February, 2007
Doctoral Dissertation

Molecular and Epidemiological Investigation of an Outbreak of Imipenem Resistant *Acinetobacter baumannii* in a University Hospital in Korea

Department of Medicine
Graduate School
Chosun University
Gwangju, Republic of Korea

Bidur Prasad Chaulagain

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2007 년 2 월
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한국의 한 대학병원에서 발생한 Imipenem 내성 *Acinetobacter baumannii* 돌발감염의 분자적 및 역학적 연구

(Molecular and Epidemiological Investigation of an Outbreak of Imipenem Resistant *Acinetobacter baumannii* in a University Hospital in Korea)

2007 년 2 월 23 일

Department of Medicine
Graduate School
Chosun University
Republic of Korea
조선대학교
대학원 의학과

Bidur Prasad Chaulagain

Molecular and Epidemiological Investigation of an Outbreak of Imipenem Resistant *Acinetobacter baumannii* in a University Hospital in Korea

Supervisor
Sook-Jin Jang, M. D., Ph. D.
Professor

A thesis submitted to the Graduate School of Chosun University in partial fulfillment of the requirements for the Degree of Doctor of Medicine

October, 2006

Submitted by

Bidur Prasad Chaulagain

Department of Medicine
Graduate School
Chosun University

This is to certify that the thesis submitted by Bidur Prasad Chaulagain has met the thesis requirement for the Degree of Doctor of Medicine of Graduate School of Chosun University

Approved by:

Committee	
	……..
chairperson	**Prof. Young-Jin Park, M. D., Ph. D.**
Committee	
	…….
member	**Prof. Dae-Soo Moon M. D., Ph. D**
Committee	
	……..
member	**Prof. Jong-Hee Shin M. D., Ph. D.**
Committee	
	…….
member	**Prof. Dong-Min Kim M. D., Ph. D.**
Committee	
	……
member	**Prof. Sook-Jin Jang M. D., Ph. D.**

December……, 2006

Department of Medicine
Graduate School
Chosun University

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ABSTRACT

Molecular and Epidemiological Investigation of an Outbreak of Imipenem Resistant Acinetobacter baumannii in a University Hospital in Korea

Bidur Prasad Chaulagain
Supervisor: Sook-Jin Jang, M.D., Ph. D.
Department of Medicine
Graduate School of Chosun University

Acinetobacter baumannii is an aerobic, glucose non-fermentative, Gram-negative coccobacillus which is widely distributed in the hospital environment. It is an important opportunistic pathogen causing a variety of nosocomial infections. Several outbreaks of *A. baumannii* in intensive care units (ICUs) of hospitals have been documented. During January 2004 to December 2004, the medical ICU and surgical ICU of Chosun University Hospital (CHU), Gwangju, Korea (Republic), a 650 bed tertiary care centre, had experienced an apparent outbreak due to the imipenem-resistant *A. baumannii*. To determine the source of the epidemic and the risk factors, and to know the clonality and features of outbreak strains, environmental sampling from the ICU, a case-control study, genotyping of *A. baumannii* by pulsed-field gel electrophoresis (PFGE) and biochemical test and PCR assay for resistance determinants were performed. Thirty imipenem resistant *A. baumannii* (IRAB) clinical isolates, 9 imipenem susceptible *A. baumannii* (ISAB) clinical isolates and 8 IRAB isolated from environmental culture (E-IRAB) collected during that outbreak period were analyzed in this study.

Environmental culture revealed widespread contamination of IRAB in the ICU environment, especially, from suction- , tracheostomy-, and patient's ventilator-associated surfaces. IRAB were isolated from health care worker's (HCW) hands also. This finding was matched well with the finding of the risk

factors which were identified by multiple logistic regression analysis were mechanical ventilation practice and time at risk. So it seemed like that multiple objects in hospital environments, especially, ventilator- and suction-related systems were important reservoir of IRAB and the spreading of IRAB was mediated by HCW's contaminated hands. Because ICU environment and HCW's hands were contaminated, prolonged stay in ICU may have contributed for the easy acquisition of IRAB, which is supported by the fact that time at risk was the important risk factor.

PFGE analysis result showed most IRAB and E-IRAB were type A, a major PFGE type in this study. Three other minor types were also observed but only in a few sporadic cases. ISAB showed 8 types which were well differentiated from IRAB types.

To determine whether the outbreak strains had carbapenemase, modified Hodge test (MHT) were carried out for the 47 *A. baumannii* isolates. All of MHT positive isolates were tested by imipenem-EDTA double disk synergy test (DDST) and several kinds of carbapenemase gene PCR. The MHT showed positive results for all of 30 IRAB and 8 E-IRAB while all of 9 ISAB were negative for MHT. The DDST showed positive results in 5 of 30 (16.7 %) of IRAB and one of eight (12.5%) of E-IRAB. Five of 30 (16.7 %) of IRAB showed blaIMP and 1 of 30 (3.33 %) of IRAB showed blaVIM PCR positive. PCR results for other carbapenemase gene such as SPM-1, GES-1, GIM-1, blaOXA-23, blaOXA-24, and blaOXA-58 were negative for all of 30 IRAB. Dot blot hybridization test by fluorescence labeled probe for blaOXA-2 gene was carried out and only 3 out of 47 isolates (6.38 %) were positive. It may be possible that another antibiotic resistant determinant genes or other resistance mechanism have contributed to the imipenem resistance of these outbreak strains. To know the prevalence of integrase genes in our samples, integrase gene PCR was carried out. Most of them were class 1 integrase gene positive while all of them were class 3 integrase gene negative. The positive rates of class 2 integrase gene were 36.7%,

22.0%, and 37.5% of IRAB, ISAB, and E-IRAB respectively.

To control the outbreak, several infection control strategy including cross-transmission prevention protocols, and restriction of the use of carbapenem was implemented. As cross-transmission prevention protocols, continued ICU personnel educational programs, thorough hand-washing procedure, rigorous open surveillance of adequate compliance with barrier precautions, proper cleaning protocols, and housekeeping procedures were used. Subsequently, steady reduction of attack rate of imipenem resistant *A. baumannii* into the basal rate was observed.

***Keywords*:** *Acinetobacter*, epidemiology, imipenem resistance, molecular typing, outbreak, PFGE

국문 초록

한국의 한 대학병원에서 발생한 Imipenem 내성 *Acinetobacter baumannii* 돌발감염의 분자적 및 역학적 연구

Bidur Prasad Chaulagain
지도교수: 장숙진, M.D., Ph. D.
조선대학교 대학원 의학과

*Acinetobacter baumannii*는 병원 환경에 널리 분포되어 있는 호기성의 포도당 비발효성 Gram 음성 구간균이다. 이는 다양한 병원감염을 일으키는 중요한 기회감염균이다. 병원의 중환자실에서 *A. baumannii*에 의한 돌발감염이 여러 차례 보고되어져 왔다. 650병상인 한국 광주 조선대 병원의 내과계 및 외과계 중환자실에서 2004년 1월부터 2004년 12월까지 imipenem 내성인 *A. baumannii*에 의한 돌발감염으로 여겨지는 감염이 발생하였다. 이 돌발감염의 근원과 위험인자를 찾아내고 돌발감염균주의 클론성과 특성을 알기 위해 중환자실의 환경배양과 환자대조군연구, pulsed-field gel electrophoresis (PFGE)에 의한 *A. baumannii* 유전형 검사, 내성 유전자에 대한 생화학적 검사 및 PCR 검사를 실시하였다. imipenem 내성인 *A. baumannii* (IRAB) 30주와 imipenem 감수성인 *A. baumannii* (ISAB) 9주, 환경배양에서 분리된 IRAB (E-IRAB) 8주를 돌발감염 기간중에 수집하여 본 연구에 사용하였다.

환경배양 결과 중환자실 환경 특히 흡인장치(suction)와 기관창냄술 (tracheostomy) 및 인공호흡기 (ventilator)에 관련된 표면에 IRAB가 광범위하게 오염되어 있는 것이 드러났다. 병원의료인 (HCW) 손에서도 IRAB가 분리되었다. 이는 다중회귀분석으로 찾아낸 위험인자가 mechanical ventilation practice와 time at risk.였던 것과 잘 맞는 소견이었다. 그래서 병원환경내 여러 물체들 특히 ventilator- 및 suction 에 관련된 체계들이 IRAB의 중요한 reservoir이고 IRAB 의 전파가 의료인의 오염된 손에 의해 매개되어 일어나는 것으로 여겨진다. 중환자실 환경과 의료인 의 손이 오염되었기 때문에 중환자실에 장기 체류하는 것이 IRAB가 쉽게 획득되게 하는데 기여했을 수도 있다. 이는 time at risk가 중요한 위험인자였다는 사실이 지지해 주는 소견이다.

PFGE 분석상 대부분의 IRAB와 E-IRAB는 주된 PFGE형인 A형이었고 소수의 산발적인 분리주가 3가지 다른 유전형을 나타냈다. ISAB는 8가지 유형을 보였고 이는 IRAB의 유형과는 잘 구분되었다.

돌발감염주가 carbapenemase를 가지고 있는지를 측정하기 위해 modified Hodge test (MHT)를 *A. baumannii* 47주를 대상으로 검사하였다. MHT에 양성인 균주들은 모두 imipenem-EDTA double disk synergy test (DDST)와 여러 종류의 carbapenemase gene PCR 을 시행하였다. The MHT 검사상 IRAB 30주와 E-IRAB 8주는 모두 양성결과를 보인 반면 ISAB는 9주 모두 음성결과를 보였다. DDST는 IRAB 30주중 5주 (16.7 %)와 E-IRAB 8주중 1주 (12.5%)에서 양성결과를 보였다. IRAB 30주중 5주 (16.7 %)가 blaIMP 를 보였고 1주(3.33 %)가 blaVIM 양성이었다. SPM-1, GES-1, GIM-1, bla_{OXA}-23, bla_{OXA}-24, and bla_{OXA}-58 등의 다른 carbapenemase gene에 대한 PCR 검사 결과 IRAB 30주 모두가 음성소견을 보였다. bla_{OXA}-2 gene에 대한 형광표지된 탐색자를 이용한 dot blot hybridization test는 47주중 3주 (6.38 %)만 양성이었다. 이들 돌발감염 균주들의 imipenem 내성에 다른 항생제 내성 유전자나 내성기전이 기여했을 가능성이 있다고 본다. 본 연구의 검체들에서 integrase genes의 보유율을 알기 위해 integrase gene PCR을 시행하였다. 그들 대부분이 class 1 integrase gene을 가지고 있었고 그들 모두가 class 3 integrase gene은 가지지 않았다. class 2 integrase gene의 양성률은 IRAB와 ISAB 및 E-IRAB에서 각각 36.7%와 22.0% 및 37.5%로 나타났다.

돌발감염을 조절하기 위해 상호전파 방지책과 carbapenem 항생제 사용제한과 같은 여러 감염관리전략을 수행하였다. 상호전파 방지책으로는 중환자실 인력의 지속적인 교육계획, 철저한 손씻기 과정, barrier precautions을 적절히 지키는지에 대해 엄격히 감시하는 것, 적절한 청소 및 관리 방법들을 사용하였다. 그 결과 IRAB의 attack rate가 꾸준히 감소하여 평소 수준으로 되돌아왔다.

***Keywords*: *Acinetobacter*, epidemiology, imipenem resistance, molecular typing, outbreak, PFGE**

I. INTRODUCTION

Acinetobacter baumannii is a non-fermentative, aerobic, Gram-negative coccobacillus which is widely distributed in the hospital environment (Coelho et al 2006, Jubelle et al, 2006; Aygun et al, 2002, Nemec et al, 2000). It is important opportunistic pathogen causing a variety of nosocomial infections (Gonzalez et al, 1996, Bergogne-Beéreézin et al 1998, and Gervich et al 1985). Several outbreaks of *Acinetobacter baumannii* in intensive care units of hospitals (ICUs) have been documented mostly occupying immunocompromised patients (Zarrilli et al, 2004, Sherertz et al, 1985, Wang et al 2003, Pilay et al, 1999). Various types of infections are caused by this microorganism, including pneumonias, bacteremias, meningitis and urinary tract infections (McDonald et al, 1998, Bergogne-Berezin and Towner, 1996, Melamed et al, 2003).

Emergence of carbapenem- and other β- lactams drug-resistance in *Acinetobacter baumannii* with increased rates of multi drug resistance has become a serious threat to the health sector (Livermore, 2003, Zarrilli et al 2004, Aygun et al 2002). The metallo beta-lactamases (MBL) resistance rates in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals (KONSAR) is quite high with the contribution of more than 6 percent by the *Acinetobacter baumannii* species alone (Lee et al, 2004). The course of resistant gene capture and transfer in the species has been suggested both vertical and horizontal. Studies for antibiotic resistance mechanisms in *A. baumannii* have demonstrated the presence of antibiotic resistance genes located either on chromosomes or on transferable plasmids, transposons and integrons (Vahaboglu et al, 1997, Koeleman et al, 2001). The presence of integrons in this species has added the dimension for its epidemic behavior and resistance to strong antimicrobials (Koeleman et al 2001).

Production of ß-lactamases and reduced expression of outer membrane protein (OMP) and penicillin binding protein are other frequently observed mechanisms of resistance to carbapenems (Fernández-Cuenca et al, 2003). Resistance to both imipenem and carbapenems in multidrug-resistant *A. baumannii* has also been found

associated with the loss of 29-kDa outer membrane protein (OMP) (Limansky et al 2002 and Walther-Rasmussen and Høiby, 2006) and disruption of carbapenem resistance associated OMP (Mussi et al, 2005) and involvement of multi-drug resistant efflux pumps (Heritier et al 2005).

A major contributing factor in the emergence of resistance of this species is the acquisition and transfer of antibiotic resistance genes on plasmids, transposons and integrons (Gombac et al, 2002; Nemec et al 2004; Ploy et al, 2000; and Vila et al 1997). *A. baumannii* strains notoriously cause hospital outbreaks, and a few lineages achieve epidemic status, reaching multiple hospitals or countries (Coelho et al, 2006). By convention, these are termed clones rather than strains when their relatedness is inferred on the basis of DNA profiles, without proven chains of site-to-site transmission (Coelho et al, 2006). *Acinetobacter baumannii* now emerged as a primary nosocomial pathogen in hospital outbreaks, and is ranked second after *Pseudomonas aeruginosa* among nosocomial aerobic nonfermentative gram-negative bacillar pathogens (Jeong et al 2006; Schreckenberger and Graevenitz, 1999 and Simor et al., 2002).

The serine carbapenemases are invariably derivatives of class A or class D enzymes and usually mediate carbapenem resistance in *Acinetobacter* spp. (Walsh et al, 2005). The emergence of carbapenem resistance in *Acinetobacter baumannii* has become a global concern since these beta-lactams are often the only effective treatment left against many multi-resistant strains. Whether these carbapenemases have been acquired or are part of the genetic make-up of this species has yet to be determined. More importantly, however, they represent an important stage in the evolution of antibiotic resistance in acinetobacters. β-Lactamase production is the most salient mechanism of acquired β-lactam resistance in gram-negative pathogens (Yong et al., 2006). Carbapenems (e.g., imipenem) 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 and Lee and Lee, 2006). Their epidemiology is also complex (Jubelle et al, 2006 and Nemec et al,

2000) and a robust and efficient molecular biological technique is demanding for its quick epidemiological study.

Acinetobacter baumannii is a pathogen that has been associated with hospital outbreaks worldwide. Understanding the fundamental mechanisms underlying *Acinetobacter* infections, including the original sources of the infecting organisms, their clonality, and geographical spread, are important requirements for the development of appropriate infection control measures (Ecker et al, 2006). Genotyping allows investigation of clonal spread and can be used to identify the source of the original infection (Ecker et al, 2006). *Acinetobacter* strain typing methods include serotyping (Traub,1989), multilocus enzyme electrophoresis (Seltmann et al , 1995), and DNA-based methods, including repetitive extragenic palindromic sequence-based PCR (Buxton et al, 1978 and Huys et al, 2005), amplified ribosomal DNA restriction analysis (ARDRA), pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) (Vaneechoutte et al, 1995), and ribotyping (Ibrahim et al, 1997, Koeleman et al, 1998 and Misbah et al, 2005) and MLST (Ecker 2006, and Bartual 2005). As part of the investigation to identify the possible source(s) of this outbreak, to determine the likely mode(s) of transmission, and to assist in generating and implementing infection control countermeasures, PFGE based typing method and conventional outbreak control measures were set out. For present investigation PFGE was chosen as the typing method for it is taken as gold standard in molecular epidemiological technique (Seifert and Gerner –Smidt, 1995).

During January 2004 to December 2004, the ICU of Chosun University Hospital (CHU), Gwangju, Korea (Republic), a 650 bed tertiary care centre, had experienced an apparent outbreak due to the imipenem-resistant *A. baumannii*. The aim of this study was to investigate the (i) imipenem resistant *Acinetobacter baumannii* outbreak by genotypic method, (ii) carbapenem resistance determinant for the outbreak, (iii) role of integrase in the outbreak, (iv) risk factor analysis for the outbreak, and (v) develop control measures for the outbreak.

II. MATERIALS AND METHODS

A. Bacteria and description of the outbreak

Chosun University Hospital (CHU) is a 650-bed tertiary care centre located at Gwangju, South Korea. An apparent nosocomial outbreak of imipenem resistant *A. baumannii* occurred in the medical intensive care unit (MICU) and surgical intensive care unit (SICU) unit of this hospital from January 2004 to December 2004 **(Figure-1).** In order to determine whether the event was a true outbreak it was designed a retrospective review of medical records of patients. Clinical and environmental isolates of *A. baumannii* collected during the period of January 2004 to December 2004 from medical intensive care unit (MICU) and surgical intensive care unit (SICU) of CHU were analysed for the present study. Strain identification and antimicrobial susceptibility test was carried out by Vitek ID 32 GN system with Vitek AST-N017 kit (bioMėrieux, Durham, NC, USA) according to the instruction of manufacturer. The minimum inhibitory concentration (MIC) levels for the tested antibiotics were determined following the NCCLS guidelines (NCCLS, 2001). The bacteria isolation history and antibiogram tested is presented in **Table-1**.

B. Environmental sample culture of *A. baumannii*

The environment surveillance culture was performed in the MICU and SICU on March 2004. An extensive environmental sampling of the ICUs were performed for the presence of *A. baumannii* and focused on various inanimate surfaces and items in frequent use and contact with patients and health care workers (HCW). A total of 88 samplings from inanimate surfaces were obtained. Hand swabs and nares scrapings were obtained from 36 samplings from the HCWs (both hands of 18 HCWS) **(Table-2).** Swabs pre-moistened with sterile normal saline were use to collect the environmental samples. The survey included the following items: DP set containing suction kit, tracheostomy site, O2 supplier, washing stands, waste water disposal sink surface, station table, dressing car or assisted trolleys, keyboard and monitor of computer, door handle, surface of suction, surface of recording sheet of patient, water

in humidifier of patient, surface of chart of patient, bed rail and sheet of bed of patient, surface of EKG monitor, water containing airway, cannular, or suction tube tip of patient, skin of patient near tracheostomy site, surface of sink, the water tap, a washing stand, disinfectant solution, receiver of telephone of ICU station, drawers of dresser, an electric wire running in the ICUs; an electric cord of EKG machine, intravenous injection fluid line, a lid and handle of canister, surface of ventilator, monitor of ventilator, fluid in trap of ventilator (collected or trapped fluid in ventilator), handle and monitor of ventilator, surface of wooden dresser in station. The same surveillance culture methods were repeated after the outbreak control.

