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Outbreaks of Imipenem-Resistant *Acinetobacter baumannii* Producing Carbapenemases in Korea

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Among 53 *Acinetobacter baumannii* isolates collected in 2004, nine imipenem-resistant isolates were obtained from clinical specimens taken from patients hospitalized in Busan, Korea. Nine carbapenemase-producing isolates were further investigated in order to determine the mechanisms underlying resistance. These isolates were then analyzed via antibiotic susceptibility testing, microbiological tests of carbapenemase activity, pI determination, transconjugation test, enterobacterial repetitive consensus (ERIC)-PCR, and DNA sequencing. One outbreak involved seven cases of infection by *A. baumannii* producing OXA-23 β-lactamase, and was found to have been caused by a single ERIC-PCR clone. During the study period, the other outbreak involved two cases of infection by *A. baumannii* producing IMP-1 β-lactamase. The two clones, one from each of the outbreaks, were characterized via a modified cloverleaf synergy test and an EDTA-disk synergy test. The isoelectric focusing of the crude bacterial extracts detected nitrocefin-positive bands with pI values of 6.65 (OXA-23) and 9.0 (IMP-1). The PCR amplification and characterization of the amplicons via direct sequencing showed that the clonal isolates harbored *bla*$_{\text{IMP-1}}$ or *bla*$_{\text{OXA-23}}$ determinants. The two clones were characterized by a multidrug resistance phenotype that remained unaltered throughout the outbreak. This resistance encompassed penicillins, extended-spectrum cephalosporins, carbapenems, monobactams, and aminoglycosides. These results appear to show that the imipenem resistance observed among nine Korean *A. baumannii* isolates could be attributed to the spread of an IMP-1- or OXA-23-producing clone. Our microbiological test of carbapenemase activity is a simple method for the screening of clinical isolates producing class D carbapenemase and/or class B metallo-β-lactamase, in order both to determine their clinical impact and to prevent further spread.

Keywords: *Acinetobacter baumannii*, carbapenemase, IMP-1, OXA-23, ERIC-PCR

Acinetobacter baumannii has emerged as a primary nosocomial pathogen in hospital outbreaks, and is ranked second after *Pseudomonas aeruginosa* among nosocomial aerobic nonfermentative gram-negative bacillar pathogens (Schreckenberger and Graevenitz, 1999; Simor *et al.,* 2002). *A. baumannii* causes respiratory and urinary tract infections, as well as meningitis, endocarditis, burn infections, and wound sepsis, especially in intensive care units (ICUs) (Chastre and Trouillet, 2000). *A. baumannii* infections often prove difficult to eradicate due to high-level resistance to a host of antibiotics as the result of both intrinsic and acquired mechanisms. β-Lactamase production is the most salient mechanism of acquired β-lactam resistance in gram-negative pathogens (Yong *et al.,* 2003). Carbapenems (e.g., imipenem and meropenem) have become the drugs of choice against *Acinetobacter* infections in a host of medical centers, but are being compromised by the emergence of carbapenem-hydrolysing β-lactamase (carbapenemase) of molecular classes B and D (Livermore, 2002; Lee and Lee,

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2006). The class B carbapenemases thus far detected in *Acinetobacter* spp. include a variety of IMP- and VIM-type metallo-β-lactamases (http://www.lahey.org/studies/webt.asp), but the majority of *Acinetobacter* spp. produce zinc-independent members of the molecular class D β-lactamases (Afzal-Shah *et al.,* 2001). The sequenced carbapenemases of this latter class from that species include three distinct clusters, as follows: (i) OXA-23-like cluster (OXA-23, -27 and -49) (Donald *et al.,* 2000; Afzal-Shah *et al.,* 2001; Bonnet *et al.,* 2002; Brown *et al.,* 2004), (ii) OXA-24-like cluster (OXA-24, -25, -26 and -40) (Bou *et al.,* 2000; Afzal-Shah *et al.,* 2001; Héritier *et al.,* 2003) and (iii) OXA-48-like cluster (OXA-48 and -54) (Poirel *et al.,* 2004). OXA-23 and OXA-27 show 99% amino acid identity, but exhibit only 60% identity with the amino acid sequences of the OXA-24-like cluster. OXA-48 shares an amino acid identity of 92% with OXA-54, but was only weakly related to other class D β-lactamases, showing an amino acid identity of less than 46% with any other OXA-type β-lactamase. Recently, new subclasses of metallo-β-lactamases have been reported, including SPM-1 and GIM-1 (Castanheira *et al.,* 2004). GES-2, GES-4 and GES-5 class A β-lactamases show weak imipenem hydrolysis (Wachino *et al.,* 2004; Jeong *et al.,* 2005).

