# Risk Factors for the Acquisition of Pandrug-Resistant Glucose Non-Fermenting Gram-Negative Bacilli and Their Resistance Mechanisms to Carbapenems

**Yoon Soo Park** 

**Department of Medicine** 

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Directed by Professor June Myung Kim

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

**Yoon Soo Park** 

December 2008

## This certifies that the Doctoral Dissertation of Yoon Soo Park is approved.

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Thesis Supervisor : June Myung Kim

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(Kyungwon Lee: Thesis Committee Member#1)

-----

(Bong Ki Lee: Thesis Committee Member#2)

-----

(Young Soo Ahn: Thesis Committee Member#3)

-----

(Hyukmin Lee: Thesis Committee Member#4)

The Graduate School

Yonsei University

December 2008

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#### ABSTRACT

### Risk Factors for the Acquisition of Pandrug-Resistant Glucose Non-Fermenting Gram-Negative Bacilli and Their Resistance Mechanisms to Carbapenems

Yoon Soo Park

Department of Medicine The Graduate School, Yonsei University

(Directed by Professor June Myung Kim)

**Background:** Infections with multidrug-resistant (MDR) glucose non-fermenting Gram-negative bacilli (GNFB) are associated with severe outcomes, including increased mortality and morbidity. Recently, pandrug-resistant (PDR) GNFB, defined as being resistant to all available anti-pseudomonal antibiotics, were reported. Because of limited therapeutic options and increased mortality and morbidity, prevention for acquisition of PDR GNFB is essential. However, risk factors for the acquisition of PDR GNFB are not yet well documented. Resistance mechanisms that are expressed frequently in GNFB include  $\beta$ -lactamases, alterations in cell-wall channels, and efflux pumps. Clinically most troubling has been GNFB's acquired  $\beta$ -lactamases, including serine and metallo- $\beta$ -lactamases (MBLs), which then confer a resistance to carbapenems, potent agents for treating infections caused by Gram-negative bacteria. However, carbapenem resistance mechanisms of PDR GNFB are not described. In this study, we investigated not only the risk factors for PDR GNFB acquisition but also their resistance mechanisms to carbapenems.

**Methods:** From June to November, 2007, isolates of PDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (PDRPA and PDRAB) were collected from patients in 8 tertiary care hospitals. Modified Hodge test, EDTA-imipenem disk synergy test, and PCR amplification and sequencing were performed to detect the presence and typing of the MBL and OXA genes. A case-case-control study was performed to determine factors associated with PDRPA and PDRAB acquisition. Data were collected retrospectively from medical records.

**Results:** During the study period, 43 isolates of PDRPA and 30 isolates of PDRAB were collected from 8 hospitals and were analyzed for carbapenem resistance mechanism. Thirty three patients with

PDRPA and 26 with PDRAB were studied for risk factor analysis. For P. aeruginosa, mechanical ventilation (OR, 6.8; 95% CI, 1.1-42; *P*=.039) and APACHE II score (OR, 1.13; 95% CI, 1.02-1.23; *P*=.019) were identified as independent risk factors for PDRPA acquisition. Mechanical ventilation (OR, 18.8; 95% CI, 3.6-99.5; P=.008) and urinary catheter usage (OR, 3.8; 95% CI, 1.01-14.6; P=.048) were identified as risk factors for imipenem-resistant P. aeruginosa acquisition, whereas mechanical ventilation (OR, 5.3; 95% CI, 1.1-24.4; P=.034) and associated pulmonary disease (OR, 4.6; 95% CI, 1.2-17.4; P=.026) for imipenem-susceptible P. aeruginosa acquisition. For A. baumannii, mechanical ventilation (OR, 11.5; 95% CI, 1.7-76.7; P=.012) and time at risk (OR, 1.04; 95% CI, 1-1.07; P=.049) were identified as independent risk factors for PDRAB acquisition. Mechanical ventilation (OR, 22.8; 95% CI, 2.4-218; P=.007) and defined daily dose of 3rd generation cephalosporin (OR, 1.2; 95% CI, 1.02-1.4; P=.028) were identified as risk factors for imipenem-resistant A. baumannii acquisition, whereas APACHE II score (OR, 1.2; 95% CI, 1.03-1.4; P=.022) and associated pulmonary disease (OR, 6; 95% CI, 1.2-30.9; P=.031) for imipenem-susceptible A. baumannii acquisition. Pulsed-field gel electrophoresis (PFGE) of PDR GNFB showed that

clonal epidemic GNFB isolates coexisted with sporadic isolates. Among 43 isolates of PDRPA, 8 isolates (19%) were shown to produce MBL; 4 were VIM-2 producers and 4 were IMP-6 producers. Of the 30 isolates of PDRAB, no MBL producer was detected. All of the PDRAB isolates contained the OXA-51 gene and IS*Aba1* was detected upstream of the OXA-51 gene in 7 (33%) isolates, among which no OXA-23 gene was found. The IS*Aba1* was detected upstream of the OXA-23 gene in 23 (77%) PDRAB isolates.

**Conclusion:** This study supports a major role for mechanical ventilation on the acquisition of PDR GNFB. Moreover, PFGE showed clonal epidemic within hospitals. Taken together, these results suggest that patient to patient transmission contributes the acquisition of PDR GNFB in Korea. Carbapenem resistance of PDRPA is mainly due to a non-carbapenemase mechanism and 19% of PDRPA produce MBL. Among PDRAB, 77% of isolates contain OXA-23 genes and 23% contain IS*Aba1*-activated OXA-51 genes. Coexistence of OXA-23 gene and IS*Aba1*-activated OXA-51 gene is not detected.

**Key words**: Pandrug resistance, glucose non-fermenting Gramnegative bacilli, risk factor, carbapenem, resistance mechanism

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#### I. INTRODUCTION

*Pseudomonas* spp. and *Acinetobacter* spp. belong to glucose nonfermenting Gram-negative bacilli (GNFB) and are important nosocomial pathogens. *Pseudomonas aeruginosa* is commonly found in soil, water and plants, and can on occasion be associated with the colonization of otherwise healthy humans and animals. *Acinetobacter* spp. is ubiquitous in nature<sup>1</sup>. GNFB are not only intrinsically resistant to several antimicrobial agents but also often acquire mechanisms of resistance to other antibiotics. Carbapenems are potent agents for treating infections caused by Gram-negative bacteria, because of their broad spectrum of activity and stability to hydrolysis by most β-lactamases including the extended-spectrum β-lactamases<sup>2</sup>. National Nosocomial Infections Surveillance System regarding intensive care unit (ICU) patients across the USA showed a significantly increasing trend of resistance to imipenem for *P. aeruginosa* and *Acinetobacter baumannii* over the study period of 1987-2003<sup>3</sup>. In Korea, increasing trends for imipenem resistance were also observed in *P. aeruginosa* and *Acinetobacter* spp. over the study period of 1997-2004<sup>4</sup>.

