(P = 0.009), longer admitted days (P <0.001), and longer treated days (P = 0.027) than those with ISAB. Comparing data of 21 mortal patients and 41 alive ones, there was no significant difference between these 2 groups in WBC, CRP or age (all P > 0.05). Results of the univariate analysis revealed significant differences between the patients with IRAB or ISAB in terms of previous ICU stay at least 5 days, use of mechanical ventilation at least 5 days, given carbapenems or extended-spectrum cephalosporines for at least 5 days, and given carbapenem (Table 1). However, only given carbapenems or extended-spectrum cephalosporines for at least 5 days was the only independent risk factor for acquiring imipenem resistance by multivariate logistic regression analysis (Odds Ratio: 342.07, 95% CI: 2.07–56514.17). Any of other risk factors was correlated with this independent risk factor (P < 0.001).

Conclusion: Patients infected with IRAB had higher mortality rate and longer hospital stay than those with ISAB. Prior use of carbapenems or extended-spectrum cephalosporines for at least 5 days was the only independent risk factor significantly correlated with IRAB infection. Therefore, restricted use of carbapenems and extended-spectrum cephalosporines is crucial to reduce acquisition of IRAB.

Table 1. Univariate analysis of risk factors for acquiring imipenem-resistant *A. baumannii* (IRAB)

Characteristic*	IRAB (N=42) n (%)	ISAB (N=20) n (%)	P-value	Odds ratio (95% CI)
Mean age, years	66.2±18.1	63.3±18.7	0.549	1.01 (0.98-1.04)
Male gender	29 (69.0)	10 (50.0)	0.150	2.23 (0.75-6.66)
Number of underlying diseases	2.3 ± 1.4	1.9 ± 1.1	0.177	1.34 (0.88-2.06)
Hospital size > 1000 beds	19 (45.2)	7 (35.0)	0.446	1.53 (0.51-4.62)
Previous ICU stay≥5 days	39 (92.9)	4 (20.0)	<0.001	52.00 (10.4-259.10)
Mechanical ventilation≥5 days	39 (92.9)	3 (15.0)	<0.001	29.60 (7.02-124.89)
Use of extended-spectrum antibiotics ≥5 days	42 (100)	3 (15)	<0.001	796.54 (24.03-26406.65)
Carbapenems	24 (57.1)	1 (5)	0.003	25.33 (3.10-207.23)
Extended-spectrum Cephalosporins	25 (59.5)	12 (60.0)	0.971	0.980 (0.33-2.91)
Piperacillin–tazobactam	8 (19.0)	0	0.132	10.34 (0.50-215.39)
Aminopenicillins	6 (14..3)	0	0.204	7.32 (0.338-158.57)
Aminoglycosides	4 (9.5)	0	0.342	4.61 (0.20-107.56)
Fluoroquinolones	14 (33.3)	3 (15.0)	0.223	2.83 (0.71-11.32)

*Unless otherwise indicated, data are the number (%) of patients with each characteristic.
IRAB, imipenem-resistant *Acinetobacter baumannii*; ISAB, imipenem-susceptible *A. baumannii*; ICU, intensive care unit.

P714 Molecular epidemiology of clinical *Acinetobacter baumannii* isolates in a Korean hospital

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Objectives: *Acinetobacter baumannii* are commonly associated with nosocomial infections, and usually multiresistant. We investigated the characteristics of 35 *A. baumannii* isolates by multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), PCR of the antimicrobial resistance determinants, and antimicrobial susceptibilities.