Fifty-three *A. baumannii* isolates were collected from 53 patients with suspected nosocomial infections. The primary objective of the present study was to investigate an outbreak of imipenem-resistant *A. baumannii* in the Republic of Korea, and to characterize the mechanisms underlying the imipenem resistance evidenced by the outbreak isolates.

Materials and Methods

Bacterial strains

A total of 53 nonrepetitive clinical isolates of *A. baumannii* were obtained between June 1st, 2004 and November 30th, 2004 at the Kosin University Gospel Hospital (Busan, Republic of Korea), a facility with 1,300 beds. These isolates were collected from different patients hospitalized in the intensive care unit (ICU) and in the general (i.e., neurology and urology) wards. The isolates were identified via conventional techniques (Schreckenberger and Graevenitz, 1999) and/or using the Vitek GNI card (bioMérieux Vitek Inc., USA). *A. baumannii* YMC02/8/ P535 (Young *et al.,* 2003) was utilized as the recipient strain for transfer via transconjugation. *Escherichia coli* ATCC 25922 was used as the quality control strain for the susceptibility tests.

Susceptibility of β-lactams

Antibiotic susceptibility was evaluated via disk diffusion tests, which were conducted in accordance with the manufacturer's instructions using BBL (USA) disks impregnated with amikacin (30 μg), gentamicin (10 μg), tobramycin (10 μg), tetracycline (30 μg), trimethoprim-sulfamethoxazole (1.25 and 23.75 μg, respectively), ciprofloxacin (5 μg), ampicillin (10 μg), ampicillin-sulbactam (10 and 10 μg, respectively), piperacillin (100 μg), piperacillin-tazobactam (100 and 10 μg, respectively), cefoxitin (30 μg), cefotetan (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), cefepime (30 μg), aztreonam (30 μg), imipenem (10 μg), and meropenem (10 μg). The disks were dispensed using a BBL Sensi-Disc 12-place dispenser. MICs were determined via agar dilution on Muller-Hinton agar plates (Becton-Dickinson, USA) containing two-fold serially-diluted β-lactams, as described previously (NCCLS 2003).

Microbiological tests of carbapenemase activity

In order to characterize imipenem inactivation by the *A. baumannii* OXA-type β-lactamase, we conducted a microbiological disk synergy test. The cloverleaf test of Hornstein *et al.* (1997) was modified by substituting *E. coli* ATCC 25922 for the imipenem-susceptible *Micrococcus luteus*. The surface of a Muller-Hinton agar plate was inoculated evenly using a cotton swab with an overnight culture suspension of *E. coli*, which was adjusted to the turbidity of the McFarland no. 0.5 tube. After a brief drying procedure, an imipenem disk (30 μg) was positioned at the center of the plate, and the imipenem-resistant test strains from the overnight culture plates were streaked from the edge of the disk to the periphery of the plate. The presence of a distorted inhibition zone after overnight incubation was interpreted as a positive modified cloverleaf synergy test, revealing imipenem had been inactivated by carbapenemase (class B and/or D).

The microbiological testing of metallo-β-lactamase activity was conducted via an EDTA-disk synergy test (Lee *et al.,* 2000), modified as follows: An overnight culture of the test strain was suspended to the turbidity of the McFarland no. 0.5 tube, and utilized to swab the inoculate on a Muller-Hinton agar plate. After drying, a 30 μg imipenem disk (BBL) and a blank filter paper disk were positioned 15 mm apart from edge to edge, and 10 μl of 0.5 M EDTA solution was then applied to the blank disk, resulting in a *ca* 1.5 mg/disk. After overnight incubation, the presence of an enlarged inhibition zone was interpreted as a positive EDTA-disk synergy test, revealing the inactivation of metallo-β-lactamase (class B) activity by EDTA.

