



# ACINETOBACTER 2010

**8th International Symposium on the Biology of  
*Acinetobacter***

**1 – 3 September 2010  
Università di Roma Tre, Rome, Italy**

**Programme and Abstract Book**



# Acinetobacter 2010

**8th International Symposium on the Biology of *Acinetobacter***  
**1 – 3 September 2010**  
**Aula Magna, Università di Roma Tre, Via Ostiense 161, Rome, Italy**

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# Acinetobacter 2010

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## Scientific Programme

### Wednesday 1 September 2010

1600-2000 Registration, Reception and Get-Together

### Thursday 2 September 2010

0900-0910 Welcome and Introduction  
 P. Visca

**SESSION 1** Chair: K. Towner

0910-0940 **Keynote Lecture:**  
**Evolutionary structure and phylogenetic lineages of *Acinetobacter***  
**S. Brisse**

0940-0955 O1 Taxonomy and nomenclature of *Acinetobacter* in the light of a “sequence-based” versus a polyphasic approach  
 P. Kämpfer

0955-1010 O2 Identification of variable-number tandem-repeats (VNTR) in *Acinetobacter baumannii* and inter-laboratory validation of an optimized multiple-locus VNTR analysis (MLVA) typing scheme  
C. Pourcel, F. Minandri, Y. Hauck, S. D’Arezzo, F. Imperi, G. Vergnaud and P. Visca

1010-1025 O3 Use of the accessory genome for characterization and typing of isolates of *Acinetobacter baumannii*  
J. F. Turton and B. Baddal

1025-1040 O4 Molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* strains in four Mediterranean countries using multilocus sequence typing and comparative genome analysis  
 M. Giannouli, A. Di Popolo, F. Rocco, P. P. Di Nocera, M. Triassi, M. Pallen, S. Brisse and R. Zarrilli

**1040-1110 Coffee break and discussions**

**SESSION 2** Chair: G. Rossolini1110-1140 **Keynote Lecture:**  
**Comparative genomic analysis of *Acinetobacter***  
**A. Carattoli**1140-1155 O5 Extensive variability is present among contemporaneous isolates of *Acinetobacter baumannii*: insights from genome sequencing  
M. D. Adams, N. Molyneaux, F. Perez and R. A. Bonomo1155-1210 O6 Exploring the evolutionary dynamics of plasmids: the *Acinetobacter* pan-plasmidome  
M. Fondi, M. C. Papaleo, A. Mengoni, I. Maida, E. Perrin, M. Vaneechoutte, L. Dijkshoorn and R. Fani1210-1225 O7 Molecular identification and characterization of *Acinetobacter* spp. clinical isolates from Norway  
N. Karah, B. Haldorsen, K. Hegstad, G. Skov Simonsen, A. Sundsfjord, Ø. Samuelsen and the Norwegian Study Group on *Acinetobacter*.1225-1240 O8 Epidemiology of *Acinetobacter baumannii* in three intensive care units in Sicily, Italy: inter-hospital and intra-hospital spread  
A. Agodi, M. Barchitta, G. Valenti, M. A. Romeo, L. Giaquinta, C. Santangelo and G. Castiglione**1240-1400 Poster Discussion Session – buffet lunch available at 1300****SESSION 3** Chair: J. Rodriguez-Baño1400-1430 **Keynote Lecture:**  
**Clinical importance of *Acinetobacter***  
**H. Seifert (DE)**1430-1445 O9 Molecular epidemiology of *Acinetobacter baumannii*-group bloodstream isolates in the United States, 1995 – 2004  
K. Janssen, H. Wisplinghoff, P. G. Higgins and H. Seifert1445-1500 O10 Do biofilm formation and interactions with human cells explain the clinical success of *Acinetobacter baumannii*?  
A. de Breij, L. Dijkshoorn, E.L. Lagendijk, J.M. van der Meer, A.J. Koster, G.V. Bloemberg, R. Wolterbeek, P.J. van den Broek and P.H. Nibbering1500-1515 O11 Surface-associated motility is a common trait of clinical *Acinetobacter* isolates  
G. Wilharm, P. Morczinek, F. Faber, T. Kerrinnes, D. Lepka, S. Gröbner, Y. Pfeifer and E. Skiebe

