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# IMP-1과 OXA β-lactamase를 생성하는 Carbapenem 내성 *Acinetobacter baumanni*의 확산

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# Dissemination of IMP-1 and OXA Type β-Lactamase in Carbapenem-resistant *Acinetobacter baumannii*

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**Background :** *Acinetobacter baumannii* is an aerobic, gram-negative, glucose-nonfermenting bacterium, which has emerged as a serious opportunistic pathogen. In recent years, the increasing instance of carbapenem-resistant *A. baumannii* producing metallo-β-lactamases (MBLs) or OXA-type β-lactamases is causing a serious clinical problem. In this study, we investigated the prevalence of Ambler class A, B, and D β-lactamases and their extended-spectrum derivatives in carbapenem-resistant *A. baumannii* isolates.

**Methods :** A total of 31 consecutive, non-duplicate, carbapenem-resistant *A. baumannii* were isolated from three university hospitals in the Chungcheong province of Korea. The modified Hodge and inhibitor-potentiated disk diffusion tests were conducted for the screening of carbapenemase and MBL production, respectively. PCR and DNA sequencing were performed for the detection of β-lactamase genes. We also employed the enterobacterial repetitive intergenic consensus (ERIC)-PCR method for the epidemiologic study.

**Results :** Twenty-three of 31 isolates harbored *bla*$_{OXA-2}$ (51.6%), *bla*$_{OXA-23}$ (22.6%), *bla*$_{IMP-1}$ (48.4%), and *bla*$_{VIM-2}$ (3.2%). All of the OXA-2-producing strains also evidenced MBLs. The strains that harbored *bla*$_{OXA-23}$ were isolated only in hospital C, and only in a limited fashion. The ERIC-PCR pattern of the five OXA-23 strains indicated that the isolates were closely related in terms of clonality. The six strains producing IMP-1 isolated from hospital A were confirmed to be identical strains.

**Conclusions :** *A. baumannii* strains harboring IMP-1 or OXA-type β-lactamases are currently widely distributed throughout the Chungcheong province of Korea. The most notable finding in this study was that a *bla*$_{OXA-2}$-producing *A. baumannii* harboring MBL, which has not been previously reported, can also lead to outbreaks. *(Korean J Lab Med 2008;28:16-23)*

**Key Words :** *A. baumannii, MBL, OXA-2*

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## INTRODUCTION

*Acinetobacter baumannii* is an aerobic, gram-negative, glucose-nonfermenting bacterium, which has recently emerged as a serious opportunistic pathogen. It causes nosocomial infections, including pneumonia, septicemia, and urinary tract and wound infections, and is also frequently involved in outbreaks[1]. The majority of clinical *A. baumannii* isolates are highly resistant to a variety of antibiotics, including carbapenems, which are currently the drugs of choice in the treatment of the severe infections caused by this organism[2].

Carbapenem resistance in *A. baumannii* is associated with a variety of combined mechanisms, including the acquisition of β-lactamases, AmpC stable derepression, decreased permeability, altered penicillin-binding proteins (PBPs), and to a small extent, efflux pump overexpression[3, 4]. In particular, Ambler class B enzymes (also referred to as metallo-β-lactamases, MBLs) and carbapenem hydrolyzing class D-β-lactamases (CHDLs) have been identified worldwide from carbapenem-resistant *A. baumannii* strains[5, 6].

MBLs are powerful carbapenemases, and can hydrolyze a wide variety of β-lactams, including penicillins, cephalosporins, and carbapenems. Since the initial isolation of carbapenem-resistant *A. baumannii* producing IMP-1 and VIM-1 type MBL in Japan and Italy, respectively, clinical isolates of these strains have been identified worldwide[7-10]. CHDLs are Ambler class D enzymes that evidenced the ability to hydrolyze imipenem, and are the most wide- spread β-lactamases with carbapenemase activity in *A. baumannii*. The CHDL OXA-23 was initially reported in carbapenem-resistant *A. baumannii* in 1995; since then, many types of CHDLs have been identified worldwide. These enzymes belong to 3 unrelated groups: OXA-23, OXA-24, and OXA-58[11-13].