Plasmid preparation and Southern blot analysis

The isolation of plasmid DNA from *A. baumannii*

clinical isolates was conducted as described by Sambrook and Russel (2001), using plasmid-safe ATP-dependent DNase (Epicentre Technology, USA) for the removal of contaminated bacterial chromosomal DNA. The prepared plasmids were separated on 1.0% agarose using a field inversion gel electrophoresis (FIGE) Mapper Electrophoresis System (Bio-Rad, USA).

DNA was transferred to nylon membrane (Hybond-N; Amersham International, England) essentially as described by Sambrook and Russell (2001). Labeling of the DNA (PCR products for bla_{IMP} and bla_{OXA} genes) probes was conducted using digoxigenin, as described by the manufacturer (Boehringer Mannheim Biochemicals,

Table 1. Nucleotide sequences of oligonucleotides used for PCR amplifications and sequencing of bla_{IMP}-, bla_{VIM}-, bla_{GIM}-, bla_{SPM}-, bla_{OXA}-, and bla_{GES}-type genes

Primer (orientation)[a]	Sequence (5'→3')	Target β-lactamase	Accession no. of *bla*[b]
IMP-1F (F)	GCTACCGCAGCAGAGTCTTTG	IMP-1, IMP-3, IMP-6, IMP-10	S71932, AB010417, AB040994, AY074433
IMP-1R (R)	CCTTTAACCGCCTGCTCTAATG		
IMP-2F (F)	ATGTTACGCAGCAGGGCAG	IMP-2, IMP-8, IMP-10, IMP-12, IMP-13	AJ243491, AF322577, AY074433, AJ420864, AJ550807
IMP-2R (R)	ATGCTCAGTCATGAGGCGC		
IMP-4F (F)	GAAGGCGTTTATGTTCATACTTCG	IMP-4, IMP-5, IMP-7, IMP-9	AF244145, AF290912, AF318077, AY033653
IMP-4R (R)	GCGTCACCCAAATTACCTAGACC		
IMP-11F (F)	GAGAAGCTTGAAGAGGGTGTTTAT	IMP-11, IMP-12, IMP-21	AB074436, AJ420864, AB204557
IMP-11R (R)	AGGTAGCCAAACCACTACGTTATC		
IMP-18F (F)	CATTGCTGCTGCAGATGATTC	IMP-18	AY780674
IMP-18R (R)	CTGCAAGAGTGATGCGTTTC		
IMP-19F (F)	GTTTTATGTGTATGCTTCCTTTGTAGC	IMP-19, IMP-20	AB201265, AB196988
IMP-19R (R)	CAGCCTGTTCCCATGTACG		
VIM-1F (F)	GTTTGGTCGCATATCGCAAC	VIM-1, VIM-4, VIM-5, VIM-7, VIM-11	Y18050, AY135661, AY144612, AJ536835, AY635904
VIM-1R (R)	AGACCGCCCGGTAGACC		
VIM-2F (F)	GTTTGGTCGCATATCGCAAC	VIM-2, VIM-3, VIM-6, VIM-8, VIM-9, VIM-10	AF191564, AF300454, AY165025, AY524987, AY524988, AY524989
VIM-2R (R)	CTACTCAACGACTGAGCGATTTGT		
GIM-1F (F)	CAGGGTCATAAACCGCTAGAAG	GIM-1	AJ620678
GIM-1R (R)	AACTTCCAACTTTGCCATGC		
SPM-1F (F)	GAGAGCCCTGCTTGGATTC	SPM-1	AY341249
SPM-1R (R)	GCGACCTTGATCGTCTTGTT		
OXA-23F (F)	ACTTGCTATGTGGTTGCTTCTC	OXA-23, OXA-27, OXA-49	AJ132105, AF201828, AY288523
OXA-23R (R)	TGTCAAGCTCTTAAATAATATTCAGC		
OXA-24F (F)	GATGAAGCTCAAACACAGGGTG	OXA-24, OXA-25, OXA-26, OXA-40, OXA-72	AJ239129, AF201826, AF201827, AF509241, AY739646
OXA-24R (R)	TTAAATGATTCCAAGATTTTCTAGC		
OXA-48F (F)	GATTATCGGAATGCCTGCGG	OXA-48, OXA-54	AY236073, AY500137
OXA-48R (R)	CTACAAGCGCATCGAGCATCA		
GES-F (F)	GTTAGACGGGCGTACAAAGATAAT	GES-1, GES-2, GES-3, GES-4, IBC-1, IBC-2	AF156486, AF326355, AB113580, AB116260, AF208529, AF329699
GES-R (R)	TGTCCGTGCTCAGGATGAGT		

[a]Orientation of each primer: F, forward; R, reverse.
[b]β-lactamase genes (*bla*) used in the multiple sequence alignment for designing each primer pair.