- 1515-1530 O12 Evaluation of cell surface properties of two *Acinetobacter baumannii* strains isolated from the same patient  
M. Kempf, M. Eveillard, C. Lefrançois, F. Kowalczyk, S. Georgeault and M. L. Joly-Guillou
- 1530-1550 Coffee break**
- SESSION 4** Chair: N. Petrosillo and P. Visca
- 1550-1620 **Keynote Lecture:**  
**Model systems for investigating the pathogenicity of *Acinetobacter***  
**A. Peleg**
- 1620-1635 O13 Distinctive infection and host defense profiles in mice intranasally infected with a hypervirulent clinical isolate of *Acinetobacter baumannii*  
R. Kuo Lee, G. Harris, H. H. Xu and W. Chen
- 1635-1650 O14 Proteomic analysis of *Acinetobacter baumannii* cell envelope from pellicle and planktonic growth states  
S. Martí, Y. Nait Chabane, J. Vila, T. Jouenne and E. Dé
- 1650-1705 O15 Mechanisms of biofilm formation in *Acinetobacter baumannii* – a proteomic perspective  
N.C. Soares, M. P. Cabral, J. Aranda, C. Rumbo and G. Bou
- 1705 End of session**
- 1830 Buses depart for tour of the Musei Capitolini and Conference Dinner at the Terrazza Caffarelli**

## Friday 3 September 2010

- SESSION 5** Chair: N. Ornston
- 0900-0930 **Keynote Lecture:**  
**Transcriptional regulation in *Acinetobacter***  
**E. Neidle**
- 0930-0945 O16 Deep sequencing and analysis of the transcriptome of *Acinetobacter baylyi* ADP1  
B. Segurens, V. de Berardinis, K. Labadie, V. Castelli, M. Durot, J. Weissenbach, M. Salanoubat
- 0945-1000 O17 How *Acinetobacter* species react to DNA damage  
T. Elam, G. Howington, S. Wheeler, A. Grice, J. Wilder and J. Hare

1000-1015 O18 Adaptation of *Acinetobacter radioresistens* S13 to hydrophobic environments: surface modification, emulsifying activities and stress responses revealed by spectroscopic and proteomic studies  
R. Mazzoli, P. Fattori, V. M. Riva, A. Molinaro, S. Leone, C. Giunta and E. Pessione

1015-1030 O19 *Acinetobacter baumannii* serine protease is required for serum resistance and biofilm dispersal  
L.S. McDaniel and L.B. King

**1030-1100 Coffee break and discussions**

**SESSION 6** Chair: J. Vila

1100-1130 **Keynote lecture:**  
**Emergence and regulation of carbapenem resistance in *Acinetobacter***  
**P. Nordmann**

1130-1145 O20 Horizontal transfer of the OXA-24 carbapenemase gene through outer membrane vesicles: a new mechanism for carbapenemase resistance gene dissemination in *Acinetobacter baumannii*  
C. Rumbo-Lorenzo, E. Fernandez-Moreira, A. Mosquera, M. Merino, M. Poza, F. Chaves and G. Bou

1145-1200 O21 Analysis of genes coding for penicillin-binding proteins in clinical isolates of *Acinetobacter baumannii* susceptible or resistant to carbapenems  
R. Cayô, M-C. Rodríguez, P. Espinal, F. Fernández-Cuenca, A. OcampoSosa, Á. Pascual, J. Vila and L. Martínez-Martínez

1200-1215 O22 Structure-function relationships of the carbapenem resistance-associated outer membrane proteins CarO from *Acinetobacter baumannii*  
M. Catel-Ferreira, V. Molle, T. Jouenne, G. Coadou and E. Dé

1215-1230 O23 Three novel variants of the AbaR3 resistance island in multidrug-resistant *Acinetobacter baumannii* strains of European clone I  
L. Krizova, L. Dijkshoorn and A. Nemeč

1230-1245 O24 Multiple antibiotic resistance in *Acinetobacter baumannii* from an Australian hospital  
V. Post, S. J. Nigro and R. M. Hall

**1245-1400 Poster Session – buffet lunch available at 1300**

**SESSION 7** Chair: E. Neidle

1400-1430 **Keynote Lecture:**  
***Acinetobacter* as a model organism for genetic analysis and genome engineering**  
**Nick Ornston (USA)**

