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


**Objectives**: Acinetobacter baumannii are commonly associated with nosocomial infections, and usually multidrug resistant. 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 carbapenemases (OXA-23, 24, 51, and 58), metallo-

**Results**: There were incubated bacteria 24 h at 35°C 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 35°C for 18-72 h. In the trials of stock strains, three genotypes of MRAB showed red and large colonies after cultivation for 18 h at 35°C. ESBL-producing enteric bacilli did not grow on the medium. However, K. pneumoniae blaKPC and E. cloacae blalMP-1 grew as small blue colonies after 18 h of cultivation. MDRP blalVIM-2 did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP blalPM-1 yielded small red colonies after 48 h of cultivation. Twenty-one MRAB were detected from clinical and environmental specimens.

**Conclusion**: The novel selective medium CHROMagar Acinetobacter demonstrated by this study was quite useful for detecting MDRP isolates, and it is a promising method for the detection of MDRP isolates in clinical and environmental samples.

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


**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 blalOXA-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 blalCTX-M-2, P mirabilis blalCTX-M-2, K. pneumoniae blalMP-1, E. cloacae blalAM-1. Multidrug-resistant P. aeruginosa (MDRP) blalMP-1, MDRP blalVIM-2, permeability decreasing MDRP, MRAB blalMP-1, MRAB blalOXA-23, MRAB blalOXA-51 like and S. maltophilia. There were incubated at 35°C 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 35°C 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 35°C. ESBL-producing enteric bacilli did not grow on the medium. However, K. pneumoniae blalKPC and E. cloacae blalPM-1 grew as small blue colonies after 18 h of cultivation. MDRP blalVIM-2 did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP blalPM-1 yielded small red colonies after 48 h of cultivation. Twenty-one MRAB were detected from clinical and environmental specimens.

**Conclusion**: The novel selective medium CHROMagar Acinetobacter supplemented with KPC was useful for detecting our cases with MDRB blalOXA-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 for one belonged to ST92. The resistance rates of CRAB isolates to all antimicrobial agents tested were higher than those of CSAB isolates.

**Conclusion**: ST92 was dominant sequence-type in a Korean hospital, and it closely correlated with carbapenem resistance and the presence of blalOXA-23.
Porins and efflux pumps are good friends

RNase-mediated post-transcriptional regulation of gene expression of Acinetobacter 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 −80°C. 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 30μM RNaseOUT RNase inhibitor, to investigate the putative role of RNases in regulation of carbapenem resistance mechanisms.

Results: Only one isolate AB133 retained stable heteroresistant 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 ATCC19606 genome. qRT-PCR showed that expression of gene ompA was 1.93 fold of change higher versus the native population, and MS/MS analysis identified this band as of lower intensity 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 6mg/L) relative to the free-of-RNaseOUT medium and imipenem MIC was 2-fold lower (from 6 to 1.5mg/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.