A pre-moistened cotton swab in normal saline was used to collect the sample. The environmental isolates collected from different sites from the hospital environment during the surveillance period of 2004-03-09 to 2004-12-10 were cultured in MH broth medium and saved for further study in 20 % LB glycerol medium at -79 degree Celsius. They were tested for imipenem resistance following the NCCLS (2001) guidelines and designated as E-IRAB for the resistant isolates for present study purpose.

C. Antibiotic susceptibility testing for clinical and environmental samples

For antibiotyping, disk-agar diffusion tests (BD BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs (catalogue no. 8840621) were used on Muellr Hinton agar (bioMerieux) with an inoculum of 2.10^6 CFU/ml. Antibiotics tested and disc potency were: amikacin (30µg), ceftazidime (30µg), ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg) and netilmicin (30µg), aztreonam(30µg), piperacillin (100µg), ticarcillin-clavulanic acid (75/10 µg) and tobramicin (10µg). MICs of imipenem were determined by the agar dilution method with Mueller-Hinton agar and an inoculum of 10^4 CFU per spot. All cultures were incubated aerobically at 37^0 C for 24 hours.

The criteria for imipenem resistance (R) was set as less or equal to 13 mm for growth inhibition zone, intermediate (I) for the zone diameter between 14mm and 15

mm and more than 16 mm inhibition zone was recorded as susceptible (S) for the imipenem. Like wise for amikacin inhibition zone determination the breakpoint of 14 mm was taken as resistant (R) while the inhibition zone between 15mm to16mm was taken as intermediate (I) and more than 17 mm zone diameter was taken as susceptible (S) to the amikacin for the *A. baumannii* organism. The criteria for antibiotics was set as follows: ciprofloxacin resistance (R), ≤15mm, 16-20mm for intermediate (I) and ≥zone diameter for susceptible (S). Like wise for aztreonam, R, ≤15, I, 16-21 and S, ≥22; gentamicin R, ≤12, I, 13-14 and S, ≥15; for netilmicin, R, ≤12, I, 13-14 and S, ≥15; piperacillin, R, ≤17, I, 18-20 and S, ≥21; ticarcillin-clavulanic acid, R, ≤14, I, 15-19 and S, ≥20; tobramicin, R ≤12, I, 13-14 and S, ≥15mm. The breakpoints used were those from published recommendations (NCCLS 2001).

D. Preparation of genomic DNA (gDNA) material

Template DNA for present work was isolated from the gDNA of freshly cultured bacteria either by enzymatic extraction method or from the bacterial lysates. For the enzymatic extraction method proteinase-K extraction method was applied. Briefly, the bacterial colonies from the overnight culture in Luria-Bertani (LB) agar plate was collected with the help of a loop and suspended in 300 microlitre Tris-EDTA sodium chloride buffer containing 20 microlitre of 20 percent sodium deodocyl sulfate solution with the enzymatic enrichment of 20ul 20mg/ml proteinase-K and the reaction mixture was incubated overnight at 54 degree Celsius. The reaction mixture was then added with 100ul 5M NaCl and vortexed and centrifuged for 13,000 rpm for 10 minutes. The resulting supernatant was collected in a new tube and 1 ml of -20 °C chilled ethyl alcohol was added and inverted for 4 to 5 time to mix it up well and allowed for storage in -20 °C for an hour and centrifuged for 15 minute at 13000 rpm at 4 degree Celsius. The obtained supernatant was discarded and the pellet was washed with 70 percent ethyl alcohol at room temp by vortexing at 1000 rpm for 1 minute and centrifugation at 7000 rpm for 5 minute at 4 °C. Then the resulting supernatant was clearly discarded and the pellet was vacuum dried to totally wipe out

the ethyl alcohol. Then the dry pellet was suspended in 100 ul double distilled and autoclaved sterilized water and incubated at 37 ℃ for overnight. The gDNA amount was determined by spectrophotometer method and aliquot were stored at -20 ℃ for further uses.

For cell-lysate template DNA extract the bacterial colonies were grown overnight in LB agar. A loopful of bacteria were then directly suspended in sterile dH2O at a McFarland density of 2 (6 × 108 cfu/mL), and lysed by heating at 95 ℃ for 20 min once and freezeed and thawed for three times, centrifuged at 12, 000 revolution per minute (rpm) for 2 minutes and then stored at − 20 ℃ as PCR template material.

E. Pulsed field gel electrophoresis (PFGE)

1. Plug preparation

Test strains were inoculated onto LB agar and incubated overnight at 36 ± 1°C in ambient air. A loopful of bacteria was taken from the agar surface with a sterile plastic 10-µl loop and suspended in an Eppendorf tube containing 1.0 ml of cell suspension buffer (100 mM Tris, 100 mM EDTA, pH 8.0). Each cell suspension was adjusted to 2 O.D. and 100 µl of the suspension was taken. This was performed by using the UV spectrophotometer. For this purpose, a 500-µl aliquot of the adjusted bacterial cell suspension was transferred to a 1.5-ml microcentrifuge tube and centrifuged at 15,000 rpm for 10 min. The pellet was resuspended by pippeting and the cell suspension was incubated at 37°C for 10 min in a water bath. An aliquot of 25 µl proteinase K (20-mg/ml stock solution in ultrapure water) was added, and the suspension was mixed gently by inverting the tube two to four times. An equal volume of melted 1% SeaKem Gold agarose (Biozym Diagnostics, Hessisch-Oldendorf, Germany)-1% sodium dodecyl sulfate in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) was added to the cell suspension, and the suspension was mixed gently by inverting the tube 10 to 12 times. The agarose-cell suspension mixture was immediately dispensed into the wells of reusable plug molds (catalog no. 170-3713;

Bio-Rad Laboratories, Munich, Germany). The agarose plugs were allowed to solidify at room temperature for 5 min and at 4°C for another 20 min.

2. Lysis of cells in the plugs and washing

The plugs were transferred to disposable screw-cap 15-ml polypropylene tubes containing 5 ml of cell lysis buffer (50 mM Tris, 50 mM EDTA (pH 8.0), 10% sarcosine) and 25 μl of proteinase K (20 mg/ml stock solution). Lysis was performed at 55°C in a shaking water bath for 16 h with constant and vigorous agitation (150 rpm). After lysis, the buffer was carefully removed and the plugs were washed five times (15 min/wash) at 55°C (two times with sterile ultra pure water and three times with TE buffer; 50 ml for each washing step) in a shaking water bath. The water and 0.5X TE buffer were preheated at 50 to 55°C before each washing step. If the plugs were not used on the same day, they were kept overnight for direct use or were stored up to several weeks at 4 to 8°C for later use.

3. Restriction digestion of gDNA and loading of the plug in the gel

A slice from each plug (4.0 by 5.5 mm) was cut with a sterile scalpel or razor blade and transferred to a microcentrifuge tube containing 200 μl of the restriction buffer provided with the enzyme (see below), with 100 μg/ml bovine serum albumin included in the buffer. The plug slices were incubated in this restriction buffer at 25°C for 15 min. Then, the restriction buffer was removed and replaced with 100 μl of fresh restriction buffer containing 30 U of Apa*I* (New England Biolabs, Frankfurt, Germany, or Promega, Madison, Wis.). The reaction tubes were shaken gently, and the plug slices were incubated at 25°C for 16 h. Prior to casting of the gel, the restriction mixture was removed from each tube and replaced with 200 μl of 0.5× TBE (10× TBE is 0.89 M Tris, 0.89 M boric acid, and 20 mM EDTA, pH 8.3). If the slices were not used on the same day, they were kept overnight or for up to several days at 4 to 8°C in a refrigerator. The plug slices were allowed to stand at room temperature for 5 min, after which they were loaded into the appropriate wells of a 1% SeaKem Gold agarose gel. The wells were made by using a 15-slot comb, with

each slot being 1.5 mm thick (catalog no. 1704324; Bio-Rad Laboratories). A slice of a bacteriophage lambda ladder PFGE marker (CHEF DNA size standard; catalog no. 170-3035; Bio-Rad Laboratories) was loaded into lane 1; and an index strain was loaded into any one lane to allow later normalization of the electrophoresis patterns across the gel.

4. Electrophoresis

Electrophoresis was performed in a contour-clamped homogeneous electric field (CHEF DR III, BIO-RAD, Hercules, CA, USA). The gels were covered with 2,000 ml of 0.5× TBE. The running temperature was set at 14°C. The total run time was 20 h, with switch times ranging from 5.3 s to 49.9 s at 12O^{o} angle. The voltage for the run was 6 V/cm or 200 V. The gels were stained for 30 min with 300 ml of ethidium bromide solution (1 mg/ml) and destained for 45 min in distilled water with gentle shaking. The gels were observed under UV illumination and images were captured and stored as TIFF files with the Gel Doc 2000 system (Bio-Rad, Hercules, CA, USA). Computer-assisted analysis of the fingerprints was performed using Bionumerics software (version 4.5; Applied Maths, Kortrijk, Belgium). The TIFF files were converted with a resolution of 400pt, normalized with resolution of 400pt, smoothing of 3pt and background subtraction of 6pt. The similarities of PFGE were calculated with the DICE coefficient correlation. Cluster analysis of similarity matrices was performed using the UPGMA algorithm (Sneath & Sokal, 1973) for gross epidemiological study.

F. Case definition, control definition, and study design

The epidemiological study was designed based on WHO guidelines and other published literatures (Beaglehole et al, 1993 and Last, 1988 and 1990).The design applied was 1:1 case-control study. The microbiology laboratory database was electronically searched to identify all the clinical cultures positive for *A. baumannii* from patients who admitted the medical intensive Case Unit (MICU) and surgical intensive care unit (SICU) between December 2003 and January 2005 at Chosun

University Hospital. Case group patients were 77 persons who included were those of patients from whom imipenem-resistant *A. baumannii* (IRAB) had been isolated in clinical cultures. Seventy-seven control group patients were selected from the patients who had not been isolated IRAB and were admitted during the same time period as the case group. Case and control patients were matched by gender and age (±5 years).

G. Risk factors analyzed

The demographic, administrative, pharmaceutical and laboratory information was collected by examining medical charts. Variables explored as possible risk factors included the presence of underlying diseases or comorbid conditions, and ICU stays prior to the outcome of interest which is referred to as the time at risk (length of stay prior to IRAB isolation for cases and the complete length of ICU stay for the controls), use of hospital equipments, number of admissions to the hospital in the prior year, and prior exposures to antimicrobial drugs.

H. Statistical analysis

All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL). Univariate analyses of qualitative variables were done by the chi-square test or Fisher's exact test. Quantitative variables were compared by using Student's t test. Variables with p-value < 0.1 in the bivariate analyses were included in a multiple logistic regression analysis, and Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. All tests were two-tailed, and a p-value of < 0.05 was considered statistically significant.

I. Infection control intervention program

Multicomponent intervention included the extensive decontamination, partial structural redesign of the units with the placement of hand washing facilities within the rooms; continued ICU personnel educational programs; rigorous open surveillance adequate compliance with barrier precautions, cleaning protocols, and

house-keeping procedures. Adequate compliance with the control program was supervised by a team comprised of a medical physician and a staff nurse who attended to the ICUs daily after the implementation of the intervention program.

J. Biochemical tests

Experiment with Modified Hodge Test (MHT) and DDST (Lee et al 2003) was carried out to find out the carbapenemase activity in imipenem resistant *A. baumannii*. An isolate with VIM β-lactamase will show an arrowhead-shaped synergistic zone, and an isolate with an IMP enzyme will show an oval synergistic zone in DDST. The general scheme for Modified Hodge Test and DDST is shown in the **Figure-2** as described by Lee et al (2003).

K. Modified Hodge test (MHT)

Acinetobacter baumannii were isolated from January 2004 to December 2004 from clinical and environmental samples and identified by conventional methods and by the ID 32 GN system (bioMerieux Vitek, Marcy-l'Etoile, France). Isolates resistant to imipenem by the disk diffusion test were kept at −76 °C in 20% LB agar glycerol until use. The Hodge test was modified by substituting *Escherichia coli* ATCC 25922 for penicillin-susceptible *Staphylococcus aureus* ATCC 25923, and 10µg imipenem disk for a 10-U penicillin disk. The imipenem disk was added with 20 µl of 50mM $ZnSO4^{++}$ solution. The surface of a Mueller–Hinton agar plate was inoculated evenly using a cotton swab with an overnight culture suspension of *E. coli*, which was adjusted to one-tenth turbidity of the McFarland no. 0.5 tube. After brief drying, an imipenem disk was placed at the center of the plate, and imipenem-resistant test strains from the overnight culture plates were streaked heavily 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 modified Hodge test positive.

L. Double disk synergy test (DDST)

For the EDTA-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-µg imipenem disk (BBL, Cockeysville, MD) and a blank filter paper disk were placed 10 mm apart from edge to edge, and 10 µL of 0.5 M EDTA solution was then applied to the blank disk, which resulted in approximately 1.5 mg/disk. After overnight incubation, the presence of an enlarged zone of inhibition was interpreted as EDTA-synergy test positive.

M. PCR and sequencing for molecular biological work

1. PCR primer

Primers used in present study were either taken from the published journals or designed at laboratory using either the DNA sequences obtained from present work or NCBI data. **Primerselect** program (Version 5.0.0, DNASTAR, inc., Madison, WI, USA) was used to design the primers.

2. Integron and integrase PCR

a. Detection of class 1 integrons

The template DNA preparation for the integron PCR was as mentioned above in proteinase-K extraction method. Primers 5'-CS (5'-GGC ATC CAA GCA GCA AG-3') and 3'-CS (5'-AAG CAG ACT TGA CCT GA-3') were used as described by Lévesque et al (1995). PCRs were carried out in 50-ul volumes using Takara buffer—I and Taq DNA polymerase with the procedure mentioned in the manufacturer's instruction manual (Takara BIO INC, Japan). The DNA in the thermal cycler, after denaturation at 94 ^{o}C for 5 minutes three-step profile of 1 min of denaturation at 94 ^{o}C, 1 min of annealing at 55 ^{o}C, and 5 min of extension at 72 ^{o}C for a total of 35 cycles was used. Twenty microlitre reaction mixture of Bioneer Accupower PCR premix kit (Bioneer Corporation, Daejoan, S. Korea) also gave the same result by using the above mentioned thermal condition, PCR specific primers and template DNA. Amplification products were separated on 0.8% agarose gel prepared in 0.5X

TBE buffer and visualized using ultraviolet light after staining with 1mg/ml ethidium bromide solution. The general scheme for integron PCR is outlined in **Figure-3**.

b. Integrase PCR

For integrase class 1 and class 2 PCR primer pairs and PCR methodology were used as described by Koeleman et al (2001) while for integrase class 3 primer sequences and PCR methodology were used as mentioned by Senda et al (1996) (**Table-3**). Amplification products were separated on 2.0 % agarose gel prepared in 0.5XTBE buffer and visualized using ultraviolet light after staining with 1mg/ml ethidium bromide solution.

c. Thermal asymmetric interlaced PCR (Tail PCR)

Thermal asymmetric interlaced PCR (Tail PCR) (Yao-Guang Liu and Ning Huang, 1995) was applied to extend the end sequence of the integron PCR sequence. For this purpose DNA walking SpeedupTM Premix Kit (Seegene Corp., S. Korea) was used as mentioned in the user's manual. Various combinations of DW-ACP1, DW-ACP2, DW-ACP3, DW-ACP4 primers and lab designed integron sequence based primers were used to run the Tail PCR. The obtained amplicon was purified with QIAquick PCR purification kit (Qiagen Co. Valencia, CA, USA) and sequenced.

3. Beta-lactamase and carbapenemase PCR

Different primers sets were used to detect the beta-lactamase genes presence in the isolates. The primers and specific target genes are mentioned in the **Table-4** and **Table-5**.

For bla- PCRs the amplification conditions were, initial denaturation at 94 °C for 5 min 30 cycles of 94 °C for 25 s, 52 °C for 40 s and 72 °C for 50 s, and a final elongation at 72 °C for 6 min. After the PCR the amplified products were gel electrophoresed in 0.5X Tris-borate EDTA buffer and stained in 1mg/ml ethidium bromide solution and visualized under UV light.

For other carbapenemase PCR in the thermal cycler, after denaturation at 94 ^{o}C for 5 minutes three-step profile of 1 min of denaturation at 94 ^{o}C, 1 min of annealing at 55 ^{o}C, and 5 min of extension at 72 ^{o}C for a total of 35 cycles was used. Twenty microlitre reaction mixture of Bioneer Accupower PCR premix kit (Bioneer Corporation, Daejoan, S. Korea) gave the result by using the above mentioned thermal condition, PCR specific primers and template DNA. Amplification products were separated on 1.5% agarose gels prepared in TBE buffer and visualized using ultraviolet light after staining with ethidium bromide.

4. Cloning and sequencing of the PCR amplicons

Amplified integron and beta-lactamse gene PCR fragments were cloned into pGEM-Teasy (Promega Corp., Madison Wi, USA) according to the manufacturer's instructions. Recombinant plasmids were introduced into *Escherichia coli* DH5α competent cells and selected on Luria–Bertani (Difco Laboratories) agar plates containing ampicillin (50 mg/L). Individual white colonies were screened for inserts by inoculating into 100 ul of water, boiling for 10 min, clearing by centrifugation, and by using 1 uil of supernatant in a PCR with primers specific for that PCR. Sizes of inserts were assessed by agarose gel electrophoresis, and colonies representative of each size class were used to prepare plasmids either with the QIAquick Qiagen Plasmid extraction kit (Qiagen, Germany) or Solgent Plasmid DNA purification kit (Solgent, S. Korea). In some cases direct sequencing was performed after purification of the PCR product using the QIAquick PCR purification kit (Qiagen Co. Valencia, CA, USA). Sequencing of cloned plasmid was done with ABI Prism 3730 DNA analyzer capillary Big dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Applied Biosystems, Roissy, France. For cloned DNA primer used were from the pGEM-T easy vector (Promega Corp. , Madison Wi)) binding the forward and reverse site by ChDc-GEM –F and CHDC _GEM –R designed by the Solgent Lab., Daejoan, S. Korea). The obtained sequences were analyzed using the SeqMan (Version 5.0.0 DNASTAR, inc., Madison, WI, USA) program and NCBI BLAST (*http://www.ncbi.nih.gov/BLAST*) program.

DNA sequences were edited and connected by using DNATAR software (Version 5.0.0 DNASTAR, inc., Madison, WI, USA). Contigs were joined. To confirm that the two independent PCR products connected during this process accurately represented the genomic DNA, primers were designed to amplify the majority of the sequenced region for each isolates. These regions were then amplified as described above.

N. DNA DIG-labeling, dot-blot hybridization and detection

To detect the specific genes in the genomic DNA of all outbreak case, control and environmental isolates DNA probe hybridization was done. For this purpose DIG- High prime DNA Labeling and detection Starter Kit II (Roche Diagnostics GmbH, Germany) was used and the method was followed as mentioned in the 'Instruction for Random primed labeling with DIG-oxigenin-dUTP, alkali –labile and chemiluminescence detection with CSPD, ready –to-use kit', (Cat no. 11 585 614 910) manual version October 2004. (Roche, Germany). The probe prepared was bla--2.

1. DIG labeling of DNA for probe preparation

To make the probe *intI1* and bla-OXA-2 PCR product was gel purified with Solgent gel purification kit. The DNA quantification was done comparing with the intensity of DNA marker run in the same gel and the amount of probe DNA was calculated. One microgram of gel purified PCR product as template DNA for each probe making template DNA were taken and suspended in 16 ul DDAW in a 1.5 ml Eppendorf tube. Then the template DNA was denatured by heating in a boiling water bath for 10 min and then by quickly chilling in an ice bath. Then 4 ul of DIG-High Prime was added, mixed thoroughly and briefly centrifuged and incubated for 20h at 37 ^{o}C. The reaction was terminated by adding 2 ul 0.2 EDTA and heating the reaction tube to 65 ^{o}C for 10 minutes.