- 1430-1445 O25 Insights into gene amplification in *Acinetobacter baylyi*: variations in the duplication frequency and genetic stability of different genomic loci  
K. T. Elliott, S. H. Craven, L. E. Cuff and E. L. Neidle
- 1445-1500 O26 The acquisition of foreign DNA by natural transformation of *Acinetobacter baylyi* ADP1  
K. Harms, P. J. Johnsen and K. M. Nielsen
- 1500-1515 O27 Large-scale experimental annotation of *Acinetobacter baylyi* ADP1 genome by a biochemical screening for large enzyme family activities  
V. de Berardinis, A. Mariage, J. L. Petit, A. Perret, J. Weissenbach and M. Salanoubat
- 1515-1530 O28 Fitness cost and stability of class I integrons  
I. Starikova, T. Munthali, K. Harms, P. Johnsen and K. Nielsen
- 1530-1545 O29 Inducible expression plasmids for *Acinetobacter baylyi* ADP1  
C. D. Murin, K. Segal, A. Bryksin and I. Matsumura

**1545-1605 Coffee break**

**SESSION 8** Chair: H. Seifert

1605-1635 **Keynote Lecture:**  
**Options for treatment of *Acinetobacter* infections**  
**J. Rodriguez-Baño**

- 1635-1650 O30 A novel mechanism of colistin resistance in *Acinetobacter baumannii*  
J. H. Moffatt, M. Harper, P. Harrison, J. D. Hale, E. Vinogradov, T. Seemann, R. Henry, B. Crane, F. St. Michael, A. D. Cox, B. Adler, R. L. Nation, J. Li and J. D. Boyce
- 1650-1705 O31 Biological cost of extreme resistance to tigecycline in *Acinetobacter baumannii*  
S. D'Arezzo, S. Fittipaldi, F. Imperi, S. Sepe, A. Di Giulio, L. Principe, N. Petrosillo and P. Visca
- 1705-1720 O32 Cloning and characterization of algC gene from *Acinetobacter baumannii*: essential for alginate synthesis in biofilms  
P. K. Sahu, K. R. Pardesi and B. A. Chopade
- 1720-1735 O33 Vaccination and immunotherapy against *Acinetobacter baumannii* using bacterial outer membrane proteins  
M. J. McConnell, J. Domínguez-Herrera, Y. Smani, R. López-Rojas, F. Docobo-Pérez and J. Pachón

**1735 Final Remarks** E. Bergogne-Bérézin

**1745 End of Symposium and Farewell** K. Towner

## Abstracts of Offered Oral Presentations

### O1 TAXONOMY AND NOMENCLATURE OF *ACINETOBACTER* IN THE LIGHT OF A “SEQUENCE-BASED” VERSUS A POLYPHASIC APPROACH

P. Kämpfer

**Institut für Angewandte Mikrobiologie, University Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany**

In the last years several new *Acinetobacter* species have been described. One reason is the detailed insight into the microbial diversity in various environments. But species descriptions are now largely based on the initial 16S rRNA gene sequencing approach, and the methods necessary for sequencing are now widely (and easily!) used in many laboratories. Several sequencing studies on housekeeping genes have furthermore allowed a more detailed insight into the so-called “phylogenetic” structure of the genus. However, despite the advantages of the sequence based approach, there appears to be a tendency to allow comparative sequence analyses of 16S rRNA gene sequence and other gene sequence data to classify new species contrary to the indications of other data. One of the problems coming along with this development is the increasing number of species, which cannot be differentiated phenotypically. In this regard the problem of species descriptions on the basis of single strains is often raised and even the necessity of the introduction of “rules” has been raised. Phenotype is still of major biological importance for taxonomy. A lot of complex genotypic information is behind phenotype (still to be discovered), and only phenotype shows, that these genes are really expressed. The criteria are used for species descriptions may change in the future, when we have a full insight into the complexity of the genomes of microorganisms. However, the biological meaning behind these sequence data is still not clear.

Experience has shown that the interplay between genetic and phenotypic datasets provides a sound basis for appreciating and describing the diversity of prokaryotes and has the potential to become the foundation of a more stable, in depth taxonomy of the prokaryotes including *Acinetobacter*. It is hoped that the recent publication of a set of guidelines (Tindall *et al.* 2010) will provide help and have also implications for other areas of microbial research.