Since the multidrug-resistant (MDR) *P. aeruginosa* strains were first reported in patients with cystic fibrosis<sup>5</sup>, there have been numerous reports for MDR GNFB. These MDR GNFB strains may be transmitted from patient to patient and sometimes lead to outbreaks among patients attending the same clinic<sup>6, 7</sup>. Infections with MDR GNFB are associated with severe outcomes, including increased mortality and morbidity<sup>8</sup>. Recently, pandrug-resistant (PDR) GNFB, defined as being resistant to all available anti-pseudomonal antibiotics, were reported and the emergence of these organisms has severely threatened therapeutic choices<sup>9, 10</sup>. Because of limited therapeutic options and increased mortality, prevention for acquisition of PDR GNFB is essential. However, risk factors for the acquisition of PDR GNFB are not yet well documented.

Resistance mechanisms that are expressed frequently in GNFB include β-

lactamases, alterations in cell-wall channels (porins), and efflux pumps. Clinically most troubling thing has been GNFB's acquired β-lactamases, including serine and metallo-β-lactamases (MBLs), which then confer a resistance to carbapenems<sup>11</sup>. The production of these MBLs by *P. aeruginosa* can lead to a resistance to imipenem and meropenem plus the antipseudomonal cephalosporins, including cefepime, and antipseudomonal penicillins<sup>12</sup>. IMP-1 was the first MBL identified in *P. aeruginosa* and first countrywide spread in Gram-negative bacilli was reported in Japan<sup>13-15</sup>. VIM-1 and VIM-2 were identified in Europe<sup>16, 17</sup>. In Korea, 9% of imipenemresistant *P. aeruginosa* isolated between 1995 and 1999 in a tertiary care hospital had VIM-2 MBL<sup>18</sup>. In PDR GNFB, however, carbapenem resistance mechanisms are not described.

The objectives of this study were to clarify the risk factors for the acquisition of PDR GNFB by Korean hospitals and to document the resistance mechanisms to carbapenems.

#### **II. MATERIALS AND METHODS**

#### 1. Bacterial strains and antimicrobial susceptibility testing

From June to November, 2007, isolates of PDR *P. aeruginosa* and *A. baumannii* (PDRPA and PDRAB) were collected from clinical specimens from patients in 8 tertiary care hospitals in Seoul, Gyeonggi, Kangwon and Busan (Severance Hospital of Yonsei University College of Medicine, Seoul; Kangnam Sacred Heart Hospital of Hallym University Medical Center, Seoul; Korea University Anam Hospital, Seoul; Myongji Hospital of Kwandong University College of Medicine, Koyang; Bundang Cha Hospital of Pochon CHA University College of Medicine, Seongnam; Soonchunhyang University Hospital, Bucheon; Yonsei University Wonju Christian Hospital, Wonju; Kosin University Gospel Hospital, Busan). Among *P. aeruginosa* and *A. baumannii*, the isolates which showed resistant or intermediate sensitivity to all tested antimicrobials (Table 1) were considered as PDR GNFB and included for further study. The MICs of antimicrobial agents were determined by an agar dilution method<sup>19</sup>.

Class	P. aeruginosa	A. baumannii
Penicillin	Piperacillin Piperacillin	
Cephalosporin	Ceftazidime, Cefepime, Ceftazidime, Cefotaxime, Ce	
Monobactam	Aztreonam	Aztreonam
Carbapenem	Imipenem, Meropenem	Imipenem, Meropenem
R Lastom/R lastomass inhibitor combination	Diporcoillin tozohootom	Piperacillin-tazobactam
p-Lactani/p-ractamase minoritor comomation	riperaciinii-tazooactaini	Ampicillin-sulbactam
Aminoglycoside	Amikacin, Gentamicin,	Amikacin, Gentamicin,
Fluoroquinolone	Ciprofloxacin	Ciprofloxacin
Others	NA	Trimethoprim-sulfamethoxazole

**Table 1.** Tested antimicrobials for the definition of pandrug-resistant *P. aeruginosa* and *A. baumannii* 

NA, not applicable

#### 2. Modified Hodge test for the screening of carbapenemases

The surface of a Mueller-Hinton agar plate was inoculated evenly using a cotton swab with an overnight culture suspension of *Escherichia coli* ATCC 25922, which was adjusted to one-tenth of the McFarland no. 0.5. After brief drying, an imipenem disk was placed at the center of the plate, and isolates from the overnight culture plates were streaked heavily. The presence of a distorted inhibition zone after overnight incubation was interpreted as Hodge test positive<sup>20</sup>.

#### 3. EDTA-imipenem disk synergy test

An overnight culture of the test strain was suspended to the turbidity of a McFarland no. 0.5 tube and used to swab inoculate a Mueller-Hinton agar plate. After drying, a 10  $\mu$ g imipenem disk (BBL, Cockeysville, MD, USA) and a blank filter paper disk were placed 10 mm apart from edge to edge, and 10  $\mu$ g of 0.5 M EDTA was applied to the blank disk. After overnight incubation, the presence of an enlarged zone of inhibition was interpreted as EDTA-imipenem disk synergy test positive<sup>20</sup>.

#### 4. PCR amplification and sequencing

#### A. MBL gene detection and sequencing in P. aeruginosa

PCR detection of the  $bla_{IMP}$  and  $bla_{VIM}$ -like alleles was carried out using the IMP1 and VIM2 primers<sup>21</sup> (Table 2). PCR was performed with 1 µl of heat-extracted DNA template, 20 pmol of each primer, and PreMix (Bioneer, Cheongwon, Korea) containing 1 U of *Taq* DNA polymerase in a total volume of 20 µl. The amplification conditions were initial denaturation at 94°C for 5 min, 25 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, and finally, 72°C for 7 min.

To determine the type of MBL genes, isolates with  $bla_{IMP}$ - and  $bla_{VIM}$ -like

alleles were used for sequencing. The primers of INT1-F, INT2-R, IMP1-F, IMP1-R, VIM2-F, and VIM2-R were used for  $bla_{IMP}$  and  $bla_{VIM}$ -like alleles sequencing. The reactions were carried out in a total volume of 50 µl, with 5 µl of heat-extracted template DNA, 20 pmol of each primer, and 3 U of LA *Taq* (Takara, Shiga, Japan). The cycling conditions were as follows: 94°C for 12 min and then 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 5 min. The extension time was increased by 5 s for each cycle. The PCR products were subjected to direct sequencing. Both strands of the PCR products were sequenced twice with an automatic sequencer (model 3730*xl*; Applied Biosystems, Weiterstadt, Germany). Sequence analysis and comparisons were performed using programs available at the NCBI (http://www.ncbi.nlm.nih.gov/).

#### B. OXA gene and insertion sequence detection in A. baumannii

Detection of the four groups of OXA-carbapenemase genes ( $bla_{OXA-23}$ ,  $bla_{OXA-24}$ ,  $bla_{OXA-51}$  and  $bla_{OXA-58}$ ) was carried out using a multiplex assay<sup>22</sup> (Table 2). Templates for the PCR amplification in the clinical isolates were made of a whole lysate. The amplification conditions were, initial denaturation at 94°C for 5 min and then 30 cycles of 94°C for 25 s, 52°C for 40 s and 72°C for 50 s, and finally, 72°C for 6 min.

The insertion sequence ISAba1 upstream of the  $bla_{OXA-23}$  and the  $bla_{OXA-51}$  genes were sought using combinations of the ISAba1F primer and the OXA23-R/OXA51-R primer. The amplification conditions for the ISAba1F/OXA23-R were, initial denaturation at 95°C for 5 min and then 35 cycles of 95°C for 45 s, 56°C for 45 s and 72°C for 3 min, and finally, 72°C for 5 min. Same conditions were used for the ISAba1F/OXA51-R, except that an annealing temperature of 58°C.