In addition to the MBLs and CHDLs, extended spectrum β-lactamases evidencing weak imipenem hydrolysis in gram-negative bacteria have also been identified[14-17]. Although many previous studies involving carbapenem-resistant *A. baumannii* have been conducted, these studies have been principally concerned with clinical isolates that generate MBL and/or CHDLs.

In this study, we assessed the prevalence of Ambler class A, B, and D β-lactamases and their extended-spectrum derivatives in clinical *A. baumannii* isolates, and characterized nosocomial outbreaks in three university hospitals located in Chungcheong province of Korea, via enterobacterial repetitive intergenic consensus (ERIC)-PCR.

## MATERIALS AND METHODS

### 1. Bacterial strains and susceptibility tests

Between March and December of 2006, a total of 31 consecutive, non-duplicate, carbapenem-resistant *A. baumannii* were isolated at three university hospitals located in Chungcheong province of Korea. The isolates were confirmed to be *A. baumannii* via biochemical profiling using conventional methods[18] and Vitek gram-negative identification (GNI) cards (bioMérieux Vitek, Inc., Hazelwood, MO, USA).

Antimicrobial susceptibilities were determined via the disk diffusion technique in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI)[19]. The following antibiotics were tested: amikacin, gentamicin, netilmicin, tobramycin, aztreonam, ceftazidime, cefepime, imipenem, meropenem, piperacillin, piperacillin-tazobactam, ticarcillin, ticarcillin-clavulanic acid, and ciprofloxacin (BBL, Cockeysville, MD, USA).

### 2. Detection of carbapenemase

In order to evaluate the inactivation of imipenem by carbapenemase, carbapenem-resistant *A. baumannii* isolates were screened via the modified Hodge test[20]. *Acinetobacter genomospecies* 3 YMC 99/11/160 and *A. baumannii* ATCC19606 were utilized as positive and negative controls, respectively. *E. coli* ATCC 25922 was cultured overnight and suspended to achieve a 0.5 McFarland standard turbidity, and was evenly inoculated onto the surface of a Mueller-Hinton agar plate, using a cotton swab. After drying, the disk containing imipenem (10 μg/mL) was positioned at the center of the plate, and the overnight-cultured test strains were heavily streaked from the center to the periphery of the plates. The presence of a distorted inhibition zone after overnight incubation was interpreted as positive on the modified Hodge test.

The inhibitor-potentiated disk diffusion (IPD) testing method was utilized for the detection of MBLs, as previously described by Oh et al.[21]. An overnight culture suspension of the test isolate equivalent to a 0.5 McFarland standard was inoculated on a Mueller-Hinton agar plate. Two imipenem disks (10 μg/mL) were positioned within a center-to-center distance of 4-5 cm on a dried agar plate and 10 μL of 0.5 M EDTA was applied to one of the disks. After overnight incubation, the presence of an enlarged inhibition zone around the imipenem disk to which 0.5 M EDTA was applied was interpreted as being IPD test positive when the width of the augmentation zone was >7 mm.

### 3. PCR amplification and sequencing

All carbapenem-resistant isolates were subjected to PCR assays for the detection of β-lactamase genes. The total DNA and plasmid DNA from the isolates were prepared as template DNA for PCR. Total DNA was extracted via 10 min of boiling, followed by centrifugation. Plasmid DNA was isolated using a Plasmid Mini-prep kit (SolGent, Daejeon, Korea) and plasmid-safe ATP-dependent DNase (Epicentre Technology, Madison, WI, USA). Specific primers for the detection of β-lactamases were utilized as described previously (Table 1).