Tindall, B.J., R. Rosselló-Móra, H.-J. Busse, W. Ludwig and P. Kämpfer. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010; 60: 249-266.

## **O2 IDENTIFICATION OF VARIABLE-NUMBER TANDEM-REPEATS (VNTR) IN ACINETOBACTER BAUMANNII AND INTERLABORATORY VALIDATION OF AN OPTIMIZED MULTIPLE-LOCUS VNTR ANALYSIS (MLVA) TYPING SCHEME**

**C. Pourcel<sup>a</sup>, F. Minandri<sup>b</sup>, Y. Hauck<sup>a</sup>, S. D'Arezzo<sup>b</sup>, F. Imperi<sup>c</sup>, G. Vergnaud<sup>a,d</sup> & P. Visca<sup>c</sup>**

<sup>a</sup>Université Paris-Sud, Institut de Génétique et Microbiologie, CNRS, Orsay, France;

<sup>b</sup>Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani IRCCS; <sup>c</sup>Dipartimento di Biologia, Università Roma Tre, Rome, Italy; <sup>d</sup>Division de Microbiologie Analytique, Centre d'Etudes du Bouchet, Vert le Petit, France

*Acinetobacter baumannii* is an opportunistic pathogen responsible for nosocomial outbreaks, mostly occurring in intensive care units. Due to the multiplicity of infection sources, reliable molecular fingerprinting techniques are needed to establish epidemiological correlations among *A. baumannii* isolates from hospital outbreaks. The multiple-locus variable number of tandem repeats (VNTR) analysis (MLVA) has proven to be a fast, reliable and cost effective typing method in several bacterial species.

Here, the utility of MLVA for *A. baumannii* typing has been investigated, using simple PCR- and agarose gel-based electrophoresis method. Ten potential polymorphic VNTRs were identified upon bioinformatics screening of the six *A. baumannii* genomic sequences so far available. A collection of 7 prototype strains plus 18 well-characterized isolates including unique types and representatives of the three International *A. baumannii* lineages was evaluated in a 2-centre study aimed at validating the MLVA assay and comparing it with other genotyping assays, namely macro-restriction analysis (MRA), and multilocus sequence typing (MLST).

The results showed that MLVA can discriminate between isolates with identical MRA and MLST type. A panel of 8 of 10 VNTR markers was selected, all showing amplifiability and good polymorphism in the majority of strains. Independently generated MLVA profiles (consisting of a string of allele numbers, corresponding to the number of repeats at each VNTR locus, separated by commas, in a predetermined order) were concordant between the 2 centres. Typeability, reproducibility, stability, discriminatory power and epidemiological concordance were excellent.

A database containing information from several *A. baumannii* strains and their MLVA profiles is available from <http://bacterial-genotyping.igmors.u-psud.fr/>.

### **O3 USE OF THE ACCESSORY GENOME FOR CHARACTERISATION AND TYPING OF ISOLATES OF *ACINETOBACTER BAUMANNII***

**J. F. Turton & B. Baddal**

**Centre for Infections, Health Protection Agency, London, UK**

Much information has become available from whole genome sequences, and the accessory genome identified provides numerous targets for informative comparative studies, which may be helpful in epidemiological investigations.

Targets based on the AbaR resistance island and on other genes found in some, but not all, sequenced isolates, were identified and primers designed such that all described variants would be amplified. Genes were sought among a panel of clinical isolates (n=38) that included multiple representatives of a number of pulsed-field gel electrophoresis (PFGE) defined strains. Each set included some that differed in their Variable Number Tandem Repeat (VNTR) profiles, which can provide discrimination within a PFGE cluster. In some instances, genetically similar isolates from more than one country were included.