Primer	Sequence (5' to 3')	Target
IMP1-F	CAT GGT TTG GTG GTT CTT GT	bla <sub>IMP-1</sub>
IMP1-R	ATA ATT TGG CGG ACT TTG GC	
VIM2-F	ATG TTC AAA CTT TTG AGT AAG	bla <sub>VIM-2</sub>
VIM2-R	CTA CTC AAC GAC TGA GCG	
INT1-F	GGC ATC CAA GCA GCA AG	
INT2-R	AAG CAG ACT TGA CCT GA	
ISAba1F	CAC GAA TGC AGA AGT TG	ISAba1
ISAba1R	CGA CGA ATA CTA TGA CAC	
OXA23-F	GAT CGG ATT GGA GAA CCA GA	bla <sub>OXA-23</sub>
OXA23-R	ATT TCT GAC CGC ATT TCC AT	
OXA24-F	GGT TAG TTG GCC CCC TTA AA	bla <sub>OXA-24</sub>
OXA24-R	AGT TGA GCG AAA AGG GGA TT	
OXA51-F	TAA TGC TTT GAT CGG CCT TG	bla <sub>OXA-51</sub>
OXA51-R	TGG ATT GCA CTT CAT CTT GG	
OXA58-F	AAG TAT TGG GGC TTG TGC TG	bla <sub>OXA-58</sub>
OXA58-R	CCC CTC TGC GCT CTA CAT AC	

Table 2.	Primers use	ed for dete	ction of car	bapenemases
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#### 5. Pulsed-field gel electrophoresis

For pulsed-field gel electrophoresis (PFGE) analysis, *Xba* I-digested and *Sma* I-digested genomic DNA of PDR *P. aeruginosa* and *A. baumannii*, respectively, were prepared according to the manufacturer's instruction (Bio-Rad, Hercules, CA, USA) and fragments were separated for 20 h at 6 V/cm at 11°C using a CHEF-DR II System (Bio-Rad, Hercules, CA, USA), with initial and final pulse times of 0.5 and 30 s, respectively<sup>18</sup>. The pattern was analyzed visually and with Fingerprinting II software (Bio-Rad, Hercules, CA, USA).

#### 6. Case-case-control study

A case-case-control study design was used. Three case-control studies were performed to determine factors associated with the PDRPA and the PDRAB acquisition, respectively. The first group of case patients included adult patients who had nosocomial isolation of PDR GNFB in clinical cultures. The second and the third group included adult patients who had nosocomial isolation of imipenem-resistant and imipenem-susceptible GNFB in clinical cultures, respectively. The second and the third group of case patients were randomly selected in the same hospital as the corresponding first case patient during the study period.

The microbiology laboratory database was electronically searched to

identify all GNFB–positive clinical cultures of samples obtained from patients admitted to the hospital during the study period. Patients who had GNFB isolates and recovered within 48 h of admission were excluded from the study. Patients in the control group were selected from among patients receiving care from the same hospital from which case patients were receiving care during the study period. Patients in the control group did not have GNFB isolated during their hospital stay. For each case patient with PDR GNFB who was selected, 2 control patients were randomly chosen. Patients who were admitted for <48 h were excluded from the control group.

Data were collected retrospectively from medical records. Variables analyzed as possible risk factors included age, sex, associated diseases or comorbid conditions, Charlson score<sup>23</sup>, ICU stay, surgery, length of hospital stay before outcome of interest (time at risk; for case patients, from admission to nosocomial isolation of GNFB; for controls, complete length of hospital stay), and severity of illness, as calculated by the Acute Physiology and Chronic Health Evaluation (APACHE) II score. The presence of a central venous catheter/arterial catheter, urinary catheter, mechanical ventilation, and naso-gastric tube was documented. Finally, all antimicrobial therapy administered before outcome of interest was ascertained.

For the measurement of antimicrobial use, the defined daily dose (DDD)

was used. Total DDD of the antimicrobial agent was calculated by dividing the total grams of the antimicrobial agent used in a hospital area by the number of grams in an average daily dose of the agent given to an adult patient (DDD). DDDs were adapted from: WHO Collaborating Centre for Drug Statistics Methodology. Anatomical Therapeutic Chemical (ATC) classification index with defined daily doses (DDD). 2007. Available from: http://www.whocc.no/atcddd/.

#### 7. Statistical analysis

All statistical analyses were performed using SPSS software, version 15 (SPSS, Chicago, IL, USA). Bivariate analyses were performed separately for each of the variables. ORs and 95% CIs were calculated for binomial variables. P values were calculated by use of Fisher's exact test, for categorical variables; and by the Student's t test, for continuous variables. Variables for which the P value was <.05 in bivariate analysis were included in a logistic regression model for multivariable analysis. A forward selection process was used. Risk factors were checked for collinearity by viewing changes in standard errors of multivariate models. All tests were 2-tailed, and a P value of <.05 was considered significant in the multivariable model.

#### **III. RESULTS**

During the study period, 43 isolates of PDRPA and 30 isolates of PDRAB were collected from 8 hospitals and were analyzed for the carbapenem resistance mechanism. Of these collected PDR GNFB isolates, 37 of *P. aeruginosa* and 27 of *A. baumannii* had the full medical record available for review. Four patients with PDRPA and one patient with PDRAB had cultures positive within the first 2 days after admission and were excluded from risk factor analysis. Finally, 33 patients with PDRPA and 26 with PDRAB were studied for risk factor analysis.

#### 1. Risk factor analysis for PDR P. aeruginosa acquisition

Of 33 patients with PDRPA acquisition, 26 were men and 7 were women, with an age of 65  $\pm$  17 years. The most common site from which PDRPA, imipenem-resistant and imipenem-susceptible *P. aeruginosa* (IRPA and ISPA) were recovered was the respiratory system (13 [39%], 19 [58%] and 21 patients [64%], respectively). The second most common site was the urinary system (12 [36%], 9 [24%] and 5 patients [15%], respectively). The hospital ward from which patients with PDRPA, IRPA and ISPA were receiving care on the date that a positive culture result was obtained included the general ward (16 [49%], 17 [51%] and 23 [70%], respectively), and ICU (17 [51%], 16 [49%] and 10 [30%], respectively).

Results of bivariate analysis for baseline demographic and clinical characteristics, associated diseases or comorbid conditions, Charlson score, ICU stay, surgery, time at risk, severity of illness, as calculated by the APACHE II score, and the receipt of specific antibiotics are presented in table 3.

Results of the multivariate analysis are presented in table 4. In the final model, mechanical ventilation (OR, 6.8; 95% CI, 1.1-42; P=.039) and APACHE II score (OR, 1.13; 95% CI, 1.02-1.23; P=.019) were identified as independent risk factors for PDRPA acquisition. Mechanical ventilation (OR, 18.8; 95% CI, 3.6-99.5; P=.008) and urinary catheter usage (OR, 3.8; 95% CI, 1.01-14.6; P=.048) were identified as risk factors for IRPA acquisition, whereas mechanical ventilation (OR, 5.3; 95% CI, 1.1-24.4; P=.034) and associated pulmonary disease (OR, 4.6; 95% CI, 1.2-17.4; P=.026) for ISPA acquisition.