PCR was conducted with 50 ng of template DNA (total DNA or plasmid DNA), 2.5 μL of 10× Taq buffer, 0.5 μL of 10 mM dNTP mix, 20 pmol of each primer, and 0.7 U of Taq DNA polymerase (SolGent) in a total volume of 25 μL. All of the β-lactamases were amplified via the pre-denaturation of the reaction mixture for 5 min at 95℃; this was followed by 35 cycles at 95℃ for 20 sec, 59℃ for 40 sec, and

Table 1. Oligonucleotides used as primers for amplification and sequencing in this study

| Class | Primer pairs | Target | Sequence (5′→3′) | Amplicon size (bp) | Reference |
|---|---|---|---|---|---|
| Class A | TEM F | TEM-1 and derivative | ATG AGT ATT CAA CAT TTC CGT | 861 | 24 |
| | TEM R | | TTA CCA ATG CTT AAT CAG TGA | | |
| | SHV F | SHV-1 and derivative | CCG GGT TAT TCT TAT TTG TCG CT | 831 | 24 |
| | SHV R | | TAG CGT TGC CAG TGC TCG | | |
| | CTX-M F | CTX-M- 1, 2, 9 group | GAT TGA CCG TAT TGG GAG TTT | 947 | 26 |
| | CTX-M R | | CGG CTG GGT AAA ATA GGT CA | | |
| | PER F | PER-1 | GTT AAT TTG GGC TTA GGG CAGA | 855 | 26 |
| | PER R | | CAG CGC AAT CCC CAC TGT | | |
| | VEB F | VEB-1 | CGA CTT CCA TTT CCC GAT GC | 650 | 25 |
| | VEB R | | GGA CTC TGC AAC AAA TAC GC | | |
| | GES F | GES-1, -2, 3, 4, IBC-1 | GTT AGA CGG GCG TAC AAA GAT AAT | 903 | 24 |
| | GES R | | TGT CCG TGC TCA GGA TGA GT | | |
| | PSE F | PSE-1 | AAT GGC AAT CAG CGC TTC | 700 | 22 |
| | PSE R | | GCG CGA CTG TGA TGT ATA | | |
| Class B | IMP F | IMP | CAT GGT TTG GTG GTT CTT GT | 488 | 20 |
| | IMP R | | ATA ATT TGG CGG ACT TTG GC | | |
| | VIM F | VIM | ATT GGT CTA TTT GAC CGC GTC | 780 | 20 |
| | VIM R | | TGC TAC TCA ACG ACT GAG CG | | |
| Class D | OXA-1F | OXA group III | AGC CGT TAA AAT TAA GCC C | 908 | 11 |
| | OXA-1R | | CTT GAT TGA AGG GTT GGG CG | | |
| | OXA-2F | OXA group II | GCC AAA GGC ACG ATA GTT GT | 700 | 22 |
| | OXA-2R | | GCG TCC GAG TTG ACT GCC GG | | |
| | OXA-10F | OXA group I | TCT TTC GAG TAC GGC ATT AGC | 760 | 25 |
| | OXA-10R | | CCA ATG ATG CCC TCA CTT TCC | | |
| | OXA-23F | OXA 23, 27, 49 | GAT GTG TCA TAG TAT TCG TCG | 1,058 | 20 |
| | OXA-23R | | TCA CAA CAA CTA AAA GCA CTG | | |
| | OXA-24F | OXA 24, 25, 26, 40, 72 | GTA CTA ATC AAA GTT GTG AA | 825 | 20 |
| | OXA-24R | | TTC CCC TAA CAT GAA TTT GT | | |
| | OXA-58F | OXA 58 | CGA TCA GAA TGT TCA AGC GC | 528 | 23 |
| | OXA-58R | | ACG ATT CTC CCC TCT GCG C | | |

Abbreviations: F, forward; R, reverse.

72℃ for 30 sec, and a final elongation for 5 min at 72℃; these reactions were conducted in a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Corp., Norwalk, CT, USA). The amplicons were purified with a PCR purification kit (SolGent) and were sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems).