Of the 30 targets initially investigated, 17 were selected as providing the most valuable information or discrimination among the panel. These were those that described the AbaR island and class 1 integron, where present, a phage gene, virulence, resistance and antigen associated genes. A sensor kinase gene, *cuss* (ABAYE3204/AB57\_0660/AIS\_2938), described as a virulence gene, was not detected in any representatives of European clone II, but was found in every representative of European clone I tested, as well as in a sporadic strain and one belonging to European clone III. Presence or absence of genes coding for a phage terminase (ACICU\_02185/AB57\_1270), a sialic acid synthase (ACICU\_0080), a polysaccharide biosynthesis protein (AB57\_0094), *aphA1*, *bla*<sup>TEM</sup>, and integron associated orfX proved the most helpful in discriminating between closely related isolates among our panel. Differences in accessory gene profile correlated with distinct VNTR types among such isolates. The most marked example of this was among a cluster described as the 'Burns Unit' strain, belonging to European clone I; representatives differed in presence or absence of an AbaR island, of an integron, *bla*<sub>OXA-23-like</sub>, AB57\_0094 and phage terminase, with each VNTR type having a distinct accessory gene profile, despite the limited number of genes investigated. These isolates shared similar, but not identical, PFGE profiles; subtle differences in band sizes between such isolates may be easily overlooked when comparing normalized gel images.

These results show that detection (or not) of accessory genes is useful for characterisation and typing and supports VNTR data in describing distinct populations of similar organisms. Such analysis, in combination with other typing methods, can inform epidemiological investigations and may lead to a better understanding of the epidemiology of this organism.

#### **O4 MOLECULAR EPIDEMIOLOGY OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII STRAINS IN FOUR MEDITERRANEAN COUNTRIES USING MULTILOCUS SEQUENCE TYPING AND COMPARATIVE GENOME ANALYSIS**

**M. Giannouli<sup>a</sup>, A. Di Popolo<sup>a</sup>, F. Rocco<sup>b</sup>, P. P. Di Nocera<sup>b</sup>, M. Triassi<sup>a</sup>, M. Pallen<sup>c</sup>, S. Brisse<sup>d</sup> & R. Zarrilli<sup>a</sup>**

**<sup>a</sup>Department of Preventive Medical Sciences, <sup>b</sup>Department of Cellular and Molecular Biology and Pathology, University of Napoli Federico II, Naples, Italy; <sup>c</sup>Department of Medical Microbiology, University Hospitals Birmingham NHS Foundation Trust, UK; <sup>d</sup>Institut Pasteur, Genotyping of Pathogens and Public Health, Paris, France**

Thirty-five multidrug-resistant *Acinetobacter baumannii* strains representative of 28 outbreaks involving 484 patients from 20 hospitals of Greece, Italy, Lebanon and Turkey from 1999 to 2009 were analyzed by multi-locus sequence typing (MLST) using the MLST scheme that is publicly available at the Institut Pasteur's MLST web site (<http://www.pasteur.fr/mlst>). Sequence type (ST) ST2, ST1, ST25, ST78 and ST20 caused 12, 4, 3, 3 and 2 outbreaks involving 227, 93, 62, 62 and 31 patients, respectively. The carbapenem-hydrolysing oxacillinase (CHDL) genes *bla<sub>OXA-58</sub>*, *bla<sub>OXA-23</sub>* and *bla<sub>OXA-72</sub>* were found in 27, 2 and 1 carbapenem-resistant strains, respectively. Thus, *A. baumannii* outbreaks in four Mediterranean countries were caused by the spread of strains belonging to genotype ST2 and to a lesser extent to genotypes ST1, ST25 and ST78; the majority of the strains showed resistance to carbapenems.

Genomes of three epidemic strains isolated in Naples, Italy, from 2006 to 2009 and assigned to ST2, ST25 and ST78 were sequenced using 454 FLX Titanium emPCR pyrosequencing. Assembled sequences were automatically annotated using xBASE2 bacterial genome annotation service (<http://xbase.bham.ac.uk>). Draft genome sequences of ST2, ST25 and ST78 strains comprised 4,013,312 bp, 4,041,331 bp and 3,964,748 bp, respectively. Comparative genome analysis showed highly homologous genome organization between ST2 strain isolated in Naples and *A. baumannii* ACICU strain previously isolated in Rome during 2005 also assigned to ST2 and let to identify several regions of unique content that we defined as genomic islands (GEIs) mostly in ST25 strain. Consistent with their close genetic relatedness, both ST2 strains contained a 15.4-kb GEI containing antimicrobial resistance genes inserted in the ATPase gene locus, confirming that MLST relatedness has high predictive power regarding overall genomic similarity/content. At the homologous location, a 12.4-kb GEI flanked by transposases but devoid of resistance genes was identified in ST78, but not in ST25 strain. Similarly to *A. baumannii* ACICU strain, the ST2 strain isolated in Naples contained two plasmids, p1-ABST2 (63,388 bp) and p2-ABST2 (21,847 bp). p1-ABST2 carried a complete *tra* locus and was highly homologous to the plasmid pACICU2, p2-ABST2 carried one copy of the *bla<sub>OXA-58</sub>* gene and was homologous to pACICU1 plasmid. ST25 strain also contained two plasmids, p1-ABST25 (15,266 bp) and p2-ABST25 (8,970 bp), that both carried one copy of the *bla<sub>OXA-72</sub>* gene. The single plasmid identified in the ST78 strain (p1-ABST78, 26,412 bp) contained one copy of the *bla<sub>OXA-58</sub>* gene.