2. Detection of labeling efficiency

Determination of yield of labeling efficiency is most important for optimal and

reproducible results. The direct detection method was applied for the determination of labeling efficiency as recommended by the manufacturer. A serials of dilutions of 1ng/ul, 10pg/u, 3pg/ul, 1pg/ul, 0.3pg/ul, 0.1pg/ul, 0.003 pg/ul, 0.01pg/ul and 0 dilutions of DIG-labeled DNA probe was prepared and 1ul spot from each dilutions were made and the same dilutions of control DNA provided by the manufacturer was also loaded side by side on a strip of the Hybond-N+ nylon membrane. Then the nucleic acid was fixed by cross linking with UV light (500 mJ/cm2 energy) for 5 min using UV cross-linker (UVP) for I minute. Then the membrane was transferred into a plastic container with 20 ml Maleic acid buffer (0.1M Maleic acid, 0.15 M NaCl, pH 7.5 with NaOH at 20 ^{o}C) and incubated for 2 min at constant shaking at 22 ^{o}C. Then the membrane was transferred and incubated for 30 minutes in 10 ml blocking solution provided by the manufacturer at 42 ^{o}C. Then the membrane was transferred into 10 ml antibody solution (anti-digoxigenin-AP, provided in the kit) and incubated for 30 minutes at 420C. The unbound antibody was then washed with 10 ml washing buffer (0.1M Maleic acid, 0.15 M NaCl, pH 7.5 with NaOH at 20 oC; 0.3% Tween 20) for 2 times for 15 minutes at 42 0C. Then the membrane was equilibrated in 10 ml detection buffer (0.1M Tris-HCl, 0.1 M NaCl, pH 9.5 at 20 ^{o}C) for 5 minutes.

The membrane was then placed with DNA side facing up on a development folder (plastic folder) and 0.1 ml CSPD ready-to-use solution was dispensed on the to the membrane evenly and immediately covered the membrane by the upper sheet of the development folder and gently squeezed to evenly distribute the CSPD solution as well as to wipe out the air bubbles from the membrane and folder interface. Then the CSPD treatment was incubated for 5 minutes at 220C. The excess of CSPD solution was then squeezed out and the folder was sealed to prevent from drying. The CSPD damped membrane was incubated then for 10 min at 37 ^{o}C to enhance the luminescent reaction. Then the DIG labeled and antibody bounded DNA probe loaded nylon membrane was exposed to X-ray film (Fuji film, Japan) for 2 hrs, 12 hrs and 24 hrs at 20 ^{o}C and the blot result was compared and appropriate amount of probe concentration was determined for dot blot hybridization.

3. Dot-blotting of genomic DNA

Dot-blotting of the genomic DNA of A. baumannii on a 12 cm X 8cm nylon membrane (Hybond-N+, Amersham Pharmacia Biotech Ltd, England) was carried out manually. The genomic DNA was prepared by incubating the DNA in 1.5 ml Eppendhorf tube in boiling water bath for 10 minutes and immediately chilling on ice. The membrane was marked with appropriate sized circle with pencil and one microlitre of proteinase-K extracted or 2 ul of water lysate or txte (Tris-EDTA-Triton-X solution) genomic DNA was dispensed on the circle of membrane as a blot. Then the DNA blotted membrane was first room temperature air dried and make wet with 10X SSC placed facing the DNA blotted surface up on Whatman 3MM-paper soaked with10X SSC (3M NaCl, 0.3 M sodium citrate, pH 7.0 at +20 ^{o}C) for 10 minutes. Then the blotted membrane was subjected to UV cross-linking (500 mJ/cm2 energy) for 5 min using UV cross-linker (UVP). After the UV-crosslinking, the membrane was rinsed briefly in double distilled autoclaved water (DDAW) and allowed to air dried at room temperature. The sample layout is outline in **Figure-4.**

4. Immunological detection

The DNA blotted nylon membrane was prehybridized in 10 ml of 42 ^{o}C preheated DIG-easy Hyb solution (DIG High Prime Kit, Roche) for 30 minute with gentle agitation in freely moving condition in an appropriate container in the Amersham Pharmacia Hybridization Chamber. The DIG-labeled DNA probes (25ng/ml DIG-easy Hyb solution) was prepared by denaturing by heating in a boiling water bath for 5 min and rapidly cooling in an ice bath and added the denatured DIG-labeled DNA probe to pre-heated DIG easy Hyb (3.5ml/96 cm2 membrane and well mixed without forming the foam). Then the prehybridization solution was poured off and probe/hybridization mixture was added to the membrane and incubated for overnight with gentle agitation at 42 ^{o}C in the hybridization chamber. After hybridization two stringency washes were carried out. The first wash was done 2 times for 5 minutes each with 2X SSC, 0.1% SDS at 25 ^{o}C under constant agitation.

The second washing was carried out also for 2 times for 15 minutes with 68 ^{o}C prewarmed 0.5X SSC, 0.1% SDS in the hybridization chamber with constant agitation.

After hybridization and stringency washes the membranes were rinsed briefly with (5 min) in washing buffer Then the membrane was transferred and incubated for 30 minutes in 100 ml **blocking solution** (provided by the manufacturer, Roche, DIG High Prime kit) at 42 ^{0}C. Then the membrane was transferred into 20 ml antibody solution (anti-digoxigenin-AP, provided in the kit) and incubated for 30 minutes at 42 ^{0}C. The unbound antibody was then washed with 100 ml **washing buffer** (0.1M Maleic acid, 0.15 M NaCl, pH 7.5 with NaOH at 20 ^{0}C; 0.3% Tween 20) for 2 times for 15 minutes at 42 ^{0}C. Then the membrane was equilibrated in 20 ml **detection buffer** (0.1M Tris-HCl, 0.1 M NaCl, pH 9.5 at 20 ^{0}C) for 5 minutes. The membrane was then placed with DNA side facing up on a development folder (plastic folder) and 1 ml **CSPD ready-to-use** solution was dispensed on the to the membrane evenly and immediately covered the membrane by the upper sheet of the development folder and gently squeezed to evenly distribute the CSPD solution as well as to wipe out the air bubbles from the membrane and folder interface. Then the CSPD treatment was incubated for 5 minutes at 22 ^{0}C. The excess of CSPD solution was then squeezed out and the folder was sealed to prevent from drying. The CSPD damped membrane was incubated then for 10 min at 37 ^{0}C to enhance the luminescent reaction. Then the DIG labeled and antibody bounded DNA probe loaded nylon membrane was exposed to X-ray film (Fuji film, Japan) for 2 hrs, 12 hrs and 24 hrs at 20 ^{0}C and the blot result recorded.

III. RESULTS

A. Description of the outbreak

The susceptibility of Gram-negative bacilli to imipenem and other antibiotics has been routinely tested out at CHU. Before the epidemic, the microbiology laboratory of CHU has detected *A. baumannii* in its basal level with decreased susceptibility to imipenem. From January 2004 to December 2004 the number of patients increased who were either infected or colonized with imipenem resistant *A. baumannii* (**Fig.1**). The source of first isolation was the sputum (1 case in February) and other subsequent sputum isolates were from the months of April, May, August, September, October and November of 2004. Other isolations were from close pus, catheterized urine and other sites which were not recorded in the medical microbiology records (**Table-1**).

B. Imipenem resistance in antibiogram of clinical isolates

All the 30 clinical isolates taken for present study of the epidemic period were resistant for imipenem test (IRAB) (**Table-1**). Imipenem susceptible isolates collected during the same time period were taken as control bacteria (ISAB).

C. Result of susceptibility test of environmental *A. baumannii*

All the environmental samples tested were resistant for imipenem (zone diameter 12 and 13 mm, MICs 32 to 64 µg /ml). No inhibition zone was recorded for *A. baumannii* against piperacillin, netilmicin, gentamicin, ciprofloxacin, aztreonam, tobramycin and ticacillin-clabulanic acid antibiotics tested showing the resistance for the tested antibiotics (Table-9).

D. IRAB in environmental samples

The environmental isolates were collected from different sites from the hospital

environment **Table-6**. A total of 124 *A. baumannii* isolates including 88 from inanimate surfaces of the ICUs and 36 from the hands of 18 health care workers isolates were recovered from the environmental sampling. They were tested for imipenem resistance following the NCCLS guidelines (NCCLS, 2001) guidelines and designated as E-IRAB for the resistant isolates for present study purpose **(Table-9)**. When the health care workers hands (left and right) were sampled 1 isolate of E-IRAB was recovered from 18 left hand samplings and 1 from 18 right hand samplings. When 10 beds were surveyed 7 E-IRAB were found. The hospital record chart and recording sheet sampling gave no isolates, but when 6 EKG (echocardiogram machine) sampled 1 of them gave 1 E-IRAB isolate. When 6 DP set containing suction kits were sampled 4 E-IRAB isolates were recovered. Six tracheostomy sites gave 3 E-IRAB isolates. One IV fluid line gave 1 E-IRAB isolate but two O2 supplier machine samplings gave no isolates. When, 13 ventilators were screened they gave 7 E-IRAB isolates. Two washing stands were negative for the bacteria sampled. Four out of four waste water disposal sink surface sampling gave 4 E-IRAB isolates positive for E-IRAB. When 11 station tables were surveyed they gave 2 E-IRAB isolates and nine dressing car survey gave 2 E-IRAB isolates. When 6 DP sets containing suction kits were surveyed they gave 4 E-IRAB isolates for E-IRAB positive. Three computers were screened and they were negative for the isolates. Two door handles, 2 canisters, 2 suction pump, and one humidifier so far sampled all of them were negative for the *A. baumannii* isolates.

When 36 samplings from 18 HCWs both right and left hands were sampled 1 isolate of E-IRAB was recovered from 18 left hand samplings and 1 E-IRAB recovered from 18 right hand samplings (**Table-6 and 9**).

E. Risk factor analysis

Data were collected from the administrative and microbiology laboratory computerized databases for the risk factor analysis. During the study period 77 patients with IRAB (case group 1) were identified and 77 control group patients were selected. IRAB was mostly isolated from sputum, and others were from close and

open pus, catheterized urine.

Results of the univariate analyses of risk factors for IRAB are shown in **Table-7**. The associated factors with occurrence of IRAB in univariate analyses were comorbidity such as having cardiac diseases or having pulmonary disease, the presence of medical instrument such as bladder catheter, mechanical ventilation or intubation, prior exposure to antimicrobial drug such as first-generation cephalosporins or third-generations cephalosporins and duration of drug use prior to occurrence of IRAB.

The results of multivariate analysis of risk factors for IRAB are outlined in **Table-8**. The risk factors which were identified by multiple logistic regression analysis were time at risk (OR, 1.29; 95% CI, 1.14-1.46; p-value, 0.000), and patient having mechanical ventilation (OR, 6.41; 95% CI, 1.71-24.06; p-value, 0.006). However, comorbidity, presence of bladder catheter, intubation and prior exposure to third-generation cephalosporin was not statistically significant with occurrence of IRAB in this study.

F. Response to infection control intervention program

The incidence rate of new patients with clinical samples positive for IRAB reached its peak in April 2004 with 12 IRAB-positive new patients. The implementation of the multicomponent intervention in late July resulted in a sharp reduction in the incidence rate of new *A. baumannii* infection or colonization of IRAB, E-IRAB or ISAB to the patients.

G. Pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis (PFGE) analysis was carried out as primary typing tool for the epidemiological study. For present investigation Apa*I* restriction enzyme digested PFGE was carried out **(Figure-5)**. A total of 47 isolates including imipenem resistant (IRAB) and susceptible clinical isolates (ISAB) and imipenem resistant environmental isolates (E-IRAB) were analysed for PFGE. Then computer-

assisted analysis of the fingerprints was performed using Bionumerics software (Version 4.5; Applied Maths, Kortrijk, Belgium) and a dendrogram was obtained **(Figure-6).**

Of the 47 isolates typed by PFGE 35 isolates of *A. baumannii* were grouped into A type while remaining 12 isolates were distributed in other types showing sporadic nature. Of the total 30 IRAB isolates type A was represented by 27 isolates showing 90 percent. Other remaining isolates from IRAB were distributed 1 in type C, 1 in type G and 1 in type J of the total types A to J (10 types). Analyzing a total of 8 imipenem susceptible *A. baumannii* (ISAB) non outbreak control isolates there was found 1 ISAB isolate in the type A group and others were type B, D, E, F, G and J representing each isolate for each of the types and type H was represented by two isolates. For the total 8 E-IRAB isolates typed majority of them, 7 of 8 (87.5 percent) were present in type A and I isolate was present in type J sharing the similarity with an ISAB isolate **(Table-9).**

H. Modified Hodge test (MHT) and DDST

A total of 47 isolates were taken for Modified Hodge Test (MHT) and imipenem-Zn EDTA double synergy test (DDST). Imipenem resistant isolates were 100 % (30 of 30) positive for IMP- Zn MHT and 19.35 % (6/30) positive EDTA-DDST. A total of 9 ISAB were tested for MHT and all of them were negative for the test. Since MHT negative have no meaning for further DDST further test was no done for DDST. Eight E-IRAB isolates were tested for MHT and DDST and for MHT and all were positive. Only 1 isolate was positive for DDST for eight E-IRAB tested. The data is presented in **Table -10.**

I. Integron study

1. Detection of class 1 integron

The template DNA preparation for the integron PCR was as mentioned above in proteinase-K extraction method which is applied to capture the possible antibiotics

resistance gene(s) inserted in the integron as described by Lévesque et al (1995). The integron gene cassette PCR amplicons were subjected for sequencing and further BLAST search. A gel electrophoresis and PCR product stained with ethidium bromide is shown in **Figure-7.**

2. Integrase gene PCR

Integrase genes *intI1, intI2, intI3* were assayed for the detection of classes 1, 2 and 3 integrons **(Figure-8 and 9; Table-11 and 12)**. Present study showed integrase class 1 (*intI1*) gene in most of the imipenem resistant outbreak, environmental and imipenem susceptible isolates**.** Integrase class 2 gene PCR showed 16 positive (34.4%) in total 47 tested isolates of which IRAB has shown 11/30 (36.66%), ISAB 2/9 (22.00%), and 3/8 (37.5%) was shown by environmental isolates (E-IRAB). Class 3 integrase PCR was negative for all the tested strains. The present integrase PCR study revealed that integrase gene inti1 is prevalent among the *A. baumannii* strains while class 2 integrase is sporadic.

3. Thermal asymmetric interlaced PCR (Tail PCR)

Tail PCR sequence analysis gave the bla-2 gene positive for the sequenced DNA obtained from genomic DNA.

J. Beta-lactamase and carbapenemase specific gene PCR

Although present imipenem resistant apparent outbreak was phentotypically related with imipenem resistance as data obtained from antibiogram and MHT and a higher rates of negative result for DDST for the beta-lactamases molecular study based on beta-lactamase and carbapenemase specific PCR were carried out **(Table-13, 14 and 15).**

When PCR screening was done for beta lactamases IMP-1, IMP-3, IMP-6, IMP-10 only one isolate was positive for the test showing the presence of bla-IMP-1. For the test of beta lactamase IMP-2, IMP-8, IMP-10, IMP-12, IMP-13 five out of 30

samples were positive (16.66 %). For the beta lactamase test of IMP-4, IMP-5, IMP-7, and IMP-9 tested for thirty samples two gave positive result (6.66 %). Beta-lactamase test of IMP-11, IMP-12, IMP-18, IMP-19, IMP-20 and IMP-21 gave negative results for all the 30 tested isolates. Beta lactamase tested for 30 isolates for the genes VIM-1, VIM-4, VIM-5, VIM-7, VIM-11 gave 1 positive result (3.33 %). Likewise for other betalactamase genes VIM-2, VIM-3, VIM-6, VIM-8, VIM-9, VIM-10 tested by PCR only 1 isolate gave positive result. Other betalactamases tested were SPM-1, GES-1, GES-2, GES-3, GES-4, IBC-1,IBC-2 and GIM and all of them were negative for the present studied isolates.

K. Probe hybridization and dot blot

Only 2 out of 30 IRAB and 1 out of 9 ISAB were positive and remaining IRAB and ISAB and E-IRAB were negative for bla--2 gene in the blotting result **(Figure-10).**

L. DNA sequence analysis of PCR products

For the detection of possible gene involved in the present *A. baumannii* isolates beta-lactamase gene specific PCR were done and the fragment were sent for sequencing and data were BLAST (http://www.ncbi.nlm.nih.gov/) searched. The obtained gene from betalactam specific PCR were cloned in *E. coli* α using pGEM-T easy vector and the plasmids obtained were sequenced. The obtained sequence from the PCR products of *A. baumannii* isolate 114 showed the presence of bla-vim-2 and -2 in the *A. baumannii* isolate 115.

The beta-lactamase gene blaVIM-2 was found in only one isolate (Acib 114) from the sequenced and blaVIM-2 specific PCR result. Another recorded gene cassettes from sequencing was *bla*--2 in the Acib 115 when the integron sequence was extended by Tail PCR (Yao-Guang Liu and Ning Huang, 1995) but could not be detected in more than four isolates of the detected 30 isolates when probe hybridized with the -2 primer applying DIG High prime kit.

IV. DISCUSSION

From January 2004 to December 2004 there had happened an imipenem resistant *A. baumannii* apparent outbreak in the MICU and SICU of Chosun University Hospital. *A. baumannii* strains notoriously cause hospital outbreaks, and a few lineages achieve epidemic status, reaching multiple hospitals or countries (Coelho 2006). By convention, these are termed clones rather than strains when their relatedness is inferred on the basis of DNA profiles, without proven chains of site-to-site transmission (Coelho 2006). *Acinetobacter baumannii* now emerged as a primary nosocomial pathogen in hospital outbreaks, and is ranked second after *Pseudomonas aeruginosa* among nosocomial aerobic nonfermentative gram-negative acillar pathogens (Jeong et al 2006, Schreckenberger and Graevenitz, 1999; Simor et al., 2002. An outbreak is defined as an increase in the number of infections caused by a particular pathogen above baseline levels (Zaza and Jarvios, 1996). *Acinetobacter baumannii* strains may vary in their epidemiologic potential, and those known to spread widely and rapidly among hospitalized patients have been designated epidemic *A. baumannii* strains (Koeleman et al., 2001). Although a number of bacterial factors may contribute to epidemic behavior (Jawad et al 1996 and Koeleman et al 2001a), antimicrobial resistance has been identified as the major risk factor (Koeleman et al., 2001a). The initial concern about carbapenem-resistant *A. baumannii* infections arose when the first hospital-wide outbreak occurred in New York City in 1991 (Go et al., 1994), and currently they are reported worldwide (Corbella et al 2000 and Takahashi et al 2000).

Carbapenems are often used as antibiotics of last resort for treating infections due to multidrug-resistant gram-negative bacilli, because they are stable even in response to extended-spectrum and AmpC β-lactamases. However, gram-negative bacilli producing the acquired metallo-β-lactamases (MBLs) IMP and VIM have been increasingly reported in Asia and Europe (Chu et al 1998, Iyobe et al 2000, Lee et al 2002), and they have also been detected in Canada (Livermore 2000) and the United States (Tolman et al 2002). The MBLs efficiently hydrolyze all β-lactams, except for aztreonam, in vitro. Therefore, detection of MBL-producing gram-

negative bacilli is crucial for the optimal treatment of patients and to control the spread of resistance (Richet, 2001). However, NCCLS documents (NCCLS 2001) do not yet contain a method for detection of MBL-producing isolates. Lee et al. (2002) have reported that the Hodge test can be used to screen carbapenemase-producing gram-negative bacilli and that the imipenem (IPM)-EDTA double-disk synergy test (DDST) can distinguish MBL-producing from MBL-non-producing gram-negative bacilli. Thus an endeavor had been made to apply this method to study the present imipenem resistant epidemic.