### 4. ERIC-PCR

Chromosomal DNA was extracted from each β-lactamase-harboring strain, using a genomic DNA purification kit (Promega, Madison, WI, USA) in accordance with the standard protocols. ERIC-PCR was conducted with a 50 μL reaction mixture containing 100 ng of chromosomal DNA, 5 μL of 10× Taq buffer, 1.0 μL of 10 mM dNTP mix, 1.5 U of Taq DNA polymerase (SolGent), and 50 pmol of each of the primers ERIC1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′)[16]. The cycling conditions were as follows: an initial denaturation step at 95℃ for 5 min; followed by 30 cycles of amplification steps at 92℃ for 50 sec, 52℃ for 55 sec, and 70℃ for 7 min; and a final extension step at 70℃ for 10 min.

Table 2. Characterization of carbapenem-resistant *A. baumannii* isolates

| Isolates | Antibiotic susceptibilities | | | | | | | | | | | Clover-leaf | IPD | β-lactamase |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AMK | GEN | TOB | ATM | CAZ | FEP | IPM | MEM | PIP | TZP | CIP | | | |
| A1 | R | R | R | R | R | R | R | R | R | I | R | + | + | IMP-1, OXA-2 |
| A3 | R | R | R | R | R | R | R | R | R | I | R | + | + | IMP-1, OXA-2 |
| A4 | R | R | R | R | R | R | R | R | R | I | R | − | − | |
| A5 | S | S | I | I | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| A6 | S | R | I | R | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| A7 | R | R | R | R | R | R | R | R | R | I | R | + | + | IMP-1, OXA-2 |
| A8 | S | R | R | R | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| A9 | R | R | R | R | R | R | R | R | I | S | R | + | + | IMP-1, OXA-2 |
| A10 | S | R | R | R | R | R | R | R | R | R | R | + | + | IMP-1, OXA-2 |
| A11 | S | S | I | R | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| A12 | S | R | R | R | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| A14 | R | R | R | R | R | R | R | R | R | R | R | − | − | |
| A15 | S | S | S | R | R | R | R | R | R | R | S | − | − | |
| A16 | S | R | R | R | R | R | R | R | R | R | I | − | − | |
| B25 | R | R | R | I | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| B75 | R | R | R | I | R | R | R | R | S | S | I | + | + | IMP-1, OXA-2 |
| B297 | R | R | R | I | R | R | R | R | R | S | S | + | + | IMP-1, OXA-2 |
| B814 | R | R | R | R | R | S | R | R | S | R | S | + | + | VIM-2, OXA-2 |
| B852 | R | R | R | I | R | I | R | R | R | S | I | + | + | IMP-1, OXA-2 |
| C1 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C2 | R | R | R | R | R | R | R | R | R | I | R | + | + | IMP-1, OXA-2 |
| C3 | R | R | R | R | R | R | R | R | R | R | R | − | − | |
| C4 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C5 | R | R | R | R | R | R | R | R | R | R | R | − | − | |
| C6 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C9 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C13 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C14 | R | R | R | R | R | R | R | R | R | I | R | − | − | |
| C15 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C16 | R | R | R | R | R | R | R | R | R | R | R | + | − | OXA-23 |
| C17 | R | R | R | R | R | R | R | R | R | I | R | − | − | |
| 19606* | | | | | | | | | | | | − | − | |
| YMC† | | | | | | | | | | | | + | + | VIM-2 |

*Non-carbapenemase producing *A. baumannii* ATCC 19606, †VIM-2-producing *A. genomospecies* 3 YMC 99/11/160.
Abbreviations: AMK, amikacin; GEN, gentamicin; TOB, tobramycin; ATM, aztreonam; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; PIP, piperacillin; TZP, piperacillin-tazobactam; CIP, ciprofloxacin.

The amplified products were separated via electrophoresis on 1.5% agarose gels containing ethidium bromide, and visualized using a BioDoc-14 Imaging system (UVP, Cambridge, UK).