Methods: This study included 16 carbapenem-resistant *A. baumannii* (CRAB) and 19 carbapenem-susceptible *A. baumannii* (CSAB) from a secondary hospital in Daejeon, Korea between January and July 2009 without any selection criteria. PCR amplification of genes for OXA-type carbpenemases (OXA-23, 24, 51, and 58), metallo-β-lactamases (IMP and VIM), ISCR1, aminoglycoside-modifying enzymes (AMEs), and armA was performed. Susceptibility to imipenem, meropenem, amikacin, tobramycin, gentamicin, trimethoprim-sulfamethoxazole, cefepime, and ceftazidime were determined by an agar dilution method, and interpretation was in accordance with the CLSI 2010 guidelines. To elucidate the clonal relatedness, MLST and PFGE were carried out.

Results: CSAB showed more various genetic mechanisms than CRAB because 9 STs were identified in CSAB while only 2 STs were identified in CRAB. ST92 was the most prominent ST, accounting for 93.8% (15/16) of CRAB and 21.1% (4/19) of CSAB isolates. A novel double-locus variant of ST92 (STn1) was another major ST, which was detected in 42.1% (8/19) of CSAB isolates and not detected in CRAB isolates. The resistance rate to carbapenem was the highest (78.9%) in ST92 isolates while only one isolate had carbapenem resistance among isolates of other STs. BlaOXA-23 was positive in 62.5% (10/16) of CRAB isolates but negative in CSAB isolates. All blaOXA-23-bearing isolates except one belonged to ST92. The resistance rates of CRAB isolates to all antimicrobial agents tested were higher than those of CSAB isolates.

Conclusions: ST92 was dorminant sequence-type in a Korean hospital, and it closely correlated with carbapenem resistance and the presence of blaOXA-23.

P715 Evaluation of a novel selective medium, CHROMagar acinetobacter with KPC supplement, for detection of multidrug-resistant *Acinetobacter baumannii* from clinical specimens in Japan

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Objectives: Multidrug-resistant *A. baumannii* (MRAB) has recently been reported in both western countries and in China. However, cases of such infections are very rare in Japan. Here, we report hospital-acquired infection by *A. baumannii* blaOXA-51-like resistant to the carbapenems imipenem or meropenem, the aminoglycoside amikacin and the fluoroquinolones levofloxacin or ciprofloxacin. We also evaluated the novel chromogenic medium, CHROMagar *Acinetobacter* (CHROMagar, France) supplemented with KPC to detect MRAB.

Methods: KPC-supplemented CHROMagar *Acinetobacter* was used for isolation of drug-resistant strains, such as *E. coli* blaCTX-M-2, *P. mirabilis* blaCTX-M-2, *K. pneumoniae* blaIMP-1, *E. cloacae* blaIM-1, Multidrug-resistant *P. aeruginosa* (MDRP) blaIMP-1, MDRP blaVIM-2, permeability decreasing MDRP, MRAB blaIMP-1, MRAB blaOXA-23, MRAB blaOXA-51-like and *S. maltophilia*. There were incubated at 35ºC for 18–72 h. For the clinical trial, 5,740 specimens from the pharyngeal swabs, urine and rectal swabs, and 6,617 swab specimens from environmental materials were plated on this medium and incubated at 35ºC for 18–72 h.

Results: In the trials of stock strains, three genotypes of MRAB showed red and large colonies after cultivation for 18 h at 35ºC. ESBL-producing enteric bacilli did not grow on the medium. However, *K. pneumoniae* blaKPC and *E. cloacae* blaIPM-1 grew as small bluecolonies after 18 h of cultivation. MDRP blaVIM-2 did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP blaIMP-1 yielded small red colonies after 48 h of cultivation.

Twenty-one MRAB were detected from clinical and environmental specimens.

Clinical and environmental isolates with carbapenems MIC ⩾2 micro g/ml (*P. aeruginosa*, *P. fluorescens*, *S. maltophilia*, *C. indologenes* and *A. xylosoxidans*) grew as small red colonies on this medium after cultivation for 24–48 h. *P. putida* isolates with carbapenems MIC <1 micro g/ml yielded small red colonies on this medium after incubation for 24 h.

Among red colonies suspected *Acinetobacter* were easily discriminated from other genus by oxidase test and Gram staining.