	PDRPA	IRPA	ISPA	Control patients
Risk factor	(n = 33)	(n = 33)	(n = 33)	(n = 66)
Age, years	$65\pm12^{\#}$	$59\pm17$	$62\pm13$	$56\pm19$
Male sex	26 (79)#	19 (58)	25 (76)#	30 (46)
Associated disease				
Diabetes mellitus	11 (33)	9 (27)	5 (15)	15 (23)
Cardiac disease	7 (21)	4 (12)	13 (39)	17 (26)
Pulmonary disease	13 (39) <sup>§</sup>	13 (39) §	14 (42) <sup>§</sup>	5 (8)
Renal disease	6 (18)	4 (12)	5 (15)	3 (5)
Neurological disease	14 (42)#	11 (33)*	6 (32)	7 (11)
Malignancy	11 (33)	8 (24)	7 (21)	22 (33)
Charlson comorbidity scale	$3.4\pm2.6^{\#}$	$2.6\pm2.2$	$2.4 \pm 2$	$2\pm2.3$
Related to hospitalization				
Time at risk, days	$51.7 \pm 64.4^{\#}$	$31.2\pm27.5*$	$19.5\pm24.1$	$17.2\pm20.1$
Intensive care unit stay, days	$20.2\pm30.1^{\#}$	$15.7 \pm 22.8^{\#}$	$9.5\pm25.6$	$2.2\pm7.3$
Surgery	14 (42)	8 (24)	9 (27)	20 (31)
Device				
Central venous/arterial catheter	19 (58) <sup>§</sup>	19 (58) <sup>§</sup>	11 (33)	12 (18)
Urinary catheter	24 (73) <sup>§</sup>	25 (76) <sup>§</sup>	13 (39)	19 (29)
Mechanical ventilation	15 (46) <sup>§</sup>	18 (55) <sup>§</sup>	11 (33) <sup>§</sup>	3 (4.5)
Naso-gastric tube	16 (49) <sup>§</sup>	17 (52) §	12 (36) #	7 (11)
Antimicrobial, DDD				
Cephalosporin				
First generation	$0.6 \pm 2.7$	$0.6 \pm 2.1$	$0.1\pm0.7$	$0.9\pm4.8$
Second generation	$4.1\pm13.0$	$1.1 \pm 2.7$	$0.5 \pm 1.9$	$1.2\pm4.6$
Third generation	$12.6\pm23.8$	$7.4\pm8.7$	$5.0 \pm 11.1$	$4.3\pm 6.4$
Penicillin with β-lactamase inhibitor	$7.6 \pm 18.3^{*}$	$2.1\pm5.9$	$3.2\pm8.5$	$0.8 \pm 4.1$

## Table 3. Bivariate analysis of risk factors for the acquisition of Pseudomonas

aeruginosa

Quinolone	$13.9\pm26.5^{\#}$	$5.6\pm12.7$	$2.3\pm7.0$	$1.1\pm3.6$
Aminoglycoside	$4.5\pm9.2$	$2.3\pm5.9$	$2.8 \pm 10.1$	$3.3\pm10.5$
Carbapenem	$2.6\pm5.7$	$4.1\pm8.0$	$2.1\pm8.7$	$1.3\pm8.4$
Glycopeptide	$7.3\pm19.6$	$3.3\pm5.8$	$2.5 \pm 5.4$	$1.1 \pm 4.3$
Septic shock/severe sepsis	7 (21)*	6 (18)	5 (15)	3 (5)
APACHE II score	$14.5\pm7.2^{\$}$	$12.3\pm7.5^{\$}$	$11.2\pm8.4^{\#}$	$6.8\pm 6$

Data are no. (%) of patients or mean  $\pm$  standard deviation. \* p<0.05 vs. control group; <sup>#</sup> p<0.01 vs. control group; <sup>§</sup> p<0.001 vs. control group. PDRPA, pandrug-resistant *P. aeruginosa*; IRPA, imipenem-resistant *P. aeruginosa*; ISPA, imipenem-susceptible *P. aeruginosa*; DDD, defined daily dose; APACHE, acute physiology and chronic health evaluation.

Disk footor	Unadjusted OR	Adjusted OR	Dualua
RISK factor	(95% CI)	(95% CI)	P value
PDRPA			
Mechanical ventilation	17.5 (4.6-67.2)	6.8 (1.1-42.0)	.039
APACHE II score		1.13 (1.02-1.23) <sup>a</sup>	.019
IRPA			
Mechanical ventilation	25.2 (6.6-96.8)	18.8 (3.6-99.5)	.008
Urinary catheter	7.7 (3.0-20.1)	3.8 (1.01-14.6)	.048
ISPA			
Mechanical ventilation	10.5 (2.7-41.1)	5.3 (1.1-24.4)	.034
Associated pulmonary disease	9.0 (2.9-28.2)	4.6 (1.2-17.4)	.026

# Table 4. Multivariate analysis of risk factors for the acquisition of Pseudomonas aeruginosa

OR, odds ratio; CI, confidence interval; PDRPA, pandrug-resistant *P. aeruginosa*; IRPA, imipenem-resistant *P. aeruginosa*; ISPA, imipenem-susceptible *P. aeruginosa*; APACHE, acute physiology and chronic health evaluation.

#### 2. Risk factor analysis for PDR A. baumannii acquisition

Of these 26 patients with PDRAB acquisition, 20 were men and 6 were women, with an age of  $63 \pm 14$  years. PDRAB, imipenem-resistant and imipenem-susceptible *A. baumannii* (IRAB and ISAB) were most frequently recovered from the respiratory system (19 [73%], 16 [70%] and 16 patients [73%], respectively).

Results of bivariate analysis for baseline demographic and clinical characteristics, associated diseases or comorbid conditions, Charlson score, ICU stay, surgery, time at risk, severity of illness, as calculated by the APACHE II score, and the receipt of specific antibiotics are presented in table 5.

Results of the multivariate analysis are presented in table 6. In the final model, mechanical ventilation (OR, 11.5; 95% CI, 1.7-76.7; P=.012) and time at risk (OR, 1.04; 95% CI, 1-1.07; P=.049) were identified as independent risk factors for PDRAB acquisition. Mechanical ventilation (OR, 22.8; 95% CI, 2.4-218; P=.007) and DDD of 3rd generation cephalosporin (OR, 1.2; 95% CI, 1.02-1.4; P=.028) were identified as risk factors for IRAB acquisition, whereas APACHE II score (OR, 1.2; 95% CI, 1.03-1.4; P=.022) and associated pulmonary disease (OR, 6; 95% CI, 1.2-30.9; P=.031) for ISPA acquisition.