## RESULTS

### 1. Properties of carbapenem-resistant *A. baumannii* isolates

A total of 31 carbapenem-resistant *A. baumannii* strains (14, 5, and 12 strains from hospitals A, B, and C, respectively) were isolated. Twenty-three of the 31 isolates (74.2%) evidenced carbapenemase activity in the modified cloverleaf test. These results were confirmed with the carbapenemase activity data acquired via spectrophotometric assays in the presence or absence of EDTA. Of the 23 carbapenemase-producing isolates, 16 (69.6%) evidenced MBL generation on the IPD test. The remaining 7 strains were isolated only in hospital C, and did not generate MBLs. All of the isolates evidenced resistance to all of the tested antibiotics with the exception of piperacillin-tazobactam (Table 2).

### 2. β-lactamase characterization

Twenty-three of 31 isolates (74.2%) harbored OXA β-lactamase genes, and 16 of 31 isolates (51.6%) harbored MBL genes. The 16 strains isolated from the three hospitals harbored *bla*$_{OXA-2}$ in addition to the MBL genes. The 7 strains isolated only from hospital C harbored *bla*$_{OXA-23}$ (Table 2, 3). The 16 strains harboring *bla*$_{OXA-2}$ included 15 *bla*$_{IMP}$ and 1 *bla*$_{VIM}$ (Table 2, 3). We were unable to detect any other OXA-type β-lactamse genes, including *bla*$_{OXA-1}$, *bla*$_{OXA-10}$, *bla*$_{OXA-24}$, and *bla*$_{OXA-58}$, and Ambler class A β-lactamases (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{PER}$, *bla*$_{VEB}$, *bla*$_{GES}$, and *bla*$_{PSE}$). The DNA sequences of the *bla*$_{OXA-2}$, *bla*$_{OXA-23}$, *bla*$_{IMP}$, and *bla*$_{VIM}$ amplicons were identical to those of *bla*$_{OXA-2}$, *bla*$_{OXA-23}$, *bla*$_{IMP-1}$, and *bla*$_{VIM-2}$, respectively.

### 3. ERIC-PCR patterns of OXA-type β-lactamase and MBL producers

ERIC-PCR analysis was conducted in an effort to elucidate the clonal characterization of the 7 and 15 isolates harboring *bla*$_{OXA-23}$ and *bla*$_{IMP-1}$, respectively. Five of the 7 OXA-23-producing strains isolated from hospital C evidenced identical patterns, and the remaining 2 were found to be identi-

Table 3. Prevalence of β-lactamases in carbapenem- resistant *A. baumannii* isolates

| Class of β-lactamase | Type of β-lactamase | N (%) of isolates | N (%) of isolates in each hospital | | |
|---|---|---|---|---|---|
| | | | A | B | C |
| Class A | TEM | 0 | 0 | 0 | 0 |
| | SHV | 0 | 0 | 0 | 0 |
| | CTX-9 | 0 | 0 | 0 | 0 |
| | PER-1 | 0 | 0 | 0 | 0 |
| | VEB | 0 | 0 | 0 | 0 |
| | GES/IBC | 0 | 0 | 0 | 0 |
| | PSE | 0 | 0 | 0 | 0 |
| Class B | IMP-1 | 15 (48.4) | 10 (32.3) | 4 (12.9) | 1 (3.2) |
| | VIM-2 | 1 (3.2) | 0 | 1 (3.2) | 0 |
| Class D | OXA-1 | 0 | 0 | 0 | 0 |
| | OXA-2 | 16 (51.6) | 10 (32.3) | 5 (16.1) | 1 (3.2) |
| | OXA-10 | 0 | 0 | 0 | 0 |
| | OXA-23 | 7 (22.6) | 0 | 0 | 7 (22.6) |
| | OXA-24 | 0 | 0 | 0 | 0 |
| | OXA-58 | 0 | 0 | 0 | 0 |