Conclusion: The novel selective medium CHROMagar *Acinetobacter* supplemented with KPC was useful for detecting our cases with MDRB blaOXA-51-like. In addition, it was especially valuable for active surveillance of specimens containing multiple bacteria, such as those from the pharynx, urine, faeces and the environment.

P716 MDR *Acinetobacter baumannii* faecal colonisation of nursing home residents of northern Portugal

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Objectives: Our work, in fecal colonization with ESBL producers in nursing home (NH) residents, showed that we could also find carbapenem-resistant isolates. The aim of our work was the detection of carbapenem-resistant Gram negatives, in the fecal flora of NH residents, in the North of Portugal, including Porto metropolitan area.

Methods: Faecal samples of NH residents from two NH of Porto Metropolitan area and one in the North of Portugal, were collected during 2008 and 2009. Samples were suspended in BHI. Isolates were selected

in MacConkey agar with ceftazidime (2mg/l), cefotaxime (2mg/l), aztreonam (2mg/l) and imipenem (1mg/l). Colonies were randomly selected and susceptibility to antimicrobial agents was determined by agar diffusion methods according to the CLSI guidelines. Carbapenem-resistant isolates were obtained from imipenem containing plates and also from other antibiotic selective media. Phenotypic identification of the selected isolates, was achieved by ID 32 GN. β-lactamases were characterized by isoelectric focusing. MICs were determined by the Etest methodology in Mueller-Hinton agar. Hodge test, was carried out according to the CLSI guidelines, bioassays and inhibition studies were done according to the literature.

Results: Our work showed the presence of 7 non fermenter isolates, identified as *Acinetobacter baumannii* by ID 32 GN, from three different nursing homes in the same region but geographically apart. Susceptibility testing showed a multi-resistant phenotype including carbapenems and fluoroquinolones and susceptibility to amikacin. MICs to imipenem were over 16 mg/l, to meropenem were over 8 mg/l and to cefotaxime were over 32 mg/l. Bioassays and Hodge test, showed carbapenem degradation in representative isolates.

Conclusions: Hospitals of Porto urban area, have already experienced the installation of outbreaks of MDR *Acinetobacter baumannii* of difficult resolution, by patients transferred from hospitals in the same metropolitan area. NH of this area interchanging patients with local hospitals, might function as reservoirs of MDRGN contributing for outbreaks in particularly important hospital wards. This question should be addressed as a relevant public health threat, once it might also contribute for community spread of MDRGN via domiciliary care of dependent people and dispersion to the healthy population. Our work seems to suggest the utility of pre-admission screening of MDRGN in the fecal flora of nursing home residents.

Porins and efflux pumps are good friends

P717 RNase-mediated post-transcriptional regulation of gene ompA seems to be the major mechanism of carbapenem heteroresistance in *A. baumannii*

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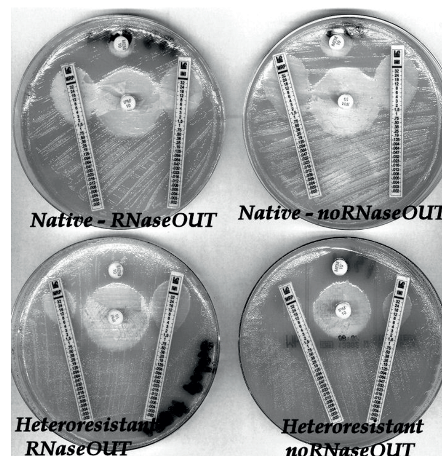
Objectives: To investigate the molecular mechanism of carbapenem heteroresistance in characterized carbapenem heteroresistant *Acinetobacter baumannii* (ChRAB) isolates.