The aim of Modified Hodge Test (MHT) and IMP EDTA-DDST study was thus centered for the screening of metallo-β-lactamase-producing strains from a large number of imipenem-resistant clinical isolates of *Acinetobacter baumannii* (Lee et al 2003). Hodge et al (1978) developed a test to detect penicillinase-producing *Neisseria gonorrhoeae* and other species of bacteria. A double disk synergy test using β-lactam and β-lactamase-inhibitor disks is a convenient method of detecting extended-spectrum β-lactamase-producing Gram-negative bacilli. EDTA inhibition of β-lactamase activity is used to differentiate a metallo-β-lactamase from other β-lactamases. A total of 47 isolates were taken for Modified Hodge Test (MHT) and imipenem EDTA double synergy test (DDST). Imipenem resistant isolates were 100 % (30 of 30) positive for MHT and 19.35 % (31/6) positive for DDST. A total of 9 ISAB were tested for MHT and all of them were negative for the test. Since MHT negative have no meaning for further DDST further test was no done for DDST. Eight E-IRAB were tested for MHT and DDST and for MHT and all were positive. Only 1 isolate was positive for DDST eight E-IRAB. Present result shows the presence of metallo-β-lactamase but negative for DDST test implying that there is possibility of other unknown betalactamases including carbapenemase genes responsible for this phenotypic imipenem resistance.

Since the DDST did not gave high positive result for the metallo-betalactamases or carbapenemase for the outbreak isolates other different betalactamase gene specific PCRs were carried out. Of the PCR screened carbapenemase bla-IMP gene was found in 5 out of 47 isolates while bla-VIM was detected in only one isolate out of 47 isolates. Bla-51 was present in all of the isolates but bla--2 was detected in 3

isolates, 2 from IRAB and 1 from ISAB. Other carbapenemase PCR SPM, GES, GIM, bla-23, bla--24 and bla--58 were negative to all the screened isolates. Carbapenemase bla--2 was revealed from the Tail PCR and sequencing analysis in the Acib114 isolate and later when it is dot blotted against the genomic DNA the gene was found in two IRAB isolates Acib 113 and Acib 114 and two ISAB isolates Acib 214 out of 47 present sample and a total of 91samples including the 47 present samples so far DIG-labeled probe hybridization blotted. There may be other novel carbapenemase genes or playing the role by agents for such imipenem resistance for which isoelectric focusing based research is needed in further to go ahead foe the detection of likely genes.

There are several reports for carbapenemase based research in these days for Gram-negative bacteria and particularly for *A. baumannii*. Despite the stability of carbapenems to common class D enzymes, carbapenem resistance in *Acinetobacter* isolates has repeatedly been associated with enzymes with strong oxacillinase activity (Livermore and Woodfortd, 2000). Several of these oxacillinases from carbapenem-resistant acinetobacters have now been sequenced and confirmed as class D enzymes. These include bla-OXA-23 (Donald, 2000), from an isolate collected in Scotland in 1985, bla-OXA -24 (Bou et al., 2000) and bla-OXA 25 from Spain, bla-OXA -26 from Belgium, and bla-OXA -27 from Singapore (Livermore and Woodfortd, Afzal-Shah 1999). Further, unsequenced OXA enzymes with carbapenemase activity are known from acinetobacters collected in Argentina (Afzal-Shah 1999and Brown, 1998), Brazil, France (Hornstein 1997) and Kuwait (Bou, 2000).

Bla-OXA -51 and -OXA-58 are the most recent discoveries (Coelho 2006). bla-OXA -51-like enzymes have carbapenemase activity in vitro (Brown 2005a and 2005b) but are very widespread in *A. baumannii*, including carbapenem-susceptible strains (Heritier et al, 2005, Woodford, in press). They may confer resistance only in particular circumstances, which have yet to be defined fully. Present IRAB and ISAB isolates all are positive for Oxa-51 like enzymes showing their intrinsic behavior in *A. baumannii*. OXA-58 is much less common and, like OXA-23 and OXA-24, is more consistently associated with non-susceptibility, as also confirmed by transfer and

inactivation studies (Heiter et al 2005a, and Heiter et al 2005b). Present strains when tested for Oxa-58 were negative and the positive isolates when checked for its conformity with the PCR product cloning in *E. coli* using pGEM T-easy vector and sequence analysed by BLAST search (*http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi*) it gave the sequence similarity with Oxa-51. Thus present outbreak is devoid of OXA-58 carbapenemase. The OXA-2 is the commonest representative of molecular class D beta-lactamase accounting for its oxacillinase activity but lacks carbapenemase activity (Livermore and Woodford, 2000, Livermore, 1995). Present 2 IRAB isolates and 1 ISAB isolate gave positive rate for -2 and sequence result with BLAST search confirmed it.

All the IMP enzymes have broad activity against β-lactams except monobactams. Apparent kinetic differences may partly reflect assay differences (Watanabe et al 1991 and Osano et al 1994, Laraki et al 1999). Correlation between carriage of bla_{IMP} alleles and of carbapenem resistance is imperfect, and some bla_{IMP} hosts appear susceptible (Senda et al 1996]. Two explanations are possible for this reason: either bla_{IMP} is not always expressed, or substantive resistance may demand reduced uptake of the carbapenem as well as the presence of β-lactamase enzyme (Livermore and Woodford, 2000). This latter model certainly applies for some isolates (Minami et al 1996) and it is notable that cloned IMP-1 enzyme gives only low-level resistance to carbapenems in *Escherichia coli*. The low level of present outbreak strains for their expression of carbapenemase gene in polymerase chain reaction should be understand in the above mentioned facts (Livermore and Woodfortd, 2000, Minami et al, 1996, Minami et al 1996).

The second family of acquired class B β-lactamases the VIM types was first reported in 1999, with the description of VIM-1 from a *P. aeruginosa* isolate collected in 1997 in Verona (Italy) (Lauretti, 1999). It subsequently became apparent that an outbreak of a serotype O12 *P. aeruginosa* strain with an unsequenced bla_{VIM} variant had been ongoing in Thessaloniki (Greece) since 1996. Over 200 producer isolates were obtained, with their prevalence declining after a reduction in imipenem usage in 1998 (Tsakris 2000). A second VIM variant, VIM-2, was described from *P. aeruginosa* isolate collected in France in 1996 (Poirel, 2000). VIM-2 has 90% amino

acid homology with VIM-1 (Livermore and Woodford, 2000). In addition, it is awful for *P. aeruginosa* strains with unsequenced VIM-related enzymes from South Korea and Oman (Livermore and Woodford, 2000, Livermore). The recognition, within four years, of VIM-producing *P. aeruginosa* strains from three extremities of Eurasia (France, Korea and Oman) poses fascinating evolutionary questions (Livermore and Woodford, 2000). Present outbreak which did not expressed VIM-related enzymes or other carbapenemases except having positive result for only one IRAB isolate 114 have posed a question to address the present outbreak

A major contributing factor in the emergence of resistance of this species is the acquisition and transfer of antibiotic resistance genes on plasmids, transposons and integrons (Gombac et al 2002, Nemec et al 2004, Ploy et al 2000 and Vila et al, 1997). Integron are genetic makeup consisting of a gene encoding an integrase flanked by an *attI* recombination site, where mobile gene cassettes, often comprising antibiotic resistance genes reside. Such gene cassettes can be inserted or excised through a site-specific recombination system called 59 base element (59-be) (Recchia et al 1995 and Recchia et al 1994). To develop an alternative but efficient screening method for the outbreak study in conjunction with antibiotics resistance mechanism integron study had been chosen due to its salient feature of encompassing diverse gene cassettes and efficient gene diffusion mechanism as mentioned by various authors (Recchia et al 1995 and Recchia et al 1994, Levesque et al 1995, Stokes, 2001, Gillings et al 2005, Torton et al 2005 and 2006). Thus integrase PCR was taken as a signature gene PCR for screening different classes of integrons as suggested by various authors (Koeleman et al, 2001, J Clin Microbiol. 1996; 34: 2909–2913). Epidemic strains of *A. baumannii* are containing significantly more integrons than non-epidemic strains (Koelman 2001 and Turton 2005). Thus present outbreak and non-outbreak isolates were tested for integrase gene PCR for the epidemic behavior of the isolates and integron PCR to detect the antibiotic resistant gene cassettes resided in the integron. No such carbapenemase resistant gene cassettes in the integron except blaVIM-2 in one isolate and d bla-2 in three isolates were revealed from the sequenced data and PCR assays. The integrase gene *intI1* for the integron class 1 was present in all of the tested isolates but *intI2* gene, an indicator for class 2

integron was found sporadically in the IRAB, E-IRAB and ISAB isolates. Present *A. baumannii* has shown epidemic behavior due to the presence of class 1 integrase gene *intI1* (Koelman et al, 2001 and Turton et al, 2005).

Acinetobacter baumannii is a pathogen that has been associated with hospital outbreaks worldwide. Understanding the fundamental mechanisms underlying *Acinetobacter* infections, including the original sources of the infecting organisms, their clonality, and geographical spread is an important requirement for the development of appropriate infection control measures (Ecker et al, 2006). Genotyping allows investigation of clonal spread and can be used to identify the source of the original infection (Ecker et al, 2006). *Acinetobacter* strain typing methods include serotyping (Traub 1989), multilocus enzyme electrophoresis (Seltmann et al , 1995), and DNA-based methods, including repetitive extragenic palindromic sequence-based PCR (Buxton et al, 1978 and Huys et al, 2005), amplified ribosomal DNA restriction analysis (ARDRA), pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) (Vaneechoutte et al, 1995), and ribotyping (Ibrahim et al, 1997, Koeleman et al, 1998 and Misbah et al, 2005) and MLST (Ecker 2006, and Bartual 2005). As part of the investigation to identify the possible source(s) of this outbreak, to determine the likely mode(s) of transmission, and to assist in generating and implementing infection control countermeasures, PFGE based typing method and conventional outbreak control measures were set out. For present investigation PFGE was chosen as the typing method for it is taken as gold standard in molecular epidemiological technique (Seifert and Gerner –Smidt, 1995).

One of the major concerns of present work is to determine the course or cause of the outbreak and formulate the control strategy. Thus present investigation was carried out using established molecular technique and epidemiology based case control study. Various genotypic methods have been developed for the typing of *Acinetobacter* species, including serotyping (Traub 1989), multilocus enzyme electrophoresis (Seltmann et al, 1995), ribotyping (Ibrahim et al, 1997, Koeleman et al, 1998 and Misbah et al, 2005), repetitive extragenic palindromic sequence-based PCR (Buxton et al, 1978 and Huys et al, 2005), amplified ribosomal DNA restriction

analysis (ARDRA), macro-restriction analysis by PFGE (Vaneechoutte et al, 1995), randomly amplified polymorphic DNA (RAPD) analysis, and total genomic fingerprinting by AFLP (amplified fragment length polymorphism analysis) (Vaneechoutte et al, 1995) and MLST (Ecker 2006, and Bartual 2005). Among these, PFGE is regarded as the "gold standard" for epidemiological typing (Seifert and Gerner –Smidt, 1995) and useful for molecular typing of *A. baumannii* (Ayan 2003). For present epidemiological study PFGE typing has been taken as the primary typing method.

Computer-assisted analysis of the PFGE fingerprints was performed and a dendrogram was obtained (Fig. 5). It grouped the outbreak into major type A covering 35 isolates out of 47 isolates and other sporadic types were B, C, D, E, F, G and H. When the PFGE data was checked with the date of isolation majority of the type A was found from those strains isolated from the major apparent outbreak peak period. The type A strains were mostly occurred from the sputum samples and from the same or similar isolation dates from different months of the year 2004. Thus it can be speculate from the hospital record data and PFGE that the IRAB epidemic had happened from the clonal strains of early days of January 2004.

The *Acinetobacter baumannii* infection that was observed at the university hospital was occurred in patients with a long treatment and stay in hospital environment. Patients tended to have risk factors of presence of medical instrument such as mechanical ventilation (p-value, 0.006) and time at risk (p-value, 0.000). Epidemiological investigations often focus on identifying a possible common environmental source. In many cases, the rise in rates of a particular pathogen are due to lapses in infection control measures, resulting in an increase in cross-transmission between patients and HCW's hands (Hartstein et al 1988, Buisson et al, 1990, Getchell-White et al 1989, D'Agata et al 1999, D'Agata et al 1999a). These lapses may be particularly remain relevant for *A baumannii,* because dissemination of this pathogen is facilitated by its prolonged survival on inanimate surfaces, high colonization rates among hospitalized patients, and frequent contamination of healthcare workers' hands (Hartstein et al 1988, Buisson, 1990, Getchell-White et al 1989, D'Agata et al 1999, D'Agata et al 1999a). The results of case-control study

underscore the opportunistic nature of this organism. The patients who acquired *A. baumannii* were more likely to have received third generation cephalosporins, a class of broad spectrum antibiotics that could have selected for this organism, which is intrinsically resistant to a large number of antibiotics (Scerpella 1995). They also were to have longer ICU stays and to have undergone invasive procedures such as intubations and catheter use. The most significant fact related with thecase-control study is the ventilator used by the patients and the occurrence of IRAB PFGE type A clone in the ventilators during the surveillance culture study and same PFGE type A clone-strain was isolated from the patients in the outbreak period.

Numerous outbreaks of nosocomial infections due to *Acinetobacter baumannii* have been described in previous reports (Erika et al 2000, Kaul et al 1996, Patterson et al, 1991, Hartstein et al, 1988; Buisson et al, 1990; Castle et al, 1978; and Mortensen et al, 1987). When these outbreaks were analysed risk factors associated with those infections included were antibiotic exposure, length of stay in intensive care units (ICUs), mechanical ventilation, and severity of underlying illness (Erika et al 2000; Kaul et al, 1996; Patterson et al, 1991; Hartstein et al, 1988; Buisson et al, 1990; Castle et al, 1978; Mortensen 1987, Scerpella 1995). Such outbreaks had been found associated with contaminated respiratory equipment, air humidifiers, mattresses, and pillows (Hartstein et al 1988, Cefai et al 1990, Smith et al 1977, Sherertz et al, 1985 and Weernink et al 1995).

Acinetobacter baumannii is known for its ability to survive for long periods in dry surfaces (Wendt et al, 1997), and outbreaks in ICUs mediated through environmental contaminations by this organism have been reported by several authors (Jawad et al 1996, Melamed et al 2003). *A. baumannii* is capable of rapidly adapting to the hospital environment, and outbreaks may result from intrinsic contamination of the medical equipment or devices used with patients for monitoring or therapy and/or from contamination of the environment (Lee et al 2004 and Brooks et al, 1996). Contamination may also result via the airborne route as well as from contact with patients (Brooks et al, 1996 and, Bergogne-Bérézin and Towner, 2000). The environmental sampling for *A. baumannii* and the imipenem resistant nature of the environmental isolates with PFGE type A outbreak may have crucial contribution

for the present outbreak supported by the E-IRAB strain positive hands of HCWs and the type A PFGE pattern obtained from the *A. baumannii* isolated from them. Thus cross contamination among the inanimate surfaces like patients' hospital equipments like ventilators and other associated environmental objects like bed sheets, hospital record sheets and waste water disposal sink surface and contaminated hands of HCWs handling these objects and patients should be speculated as significant contributing factors for the present outbreak.

Epidemic strain spread may be explained by their ability to survive within the nosocomial environment, and to colonize patients and hospital personnel, in addition to selective antibiotic pressure (Jawad et al 1996 and Koeleman et al 2001a). Many investigators have isolated *Acinetobacter baumannii* from the hospital environment and from the hands of health care workers (Ayan 2003,). It is assumed that the pathogen initially introduces via these routes and then spreads through the ICUs (Villers, 1998). Transmission of *A. baumannii* by contaminated hands and gloves has been suggested in previous reports (Scerpella 1995). In present environment surveillance culture E-IRAB were recorded from different hospital equipments and the hands of health care workers. All isolates were PFGE type A clone except one isolate and the outbreak clone is leaded by type A clone supported by the previous statement.

Acinetobacter epidemics tend to rise worldwide in the late summer months (Corbella et al 2000) but it is a surprising epidemic behavior of the imipenem resistant *A. baumannii* in CHU ICUs that the trend of increase of *A. baumannii* epidemic was in its brisk rise during the winter months of January-February and in its peak in the late winter (March) to mid-spring of 2004. After applying the intervention measures including strict hand washing, health hygiene, decontamination of floors and associated objects followed by surveillance culture in the July 2004 it reduces the IRAB and ISAB occurrence in new patients in the summer months where there is global rise in *Acinetobacter* epidemics in the summer. It is thus believed that the proportions of *A. baumannii* isolates were reduced similarly among the IRAB and ISAB groups (both clinical and environmental) and it strongly reinforces the roles of adequate compliance of outbreak intervention strategy and surveillance including

hand-washing procedures, the use of barrier precautions and cleaning procedures to control the present *A. baumannii* outbreak. This case should be interpreted as a successful intervention method with the cause of strict control measures applied in the ICUs and which worked against the environmentally favorable season for the *A. baumannii* epidemic (Corbella et al, 2000). Relating the present genotyping, surveillance culture report and interventions measures with the univariate and multivariate result of case control study it can be drawn a conclusion that time at risk (p-value,0.000), and the duration of hospital stay coupled with use of mechanical ventilator by the patients (p-value,0.006) contaminated by PFGE type A clone of IRAB isolates were the underplaying causes for the present outbreak.

The precise identification of individual isolates of a given microorganism beyond the species level provides valuable information in outbreak situations (Scerpella et al, 1995). Such analysis of chromosome restriction fragment patterns by PFGE has been applied previously to the epidemiological study of *A. baumannii* and PFGE is increasingly being viewed as a very promising and useful technique for the epidemiological analysis of nosocomial infection (Scerpella et al 1995). Present study using PFGE enabled us to define and identify the outbreak strain and demonstrated that contamination and transmission of a single clone (PFGE type A) of imipenem-resistant *A. baumannii* was the main cause of present outbreak. Comparison between the outbreak strain and non outbreak control isolates from the same ICU and other parts of hospital revealed marked strain differences. This clearly show that with an entirely new and unique clone that was introduced to the ICU and spread among the patients.

V. CONCLUSION

Present investigation is primarily based on the molecular and epidemiological study. For the mechanism of carbapenem resistance study beta-lactamase gene specific PCR like IMP, VIM, OXA, SPM, GES, OXA were applied of which IMP-1, VIM-2, have been found sporadically in very few isolates and like wise the case for the bla-OXA-2. Although the present carbapenem resistance outbreak has not been found related with particular beta-lactamase or carbapenemase genes molecular epidemiological based research was carried out to find the cause of outbreak and develop control measures.

Comparing the association of risk factors for the acquisition of imipenem resistant *A. baumannii* the nosocomial occurrence of IRAB is found strongly related to the ICU stay and the use of mechanical ventilators used by the patients contaminated with the IRAB. Surveillance culture including the hospital equipments and related objects along with health care worker's hands were found positive for IRAB and PFGE based genotyping supported the transmission of PFGE type A clone of IRAB among the ICU patients. The study thus presumed that the outbreak was spread from a single clone of *A. baumannii* due to the transmission through the IRAB contaminated hospital equipments and environment via the health care worker's hands. Although the present outbreak is phenotypically related with carbapenem resistance they did not produced carbapenemase as revealed from the PCR methods, the mechanism of imipenem resistance should be a subject of further investigation and such type of cases were also experienced by other workers in the past (Turton et al, 2004).