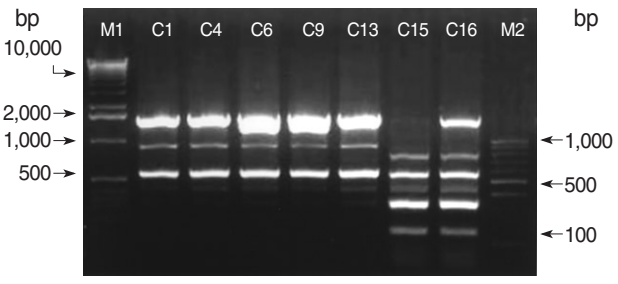


Fig. 1. ERIC-PCR patterns of genomic DNA from seven clinical isolates of *A. baumannii* harboring *bla*$_{oxa-23}$. Lane M1 and M2 are 1 kb and 100 bp DNA size markers, respectively.

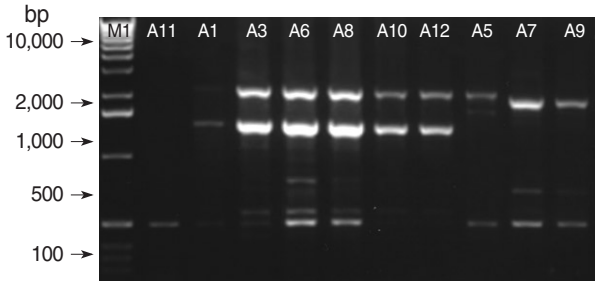


Fig. 2. ERIC-PCR patterns of genomic DNA from ten clinical isolates of *A. baumannii* harboring *bla*$_{IMP-1}$. Lane M1 is a 1 kb DNA size marker.

cal with each other (Fig. 1). ERIC-PCR conducted with the 15 IMP-1-producing isolates revealed seven different banding patterns. One characteristic identical pattern was observed in 6 strains, which were isolated only from hospital A (Fig. 2).

## DISCUSSION

Carbapenem resistance in *A. baumannii* has been increasingly detected worldwide, and this is also the case in Korea [27]. Carbapenems (e.g., imipenem and meropenem) have become the drugs of choice in the treatment of *Acinetobacter* infections in a host of medical centers, but are being compromised by the emergence of carbapenem-hydrolyzing β-lactamase (carbapenemase) of molecular classes B and D. Furthermore, several studies have indicated that imipenem may be hydrolyzed by the extended-spectrum β-lactamases of gram-negative bacteria[15, 16].

In this study, the Ambler class D β-lactamases were detected more frequently than MBLs in carbapenem-resistant *A. baumannii*, although MBLs evidence powerful carbapenemase activity. 23 (74.2%) of the 31 isolates harbored class D β-lactamases, and carbapenemase activity was noted in all of them. In particular, the largest proportion of β-lactamases was OXA-2 β-lactamase, and this was consistently accompanied by *bla*$_{IMP-1}$ or *bla*$_{VIM-2}$ in the *A. baumannii* isolates. An increasing frequency of OXA-2 β-lactamase has been reported in *Pseudomonas aeruginosa*[28], and has also been observed in Korea[29]. However, there have been no reports as yet concerning OXA-2 β-lactamase production in *A. baumannii* strains. This is, to the best of our knowledge, the first report of *bla*$_{OXA-2}$-producing *A. baumannii* harboring MBLs. The coexistence of OXA-2 β-lactamase with MBL compels us to surmise that the carbapenem-resistant *A. baumannii* isolates may harbor an integron with the two β-lactamase gene cassettes. Further studies regarding the relationship between MBL and OXA-2 β-lactamase will be required to confirm this supposition.

In this study, 15 of 16 *A. baumannii* with MBL genes harbored *bla*$_{IMP-1}$, and only 1 of these isolates harbored *bla*$_{VIM-2}$. In particular, 6 strains of 10 IMP-1-producing *A. baumannii* strains isolated from the same hospital evidenced identical banding patterns on their ERIC-PCR profiles, thereby indicating clonal relation and horizontal spreading in the hospital. The dissemination of IMP-1-producing *Acinetobacter* was recently reported in Korea[27] and Brazil[30]. Our results are contrary to previous reports showing that *bla*$_{VIM-2}$ was the most prevalent MBL among clinical isolates of *A. baumannii* from Korean hospitals[21]. This indicates that the proportion of IMP-1 producing isolates is increasing, along with that of the VIM-2-producing isolates.