	PDRAB	IRAB	ISAB	Control patients
Risk factor	(n = 26)	(n = 23)	(n = 22)	(n = 52)
Age, years	$63\pm14^{\#}$	$67\pm13^{\$}$	$63\pm15^{\#}$	51 ± 18
Male sex	20 (77) <sup>#</sup>	14 (61)	19 (86) <sup>§</sup>	23 (44)
Associated disease				
Diabetes mellitus	5 (19)	6 (26)	3. (17)	11 (21)
Cardiac disease	12 (46)	11 (48)	9 (41)	12 (23)
Pulmonary disease	9 (35)*	17 (23) <sup>§</sup>	9 (41) <sup>#</sup>	5 (10)
Renal disease	3 (12)	2 (9)	1 (6)	4 (8)
Neurological disease	9 (35) <sup>#</sup>	6 (26)	8 (36) <sup>#</sup>	4 (8)
Malignancy	4 (15)	7 (30)	3 (14)	11 (21)
Charlson comorbidity scale	$2.3\pm2.1$	$3.3\pm2.6^{\#}$	$2.1 \pm 1.8$	$1.6\pm1.9$
Related to hospitalization				
Time at risk, days	$32.4\pm33.2^{\$}$	$21.7\pm21.9*$	$21\pm26.1$	11.3 ± 14.5
Intensive care unit stay, days	$28\pm29^{\$}$	$17.8\pm25.8^{\#}$	$14.4\pm8.1*$	$0.13\pm0.6$
Surgery	10 (39)*	3 (13)*	6 (27)	21 (40)
Device				
Central venous/arterial catheter	8 (31)*	7 (30)*	5 (23)	4 (8)
Urinary catheter	15 (58) <sup>§</sup>	10 (44)#	9 (41) <sup>#</sup>	5 (10)
Mechanical ventilation	12 (46) <sup>§</sup>	14 (61) <sup>§</sup>	5 (23)*	2 (3.8)
Naso-gastric tube	15 (58) <sup>§</sup>	10 (44) <sup>§</sup>	8 (6)#	3 (6)
Antimicrobial, DDD				
Cephalosporin				
First generation	$0.1\pm0.3$	0	$0.9 \pm 3.3$	$0.3 \pm 1.1$
Second generation	$0.3\pm1.2$	$0.5 \pm 1.4$	$0.7\pm2.4$	$1.1\pm2.9$
Third generation	$9.3 \pm 13.1 *$	$7.6\pm8.6^{\#}$	$5.1\pm8.0$	$2.3 \pm 3.7$
Penicillin with $\beta$ -lactamase inhibitor	$4.3 \pm 16.8$	$4.4 \pm 13.9$	$0.2\pm0.5$	0

Table 5. Bivariate analysis of risk factors for the acquisition of Acinetobacter

baumannii

Quinolone	$3.0\pm5.8$	$3.4\pm6.4$	$1.7\pm4.3$	$0.9\pm2.2$
Aminoglycoside	$5.8\pm7.4$	$3.9\pm 6.2$	$5.1\pm15.2$	$3.3 \pm 11.1$
Carbapenem	$4.0\pm9.4*$	$2.1\pm5.5$	$1.7\pm5.4$	0
Glycopeptide	$5.9 \pm 13.4$	$3.5\pm5.8^{\ast}$	$3.4 \pm 12$	$0.6 \pm 3$
Septic shock/severe sepsis	7 (27) <sup>§</sup>	6 (26)#	2 (9)	0
APACHE II score	$14.2\pm8.7^{\$}$	$15.8\pm8.3^{\$}$	$10.1\pm5.4^{\$}$	$4.4 \pm 4$

Data are no. (%) of patients or mean  $\pm$  standard deviation. \* p<0.05 vs. control group; <sup>#</sup> p<0.01 vs. control group; <sup>§</sup> p<0.001 vs. control group. PDRAB, pandrug-resistant *A. baumannii*; IRAB, imipenem-resistant *A. baumannii*; ISAB, imipenem-susceptible *A. baumannii*; DDD, defined daily dose; APACHE, acute physiology and chronic health evaluation.

Disk factor	Unadjusted OR	Adjusted OR	Dualua
KISK Tactor	(95% CI)	(95% CI)	P value
PDRAB			
Mechanical ventilation	21.4 (4.3-107)	11.5 (1.7-76.7)	.012
Time at risk (days)		1.04 (1-1.07) <sup>a</sup>	.049
IRAB			
Mechanical ventilation	38.9 (7.5-201)	22.8 (2.4-218)	.007
Third generation cephalosporin (DDD)		1.2 (1.02-1.4) <sup>a</sup>	.028
ISAB			
APACHE II score		1.2 (1.03-1.4) <sup>a</sup>	.022
Associated pulmonary disease	6.5 (1.9-22.8)	6.0 (1.2-30.9)	.031

# Table 6. Multivariate analysis of risk factors for the acquisition of Acinetobacter baumannii

OR, odds ratio; CI, confidence interval; PDRAB, pandrug-resistant *A. baumannii*; IRAB, imipenem-resistant *A. baumannii*; ISAB, imipenem-susceptible *A. baumannii*; DDD, defined daily dose; APACHE, acute physiology and chronic health evaluation.

#### 3. PFGE profile of PDR P. aeruginosa and A. baumannii

PFGE of *Xba* I-digested and *Sma* I-digested genomic DNA of PDRPA and PDRAB revealed that clonal epidemic PDR GNFB isolates coexisted with sporadic isolates (Figure 1, 2). Among PDRPA isolates from the same hospital, PFGE patterns were genetically related (Dice coefficient > 80%); 3 of 4 isolates from hospital HA, 3 of 6 isolates from hospital HE, 5 of 6 isolates from hospital HH. For the PDRAB isolates, all 9 of the isolates from hospital HH were genetically related, whereas 5 of 9 from hospital HD were genetically related.



**Figure 1.** Pulsed-field gel electrophoresis dendrogram of pandrug-resistant *Pseudomonas aeruginosa.* Ten of 12 isolates from hospital HF were available for analysis.



**Figure 2.** Pulsed-field gel electrophoresis dendrogram of pandrug-resistant *Acinetobacter baumannii*.

#### 4. Antimicrobial susceptibility of PDR P. aeruginosa and A. baumannii

All PDRPA and PDRAB showed intermediate or resistant to all tested antimicrobials according to the definition of PDR GNFB. MIC ranges of imipenem for PDRPA and PDRAB were 8 to 128 and 8 to 32  $\mu$ g/ml, respectively (Table 7). MICs of all of the other  $\beta$ -lactams for the isolates were  $\geq$ 16  $\mu$ g/ml. MIC ranges of colistin for PDRPA and PDRAB were 0.5 to 2 and 0.5 to 1  $\mu$ g/ml, respectively, and all isolates were susceptible to colistin. For tigecycline, PDRAB MIC range, MIC<sub>50</sub> and MIC<sub>90</sub> were 1 to 8, 2 and 4  $\mu$ g/ml, respectively.

 Table 7. Antimicrobial susceptibility of pandrug-resistant Pseudomonas

 aeruginosa and Acinetobacter baumannii

	P. aerugi	P. aeruginosa (n = 43)				A. baumannii (n = 30)		
Antimicrobial agent	MIC range*	MIC <sub>50</sub> *	MIC <sub>90</sub> *	MIC range*	MIC <sub>50</sub> *	MIC <sub>90</sub> *		
Piperacillin	128->256	256	>256	>256	>256	>256		
Piperacillin/tazobactam	128->256	256	>256	>128	>128	>128		
Ampicillin/sulbactam	NT	NT	NT	32->128	64	64		
Cefotaxime	NT	NT	NT	>128	>128	>128		
Ceftazidime	16->128	64	>128	128->128	>128	>128		
Cefepime	32->128	>128	>128	32->128	>128	>128		
Aztreonam	16->128	128	>128	NT	NT	NT		
Imipenem	8->128	16	64	8-32	32	32		
Meropenem	8->128	32	>128	16-64	32	32		
Amikacin	64->128	>128	>128	>128	>128	>128		
Gentamicin	32->128	>128	>128	>128	>128	>128		
Ciprofloxacin	8->128	32	64	32-128	64	128		
Cotrimoxazole	NT	NT	NT	16->128	>128	>128		
Colistin	0.5-2	1	2	0.5-1	0.5	1		
Tigecycline	NT	NT	NT	1-8	2	4		

\* Values are in  $\mu$ g/ml; MIC<sub>50</sub> and MIC<sub>90</sub>, MICs for 50% and 90% of strains, respectively. MIC,

minimum inhibitory concentration; NT, not tested.