IN). Hybridization was conducted at 68°C with the buffers recommended in the manual of the digoxigenin kit purchased from Boehringer Mannheim Biochemicals.

Transconjugation experiments

Curing was attempted via the overnight growth of the cultures in nutrient broth containing ethidium bromide (Sigma-Aldrich, USA) at 0.25-0.5 times the MIC, followed by replica plating onto Muller-Hinton agar plates, with and without imipenem at concentrations of 2 μg/ml or 10 μg/ml. The transconjugation experiments were conducted as described previously (Oh *et al.,* 2003), using a rifampin-resistant *A. baumannii* YMC02/8/P535 as the recipient. The transconjugants were selected on Muller-Hinton agar supplemented with rifampin (Sigma, USA) (100 μg/ml) to inhibit the growth of the donor strain, and with imipenem (1 μg/ml) to inhibit the growth of the recipient strain.

Isoelectric focusing analysis

The crude bacterial extracts were acquired from *A. baumannii* clinical isolates after the centrifugation of the sonicated culture, as described previously (Lee *et al.,* 2002). Sonic extracts were employed in the determination of isoelectric points (pIs) and β-lactamase activity. Isoelectric focusing (IEF) was conducted on Ready Gel precast IEF polyacrylamide gels (Bio-Rad, USA) as described previously (Lee *et al.,* 2002). Gels were developed with 0.5 mM nitrocefin (Merck, USA).

PCR amplification and DNA sequencing

Unless stated otherwise, molecular biological reagents and restriction enzymes were acquired from Sigma-Aldrich. The genomic DNA of the clinical isolates was prepared using a Wizard Genomic DNA Purification Kit (Promega, USA), and used as template DNA in the PCR amplification procedure. The PCR amplification primers were designed via the selection of consensus sequences in the multiple-nucleotide alignment of IMP-type β-lactamase genes (bla_{IMP}), VIM-type β-lactamase genes (bla_{VIM}), GIM-1 β-lactamase gene (bla_{GIM-1}), SPM-1 β-lactamase gene (bla_{SPM-1}), OXA-type β-lactamase genes (bla_{OXA}) and GES-type β-lactamase genes (bla_{GES}), using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The primers are described in Table 1. PCR amplifications were conducted as described previously (Lee *et al.,* 2000, 2001, 2005b; Jeong *et al.,* 2003*,* 2004; Song *et al.,* 2005*,* 2006).

DNA sequencing was conducted via direct sequencing, using an automatic sequencer (ABI PRISM3100; Applied Biosystems, Germany), as described previously (Lee *et al.,* 2001). DNA sequence analysis was performed using DNASIS for Windows (Hitachi Software Engineering America Ltd., USA). Database similarity searches for both the nucleotide sequences and the deduced protein sequences were conducted via BLAST at the National Center for Biotechnology Information website (http:// www.ncbi.nlm.nih.gov).

Enterobacterial repetitive consensus PCR

Enterobacterial repetitive consensus (ERIC)-PCRs were conducted in 50 μl volumes, each containing 10 ng of genomic DNA from the *A. baumannii* clinical isolates, 4 mM $MgCl_2$, 50 pM of each primer: ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Versalovic *et al.,* 1991), 1.25 U of TaKaRa Ex *Taq* polymerase (TaKaRa, Japan), 0.2 mM of each dATP, dCTP, dGTP and dTTP in 25 mM TAPS [N-Tris(hydroxy)methyl-3-amino-propane sulfonic acid pH 9.3], 50 mM KCl, and 1 mM 2-mercaptoethanol. Amplification was carried out with the following program: 95°C for 5 min followed by 35 cycles of 1 min at 52°C, 5 min at 70°C, and 1 min at 92°C. A final extension step was run at 70°C for 10 min. The amplified products (10 μl aliquots) were analyzed in 2% Seakem LE agarose (BMA, USA). For pulsed-field gel electrophoresis (PFGE), the *Sma*I-digested genomic DNA was prepared in accordance with the instructions provided by Bio-Rad (USA), and the fragments were separated for 20 h at 6 V/cm at 11°C, using a CHEF-DRII system (Bio-Rad), with initial and final pulse times of 0.5 and 60 sec, respectively. DNA fingerprints were interpreted in accordance with the recommendations of Zarrilli *et al.* (2004).

