lee K. Carbapenem-non-susceptible *Acinetobacter baumannii* of sequence type 92 or its single-locus variants with a G428T substitution in zone 2 of the *rpoB* gene. J Antimicrob Chemother 2011;66:66-72.

Lee Y, Bae IK, Kim J, Jeong SH, Lee K. Dissemination of ceftazidime-resistant *Acinetobacter baumannii* clonal complex 92 in Korea. J Appl Microbiol 2012;112:1207-11.