## **O5 EXTENSIVE VARIABILITY IS PRESENT AMONG CONTEMPORARANEOUS ISOLATES OF *ACINETOBACTER BAUMANNII*: INSIGHTS FROM GENOME SEQUENCING**

**Mark D. Adams<sup>a</sup>, Neil Molyneaux<sup>a</sup>, Federico Perez<sup>b</sup> & Robert A. Bonomo<sup>b</sup>**

**<sup>a</sup>Department of Genetics, Case Western Reserve University, Cleveland, OH, US; <sup>b</sup>Louis Stokes Cleveland Dept. of Veterans Affairs Medical Center, Cleveland, OH, USA**

Multidrug resistant *Acinetobacter baumannii* has increased markedly in the last decade. Resistance genes are often found on mobile genetic elements including plasmids and resistance islands, but little is known about the frequency of lateral gene transfer. We examined the extent of microevolution by assessing genome-wide change occurring during an *A. baumannii* outbreak in a single hospital over a period of six months.

Over 100 *A. baumannii* isolates obtained over a 6 month period were sequence typed by PCR-ESI/MS. 60% of isolates were from a single sequence type (ST) belonging to European clone type II. Draft genome sequences were obtained for four isolates from the dominant ST by paired-end sequencing on an Illumina GAI. Sequences were compared with one another and to available complete *A. baumannii* genomes. The presence and genomic organization of specific resistance genes was tested in additional isolates by PCR.

Each of the four genomes is distinct. Phylogenetic analysis demonstrated that at least two distinct founders contributed to the outbreak. Single nucleotide variants clustered within several hundred kilobases of the origin of replication and at additional smaller regions around the genome, providing the first evidence for recombination among *A. baumannii* strains. The resistance island (RI) structure in these isolates differs markedly from those previously described. Two strains have a second IS26-associated RI that carries three aminoglycoside resistance genes. Two strains from different lineages share the same plasmid that carries both *bla*<sub>OXA-23</sub> and *aphA6*. Genomes also differ in the locations of insertion sequences (IS elements), but five IS*Aba1* sites are common to all four strains, including one copy adjacent to *bla*<sub>ampC</sub>, suggesting that they may contribute to resistance or pathogenicity. Testing of additional isolates by PCR revealed additional variability in IS distribution. In summary, microevolution of antibiotic resistance in *A. baumannii* is rapid, leading to changes in the resistance genotype and phenotype across the span of an outbreak in a single hospital.

## O6 EXPLORING THE EVOLUTIONARY DYNAMICS OF PLASMIDS: THE *ACINETOBACTER* PAN-PLASMIDOME

M. Fondi<sup>a</sup>, M. C. Papaleo<sup>a</sup>, A. Mengoni<sup>a</sup>, I. Maida<sup>a</sup>, E. Perrin<sup>a</sup>, M. Vaneechoutte<sup>b</sup>, L. Dijkshoorn<sup>c</sup> & R. Fani<sup>a</sup>

<sup>a</sup>Laboratory of Microbial and Molecular Evolution, Dept. of Evolutionary Biology, Via Romana 17- 19, University of Florence, Florence, Italy; <sup>b</sup>Laboratory Bacteriology Research, Faculty Medicine & Health Sciences, University of Ghent, Belgium; <sup>c</sup>Dept. of Infectious Diseases, Leiden University Medical Center, P.O. Box 9600, Leiden, The Netherlands