Many studies had provided the evidence that the fateful trend to-ward antibiotic resistance *in A. baumannii* may finally include carbapenems; the last recognized antibiotic alternative for most strains isolated worldwide, during large and sustained hospital outbreaks (Corbella et al, 2000). Management of this emerging organism is complex and requires a combination of molecular biological techniques and epidemiological studies. High-level and extended environmental contamination,

close contact between colonized patients and health care workers, and wide spread imipenem use were the main determinant factors that promoted rapid clonal dissemination of IRAB strains throughout the hospital ICUs.

Strict compliance with basic infection control measure may have a strong impact on controlling such *A. baumannii* outbreaks. By comparing the *A. baumannii* strains typed and the case control study it can be concluded that the outbreak was due to the transmission of IRAB clone A through the medical equipments and hands of health care workers with patients having time at risk. An intervention program applying molecular technique and strict infection control measures enabled to bring the ill-fated imipenem resistant *A. baumannii* outbreak into control. Thus, in further, a detailed molecular biological approach with the development of efficient molecular typing technique along with identification of responsible carbapenemase determinant and follow-up of strict hospital health hygiene measures are required to address the problem.

Table 1. Site and date of isolation, antibiotic tested and PFGE types of imipenem-resistant *A. baumannii* (IRAB)

Isolate no.	PFGE Type	date of isolation	specimen name	AN	CAZ	CIP	GM	IPM
133	A	2004-02-21	Sputum	I	R	R	R	R
121	A	2004-03-10	Sputum	R	R	R	R	R
130	A	2004-03-10	Sputum	I	R	R	R	R
141	A	2004-03-25	Sputum	R	R	R	R	R
119	A	2004-04-01	Sputum	R	R	R	R	R
135	A	2004-04-01	Sputum	I	R	R	R	R
134	A	2004-04-13	Sputum	S	R	R	R	R
140	A	2004-05-20	Sputum	R	R	R	R	R
144	A	2004-05-20	Closed pus	R	R	R	R	R
145	A	2004-05-20	Open pus	I	R	R	R	R
137	A	2004-05-21	Sputum	R	R	R	R	R
139	A	2004-05-21	Sputum	R	R	R	R	R
124	A	2004-07-05	Sputum	R	R	R	R	R
129	A	2004-07-08	Sputum	R	R	R	R	R
143	A	2004-08-20	Open pus	R	R	R	R	R
125	A	2004-08-21	Open pus	R	R	R	R	R
118	A	2004-08-24	Closed pus	S	R	R	R	R
131	A	2004-08-26	Sputum	R	R	R	R	R
112	A	2004-08-30	Sputum	R	R	R	R	R
128	A	2004-09-15	Sputum	R	I	S	R	R
115	A	2004-10-02	Catheterized urine	S	R	S	S	R
106	A	2004-10-04	Sputum	R	R	R	R	R
114	A	2004-10-18	Sputum	I	R	R	R	R
113	A	2004-10-19	Not known	S	R	S	S	R
108	A	2004-11-02	Not known	R	R	R	I	R
110	A	2004-11-02	Not known	R	R	R	I	R
117	A	2004-11-10	Not known	S	R	S	S	R
111	C	2004-11-04	Not known	S	R	R	R	R
101	G	2004-07-30	Sputum	R	R	R	R	R
136	J	2004-04-09	Closed pus	R	R	R	R	R

Abbreviations, AN,amikacin; CAZ, ceftazidime; CIP, Ciprofloxacine; GM, Gentamicin; IPM, imipenem. Antibiogram result of the antibiotics tested for *A. baumannii* outbreak isolates. MIC level is based on the NCCLS guidelines (NCCLS, 2001), (see materials and methods).

Table 2. Important environmental sampling sites for *A. baumannii* isolates

Site of the sampling		Number
Health care worker	Left hand	18
	Right hand	18
Bed		10
Chart		3
Recording sheet		4
EKG		6
DP set containing suction kit		6
Tracheostomy site		6
IV fluid line		1
O2 supplier		2
Ventilator		13
Washing stands		2
waste water disposal sink surface		4
Station table		11
dressing car or assisted trolleys		9
Computer		3
Door handle		2
Canister		2
Suction		2
Humidifier		2
Total		124

Table 3. List of primers for integron, integrase and Tail PCR

Gene or target site	PCR name	Primer sequence	Reference
Integron class 1 PCR	5'-CS	5'-GGC ATC CAA GCA GCA AG-3'	Lévesque et al 1995
	3'-CS	5'-AAG CAG ACT TGA CCT GA-3'	
Integrase class 1 PCR	Int1F	5'-CAG TGG ACA TAA GCC TGT TC -3'	Koeleman et al, 200
	Int1R	5'-CCC GAG GCA TAG ACT GTA -3'	
Integrase class 2 PCR	Int2F	5'-TTG CGA GTA TCC ATA ACC TG -3'	Koeleman et al, 2001
	Int2R	5'-TTA CCT GCA CTG GAT TAA GC-3'	
Integrase class 3 PCR	IntI3F	5'-GCA GGG TGT GGA CGA ATA CG-3'	
	IntI3R	5'-ACA GAC CGA GAA GGC TTA TG-3'	Senda et al 1996
Integron Tail PCR-Forward	115 T1-F1	5'-TGTACGTCCCGTCTGCGAGTGT-3'	
	115 T1-F2	5'-ACAGCGTGGGGTGCGAAAAACA-3'	
	115-T1F3	5'-AACGATGTTACGCAGCAGGGCAG-3'	This work
Integron Tail PCR-Backward	115-T1-R1	5'-TGACCGTAAGGCTTGATGAAACA-3'	
	115-T1-R2	5'-TGGAGAATGGCAGCGCAATGACA-3'	
	115-T1-R3	5'-CCCGCGCAGATCAGTTGGAAGA-3'	This work

Table 4. Primers for carbapenemase and other betalactamases gene PCR

gene	Sequence	Target β-lactamase	Accession no. of bla-genes
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, , AY074433,AJ420864, AJ550807, AF322577,
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,
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		
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		

Table 5. Primers for carbapenemase gene and other betalactamases (oxacillinase) PCR

gene	Primers	primer sequence	size	Reference
bla-oxa-2 like	bla-oxa-2 (F)	5'-TTC AAG CCA AAG GCA CGA TAG-3'		
	bla-oxa-2 (R)	5'-TCC GAG TTG ACT GCC GGG TTG-3'	650	Steward et al, 2001
OXA-23-like	bla-oxa-23 F	5′-GAT CGG ATT GGA GAA CCA GA -3'		
	bla-oxa-23 R	5′-ATT TCT GAC CGC ATT TCC AT	501 bp	Woodford et al, 2006
OXA-24-like	bla-oxa-24 F	5′-GGT TAG TTG GCC CCC TTA AA		
	bla-oxa-24 R	5′-AGT TGA GCG AAA AGG GGA TT-3'	246 bp	Woodford et al, 2006
OXA-51-like	bla-oxa-51 R	5′-TGG ATT GCA CTT CAT CTT GG-3'	353 bp	Woodford et al, 2006
OXA-58-like	bla-oxa- 58 F	5′-AAG TAT TGG GGC TTG TGC TG		
	bla-oxa-58 R	5′-CCC CTC TGC GCT CTA CAT AC-3'	599 bp	Woodford et al, 2006

Table 6. Environmental sampling result for imipenem-resistant *A. baumannii* isolates.

Site of the sampling		Number	*A. baumannii*
Health care worker	Left hand	18	1
	Right hand	18	1
Bed		10	7
Chart		3	0
Recording sheet		4	0
EKG		6	1
DP set containing suction kit		6	4
Tracheostomy site		6	3
IV fluid line		1	1
O2 supplier		2	0
Ventilator		13	7
Washing stands		2	0
waste water disposal sink surface		4	4
Station table		11	2
dressing car or assisted trolleys		9	2
Computer		3	0
Door handle		2	0
Canister		2	0
Suction		2	0
Humidifier		2	0
Total		124	33

Table 7. Results of univariate analysis for the occurrence of imipenem resistant *A. baumannii* (case vs. no growth)

Variable	case (n=77)	control (n=77)	p-value
Demographics			
male gender	58(75.3)	57(74.0)	1.000
age(years)*	56.0(15.8)	55.6(16.7)	0.866
Comorbidity			
cardiac disease	4(5.2)	20(26.0)	0.001
diabetes	13(16.9)	9(11.7)	0.490
malignancy	5(6.5)	2(2.6)	0.442
CVA	17(22.1)	0(0.0)	0.000
pulmonary disease	17(22.1)	5(6.5)	0.010
hepatic disease	5(6.5)	7(9.1)	0.765
renal disease	2(2.6)	0(0.0)	0.497
Related to hospitalization			
time at risk(days)	24.2(32.4)	6.2(4.5)	0.000
surgery	48(62.3)	35(45.5)	0.052
bladder catheter	77(100.0)	58(75.3)	0.000
IV catheter	46(60.5)	34(44.2)	0.052
mechanical ventilation	52(67.5)	8(10.4)	0.000
intubation	67(87.0)	32(41.6)	0.000
Antibiotics			
imipenem	10(13.0)	5(6.5)	0.277
piperacillin	0(0.0)	3(3.9)	0.245
ampicillin-sulbactam	1(1.3)	0(0.0)	1.000
vancomycin	8(10.4)	13(10.4)	0.348
cephalosporins			
1st generation	0(0.0)	15(19.5)	0.000
2nd generation	35(45.5)	35(45.5)	1.000
3rd generation	18(23.4)	36(46.8)	0.004
aminoglycosides	46(59.7)	50(65.8)	0.505
quinolone	4(5.2)	5(6.5)	1.000
carbapenem	6(7.8)	1(1.3)	0.116
duration of antibiotics use(days)	10.8(10.0)	15.3(12.9)	0.008

Table 8. Results of multivariate analysis for the occurrence of imipenem resistant *A. baumannii*

Variable	Crude OR(95% CI)	Adjusted OR* (95% CI)	p-Value
Comorbidity			
Cardiac disease(yes/no)	0.16(0.05-0.48)	0.32(0.06-1.58)	0.163
Pulmonary disease(yes/no)	4.08(1.42-11.71)	6.03(0.88-41.08)	0.067
Related to hospitalization			
Time at risk(days)	1.31(1.19-1.45)	1.29(1.14-1.46)	0.000
Surgery(yes/no)	1.99(1.04-3.78)	2.13(0.65-6.93)	0.210
IV catheter(yes/no)	1.94(1.02-3.69)	0.42(0.12-1.45)	0.170
Mechanical ventilation(yes/no)	17.94(7.49-42.98)	6.41(1.71-24.06)	0.006
Intubation(yes/no)	9.42(4.21-21.06)	2.55(0.69-9.44)	0.161
Antibiotics			
3rd generation(yes/no)	0.35(0.17-0.69)	0.34(0.11-1.07)	0.065

Note, asterisk*, Adjusted ORs were adjusted for comorbid condition such as cardiac disease and pulmonary disease, time at risk, surgery, IV catheter, intubation, and used antibiotics in a logistic regression model.

Table 9. Susceptibility test of environmental isolates of *A. baumannii* and PFGE types

Isolates	AN	ATM	CIP	GM	IPM	NET	PIP	TIM	NN	PFGE Type
302	R	R	R	R	R	R	R	R	R	A
303	R	R	R	R	R	R	R	R	R	A
304	R	R	R	R	R	R	R	R	R	A
305	R	R	R	R	R	R	R	R	R	K
306	R	R	R	R	R	R	R	R	R	A
307	R	R	R	R	R	R	R	R	R	A
308	R	R	R	R	R	R	R	R	R	A
309	R	R	R	R	R	R	R	R	R	A
310	R	R	R	R	R	R	R	R	R	A
311	R	R	R	R	R	R	R	R	R	A
312	R	R	R	R	R	R	R	R	R	A
313	R	R	R	R	R	R	R	R	R	A
314	R	R	R	R	R	R	R	R	R	A
315	R	R	R	R	R	R	R	R	R	A
316	R	R	R	R	R	R	R	R	R	J
318	R	R	R	R	R	R	R	R	R	A
319	R	R	R	R	R	R	R	R	R	A
320	R	R	R	R	R	R	R	R	R	A
321	R	R	R	R	R	R	R	R	R	A
322	R	R	R	R	R	R	R	R	R	A
324	R	R	R	R	R	R	R	R	R	A
325	R	R	R	R	R	R	R	R	R	A
326	R	R	R	R	R	R	R	R	R	A

Note:

1. Abbreviations, AN, amikacin; ATM, aztreonam; CIP, Ciprofloxacine; GM, Gentamicin; IPM, imipenem. NET, netilmicin; PIP, piperacillin; TIM, ticarcillin-clavulanic acid; NN, tobramicin.
2. Antibiogram result of the antibiotics tested for *A. baumannii* outbreak isolates. MIC level is based on the NCCLS guidelines (NCCLS, 2001), (see materials and methods).
3. Criteria for inhibition zone are based on NCCLS (2001) guidelines (see materials and methods)
4. R, resistant
5. PFGE type, see materials and methods and result

Table-10. Modified Hodge test and IMP-EDTA DDST

Isolate	MHT	DDST	Isolate	MHT	DDST	Isolate	MHT	DDST
101	p	p	203	n	nt	302	p	n
106	p	n	213	n	nt	304	p	n
108	p	n	214	n	nt	306	p	n
110	p	p	220	n	nt	309	p	n
111	p	p	224	n	nt	312	p	n
112	p	n	225	n	nt	314	p	n
113	n	n	226	n	nt	316	p	n
114	p	p	227	n	nt	319	p	p
115	p	n	230	n	nt			
117	p	n						
118	p	n						
119	p	n						
121	p	n						
124	p	n						
125	p	n						
128	p	p						
129	p	n						
130	p	n						
131	p	n						
133	p	n						
134	p	n						
135	p	n						
136	p	n						
137	p	n						
139	p	n						
140	p	n						
141	p	n						
143	p	n						

Note, nt, not tested for MHT negative result

Table 11. Integrase class 2 gene (*inti2*) PCR result for IRAB, ISAB E-IRAB isolates. Integrase class 3 gene *intI3* PCR was negative for all the tested isolates.

Isolates	**Gene**		**isolates**	**gene**		**isolates**	**gene**	
IRAB	***inti1***	***inti2***	**ISAB**	***inti1***	***inti2***	**E-IRAB**	***inti1***	***inti2***
101	p	p	203	p	n	302	p	n
106	p	p	213	p	n	304	p	n
108	p	n	214	p	n	306	p	n
110	p	n	220	p	n	309	p	p
111	p	n	224	p	p	312	p	p
112	p	n	225	p	p	314	p	n
113	p	n	226	p	n	316	p	p
114	p	n	227	p	n	319	p	n
115	p	p	230	p	n			
117	p	p						
118	p	n						
119	p	n						
121	p	n						
124	p	n						
125	p	n						
128	p	p						
129	p	p						
130	p	n						
131	p	n						
133	p	n						
134	p	p						
135	p	p						
136	p	n						
137	p	p						
139	p	n						
140	p	p						
141	p	n						
143	p	n						
144	p	p						
145	p	n						

Note, Integrase class 3 gene *intI3* PCR was negative for all the tested isolates.

Table 12. Different class integrase gene PCR

Isolates	**Inti1 positive/total (percent)**	**IntI2 positive/total (percent)**	**IntI3 positive/total (percent)**
IRAB	30/30 (100)	11/30 (36.66 %)	0
ISAB	9/9 (100)	2/9 (22.00)	0
Environmental	8/8 (100)	3/8 (37.5)	0
total	47/47 (100)	16/47 (34.04 %)	0

Table 13. PCR result of IRAB for carbapenemase and other betalactamase genes

isolates	IMP-1	IMP-2	IMP-4	Imp-11	Imp-18	Imp-19	VIM1	Vim-2	GIM1	SPM1	GES	Oxa-2	Oxa-23	Oxa-24	Oxa-51	Oxa-58
101	n	p	n	n	n	n	n	n	n	n	n	n	n	n	p	n
106	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
108	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
110	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
111	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
112	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
113	n	p	p	n	n	n	n	n	n	n	n	p	n	n	p	n
114	n	n	n	n	n	n	p	p	n	n	n	n	n	n	p	n
115	p	p	p	n	n	n	n	n	n	n	n	p	n	n	p	n
117	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
118	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
119	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
121	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
124	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
125	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
128	n	p	n	n	n	n	n	n	n	n	n	n	n	n	p	n
129	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
130	n	p	n	n	n	n	n	n	n	n	n	n	n	n	p	n
131	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
133	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
134	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
135	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
136	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
137	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
139	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
140	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
141	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
143	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
144	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
145	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n

Table-14. PCR result of ISAB for carbapenemase and other betalactamase genes PCR

isolates	IMP-1	iMP-2	IMP-4	Imp-11	Imp-18	Imp-19	VIM1	Vim-2	GIM1	SPM1	GES	Oxa-2	Oxa-23	Oxa-24	Oxa-51	Oxa-58
203	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
213	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
214	n	n	n	n	n	n	n	n	n	n	n	P	n	n	p	n
220	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
224	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
225	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
226	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
227	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
230	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n

Table 15. PCR result of E-IRAB for carbapenemase and other betalactamase genes

isolates	IMP-1	iMP-2	IMP-4	Imp-11	Imp-18	Imp-19	VIM1	Vim-2	GIM1	SPM1	GES	Oxa-2	Oxa-23	Oxa-24	Oxa-51	Oxa-58
302	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
304	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
306	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
309	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
312	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
314	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n
316	n	n	n	n	n	n	n	n	n	n	n	n	n	n	p	n

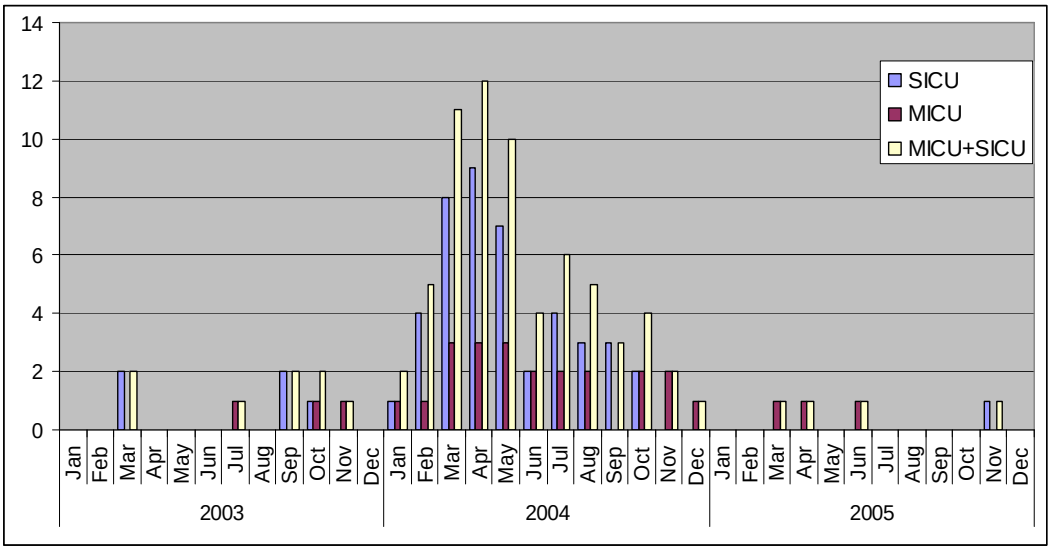


Figure 1. Epidemic curve showing the number of SICU plus MICU patients colonizing or infecting with imipenem resistant *A. baumannii* at 1-month intervals from January 2004 to December 2004