Methods: Carbapenem Etest minimal inhibitory concentrations (MICs) of the native and the respective meropenem heteroresistant populations of 13 previously characterized ChRAB clinical isolates were tested after defrosting from one year storage at −80ºC. Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of protein crude extract, in-gel protein digestion of bands of interest, nano-high performance liquid chromatography and mass spectrometer (MS/MS) analysis for protein identification as well as quantitative real-time reverse transcriptase PCR (qRT-PCR) for the quantification of expression of gene ompA were performed. All molecular techniques were applied to both the native and the meropenem heteroresistant populations. Etest carbapenem MICs of the native and the respective meropenem heteroresistant populations were tested in Mueller-Hinton agar plates containing 30U/mL RNaseOUT RNase inhibitor, to investigate the putative role of RNases in regulation of carbapenem resistance mechanisms.

Results: Only one isolate AB133 retained stable heteroresistant subpopulations and was further investigated whereas the respective heteroresistant subpopulations of the other isolates returned to the native susceptible phenotype. SDS-PAGE showed a band of approximately 35 to 40kD to be of lower intensity in the heteroresistant population versus the native population, and MS/MS analysis identified this band as the outer membrane protein A (OmpA) when the amino acid sequence was compared against ATCC17978 genome. qRT-PCR showed that expression of gene ompA was 1.93 fold of change higher in the heteroresistant population. For the heteroresistant population, meropenem MIC in RNaseOUT inoculated Mueller-Hinton agar plates was more than 3-fold lower (from >32 to 6 mg/L) relative to the free-of-RNaseOUT medium and imipenem MIC was 2-fold lower (from 6 to 1.5 mg/L). Native population showed no variations in carbapenem MICs when RNaseOUT was inoculated in the medium. When RNaseOUT treated heteroresistant population was analyzed by SDS-PAGE, a band of approx. 35 to 40kD was present and was identified as OmpA by MS/MS analysis.

Conclusions: RNase-mediated post-transcriptional regulation of gene ompA seems to be the major mechanism of carbapenem heteroresistance in *A. baumannii*.



P718 Effect of Sub-MIC concentrations of biocides on the expression of genes coding for efflux pumps and porins in *Acinetobacter baumannii* ATCC 19606

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Objective: We analyzed the ability of sub-MICs concentrations of five biocides commonly used in hospitals to affect the expression of genes coding for efflux systems and porins related with antimicrobial resistance and/or virulence in *Acinetobacter baumannii* ATCC 19606 (Ab).

Methods: The biocides evaluated were domestic bleach (DB), Sterillium (ST; propan-2-ol, propan-1-ol, mecetronium ethyl sulfate), Bdine (BT; povidone iodine), chlorhexidine digluconate (CHX) and benzalkonium chloride (BKC). The Ab was grown in i) Mueller-Hinton broth containing concentrations of biocides equivalent to the respective 0.25x MIC and ii) Mueller-Hinton broth without biocides (control). Expression of genes coding for adeB, adeJ, abeM, OmpA, CarO, OprD-like and the Omp 33–36 kDa was determined by real time RT-PCR in a Light Cycler 2.0 using specific oligonucleotides and SYBR Green I. Gene expression was normalised with the 16S rDNA. Relative gene expression was calculated using the 2(-DeltaDeltaCt) method. Expression of genes in Ab grown with no biocides was set as 1 (reference).

Results: See table.

Table. Relative expression of efflux and porin genes.

Gene	DB	ST	BT	CHX	BKC
adeB	2,3	16	6,5	19,7	90,5
adeJ	1,7	1,9	3,2	3,5	4,6
abeM	3,2	4,3	2,6	3,2	22,6
ompA	1,5	3,2	3	4	6,5
carO	2,6	5,2	1,2	2,5	3,5
oprD-like	4,6	3,5	1,6	3,5	97,1
omp 33-36	3,2	1,6	3,7	0,8	6,9

Conclusions:

1. Sub-MICs concentration of biocides increased the expression of most of the efflux-related genes analysed, particularly adeB and abeM.