Results

Phenotypic properties of imipenem-resistant isolates

Thirty-one (59%), 15 (28%), five (9%) and two (4%) among 53 nonrepetitive *A. baumannii* isolates were recovered from ICU, urology ward and neurology ward, respectively. 26 (49%), 21 (40%), four (7%) and two (4%) isolates among a total of 53 were obtained from sputum, urine, pus, and blood samples, respectively. This result shows that sputum and urine may be more important sources of spread than are pus and blood. The results of our antimicrobial susceptibility testing of 53 isolates revealed that the prevalences of resistance to ampicillin, cefoxitin, cefotetan and aztreonam were high (94.3, 86.8, 94.3 and 64.2%, respectively). The prevalence of isolates resistant to all other tested antimicrobial agents was determined to be less than 26.4% (see Table 2). We detected a significant amount (17.0%, 9 of 53) of imipenem-resistant isolates. In Korea, the incidence of imipenem resistance among *A. baumannii* from June

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

Antimicrobial agent	Resistance (%)[a]		
	IMP-S (n=44)	IMP-R (n=9)	Total (n=53)
Ampicillin	93.2	100	94.3
Ampicillin-sulbactam	0	22.2	3.8
Piperacillin	2.3	100	17.0
Piperacillin-tazobactam	2.3	100	13.2
Cefoxitin	86.4	88.9	86.8
Cefotetan	97.9	77.8	94.3
Ceftazidime	4.5	100	18.9
Cefotaxime	13.6	100	26.4
Cefepime	2.3	100	15.1
Aztreonam	63.6	100	64.2
Imipenem	0	100	17.0
Meropenem	0	100	17.0
Amikacin	6.8	88.9	20.8
Gentamicin	9.1	88.9	22.6
Tobramycin	9.1	88.9	22.6
Tetracycline	6.8	55.6	15.1
Trimethoprim-sulfamethoxazole	11.4	55.6	18.9
Ciprofloxacin	4.5	77.8	17.0

[a]IMP-S, imipenem-susceptible; IMP-R, imipenem-resistant.

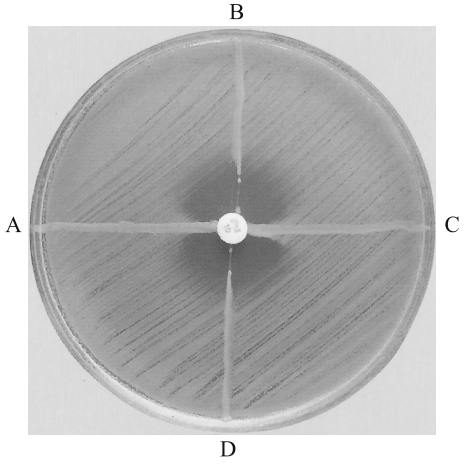

Fig. 1. Modified cloverleaf test results for a representative OXA-23-producing isolate (*A. baumannii* K0420859) (A), an imipenem-susceptible isolate producing neither IMP-1 nor OXA-23 (*A. baumannii* K0420039) (B), a representative IMP-1-producing isolate (*A. baumannii* K0420673) (C), and a negative control (*A. baumannii* ATCC 19606) (D). A Mueller-Hinton agar plate was incubated with *E. coli* ATCC 25922. An imipenem disk was positioned in the center, and test isolates were streaked from the edge of the disk to the periphery of the plate, followed by overnight incubation.