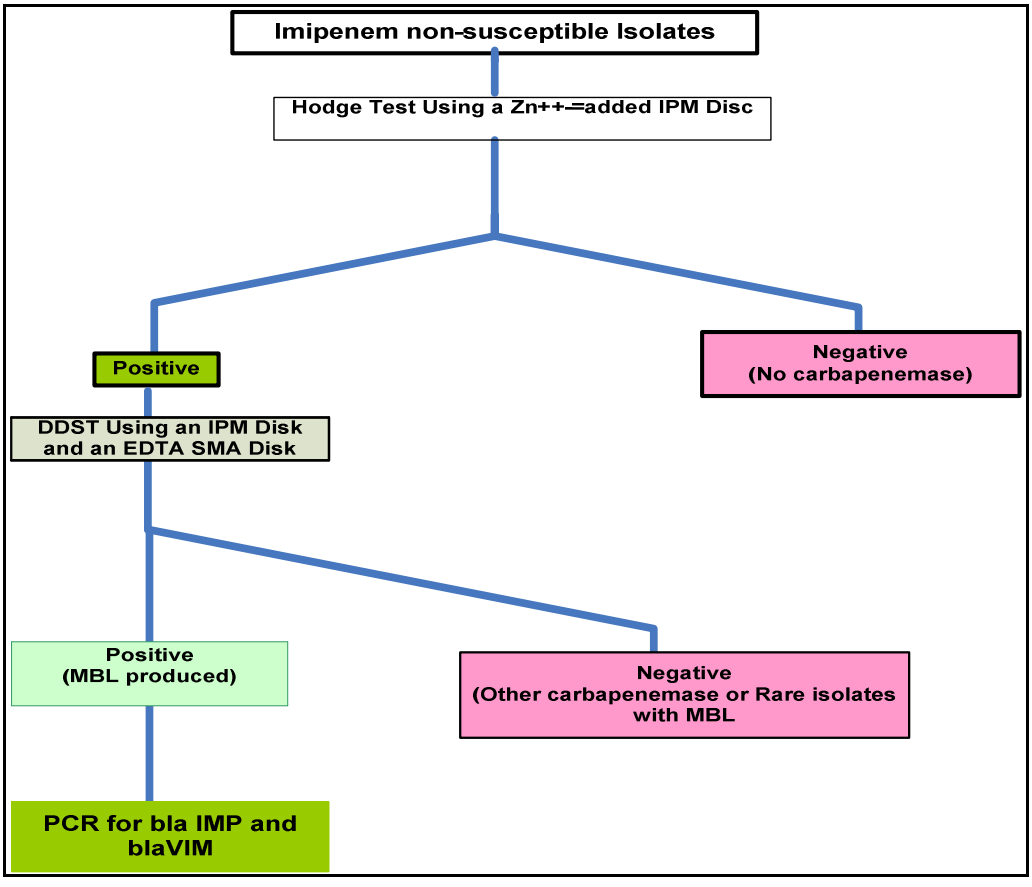


Figure 2. Schematic diagram for modified Hodge test and IMP-EDTA DDST (Ref. Lee et al 2003)

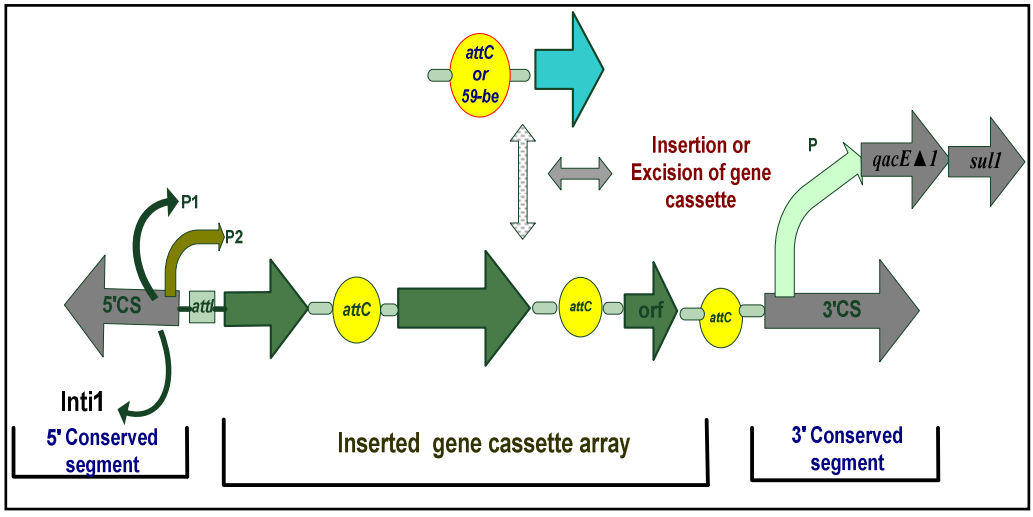
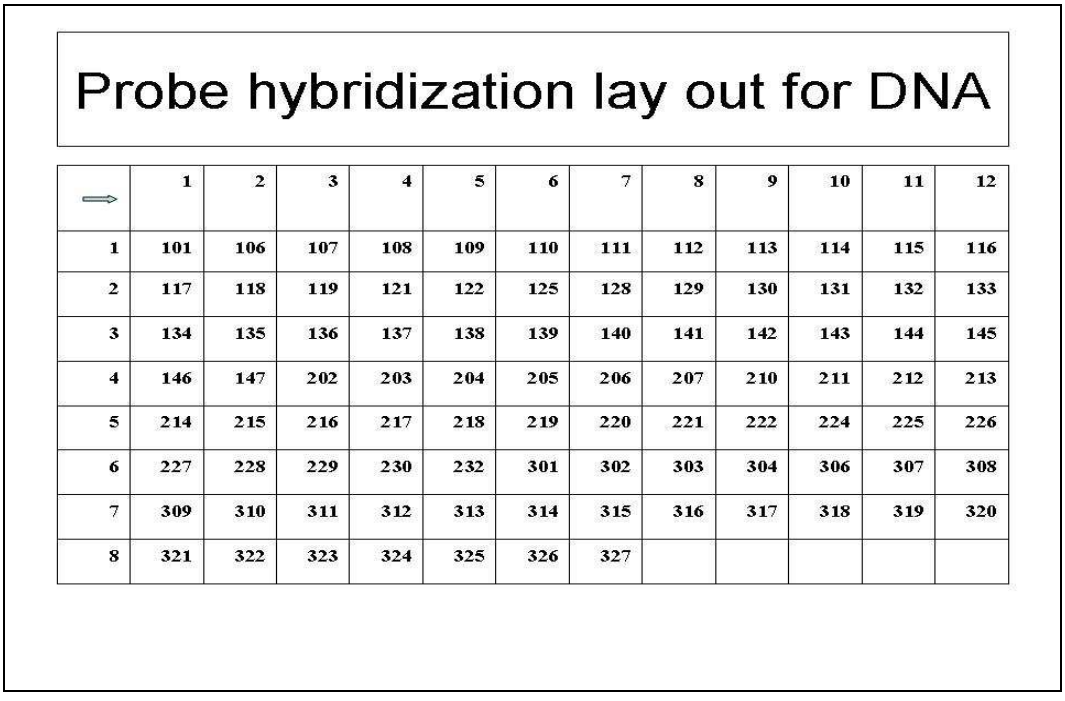


Figure 3. General structure and scheme for an integron and integrase PCR (Ref. Levesque *et al* 1995)



⇨	1	2	3	4	5	6	7	8	9	10	11	12
1	101	106	107	108	109	110	111	112	113	114	115	116
2	117	118	119	121	122	125	128	129	130	131	132	133
3	134	135	136	137	138	139	140	141	142	143	144	145
4	146	147	202	203	204	205	206	207	210	211	212	213
5	214	215	216	217	218	219	220	221	222	224	225	226
6	227	228	229	230	232	301	302	303	304	306	307	308
7	309	310	311	312	313	314	315	316	317	318	319	320
8	321	322	323	324	325	326	327					

Figure 4. A DIG-labeled probe hybridization dot-blot DNA sample layout for the gene inti1 and oxa-2

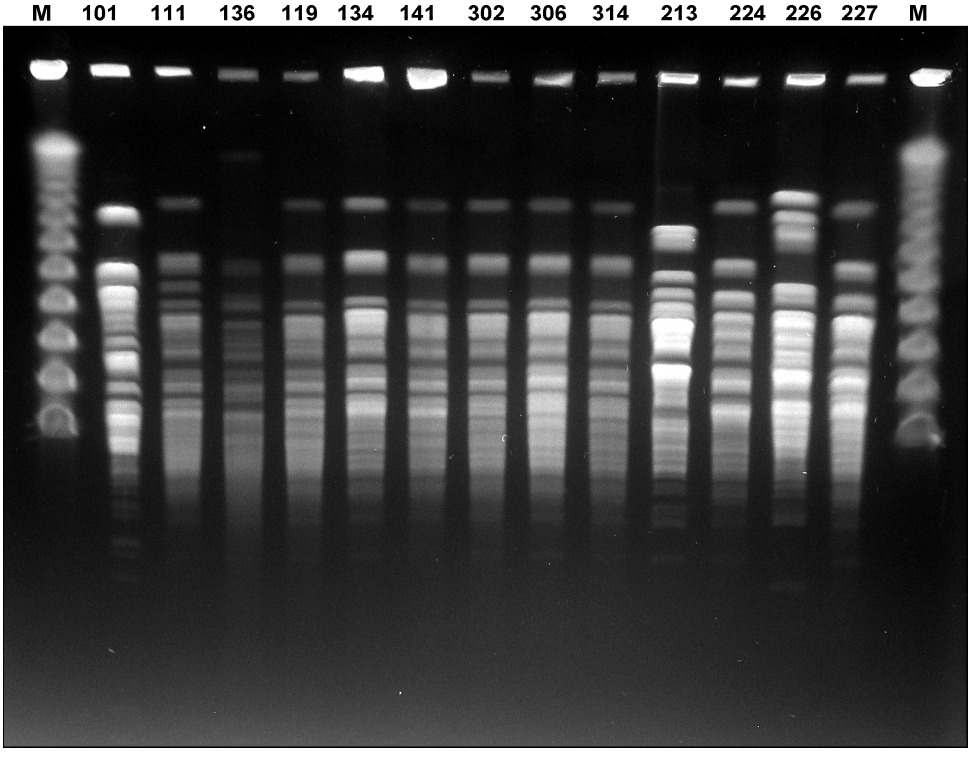


Figure 5. Representative PFGE patterns of Apa*I* digested genomic DNA from the imipenem resistance outbreak (IRAB), environmental (E-IRAB) and imipenem susceptible non-outbreak (ISAB) strains of *A. baumannii*. Lanes: 1 and 15 molecular size marker; lanes 2 to 7, clinical outbreak isolates from the MICU/SICU; lanes 8 to 10, environmental outbreak isolates; lanes 11 to 14, clinical non-outbreak isolates from the ICU/NICU. Isolates identification, lane 2-101, lane 3-111, lane 4-136, lane 5-119, lane 6-134, lane 7-141, lane 8-302, lane 9-306, lane 10-314, lane 11-213, lane 12-224, lane 13-226 and lane 14-227.

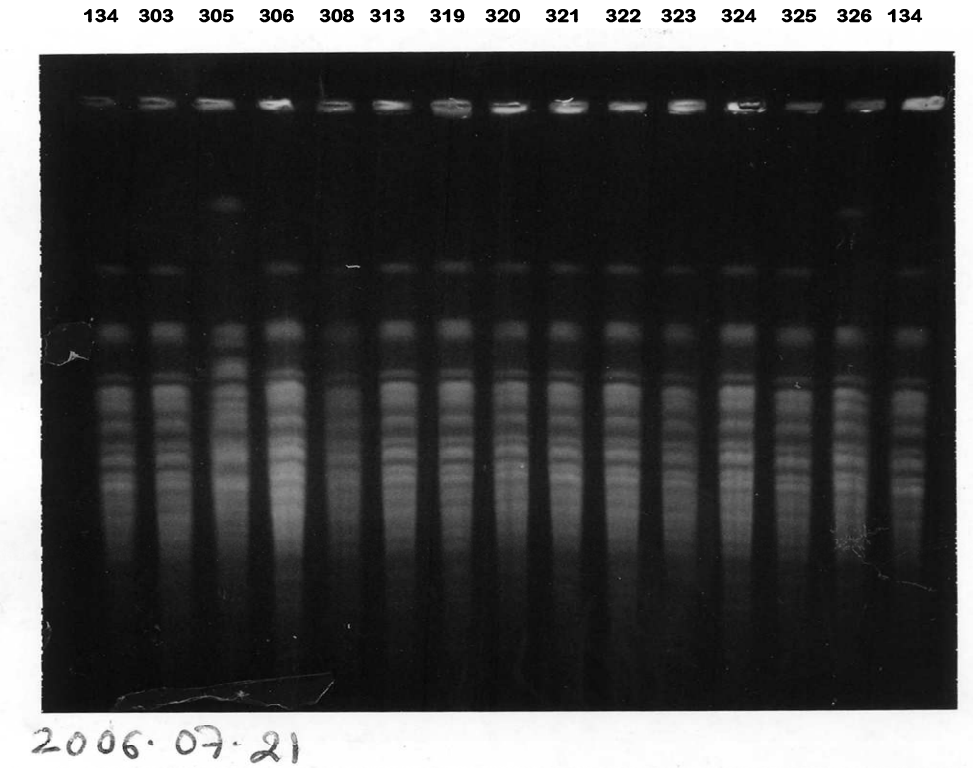


Figure 5 (a). Representative PFGE patterns of Apa*I* digested genomic DNA from the environmental (E-IRAB) strains of *A. baumannii*. Lanes: 1 and 15 index strain (IRAB isolate 134);, 134, 2 to 14, environmental isolates from the MICU/SICU; Isolates identification, lane 1 134, lane 2-303, lane 3-305, lane 4-306, lane 5-308, lane 6-313, lane 7-319, lane 8-320, lane 9-321, lane 10-322, lane 11-323, lane 12-324, lane 13-325, lane 14-326 and lane 15-134. Lane 3, imipenem susceptible strain of *A. baumannii* has band difference in comparison to other imipenem resistant strain of *A. baumannii*

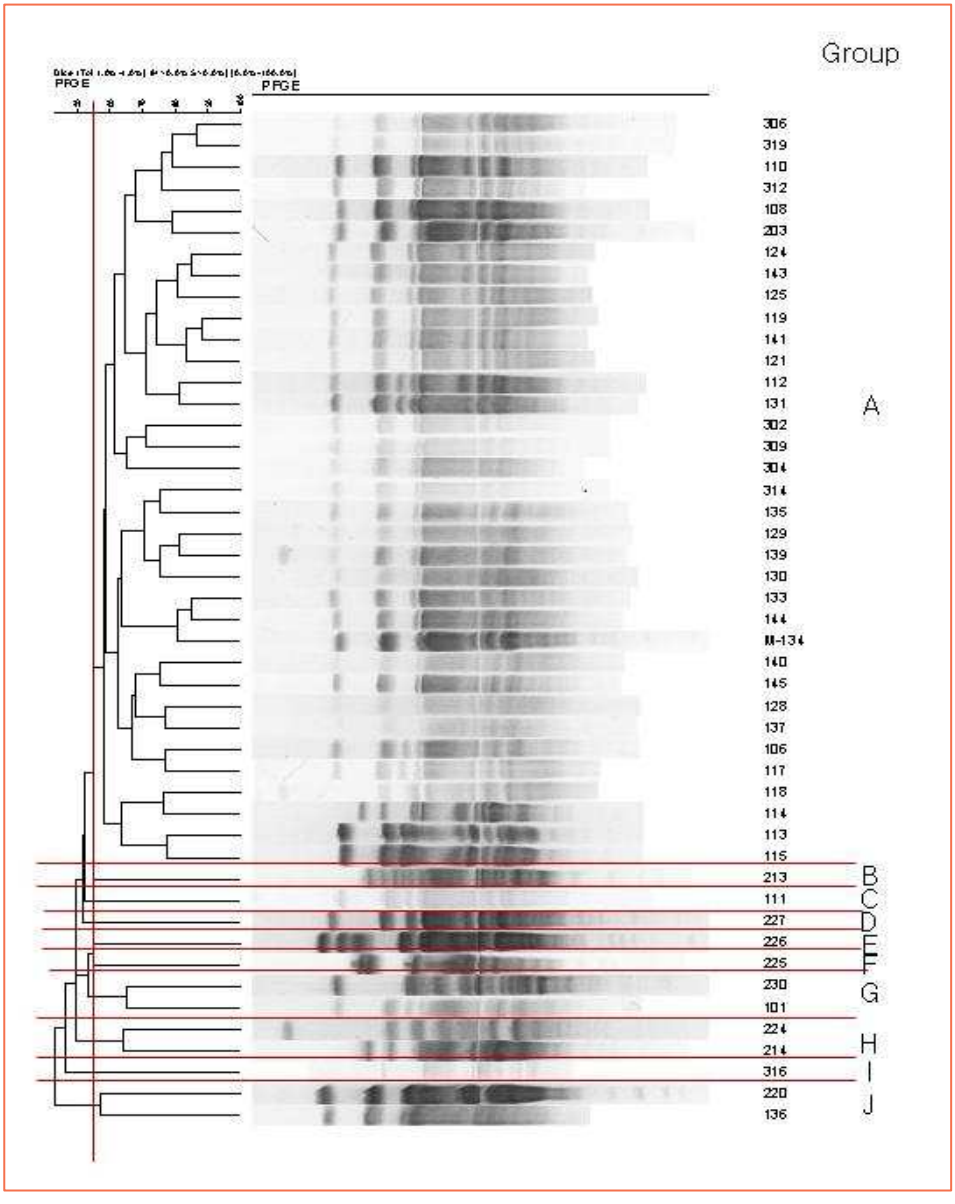


Figure 6. A dendrogram showing relationships between strains based on pulse field gel electrophoresis (PFGE). Similarity values were derived by using the Dice coefficient and the UPGMA algorithm. The cut-off point for the recognition of clusters was set at the 55% similarity value.

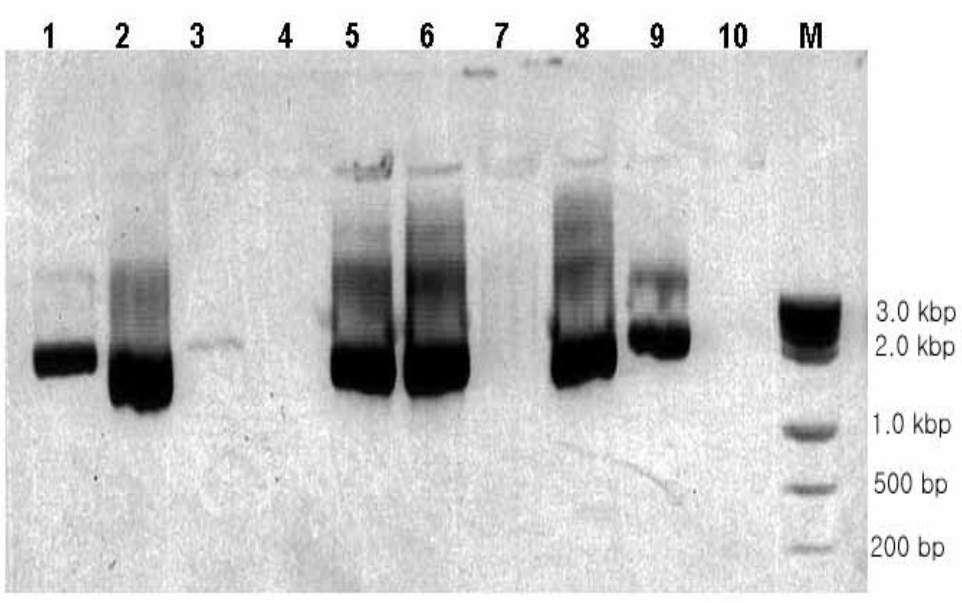


Figure 7. Class 1 integron PCR. The template DNA preparation for the integron PCR was from proteinase-K extraction method. Primers 5'-CS (5'-GGC ATC CAA GCA GCA AG-3') and 3'-CS (5'-AAG CAG ACT TGA CCT GA-3') were used as described by Lévesque et al (1995). Lane M, molecular marker: lanes 1 to 8, imipenem-resistant *A. baumannii;* lane 9, imipenem susceptible *A. baumannii,* lane 10, negative. Lane identification for isolates, lane 1-101, lane 2-106, lane 3-108-, 4- 112, 5-114, 6-115, 7-125, 8-128, lane 9-134.