Five of the OXA-23 producing strains evidenced the same type of ERIC-PCR patterns, and they also exhibited very similar antibiotic resistance profiles. This is suggestive of the horizontal mobility of *bla*$_{OXA-23}$. In particular, the detection of OXA-23 β-lactamases (only in hospital C) indicated colonization, as well as clonal expansion. Clonal outbreaks attributable to OXA-23-producing isolates were previously reported in Brazil and Korea[20, 27, 31].

The spread of IMP-1 and OXA-23-producing clones in different university hospitals at the same time illustrated that each of the clones was nosocomially colonized and infected. *A. baumannii* colonization and infection has also been reported in London and Australia[32, 33]. In order to verify the colonization of the clones detected in the present study, an epidemiological study will be required.

In conclusion, *A. baumannii* strains harboring IMP-1 and OXA-type β-lactamases are widespread in the Chungcheong province of Korea, and have been shown to cause outbreaks in university hospitals. The most notable finding was that a *bla*$_{OXA-2}$-producing strain of *A. baumannii* harbored MBL, which had not been previously reported. In order to prevent new and further clonal spread, it is apparent that β-lactamase-producing strains should be detected more rapidly by the confirmation of clonal expansion via epidemiological evaluations of both colonized patients and environmental sources.

## 요 약

**배경 :** *Acinetobacter baumannii*는 포도당 비발효 그람음성 호기성 간균으로 심각한 기회감염균 중 하나이다. 최근 metallo- β-lactamases (MBLs) 또는 OXA-type β-lactamases 생성으로 인

한 carbapenem 내성 *A. baumannii*가 증가하고 있어 임상적으로 큰 문제가 되고 있다. 본 연구에서는 임상적으로 분리된 carbapenem 내성 *A. baumannii*를 대상으로 Ambler class A, B, D에 해당하는 β-lactamases 생성 현황과 유전형을 규명하고자 하였다.

**방법 :** 2006년 3월에서 10월까지 충청지역의 3개의 대학병원 환자의 임상 검체에서 분리된 *A. baumannii*를 대상으로 imipenem에 대한 감수성을 조사하였다. Carbapenemase와 metallo-β-lactamase 생성균주의 선별을 위해서 Hodge 변법과 inhibitor-potentiated disk diffusion 시험을 수행하였다. 중합연쇄반응과 유전자의 염기서열 분석을 통하여 β-lactamase 유전자를 검출하였고 분자역학조사을 위해 enterobacterial repetitive intergenic consensus (ERIC)-PCR을 수행하였다.

**결과 :** 시험기간 중 총 31주의 carbapenem 내성 *A. baumannii*가 임상검체에서 분리되었다. 이 중 23주에서 $bla_{OXA-2}$ (51.6%), $bla_{OXA-23}$ (22.6%), $bla_{IMP-1}$ (48.4%), 그리고 $bla_{VIM-2}$ (3.2%) 등의 β-lactamase가 검출되었다. OXA-2 β-lactamase 생성균주는 모두 MBLs를 동시에 생성하였다. $bla_{OXA-23}$을 생성하는 균주는 모두 C병원에서만 분리되었으며 그 중 5주는 ERIC-PCR 결과 모두 같은 양상을 보여 유전적으로 매우 밀접한 관련이 있는 것으로 확인되었다. A병원에서 분리된 6주의 IMP-1 생성균주는 유사한 염색체형을 지니고 있음이 확인되었다.

**결론 :** IMP-1 또는 OXA-type β-lactamases를 생성하는 *A. baumannii* 균주가 충청지역에서 광범위하게 확산되고 있음이 확인되었다. 특히 $bla_{OXA-2}$ 생성 *A. baumannii*가 MBLs도 동시에 생성함이 본 연구에서 처음 밝혀졌다.

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