Prokaryotic plasmids have a dual importance in the microbial world: first they have a great impact on the metabolic functions of the host cell, providing additional traits that can be accumulated in the cell without altering the gene content of the bacterial chromosome. Additionally and/or alternatively, from a genome perspective, plasmids can provide a basis for genomic rearrangements via homologous recombination and so they can facilitate the loss or acquisition of genes during these events, which eventually may lead to horizontal gene transfer (HGT). Given their importance for conferring adaptive traits to the host organisms, the interest in plasmid sequencing is growing and now many complete plasmid sequences are available online. By using the newly developed Blast2Network bioinformatic tool, a comparative analysis was performed on the plasmid and chromosome sequence data available for bacteria belonging to the genus *Acinetobacter*. The *Acinetobacter* “pan-plasmidome”, that is the complete set of plasmids harbored by members of this genus (comprising plasmids isolated from both pathogenic and environmental strains), is particularly attractive to study its evolutionary dynamics because of the eclectic lifestyle of their host strains and the possible frequent genetic exchanges between its members. In fact, it has been reported that several *Acinetobacter* strains, especially those sharing particular ecological niches that require specific adaptations, like polluted environments and bioreactors, harbor plasmid molecules of different sizes undergoing frequent molecular rearrangements

Data obtained showed that, although most of the plasmids lack mobilization and transfer functions, they have probably a long history of rearrangements with other plasmids and with chromosomes. Indeed, traces of transfers between different species can be disclosed. We show that, by combining plasmid and chromosome similarity, identity based, network analysis, an evolutionary scenario can be described even for highly mobile genetic elements that lack extensively shared genes. In particular we found that transposases and selective pressure for mercury resistance seem to have played a pivotal role in plasmid evolution in *Acinetobacter* genomes sequenced so far.

## **O7 MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF ACINETOBACTER SPP. CLINICAL ISOLATES FROM NORWAY**

**Nabil Karah<sup>a,b</sup>, Bjørg Haldorsen<sup>a</sup>, Kristin Hegstad<sup>a,b</sup>, Gunnar Skov Simonsen<sup>a,b,c</sup>, Arnfinn Sundsfjord<sup>a,b</sup>, Ørjan Samuelsen<sup>a</sup> & the Norwegian Study Group on *Acinetobacter***

<sup>a</sup>Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway; <sup>b</sup>Research Group for Host Microbe Interactions, Department of Medical Biology, University of Tromsø, Tromsø, Norway; <sup>c</sup>Norwegian Institute of Public Health, Oslo, Norway

33 genomic species of the *Acinetobacter* genus have been currently identified. Of these, *Acinetobacter baumannii*, *Acinetobacter* gen. sp. 3 and *Acinetobacter* gen. sp. 13TU have been the most clinically relevant species. The aim of this project was to analyse a national blood culture collection of *Acinetobacter* spp. with respect to precise species identification, phenotypic susceptibility patterns and genotypic resistance characteristics.

The study included 120 consecutive blood culture isolates of *Acinetobacter* spp. collected between 2005 and 2007 throughout Norway. Species identification of isolates was performed genotypically by partial *rpoB* gene sequence analysis (zone 1, 352 bp located between positions 2916 and 3267). *16S rDNA* and *recA* sequence analyses were used to verify results of identification. Susceptibility testing was performed by agar disk diffusion and Etest. The occurrence of OXA-carbapenemase genes and mutations in quinolone resistance determining regions (QRDRs) was examined by PCR assays and sequencing. Furthermore, PCR assays were used to determine the distribution of *ISAbal* and *ISAbas3* and clonal lineage of *A. baumannii* isolates.

The most prevalent species in our collection were *Acinetobacter* gen. sp. 13TU (47.5%) and *Acinetobacter* gen. sp. 3 (19.2%), followed by *A. baumannii* (8.3%) and *A. lwoffii/Acinetobacter* gen. sp. 9 (7.5%). Sequence analyses of *16S rRNA* and/or *recA* genes verified results of *rpoB* identification. Only 4/120 (3.3%) of isolates showed resistance to one or more of the antimicrobial agents included in the study. *bla*<sub>OXA-51-like</sub> and *bla*<sub>OXA-23-like</sub> genes were detected in all *A. baumannii* and *A. radioresistens* isolates, respectively. In addition, *bla*<sub>OXA-23-like</sub>, associated with an upstream *ISAbal*, was responsible for carbapenem-resistance in 1/10 of *A. baumannii* isolates. Resistance to quinolones was related to point mutations in *gyrA* and *parC*. A novel mutation, Ser-80 to Tyr, in the QRDR of *parC* was detected. *ISAbal* and *ISAbas3* were present in 20/120 and 49/120 of isolates and were distributed among 6/13 and 10/13 of *Acinetobacter* species, respectively. None of our *A. baumannii* isolates belonged to any of the three most common clones of *A. baumannii* disseminating in Europe.