to September 2004 was lower than the incidence (26.9%) in 2003, which may be the result of a reduction in the prescription of imipenem, as well as the generally more judicious use of imipenem (Jeon *et al.,* 2005; Lee *et al.,* 2005a). A total of nine imipenem-resistant *A. baumannii* isolates were analyzed via the modified cloverleaf synergy test and the EDTA-disk synergy test. Metallo-β-lactamase generation (EDTA-disk synergy test positive) was detected in two isolates. The prevalence of carbapenemase-producing isolates (modified cloverleaf synergy test positive) was 100% (9 of 9) (see Fig. 1 and Table 3). These results were confirmed with the carbapenemase activity data via spectrophotometric assays, in the presence and absence of EDTA. These results indicate that two imipenem-resistant *A. baumannii* isolates do produce class B metallo-β-lactamases, and seven isolates produce other type β-lactamases. Nine β-lactamase-producing *A. baumannii* showed high levels of resistance to ampicillin, piperacillin, piperacillin-tazobactam, ceftazidime, ceftazidime-clavulanic acid, cefepime, imipenem, and meropenem (see Table 3). All nine β-lactamase-producing *A. baumannii* isolates only evidenced intermediate resistance to ampicillin-sulbactam,

Table 3. Characterization of imipenem-resistant *A. baumannii* isolates

Isolate	Age/Sex	Type of specimen	Ward[c]	MICs (μg/ml) of β-lactams[c]									β-Lactamase	Synergy test	
				AMP	SAM	PIP	TZP	CAZ	CAZ-CLA	FEP	IMP	MEM		Cloverleaf	EDTA-disk
K0420673	71/M	Urine	NW	>256	16	>256	>256	>256	>256	128	32	32	IMP-1	+	+
K0421793	46/M	Urine	NW	>256	16	>256	>256	>256	>256	256	32	64	IMP-1	+	+
K0420859	65/F	Urine	NW	>256	16	>256	128	256	256	32	16	16	OXA-23	+	–
K0418396	61/F	Pus	NW	>256	16	>256	128	256	256	64	16	16	OXA-23	+	–
K0418480	64/M	Sputum	ICU	>256	32	>256	128	256	256	64	16	16	OXA-23	+	–
K0418919	72/F	Sputum	ICU	>256	16	>256	128	256	256	64	32	16	OXA-23	+	–
K0419543	79/M	Sputum	ICU	>256	16	>256	128	>256	>256	64	32	16	OXA-23	+	–
K0419583	56/F	Sputum	NW	>256	16	>256	128	>256	>256	64	16	16	OXA-23	+	–
K0425685	1/M	Urine	UW	>256	16	>256	128	256	256	32	16	16	OXA-23	+	–
K0420039[a]	58/F	Sputum	NW	>256	16	>256	64	>256	>256	>256	1	1	ND[b]	–	–

[a]Imipenem-susceptible strain.
[b]ND, not detectable for carbapenemase.
[c]Abbreviation: AMP, ampicillin; SAM, ampicillin-sulbactam (2:1 ratio of β-lactam to inhibitor); PIP, piperacillin; TZP, piperacillin-tazobactam (inhibitor fixed at 4 μg/ml); CAZ, ceftazidime; CAZ-CLA, ceftazidime-clavulanic acid (inhibitor fixed at 4 μg/ml); FEP, cefepime; IMP, imipenem; MEM, meropenem; ICU, intensive care unit; NW, neurology ward; UW, urology ward.

relative to the other tested antibiotics. Two EDTA-disk and modified cloverleaf synergy test positive isolates were shown to produce β-lactamase with an apparent pI of 9.0, and seven modified cloverleaf synergy test positive isolates produced a β-lactamase with an apparent pI of 6.65 (data not shown). On the basis of the IEF results, as well as on the results of previous studies of IMP-1-producing *P. aeruginosa* (Laraki *et al.,* 1999) and OXA-23-producing *A. baumannii* (Donald *et al.,* 2000) and *Proteus mirabilis* (Bonnet *et al.,* 2002), we suspected that the IMP-1 (class B) and OXA-23 (class D) carbapenemases might be relevant to imipenem resistance.