# 5. Carbapenem resistance mechanism of PDR *P. aeruginosa* and *A. baumannii*

Of the 43 isolates of PDRPA, 11 were positive for the modified Hodge test. Among them, the 8 isolates (19%) were shown to produce MBL by the EDTA-imipenem disk synergy test and PCR; 4 were VIM-2 producers and 4 were IMP-6 producers by PCR and sequencing analysis. MBL producers were detected from 4 hospitals (Table 8).

Table8.Carbapenemresistancemechanismofpandrug-resistantPseudomonas aeruginosa

Hospital	No. of Hodge test		EDTA-imipenem disk	MBL production (%)	
	isolates	positive (%)	synergy test positive (%)	VIM-2	IMP-6
HA	4	1	1	1	0
HB	1	1	0	0	0
НС	1	0	0	0	0
HD	1	0	0	0	0
HE	6	1	1	0	1
HF	12	7	5	2	3
HG	12	1	1	1	0
HH	6	0	0	0	0
Total	43	11 (26)	8 (19)	4 (9)	4 (9)

MBL; Metallo-β-lactamase

Of the 30 isolates of PDRAB, 15 were positive for the modified Hodge test. However, no MBL producer was detected by the EDTA-imipenem disk synergy test and no further PCR assay for MBL was performed. All of the 30 PDRAB isolates contained the OXA-51 gene and IS*Aba1* was detected upstream of the OXA-51 gene in 7 (33%) isolates, among which no OXA-23 gene was found. The OXA-23 gene was detected in 23 (77%) PDRAB isolates, and IS*Aba1* was detected upstream of the OXA-23 gene in all of the PDRAB isolates (Table 9). Coexistence of OXA-23 gene and IS*Aba1*activated OXA-51 gene was not detected. Genes encoding the OXA-24 and OXA-58 carbapenemases were not detected.

Hospital	No. of isolates	Hodge test positive (%)	EDTA-imipenem disk synergy test positive	OXA-23 positive (%)	ISF/OXA23R <sup>*</sup> (%)	ISF/OXA51R <sup>*</sup> (%)
HA	2	2	0	2	2	0
HB	0	0	0	0	0	0
HC	3	0	0	0	0	3
HD	9	9	0	9	9	0
HE	2	2	0	2	2	0
HF	2	0	0	0	0	2
HG	3	2	0	1	1	2
HH	9	0	0	9	9	0
Total	30	15 (50)	0	23 (77)	23 (77)	7 (33)

**Table 9.** Carbapenem resistance mechanism of pandrug-resistantAcinetobacter baumannii

\* Results of PCRs carried out using ISAba1 forward primer (ISAba1F) and OXA-23 gene

(OXA23-R) or OXA-51 gene (OXA51-R) reverse primers

#### **IV. DISCUSSION**

There is an association between the development of antimicrobial resistance in *Staphylococcus aureus*, enterococci, and Gram-negative bacilli and increases in mortality, morbidity, length of hospitalization, and cost of health care<sup>24</sup> and infections caused by MDR Gram-negative bacilli have become a growing problem<sup>25</sup>. *P. aeruginosa* and *Acinetobacter* spp. are important nosocomial pathogens and specifically addressed as categories as MDR Gram-negative bacilli by a recent report of the Infectious Diseases Society of America<sup>26</sup>. Unfortunately, and contrary to what happened with Gram-positive bacteria, no antibiotic, with the exception of tigecycline, from a new class has been developed specifically for MDR Gram-negative bacilli. Moreover, there are now a growing number of reports of cases of infections caused by Gram-negative organisms for which no adequate therapeutic options exist<sup>9</sup>. Thus, strategies to prevent the nosocomial emergence and spread of MDR Gram-negative bacilli are essential.

Elucidation of the risk factors associated with the acquisition of antimicrobial resistant *P. aeruginosa* and *A. baumannii* has been an area of active research and there have been numerous reports. The length of hospital stay<sup>27</sup>, prior admission history<sup>28</sup>, hemodialysis<sup>28</sup>, prior antimicrobial usage<sup>28-31</sup>,

invasive therapeutic intervention<sup>28, 30</sup> were all known as risk factors for the acquisition of IRPA and the length of hospital stay<sup>32</sup>, ICU admission<sup>32</sup>, prior antimicrobial usage<sup>32-35</sup>, and invasive therapeutic intervention<sup>33, 34</sup> were for IRAB.

The analysis of 17 studies on MDR P. aeruginosa using multivariate analysis revealed that prior use of antibiotics was a very important risk factor in almost all of them (15/17 studies); carbapenems (6 studies) and fluoroquinolones (6 studies) were most commonly implicated, followed by 3rd generation cephalosporins (3 studies) and broad-spectrum beta-lactam antimicrobials (3 studies). Other independent risk factors were mechanical ventilation (5 studies), length of ICU and hospital stay (6 studies), comorbidities (diabetes mellitus, chronic obstructive pulmonary disease), etc<sup>36</sup>. Analysis of 20 studies on MDR A. baumannii using multivariate analysis revealed that prior antibiotic use was the most common risk factor for the acquisition of MDR A. baumannii, described in more than half of these studies (11/20 studies). Carbapenems and 3rd generation cephalosporins were the most commonly implicated antibiotics (4/11 studies for both), followed by fluoroquinolones, aminoglycosides and metronidazole (each implicated in one study). The second most commonly arising risk factor in case-control studies was mechanical ventilation, described in 25% of the studies (5/20 studies).

Other risk factors were: stay in an ICU and the length of ICU and hospital stay (3/20 studies), the severity of illness (expressed by the APACHE score) (2/20 studies), gender, certain therapeutic interventions such as tracheostomy (2 studies), hydrotherapy (2 studies), transfusions (one study), placement of arterial and central venous catheters, Foley catheters, etc<sup>36</sup>. These analyses for risk factors of MDR GNFB have some limitations due to heterogeneity of MDR definition and control group selection among studies. For PDR GNFB, a few descriptive studies were reported<sup>9, 10</sup>. However, the risk factors for isolates to become resistant to all beta-lactam antimicrobials, aminoglycosides, and fluoroquinolones are not yet well described. In addition to these risk factors, there are many reports of MDR GNFB outbreaks. P. aeruginosa and A. baumannii are not only ubiquitous in nature but widespread in the hospital environment<sup>1</sup> and the extensive environmental contamination revealed during outbreak investigations implies that cross transmission may be a very important mechanism for acquisition of a MDR strain; in fact, epidemiological studies using sophisticated methods of DNA fingerprinting have shown that the development of multi-drug resistance may occur by both acquisition of drug resistance by existing strains and by cross-infection with resistant strains<sup>37-39</sup>. The relative contributions of antimicrobial selective pressure and transmission between patients on the emergence of MDR GNFB

are unknown at this time. In this study, independent risk factors were mechanical ventilation and APACHE II score for the acquisition of PDRPA and mechanical ventilation and time at risk for PDRAB. Surprisingly, antimicrobial was not documented as a risk factor for either PDRPA or PDRAB. Although epidemiological study and outbreak investigation were not performed, there might be cross transmission among the patients receiving care in the same hospital because mechanical ventilation was documented as risk factor and clonality was observed on PFGE. This finding suggests that, during the study period, patient to patient transmission contributed more for the acquisition of PDR GNFB than antimicrobial selective pressure did.