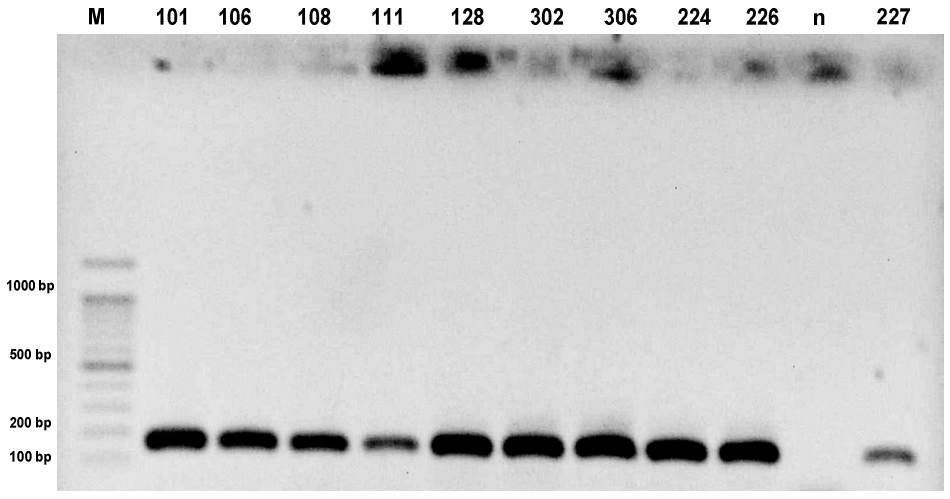


Figure 8. Integrase class 1 gene (intI1) PCR. Lane 1, molecular marker, lanes 2 to 6, imipenem resistant *A. baumannii; ;* lane 7 and 8, imipenem resistant environmental isolate, lane 9, 10 and 12 imipenem susceptible *A. baumannii*, lane 11, negative. Description of isolates: lane 2-101, lane 3-106, lane 4-108, lane 5-111, lane 6-128, lane 7-302, lane 8-306, lane 9-224, lane 10-226 and lane 12-227.

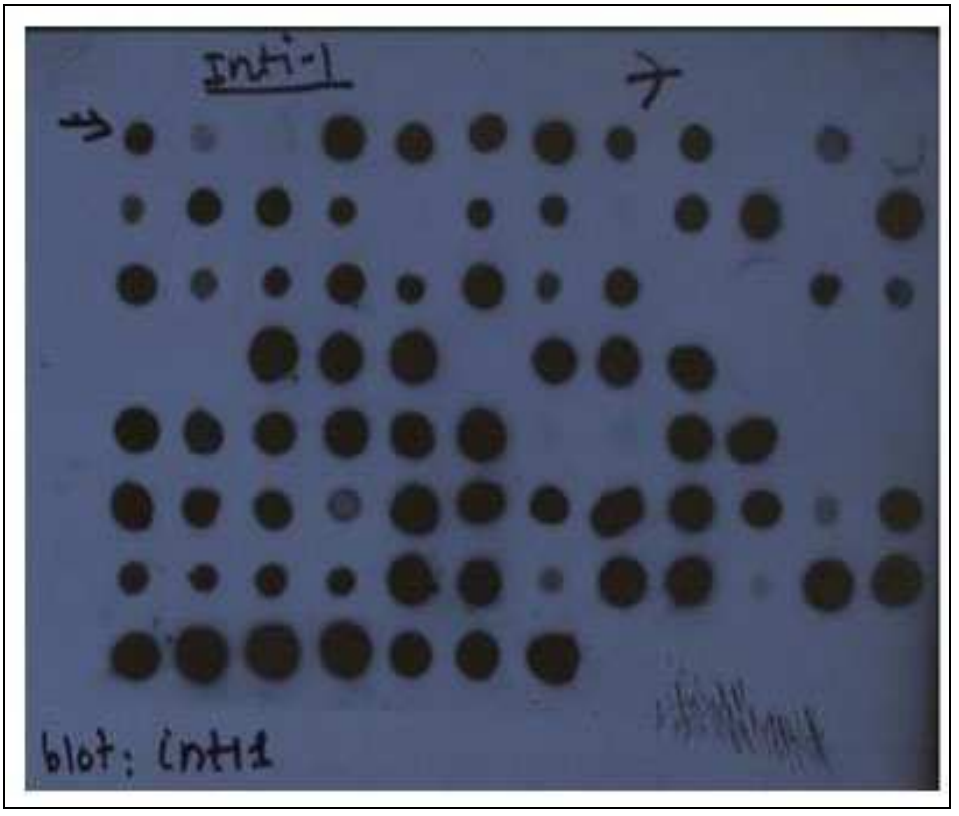


Figure 9. Probe hybridization dot-blot for class 1 integrase -*intI*. Sample layout is mentioned in Figure- 4.

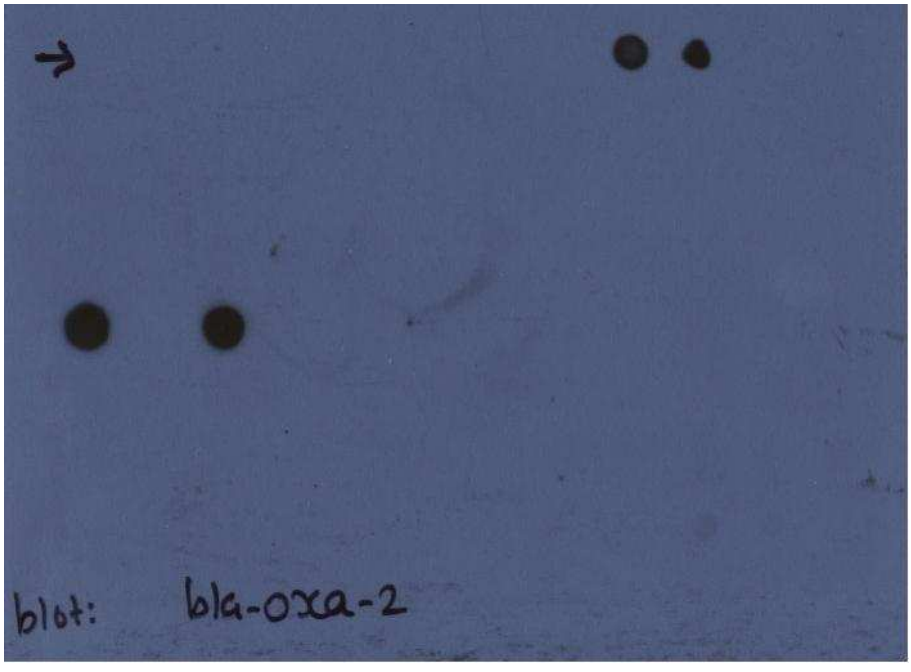


Figure 10. Probe hybridization dot-blot for bla-oxa-2. Sample layout is mentioned in Figure 4.

VI. REFERENCES

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer,Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res.1997, 25:3389-3402.

Afzal-Shah, M., H.E. Villar and D.M. Livermore, Biochemical characteristics of a carbapenemase from an *Acinetobacter baumanii* isolate collected in Buenos Aires, Argentina. *J Antimicrob Chemother* 1999, 43: 127–131.

Arakawa, Y., N. Shibata, K. Shibayama, H. Kurokawa, T. Yagi, H. Fujiwara, and M. Goto. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J. Clin. Microbiol.2000, 38:40-43.

Ayan, M.; Durmaz R.; Aktas E. ; Durmaz B. Bacteriological, clinical and epidemiological characteristics of hospital-acquired *Acinetobacter baumannii* infection in a teaching hospital. J. hosp. infect. 2003, 54: 39-45.

Aygun, G. O. Demirkiran and T. Utku *et al.*, Environmental contamination during a carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit, J Hosp Infect. 2002, 52: 259–262.

Bartual, S. G., H. Seifert, C. Hippler, M. A. Luzon, H. Wisplinghoff, and F. Rodriguez-Valera. 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J. Clin. Microbiol.2005, 43:4382-4390.

Beaglehole, R., Bonita R., and T. Kjellstrom. Basic Epidemiology. 1993. World Health Organization, Geneva, Switzerland.

Bergogne-Bérézin, E., and K. J. Towner.. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. 1996Clin. Microbiol. Rev. 9:148-165.

Bou, G., Olive, A. and J. Martinez-Beltran, A novel class D β-lactamase (OXA-24) with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob Agents Chemother 2000,44:1556–1561.

Brooks, S. E., M. A. Walczak, and R. Hameed. Are we doing enough to contain *Acinetobacter* infections? Infect. Control Hosp. Epidemiol. 2000. 21:304.

Brown, S. C. Bantar, H.K. Young and S.G.B. Amyes, Limitation of *Acinetobacter baumannii* treatment by plasmid-mediated carbapenemase ARI-2. Lancet 1998, 351:186–187.

Brown, S., and S. G. Amyes.. The sequences of seven class D β-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. Clin. Microbiol. Infect. 2005a., 11:326-329.

Brown, S., H. K. Young, and S. G. Amyes. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin. Microbiol. Infect. 2005.b. 11:15-23.

Buisson Y, Tran Van Nhieu G, Ginot L, Bouvet P, Schill H, Driot L, et al. Nosocomial outbreaks due to amikacin-resistant tobramycin-sensitive *Acinetobacter* species: correlation with amikacin usage. *J Hosp Infect* 1990;15:83-93.

Buxton, A. E., R. L. Anderson, D. Werdegar, and E. Atlas. 1978. Nosocomial respiratory tract infection and colonization with *Acinetobacter calcoaceticus.* Epidemiologic characteristics. Am. J. Med. 1978, 65**:**507-513.

Castle M, Tenney JH, Weinstein MP, Eickhoff TC. Outbreak of a multiply resistant *Acinetobacter* in a surgical intensive care unit: epidemiology and control. *Heart Lung* 1978; 7:641-644.

Cefai CJ, Richards J, Gould FK, McPeake P. An outbreak of *Acinetobacter* respiratory tract infection resulting from incomplete disinfection of ventilatory equipment. J Hosp Infect 1990;15:177-182.

Chu, Y.-W., M. Afzal-Shah, E. T. S. Houang, M.-F. I. Palepou, D. J. Lyon, N. Woodford, and D. M. Livermore.. IMP-4, a novel metallo-β-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob. Agents Chemother. 2001, 45:710-714.

Corbella, X., Montero, A., Pujol, M., Domínguez, M.A., Ayats, J., Argerich, M.J., Garrigosa, F., Ariza, J. and Gudiol, F.,. Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant *Acinetobacter baumannii*. J Clin Microbiol . 2000, 38: 4086–4095.

D'Agata, E., Venkataraman L, DeGirolami P, Burke P, Eliopoulos G, Karchmer AW, et al. Multidrug resistant gram-negative bacilli: epidemiology and risk factors for colonization. Crit Care Med 1999a; 27:1090-1095.

D'Agata EMC, Venkataraman L, DeGirolami P, Samore M. The molecular epidemiology of ceftazidime-resistant gram-negative bacilli on inanimate surfaces and their role in cross-transmission during non-outbreak periods. *J Clin Microbiol* 1999;37:3065-3067.

David, M., Livermore and Neil Woodford. Carbapenemases: a problem in waiting? Current Opinion in Microbiology. 2000. 3: 489-495.

Donald, H.M., W. Scaife, S.G.B. Amyes and H.K. Young, Sequence analysis of ARI-1, a novel OXA β-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob Agents Chemother 2000, 44: 196–199.

Ecker, Joseph A., Christian Massire, Thomas A. Hall, Raymond Ranken, Thuy-Trang D. Pennella, Cristina Agasino Ivy, Lawrence B. Blyn, et al. 2006. Identification of *Acinetobacter* Species and Genotyping of *Acinetobacter baumannii* by Multilocus PCR and Mass Spectrometry. J Clin Microbiol. 2006, 44: 2921–2932.

Ecker, Joseph A., Christian Massire, Thomas A. Hall, Raymond Ranken, Thuy-Trang D. Pennella, Cristina Agasino Ivy, Lawrence B. Blyn, et al,. Characterization of the 6'-*N*-Aminoglycoside Acetyltransferase Gene *aac(6')-Il* Associated with a *sulI*-Type Integron. Antimicrob. Agents Chemother. 1997. 41: 314-318.

Erika, M.C. D'Agata, MD, MPH; Valerie Thayer, RN; William Schaffner, MD. An Outbreak of *Acinetobacter baumannii*: The Importance of Cross-Transmission. *Infect Control Hosp Epidemiol* 2000; 21:588-591.

Fernández-Cuenca F, Martínez-Martínez L, Conejo MC, Ayala JA, Perea EJ, Pascual A. Relationship between β-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of Acinetobacter baumannii. J Antimicrob Chemother. 2003, 51:565–74.

Gonzalez, G., K. Sossa, H. Bello, M. Dominguez, S. Mella and R. Zemelman, Presence of integrons in isolates of different biotypes of *Acinetobacter baumannii* from Chilean hospitals, *FEMS Microbiol Lett* 1998, 161: 125–128.

Gibb, A. P., C. Tribuddharat, R. A. Moore, T. J. Louie, W. Krulicki, D. M. Livermore, M.-F. I. Palepou, and N. Woodford. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla_{IMP} allele, bla_{IMP-7}. Antimicrob. Agents Chemother. 2002, 46:255-258.

Go, E.S., Urban, C., Burns, J., Kreiswirth, B., Eisner, W., Mariano, N., Mosinka-Snipas, K. and Rahal, J.J.,. Clinical and molecular epidemiology of *Acinetobacter* infections sensitive to polymyxin B and sulbactam. Lancet 1994, 350: 1329–1332.

Gombac, F., M.L. Riccio and G.M. Rossolini *et al.*, Molecular characterization of integrons in epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* from Italian hospitals reveals a limited diversity of gene cassette

arrays, Antimicrob Agents Chemother. 2002, 46:3665–3668.

Hartstein AI, Rashad AL, Liebler JM, Actis LA, Freeman J, Rourke JW, et al. Multiple intensive care unit outbreak of *Acinetobacter calcoaceticus* subspecies anitratus respiratory infections and colonization associated with contaminated, reusable ventilator circuits and resuscitations bags. Am J Med 1988;85:624-631.

Heidelberg, J. F., Eisen, J. A., Nelson, W. C., Clayton, R. A., Gwinn, M. L., Doson, R. J., Haft, D. H., Hickey, E. K., Peterson, J. D., Umayam, L., et al.. Nature, 2000, 406: 477–483.

Heritier, C., A. Dubouix, L. Poirel, N. Marty, and P. Nordmann.. A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem-hydrolysing oxacillinase OXA-58. J. Antimicrob. Chemother. 2005, 55:115-118.

Heritier, C., L. Poirel, T. Lambert, and P. Nordmann. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 2005b. 49:3198-3202.

Holmes, A. J., Gillings, M. R., Nield, B. S., Mabbutt, B. C., Nevalainen, K. M. H. & Stokes, H. W. Env. Microbiol. 2003.5, 383–394.

Hornstein, M., C. Sautjeau-Rostoker, J. Peduzzi, A. Vessieres, L.T.H. Hong, M. Bartheleny, M. Scavizzi and R. Labia, Oxacillin-hydrolyzing β-lactamase involved in resistance to imipenem in *Acinetobacter baumannii*. *FEMS Microbiol Lett* (1997,153: 333–339

Huys, G., M. Cnockaert, A. Nemec, L. Dijkshoorn, S. Brisse, M. Vaneechoutte, and J. Swings. 2005. Repetitive-DNA-element PCR fingerprinting and antibiotic resistance of pan-European multi-resistant *Acinetobacter baumannii* clone III strains. J. Med. Microbiol. 2005, 54:851-856.

Ibrahim, A., P. Gerner-Smidt, and W. Liesack. 1997. Phylogenetic relationship of the twenty-one DNA groups of the genus *Acinetobacter* as revealed by 16S ribosomal DNA sequence analysis. Int. J. Syst. Bacteriol. 1997,47:837-841.

Iyobe, S., H. Kusadokoro, J. Ozaki, N. Matsumura, S. Minami, S. Haruta, T. Sawai, and K. O'Hara.. Amino acid substitution in a variant of IMP-1 metallo-β-lactamase. Antimicrob. Agents Chemother. 2000. 44:2023-2027.

Jawad, A, J Heritage, AM Snelling, DM Gascoyne-Binzi and PM Hawkey Influence of relative humidity and suspending menstrua on survival of *Acinetobacter* spp. on dry surfaces. Journal of Clinical Microbiology.1996, 34:2881-2887.

Jubelle K. Valenzuela, Lee Thomas, Sally R. Partridge, Tanny van der Reijden , Lenie

Dijkshoorn3, and Jon Iredell. Horizontal gene transfer within a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. J Clin Microbiol. 2006 Nov 15; [in Press].

Juliana Coelho, Neil Woodford, Mariya Afzal-Shah, and David Livermore. Occurrence of OXA-58-Like Carbapenemases in *Acinetobacter* spp. Collected over 10 Years in Three Continents. Antimicrob Agents Chemother. 2006; 50: 756–758.

uliana M. Coelho, Jane F. Turton,[2] Mary E. Kaufmann,[2] Judith Glover,[2] Neil Woodford, Marina Warner, Marie-France Palepou,[1] Rachel Pike,[1] Tyrone L. Pitt,[2] Bharat C. Patel, and David M. Livermore. Occurrence of Carbapenem-Resistant *Acinetobacter baumannii* Clones at Multiple Hospitals in London and Southeast England. Journal of Clinical Microbiology, 2006, 44: 3623-3627.

Kaul R, Burt J, Cork L, Dedier H, Garcia M, Kennedy C, et al. Investigation of a multiyear multiple critical care unit outbreak due torelatively drug-sensitive *Acinetobacter baumannii:* risk factors andattributable mortality. J Infect Dis 1996; 174:1279-1287.

Koeleman, J. G. M., J. Stoof, D. J. Biesmans, P. H. M. Savelkoul, and C. M. J. E. Vandenbroucke-Grauls. 1998. Comparison of amplified ribosomal DNA restriction analysis, random amplified polymorphic DNA analysis, and amplified fragment length polymorphism fingerprinting for identification of Acinetobacter genomic species and typing of *Acinetobacter baumannii*. J. Clin. Microbiol.1998, 36:2522-2529.

Koeleman, J.G., J. Stoof, M.W. Van Der Bijl, C.M. Vandenbroucke-Grauls and P.H. Savelkoul, Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR, *J Clin Microbiol.* 2001, 39: 8–13.