Molecular characterization of IMP-1 or OXA-23-producing A. baumannii isolates

Neither the transfer nor the curing of imipenem resistance was achieved with any of the imipenem-resistant isolates, despite multiple attempts. $bla_{\text{IMP-1}}$- or $bla_{\text{OXA-23}}$-carrying plasmid DNA was apparently undetectable, either in plasmid preparation without contaminated bacterial chromosomal DNA by plasmid-safe ATP-dependent DNase, or in the whole genomic DNA preparations from nine isolates. In a Southern blot experiment conducted with the genomic DNA of nine of the isolates, the $bla_{\text{IMP-1}}$ and $bla_{\text{OXA-23}}$ probes were hybridized to the band of chromosomal DNA. Nine clinical isolates yielded PCR products with primer pairs for the $bla_{\text{IMP-1}}$ and $bla_{\text{OXA-23}}$ genes, but not with other primer pairs for bla_{VIM}, bla_{GIM}, bla_{SPM}, bla_{GES}, $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-48}}$. Considering the resistance phenotypes of the nine clinical isolates, the resistance genotypes of these isolates were analyzed via the direct sequencing of the PCR-amplified fragments specific for the $bla_{\text{IMP-1}}$ and $bla_{\text{OXA-23}}$ genes. The DNA sequences of the PCR-amplified fragments specific for the $bla_{\text{IMP-1}}$ and $bla_{\text{OXA-23}}$ genes were found to be identical to the entire sequences for the $bla_{\text{IMP-1}}$ (GenBank accession number S71932) and $bla_{\text{OXA-23}}$ (GenBank accession number AJ132105) genes, respectively. Based on the DNA sequencing results, two EDTA-disk synergy test positive and seven modified cloverleaf synergy test positive clinical isolates were determined to harbor the $bla_{\text{IMP-1}}$ gene and the $bla_{\text{OXA-23}}$ gene, respectively. The IMP-1 and OXA-23 carbapenemases were involved in imipenem resistance, as described previously (Donald *et al.,* 2000; Dalla-Costa *et al.,* 2003; Laraki *et al.,* 2004).

ERIC-PCR analysis

ERIC is a technique predicated on the analysis of repetitive chromosomal sequences, and has recently been employed in the clonal characterization of intra- and inter-species of Enterobacteriaceae. Dispersed repetitive DNA sequences within the genomes of bacteria, using conserved primers corresponding to the ERIC sequences, was also assessed via PCR. ERIC-PCR generated a characteristic genomic fingerprint, which could be used to determine intra- and inter-

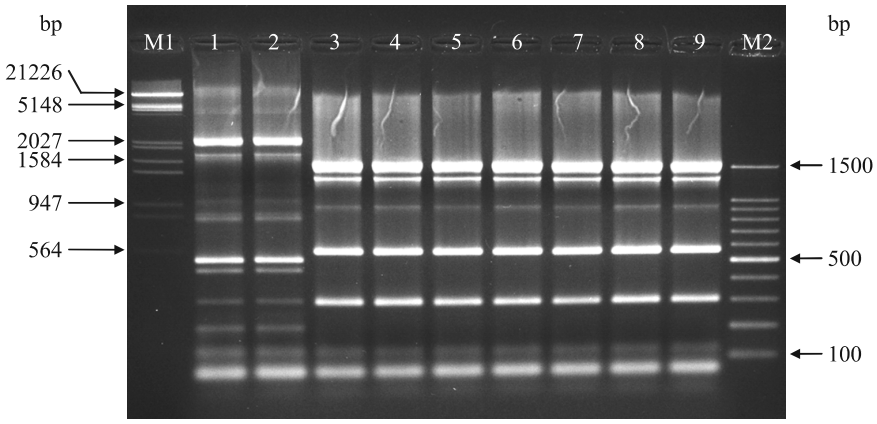

Fig. 2. ERIC-PCR patterns of genomic DNA from nine clinical isolates of *A. baumannii* : K0420673 (lane 1), K0421793 (lane 2), K0420859 (lane 3), K0418396 (lane 4), K0418480 (lane 5), K0418919 (lane 6), K0419543 (lane 7), K0419583 (lane 8), and K0425685 (lane 9). Lanes M1 (*Hin*dIII/*Eco*RI-digested phage λ) and M2 (100 bp stepwise ladder) show band patterns of DNA marker fragments (sizes in bp are indicated on the edge of the gel). ERIC-PCR was conducted with ERIC2 and ERIC1R primers.