The rationale for using a case-case-control study design was the advantage offered by the comparison of 2 multivariable models: risk factors for acquisition of antimicrobial resistant organism and risk factors for acquisition of antimicrobial susceptible organism. The first model identifies risk factors for antimicrobial resistant organism among nosocomially acquired patients, compared with those receiving care from the same hospitals, and the second model identifies risk factors for antimicrobial susceptible organism. When patients infected with the antimicrobial susceptible organism are used as control patients, these "susceptible" control patients are not representative of the "source" population for antimicrobial resistant organism. In a given patient with a vancomycin resistant enterococci (VRE) infection, the VRE does not exclusively arise from an endogenous vancomycin susceptible enterococci (VSE) strain. Typically, VRE is acquired from an exogenous source. Moreover, the control group as the patients harboring antimicrobial susceptible organism may lead to an overestimation of the association between antimicrobial exposure and cases: as prior active antibiotic exposure would eradicate susceptible organisms, the remaining potential control patients would be those patients who have not received certain antimicrobials of interest<sup>40-42</sup>. In this study, I performed 3 case-control studies with same control group for the meaningful comparison of 3 risk models. Mechanical ventilation was documented as common risk for PDRPA, IRPA, and ISPA and considered as risk factor for P. aeruginosa acquisition in general. The unique risk factor for PDRPA acquisition was APACHE II score. For A. baumannii acquisition, mechanical ventilation was a common risk factor for PDRAB and IRAB, and considered as risk factor for IRAB in general. The unique risk factor for PDRAB acquisition was time at risk.

The limitation of this study is that the control group does not represent the exact source population for the PDR GNFB case group because the control group did not include imipenem-susceptible and resistant GNFB. In addition, because the control patients were not screened by an active surveillance

culture for the presence of GNFB, it is possible that some of these patients actually might have been case patients. However, this type of misclassification would make the groups of case patients and control patients more similar by including case patients in the control groups, and it would only serve to underestimate the associations noted in this study.

Resistance mechanisms that are expressed frequently in nosocomial strains of GNFB include  $\beta$ -lactamases, alterations in cell-wall channels (porins), and efflux pumps. GNFB can become resistant to quinolones through mutations in the genes gyrA and parC and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes. However, in this study, I focused on the carbapenem resistance mechanisms of PDR GNFB because the clinically most troubling thing has been GNFB's acquired  $\beta$ -lactamases, which confer a resistance to carbapenems<sup>11</sup>. For *P. aeruginosa*, carbapenem resistance attributed to  $\beta$ -lactamases is due to metallo- $\beta$ -lactamases in general. The major types that have been identified are IMP, VIM, SPM, GIM and SIM. Since IMP-1 was first reported in Serratia marcescens and P. *aeruginosa* isolates<sup>13-15</sup>, the IMP-type  $\beta$ -lactamases have a global distribution. Numerous outbreaks of infection with VIM-2 have also been described. In Korea, VIM-2-like enzymes were the majority of MBL in P. aeruginosa and 9% of IRPA isolated between 1995 and 1999 in a tertiary care hospital had

VIM-2 MBL<sup>18</sup>. In this study, I found that 9% of PDRPA had VIM-2 gene and another 9% of PDRPA carried IMP-6 genes. In Korea, the IMP-type  $\beta$ -lactamases have rarely been reported in *P. aeruginosa* except a recent report of IMP-1 carrying *P. aeruginosa* outbreak<sup>43</sup>.

For A. baumannii, the most problematic recent occurrence is the emergence of numerous OXA enzymes that confer  $\beta$ -lactam resistance and the first description of a serine carbapenemases in A. baumannii was OXA-23<sup>44</sup>. Although OXA carbapenemases may not robustly hydrolyze imipenem, their presence in an organism that may have an insertion sequence (IS) element that acts as a promoter can result in imipenem resistance<sup>45</sup>. Two major MBLs have been reported in *Acinetobacter* spp.: IMP and VIM type. VIM-2  $\beta$ -lactamases detected in A. baumannii isolates from Korea confer significant levels of resistance to carbapenems<sup>46, 47</sup> and the distribution of MBLs in A. baumannii vary in different countries<sup>48</sup>. In this study, no MBL was detected in PDRAB because all isolates showed negative for the EDTA-imipenem disk synergy test and this result is compatible with the recent report that the majority of MBL-producing Acinetobacter are non-baumannii Acinetobacter strains<sup>49</sup>. All of the 30 PDR Acinetobacter spp. contained the OXA-51 gene, identified as A. baumannii and ISAba1 was detected upstream of the OXA-51 gene in 7 (33%) isolates. The OXA-23 gene was detected in 23 (77%) PDRAB isolates,

and IS*Aba1* was detected upstream of the OXA-23 gene in all of the PDRAB isolates. Although all of these isolates were also PCR positive for OXA-51 gene, none gave a band in the PCR using the IS*Aba1*F/OXA51-R primers. These findings suggest that IS*Aba1* was only associated with OXA-23 gene in the isolates, which were PCR positive for both the OXA-51 gene and the OXA-23 gene.

#### **V. CONCLUSION**

The risk factors for acquisition of PDR GNFB in Korean hospitals were investigated in this study. In addition, to document the resistance mechanisms to carbapenems, the presence of MBL and OXA carbapenemase genes was examined.

- Mechanical ventilation (OR, 6.8; 95% CI, 1.1-42; P=.039) and APACHE II score (OR, 1.13; 95% CI, 1.02-1.23; P=.019) were identified as independent risk factors for PDRPA acquisition.
- Mechanical ventilation (OR, 18.8; 95% CI, 3.6-99.5; P=.008) and urinary catheter usage (OR, 3.8; 95% CI, 1.01-14.6; P=.048) were identified as risk factors for IRPA acquisition, whereas mechanical ventilation (OR, 5.3; 95% CI, 1.1-24.4; P=.034) and associated pulmonary disease (OR, 4.6; 95% CI, 1.2-17.4; P=.026) for ISPA acquisition.
- Mechanical ventilation (OR, 11.5; 95% CI, 1.7-76.7; P=.012) and time at risk (OR, 1.04; 95% CI, 1-1.07; P=.049) were identified as independent risk factors for PDRAB acquisition.
- 4. Mechanical ventilation (OR, 22.8; 95% CI, 2.4-218; *P*=.007) and DDD of 3rd generation cephalosporin (OR, 1.2; 95% CI, 1.02-1.4; *P*=.028)

were identified as risk factors for IRAB acquisition, whereas APACHE II score (OR, 1.2; 95% CI, 1.03-1.4; P=.022) and associated pulmonary disease (OR, 6; 95% CI, 1.2-30.9; P=.031) for ISPA acquisition.