Koeleman, J.G.M., van der Bijl, M.W., Stoof, J., Vandenbroucke-Grauls, C.M.J.E. and Savelkoul, P.H.M., 2001. Antibiotic resistance is a major risk factor for epidemic behavior of *Acinetobacter baumannii*. *Infect Control Hosp Epidemiol, 2001,* 22: 284–288

Laraki, N. N., Franceschini, G.M. Rossolini, P. Santucci, C. Meunier, E. de Pauw, G. Amicosante, J.-M. Frere and M. Galleni, Biochemical characterization of the *Pseudomonas aeruginosa* 101/1477 metallo-β-lactamase IMP-1 produced by *Escherichia coli*. *Antimicrob Agents Chemother* (1999, 43:902–906 .

Last, J.M. 1988. A dictionary of epidemiology. 2nd Edition. Oxford, Oxford University Press.

Last, J.M. 1990. Guidelines on ethics for epidemiologists. International epidemiological Association, 1990 (Unpublished discussion document).

Lauretti, L., M.L. Riccio, A. Mazzariol, G. Cornaglia, G. Amicosante, R. Fontana and G.M. Rossolini, Cloning and characterization of *bla*$_{VIM}$, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother* (1999,43:1584–1590.

Lee, K., Y. Chong, H. B. Shin, Y. A. Kim, D. Yong and J. H. Yum. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and *Acinetobacter* species. Clinical Microbiology & Infection. 2001,7:141-147.

Lee, K., J. B. Lim, J. H. Yum, D. Yong, Y. Chong, J. M. Kim, and D. M. Livermore. *bla*$_{VIM-2}$ cassette-containing novel integrons in metallo-β-lactamase-producing *Pseudomonas aeruginosa* and *Pseudomonas putida* isolates disseminated in a Korean hospital. Antimicrob. Agents Chemother. 2002, 46:1053-1058.

Lee, K., Y. S. Lim, D. Yong, J. H. Yum, and Y. Chong. Evaluation of the Hodge Test and the Imipenem-EDTA Double-Disk Synergy Test for Differentiating Metallo-β-Lactamase-Producing Isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol. 2003, 41: 4623–4629.

Lee, K., Y. S. Lim, D. Yong, J. H. Yum, and Y. Chong. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. 2003. 41:4623-4629.

Lee, K., Yum, J. H., Yong, D., Lee, H. M., Kim, H. D., Docquier, J.-D., Rossolini, G. M., Chong, Y. (2005). Novel Acquired Metallo-{beta}-Lactamase Gene, blaSIM-1, in a Class 1 Integron from Acinetobacter baumannii Clinical Isolates from Korea. *Antimicrob. Agents Chemother.* 2005. 49: 4485-4491.

Lee, Kyungwon , Gyoung Yim Ha , Bo-Moon Shin , Jin Ju Kim , Jung Oak Kang , Sook Jin Jang , et al.,. Metallo-beta-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing Pseudomonas spp. and increase of IMP-producing Acinetobacter spp. Diagn Microbiol Infect Dis. 2004; 50:51-8.

Limansky, A. S., Mussi, M. A., Viale, A. M. Loss of a 29-Kilodalton Outer Membrane Protein in *Acinetobacter baumannii* is Associated with Imipenem Resistance. *J. Clin. Microbiol.* 2002, 40: 4776-4778.

Livermore, D. M. β-lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557–584

Livermore, DM. The threat from the pink corner. *Ann Med Int* 2003; 35: 226–234.

M.C. Ploy, F. Denis, P. Courvalin and T. Lambert, Molecular characterization of

integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron, *Antimicrob Agents Chemother* 2000, 44:2684–2688.

Marie Pe, Joel Fishbain, David Craft, Gregory Deye, Scott Riddell, Eric Milstrey, Bruno Petruccelli,Sylvain Brisse, Vanessa Harpin, Amy Schink, David J. Ecker, Rangarajan Sampath, and Mark W. Eshoo. Identification of Acinetobacter species and genotyping of Acinetobacter baumannii by multilocus PCR and mass spectrometry. : J Clin Microbiol. 2006, 44:2921-32.

McDonald LC, Walker M, Carson L, et al. Outbreak of *Acinetobacter* spp. bloodstream infections in a nursery associated with contaminated aerosols and air conditioners. Pediatr Infect Dis J 1998; 17:716–22.

Melamed, R. D. Greenberg, N. Porat, M. Karplus, E. Zmora, A. Golan, P. Yagupsky and R. Dagan. Successful control of an *Acinetobacter baumannii* outbreak in a neonatal intensive care unit. J Hosp Infect. 2003, 53:31-.8

Minami, S. M. Akama, H. Araki, Y. Watanabe, H. Narita, S. Iyobe and S. Mitsuhashi, Imipenem and cephem resistant *Pseudomonas aeruginosa* carrying plasmids coding for class B β-lactamase. *J Antimicrob Chemother* (1996, 37: 433–444.

Misbah, S., H. Hassan, M. Y. Yusof, Y. A. Hanifah, and S. Abubakar. 2005. Genomic species identification of *Acinetobacter* of clinical isolates by 16S rDNA sequencing. Singapore Med., J. 2005, 46:461-464.

Mortensen JE, LaRocco MT, Steiner B, Ribner B. Protein fingerprinting for the determination of relatedness in *Acinetobacter calcoaceticus* subspecies *anitratus* isolated from patients in a surgical intensive care unit. Infect Control 1987; 8:512-515.

Mussi, M. A, Adriana S. Limansky, and Alejandro M. Viale. Acquisition of Resistance to Carbapenems in Multidrug-Resistant Clinical Strains of *Acinetobacter baumannii*: Natural Insertional Inactivation of a Gene Encoding a Member of a Novel María Soledad Ramírez, Cecilia Quiroga, and Daniela Centrón Antimicrob Agents Chemother. 2005; 49:5179-81.

NCCLS, 2001. National Committee for Clinical Laboratory Standards.. Performance standards for antimicrobial susceptibility testing; 11th informational supplement. M100-S11. 2001 .National Committee for Clinical Laboratory Standards, Wayne, Pa.

Nemec, A., L. Dijkshoorn, and P. Jezek. Recognition of two novel phenons of the genus *Acinetobacter* among non-glucose-acidifying isolates from human specimens. J Clin Microbiol. 2000, 38:3937-3941.

Nield, B. S., Holmes, A. J., Gillings, M. R., Recchia, G. D., Mabbutt, B. C., Nevalainen, K. M. H. & Stokes, H. W. *FEMS* Microbiol. Lett. 2001,195: 59–

65.

Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura and N. Kato, Molecular characterization of an enterobacterial metallo-β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother 1994, 387: 71–78.

Partridge, S. R., H. J. Brown, and R. M. Hall. Characterization and movement of the class 1 integron known as Tn*2521* and Tn*1405*. Antimicrob Agents Chemother 2002. 46:1288-94.

Pillay, T., D. G. Pillay, M. Adhikari, A. Pillay, and A. W. Sturm. An outbreak of neonatal infection with *Acinetobacter* linked to contaminated suction catheters. J. Hosp. Infect. 1999. 43:299-304.

Ploy, M.C., F. Denis, P. Courvalin and T. Lambert, Molecular characterization of integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron, *Antimicrob Agents Chemother* 2000, 44: 2684–2688.

Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D. Cavallo and P. Nordmann, Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother*. 2000. 44, 891–897.

Recchia, G. D. and R.M. Hall, Gene cassettes: a new class of mobile element, *Microbiology* 1995,141: 3015–3027.

Recchia, G. D., H.W. Stokes and R.M. Hall, Characterisation of specific and secondary recombination sites recognised by the integron DNA integrase, *Nucleic Acids Res* 1994, 22 : 2071–2078.

Richet, H. M., J. Mohammed, L. C. McDonald, and W. R. Jarvis.. Building communication networks: International Network for the Study and Prevention of Emerging Antimicrobial Resistance. Emerg. Infect. Dis. 2001, 7:319-322.

Rowe-Magnus, D. A., Guerot, A. M., Ploncard, P., Dychinco, B., Davies, J. & Mazel, D. *Proc. Natl. Acad. Sci. USA*, (2001, 98: 652–657.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual Cold Spring Harbor Lab. Press, Plainview, NY, 2nd Ed.

Sang-Oh Lee, Nam Joong Kim, Sang-Ho Choi, Tae Hyong Kim, Jin-Won Chung, Jun-Hee Woo, Jiso Ryu, and Yang Soo Kim. 2004. Risk Factors for Acquisition of Imipenem-Resistant *Acinetobacter baumannii*: a Case-Control Study. Antimicrobial Agents and Chemotherapy, January 2004, 48, 224-228.

Scerpella EG, Wanger AR, Armitige L, Anderlini P, Ericsson CD. Nosocomial outbreak caused by a multiresistant clone of *Acinetobacter baumannii:* results

of the case-control and molecular epidemiologic investigations. *Infect Control Hosp Epidemiol* 1995;16:92-97.

Scerpella EG, Wanger AR, Armitige L, Anderlini P, Ericsson CD. Nosocomial outbreak caused by a multiresistant clone of Acinetobacter baumannii: results of the case-control and molecular epidemiologic investigations. Infect Control Hosp Epidemiol. 1995, 16:92-7.

Seifert and Gerner –Smidt, Comparison of ribotyping and pulsed-field gel electrophoresis for molecular typing of *Acinetobacter baumannii* isolates. J. Clin. Microbiol. 1995, 33:1402-1407.

Seltmann, G., W. Beer, H. Claus, and H. Seifert. 1995. Comparative classification of *Acinetobacter baumannii* strains using seven different typing methods. Zentbl. Bakteriol. 282:372-383.

Senda, K., Y Arakawa, S Ichiyama, K Nakashima, H Ito, S Ohsuka, K Shimokata, N Kato, and M Ohta. PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. J Clin Microbiol. 1996 34: 2909–2913.

Senda, K., Y. Arakawa, K. Nakashima, H. Ito, S. Ichiyama, K. Shimokata, N. Kato and M. Ohta, Multifocal outbreaks of metallo-β-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum β-lactams, including carbapenems. Antimicrob Agents Chemother 1996,40: 349–353.

Seok Hoon Jeong, Il Kwon Bae, Kwang Ok Park, Young Jun An, Seung Ghyu Sohn, Seon Ju Jang, Kwang Hoon Sung, Ki Suk Yang, Kyungwon Lee, Dongeun Young, and Sang Hee Lee. Outbreaks of Imipenem-Resistant *Acinetobacter baumannii* Producing Carbapenemases in Korea. The Journal of Microbiology, 2006; 44,:423-431.

Seok Hoon Jeong, Il Kwon Bae, Kwang Ok Park, Young Jun An, Seung Ghyu Sohn, Seon Ju Jang, Kwang Hoon Sung, Ki Suk Yang, Kyungwon Lee, Dongeun Young, and Sang Hee Lee. Outbreaks of Imipenem-Resistant Acinetobacter baumannii Producing Carbapenemases in Korea. The Journal of Microbiology, 2006;44:423-431.

Sherertz RJ, Sullivan ML. An outbreak of infections with *Acinetobacter calcoaceticus* in burn patients: contamination of patients' mattresses. J Infect Dis 1985;151:252-258.

Sherertz, R.J. and M.L. Sullivan, An outbreak of infections with *Acinetobacter calcoaceticus* in burn patients: contamination of patients' mattresses, J Infect Dis 1985, 151: 252–258.

SHI Wei-feng, JIANG Jian-ping , MI Zu-huang. Relationship between antimicrobial

resistance and aminoglycoside- modifying enzyme gene expressions in Acinetobacter baumannii. Chinese Medical Journal 2005;118(2) :141-145.

Smith PW, Massanari M. Room humidifiers as the source of *Acinetobacter* infections. *JAMA* 1977; 237:795-797.

Sneath , P. H. A. and R. R. Sokal. 1973. Numerical Taxonomy: The Principles and Practice of Numerical Classification. 1973. WH Freeman , Calif. USA.

Steward, C. D., Rasheed, J. K., Hubert, S. K., Biddle, J. W., Raney, P. M., Anderson, G. J., Williams, P. P., Brittain, K. L., Oliver, A., McGowan, J. E. Jr., Tenover, F. C. (2001). Characterization of Clinical Isolates of Klebsiella pneumoniae from 19 Laboratories Using the National Committee for Clinical Laboratory Standards Extended-Spectrum {beta}-Lactamase Detection Methods. *J. Clin. Microbiol.* 39: 2864-2872.

Stokes, H. W., Andrew J. Holmes,, Blair S. Nield, Marita P. Holley, K. M. Helena Nevalainen, Bridget C. Mabbutt, and Michael R. Gillings., Gene Cassette PCR: Sequence-Independent Recovery of Entire Genes from Environmental DNA. Applied and Environmental Microbiology, 2001, 67: 5240-5246.

Takahashi, A., Yomoda, S., Kobayashi, I., Okubo, T., Tsunoda, M. and Iyobe, S., 2000. Detection of carbapenemase-producing *Acinetobacter bauamnnii* in a hospital. *J Clin Microbiol* 38, pp. 526–529.

Tankovic, J., P Legrand, G De Gatines, V Chemineau, C Brun-Buisson and J Duval. Characterization of a hospital outbreak of imipenem-resistant *Acinetobacter baumannii* by phenotypic and genotypic typing methods. J Clin Microbiol. 1994; 32(11): 2677-2681.

Tenover, F. C., R. D. Arbeit, and R. V. Goering. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Infect. Control Hosp. Epidemiol. 1997. 18:426-439.

Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233-2239.

Tolman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh, Abstr. 42nd Intersci. Conf. Antimicrob. 2002.

Traub, W. H. 1989. *Acinetobacter baumannii* serotyping for delineation of outbreaks of nosocomial cross-infection. J. Clin. Microbiol. 1989, **27:**2713-2716.

Tsakris, A., S. Pournaras, N. Woodford, M.-F.I. Palepou, G.S. Babini, J. Douboyas and D.M. Livermore, Outbreak of infections caused by *Pseudomonas aeruginosa*

producing VIM-1 carbapenemase in Greece. *J Clin Microbiol*.(2000, 38: 1290–1292 .

Turton J. F., M. E. Kaufmann, M. Warner, J. Coelho, L. Dijkshoorn, T. van der Reijden and T. L. Pitt. A prevalent, multiresistant clone of *Acinetobacter baumannii* in Southeast England. Journal of Hospital Infection. 2004, 58:170-179.

Turton, Jane F, M. E. Kaufmann, J. Glover, J. M. Coelho, M. Warner, R. Pike, and T. L. Pitt. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. J Clin Microbiol 2005.43:3074-3082.

Turton, Jane F., Mary E. Kaufmann, Martin J. Gill, Rachel Pike, Paul T. Scott,4 Joel Fishbain, David Craft, Gregory Deye, Scott Riddell,6 Luther E. Lindler, and Tyrone L. Pitt . Comparison of *Acinetobacter baumannii* Isolates from the United Kingdom and the United States That Were Associated with Repatriated Casualties of the Iraq Conflict. Journal of Clin. Microbiol. 2006, 44: 3623-3627.

Vahaboglu, H., R. Ozturk, G. Aygun, F. Coskunkan, A. Yaman, A. Kaygusuz, H. Leblebicioglu, I. Balik, K. Aydin, and M. Otkun.. Widespread detection of PER-1-type extended-spectrum ß-lactamases among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. Antimicrob. Agents Chemother. 1997; 41:2265-2269.

Vaneechoutte, M., L. Dijkshoorn, I. Tjernberg, A. Elaichouni, P. de Vos, G. Claeys, and G. Verschraegen. 1995. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. 1995 33:11-15.

Vila, J., M. Navia, J. Ruiz and C. Casals, Cloning and nucleotide sequence analysis of a gene encoding an OXA-derived β-lactamase in *Acinetobacter baumannii*, Antimicrob Agents Chemother 1997, 41:, 2757–2759.

Villers, D., E Espaze and M Coste-Burel , Nosocomial *Acinetobacter baumannii* infections: microbiological and clinical epidemiology. *Ann Intern Med* . 1998. 129 : 182–189.

Walsh T. R. The emergence and implications of metallo-β-lactamases in Gram-negative bacteria Clinical Microbiology & Infection. Clin Microbiol Infect 2005; 11 (Suppl. 6): 2–9.

Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann. 2005. Metallo-ß-Lactamases: the Quiet before the Storm? Clinical Microbiology Reviews. 2005, 18:306-325.

Walther-Rasmussen Jan and Niels Høiby. OXA-type carbapenemases. Journal of Antimicrobial Chemotherapy 2006, 57:373-383.

Wang, S. H., Sheng, W. H., Chang, Y. Y. & 7 other authors Healthcare-associated outbreak due to pan-drug resistant *Acinetobacter baumannii* in a surgical intensive care unit. J Hosp Infect 2003. 53, 97–102.

Watanabe, K., S. Iyobe, M. Inoue and S. Mitsuhashi, Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991, 35: 147–151.

Wavel, Hodge, Jennie Clak, and E. C. Tramont. J Clin Microbiol 1978; 7: 102–3.

Webster, C. A.,, M. Crowe1, H. Humphreys1 and K. J. Towne. Surveillance of an adult intensive care unit for long-term persistence of a multi-resistant strain of *Acinetobacter baumannii.* European Journal of Clinical Microbiology & Infectious Diseases 1998, 17: 171-176.

Weernink A, Severin WPJ, Tjernberg I, Dijkhoorn. Pillows, an unexpected source of *Acinetobacter. J Hosp Infect* 1995; 29:189-199.

Wendt, C, B Dietze, E Dietz and H Ruden. Survival of *Acinetobacter baumannii* on dry surfaces. Journal of Clinical Microbiology, 1997, 35:1394-1397.

Woodford, Neil, Matthew J. Ellington, Juliana M. Coelho, Jane F. Turton, M. Elaina Ward, Susan Brown, Sebastian G.B. Amyes and David M. Livermore. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. International J.of Antimicrob. Agents. 2006. 27: 351-353.

Yao-Guang Liu and Ning Huang, 1995. Efficient Amplification of Insert End Sequences from Bacterial Artificial Chromosome Clones by Thermal Asymmetric Interlaced PCR Plant Mol.. Biol. Reporter. 1998, 16.175-8.

Yong, D., Choi, Y. S., Roh, K. H., Kim, C. K., Park, Y. H., Yum, J. H., Lee, K., Chong, Y. (2006). Increasing Prevalence and Diversity of Metallo-{beta}-Lactamases in Pseudomonas spp., *Acinetobacter* spp., and Enterobacteriaceae from Korea.. Antimicrob. Agents Chemother. 50: 1884-1886.

Zarrilli, R., Crispino, M., and M. Bagattini *et al.*, Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance, J Clin Microbiol . 2004, 42: 946–953.

Zaza, S. and Jarvis, W. R. Investigations of outbreaks. In: Mayhall CG, ed. *Hospital Epidemiology and* Infection Control. 1996:106-113.Baltimore, MD: Williams & Wilkins;

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Bidur Prasad Chaulagain
December 27, 2006
Department of Medicine, Graduate School,
Chosun University,
Gwangju, Republic of Korea

저작물 이용 허락서

학 과	**의 학 과**	학 번	20047557	과 정	석사, 박사
성 명	한글: 비듀 프라사드 찰라가인			영문 : **Bidur P. Chaulagain**	
주 소	광주광역시 동구 서석동 조선대학교 의과대학 의학연구소				
연락처	062-230-6040	E-MAIL	chaulagain@gmail.com		
논문제목	한글 : **한국의 한 대학병원에서 발생한 Imipenem 내성 *Acinetobacter baumannii* 돌발감염의 분자적 및 역학적 연구**				
	영어 : **Molecular and Epidemiological Investigation of an Outbreak of Imipenem Resistant *Acinetobacter baumannii* in a University Hospital in Korea**				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 -조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB 구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5 년으로 하고, 기간종료 3 개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1 개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(o) 반대( )

2006 년 12 월 일

저작자: **Bidur Prasad Chaulagain** (서명 또는 인)

조선대학교 총장 귀하