species genotypic variations among the Enterobacteriaceae (Dalla-Costa *et al.,* 1998), *Ps. aeruginosa* (Luzzaro *et al.,* 2001) and *A. baumannii* (Luzzaro *et al.,* 2001; Oh *et al.,* 2003; Silbert *et al.,* 2004). Nine clinical isolates, when evaluated via ERIC-PCR, generated distinct bands ranging in size from 0.1 to 35.5 kb and varying from 7 to 12 bands (see Fig. 2). The ERIC-PCR fingerprints of two IMP-1-producing *A. baumannii* isolates were identical. The seven OXA-23-producing *A. baumannii* isolates all evidenced identical patterns. Two different banding patterns were detected between two IMP-1-producing isolates and seven OXA-23-producing isolates. These results were verified by PFGE analysis with *Sma*I (data not shown).

Discussion

Since 2003, a high incidence of imipenem resistance has been observed among nosocomial *A. baumannii* isolates in Korea. Therefore, the objective of this study was to determine whether this resistance was the result of the dissemination of an imipenem resistance determinant, and/or the spread of one *A. baumannii* clone. Microbiological assays indicated that imipenem was deactivated by seven imipenem-resistant *A. baumannii* isolates. No alterations in carbapenemase activity were detected in the presence of EDTA, thereby pointing to the production of a non-metallo-β-lactamase. OXA class D carbapenemases have been identified in *A. baumannii* collected in the UK (OXA-23) (Donald *et al.,* 2000), Spain (OXA-24, 25 and 40) (Bou *et al.,* 2000; Donald *et al.,* 2000; Afzal-Shah *et al.,* 2001; Héritier *et al.,* 2003), Belgium (OXA-26) (Silva *et al.,* 2004) and Singapore (OXA-27) (Silva *et al.,* 2004). *A. baumannii* is a nosocomial pathogen which is emerging, in part, as the consequence of the capacity of the pathogen to acquire resistance against multiple antimicrobial agents. As OXA-23-producing *A. baumannii* strains confer resistance against the majority of β-lactams, including imipenem, aztreonam, ceftazidime, and cefepime, a limited number of antimicrobial agents, including polymyxin, sulbactam and minocycline, maintain reliable levels of activity against OXA-23-producing *A. baumannii* (Dolla-Costa *et al.,* 2003). However, the results of our studies showed that 85.7% of OXA-23-producing *A. baumannii* isolates evidenced an intermediate degree of resistance against ampicillin-sulbactam. Thus, it is important to monitor and control the spread of OXA-23-producing *A. baumannii,* which is resistant to most β-lactams. Two isolates produced a β-lactamase with an apparent pI of 9.0. On the basis of the IEF results, as well as previous reports regarding IMP-1-producing *A. baumannii* in Italy (Riccio *et al.,* 2000), IMP-1 metallo-β-lactamase was also involved in imipenem resistance.

We detected nine different isolates of imipenem-resistant *A. baumannii* from Korean patients. Among nine isolates, seven produced OXA-23. The clonal relatedness of *A. baumannii* Korean isolates was assessed via ERIC-PCR. All OXA-23-producing isolates exhibited very similar antibiotic resistance profiles, and very similar DNA fingerprinting patterns. Two IMP-1-producing isolates evidenced identical patterns. Such a correspondence of phenotypic and genotypic characteristics can be attributed to a common clonal origin. Our results show that the imipenem resistance observed among nine Korean *A. baumannii* isolates is attributable to the spread of two IMP-1- or OXA-23-producing clones. A method by which metallo-β-lac-

tamase (class B or D) could be easily type-identified can facilitate the control of hospital infection, and bolster the ability of the physician to prescribe the most appropriate antibiotic, thus reducing selective pressure and antibiotic resistance (Lee *et al.,* 2005a). Our microbiological test of carbapenemase activity allows for such easy identification, and constitutes a simple method for the screening of clinical isolates producing class D carbapenemase and/or class B metallo-β-lactamase, both to determine their clinical impact and to prevent further spread. The clinical significance of these isolates, which are emerging in Korea, is of great importance, as clinicians have been advised against the use of extended-spectrum cephalosporins, aztreonam, imipenem, and aminoglycosides. This observation underlines the importance of establishing effective control measures in Asian hospitals, including the early detection of colonized patients, isolation procedures, and the judicious use of antibiotics.

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