- 5. PFGE of PDRPA and PDRAB showed that clonal epidemic PDRAB isolates coexisted with sporadic isolates.
- 6. Of the 43 isolates of PDRPA, the 8 isolates (19%) were shown to produce MBL; 4 were VIM-2 producers and 4 were IMP-6 producers. Of the 30 isolates of PDRAB, no MBL producer was detected. Twenty-three (77%) isolates contained OXA-23 genes and 7 (23%) contained IS*Aba1*-activated OXA-51 genes. Coexistence of OXA-23 gene and IS*Aba1*-activated OXA-51 gene was not detected

In summary, this study supports a major role for mechanical ventilation on the acquisition of PDR GNFB. Moreover, PFGE revealed clonal epidemics within hospitals. Taken together, these results suggest that patient to patient transmission contributes the acquisition of PDR GNFB in Korea.

Carbapenem resistance of PDRPA is mainly due to a non-carbapenemase mechanism and 19% of PDRPA produce MBL. Among PDRAB, 77% of isolates contains OXA-23 gene and IS*Aba1* is only associated with OXA-23 gene in the *A. baumannii*, which contain both OXA-51 and OXA-23 gene.

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#### **ABSTRACT (In Korean)**

## Pandrug-resistant 포도당 비발효 그람음성 간균 획득의

위험요인 및 카바페녬에 대한 내성 기전

#### <지도교수 김 준 명>

#### 연세대학교 대학원 의학과

#### 박 윤 수

서론: 다제내성 포도당 비발효 그람음성 간균에 의한 감염은 환자의 사망률과 유병률 등의 예후를 악화시킨다. 최근에는 사용가능한 모든 항생제에 내성인 pandrug-resistant 포도당 비발효 그람음성 간균도 보고되고 있다. 이러한 균주에 감염되었을 경우 예후가 좋지 않을 뿐 아니라 사용할 수 있는 항생제가 제한적이기 때문에 균주가 획득되지 않도록 예방하는 것이 중요하다. 그러나 pandrug-resistant 포도당 비발효 그람음성 간균 획득의 위험인자는 알려져 있지 않다. 포도당 비발효 그람음성 간균의 항균제 내성의 기전은 βlactamase와 같은 효소의 생산, 세포벽의 투과성 변화, 유출

펌프에 의한 경우이다. 이중 임상적으로 가장 문제가 되는 것은 포도당 비발효 그람음성 간균이 serine β-lactamase나 metallo-β-lactamase (MBL)를 획득하여 카바페넴에 내성을 갖게 되는 경우이다. 그러나 pandrug-resistant 포도당 비발효 그람음성 간균의 카바페넴 내성 기전에 대해서는 알려져 있지 않다. 본 연구에서는 포도당 비발효 그람음성 간균 획득의 위험인자 및 카바페넴 내성 기전에 대해 알아보고자 하였다. **방법:** 2007년 6월부터 11월까지 전국의 8개 병원에서 pandrug-

resistant *Pseudomonas aeruginosa*와 *Acinetobacter baumannii* (PDRPA, PDRAB)를 수집하였다. MBL과 OXA 유전자의 존재 및 종류를 알기 위해 Hodge 변법, EDTA-imipenem disk 시험, 중합연쇄반응을 시행하였다. PDRPA와 PDRAB의 위험인자 분석을 위해 환자-환자-대조군 연구를 시행하였다.

결과: 연구기간 동안 PDRPA 43주와 PDRAB 32주를 수집하여 카바페넴 내성기전을 분석하였다. 수집된 균주 중 PDRPA 분리 환자 33명과 PDRAB 분리 환자 26명의 의무기록을 조사하여 위험인자를 분석하였다. PDRPA 획득의 독립적 위험인자는 기계호흡(OR, 6.8; 95% CI, 1.1-42; *P*=.039), APACHE II 점수(OR, 1.13; 95% CI, 1.02-1.23; *P*=.019)였다. 기계호흡(OR, 18.8; 95% CI, 3.6-99.5; *P*=.008)과 도뇨관 사용(OR, 3.8; 95% CI,

1.01-14.6; P=.048)<sup>o</sup>] imipenem-resistant *P. aeruginosa* (IRPA) 획득의 위험인자였으며 기계호흡(OR, 5.3; 95% CI, 1.1-24.4; P=.034)과 동반된 호흡기 질환(OR, 4.6; 95% CI, 1.2-17.4;  $P=.026)^{\circ}$  imipenem-susceptible *P. aeruginosa* acquisition (ISPA) 위험인자였다. PDRAB 획득의 독립적 획득의 위험인자는 기계호흡(OR, 11.5; 95% CI, 1.7-76.7; P=.012), 위험노출기간(OR, 1.04; 95% CI, 1-1.07; P=.049)이었다. 기계호흡(OR, 22.8; 95% CI, 2.4-218; P=.007)과 3세대 세괄로스포린 사용량(OR, 1.2; 95% CI, 1.02-1.4; P=.028)이 imipenem-resistant A. baumannii (IRAB) 획득에 위험인자였으며 APACHE II 점수(OR, 1.2; 95% CI, 1.03-1.4; P=.022)와 동반된 호흡기 질환(OR, 6; 95% CI, 1.2-30.9; P=.031)이 imipenem-susceptible A. baumannii (ISAB) 획득의 위험인자였다. Pulsed-field gel electrophoresis (PFGE) 결과 병원내에 같은 pandrug-resistant 포도당 비발효 그람음성 클론의 간균의 유행이 있었다. PDRPA 균주 43주중에서 8주(19%)가 MBL을 생성하는 균주였다; VIM-2 생성균주 4주, IMP-6 생성균주 4주. 30주의 PDRAB 균주 중 MBL을 생성하는 균주는 없었다. 모든 PDRAB 균주는 OXA-51 유전자를 가지고 있었으며 7균주에서 ISAbal이 OXA-51 유전자에서 발견되었다. OXA-23 유전자를 가지고 있는 PDRAB 23 균주는 모두 OXA-51 유전자를 같이

가지고 있음에도 불구하고 ISAbal이 OXA-23 유전자에서만 발견되었다.

결론: 기계호흡이 pandrug-resistant 포도당 비발효 그람음성 간균 획득의 공통된 위험인자였으며 PFGE상 병원내에 같은 클론의 유행이 의심되었다. 이상의 결과들을 종합해 볼 때, 환자간의 전파가 pandrug-resistant 포도당 비발효 그람음성 간균 획득에 기여하는 것으로 판단된다. PDRPA는 주로 carbapenemase 이외의 기전으로 카바페넴에 내성을 나타내며 19%에서 MBL을 생산하였다. PDRAB 균주의 77%에서 OXA-23 유전자를 가지고 있으며 23%는 ISAba1에의해 활성화된 OXA-51 유전자를 가지고 있었다. OXA-23 유전자와 ISAba1에의해 활성화된 OXA-51 유전자를 같이 가지고 있는 균주는 없었다.

핵심 되는 말: Pandrug resistance, 포도당 비발효 그람음성 간균, 위험인자, 카바페넴, 내성 기전