Most importantly, the study pointed out the significance of genotypic identification to determine the precise epidemiology of *Acinetobacter* species other than *A. baumannii*. Our study maintained the facts that *bla*<sub>OXA-51-like</sub> and *bla*<sub>OXA-23-like</sub> are intrinsic genes in *A. baumannii* and *A. radioresistens*, respectively. Furthermore, the study detected a novel QRDR mutation in *ParC* that could be involved in fluoroquinolone-resistance in *A. baumannii*.

## **O8 EPIDEMIOLOGY OF *ACINETOBACTER BAUMANNII* IN THREE INTENSIVE CARE UNITS IN SICILY, ITALY: INTER-HOSPITAL AND INTRA-HOSPITAL SPREAD**

**Antonella Agodi<sup>a</sup>, Martina Barchitta<sup>a</sup>, Giovanna Valenti<sup>a</sup>, Maria Antonietta Romeo<sup>b</sup>, Loredana Giaquinta<sup>b</sup>, Carmela Santangelo<sup>c</sup> & Giacomo Castiglione<sup>c</sup>**

**<sup>a</sup>Department of Biomedical Sciences, University of Catania, Italy; <sup>b</sup>Azienda Ospedaliera Cannizzaro, Catania, Italy; <sup>c</sup>Azienda Ospedaliero - Universitaria "Policlinico - Vittorio Emanuele", Catania, Italy**

*Acinetobacter baumannii* infection is a leading cause of morbidity and mortality in the hospital setting, especially among critically ill patients in the intensive care units (ICUs). In a previous study we have confirmed that among different risk factors, mechanical ventilation is associated to *A. baumannii* infection in ICU patients. Results from the first edition of the Italian Nosocomial Infections Surveillance in Intensive Care Units (ICUs) (SPIN-UTI) project (2006 – 2007), show *A. baumannii* as the third most common microorganisms in ICU-acquired infections (7.5% of all infection-associated microorganisms). The most recent data from the European HELICS-ICU survey 2004-05 report marked differences between countries in the relative frequencies of isolated microorganisms and *A. baumannii* ranked 10th, with the highest frequencies reported from Spain and Lithuania. As such, in order to take into account the growing problem of *A. baumannii* acquisition in the ICU, in the framework of the second edition of the SPIN-UTI project, a specific surveillance program for *A. baumannii* acquisition was implemented in three participating ICUs, in order to provide an in-depth epidemiological characterization of *A. baumannii* strains, and define quantitative parameters for control interventions.

The study design integrated both the SPIN-UTI patient-based and the laboratory-based surveillance approaches at three Hospital ICUs in Sicily and was conducted from October 2008 to May 2009, including six-months of the SPIN-UTI project. Patterns of *A. baumannii* acquisition in the ICUs during the period of the survey were carriage, colonization and infection. Characterization of *A. baumannii* was performed by Amplified rDNA Restriction Analysis (ARDRA) and genotyping by Pulsed-Field Gel Electrophoresis (PFGE) of the *ApaI*-digested genomic DNA. A cross-transmission episode was assumed when two patients had indistinguishable isolates. During the study period a total of 149 isolates, all identified as *A. baumannii* by ARDRA, were collected from 84 patients. Carriage was associated to 3.3% of isolates; colonization to 25.6%, colonization/infection to 47.9%, and infection accounted for the remaining 23.2% of isolates. Pneumonia were the most encountered infection type (53.6% of all infection episodes), followed by bloodstream infection (32.1%).

PFGE analysis of *A. baumannii* isolates led to the identification of a total of 12 unrelated pulse-types. Particularly, 6 clones were associated to cross-transmission and 6 were single patterns associated with sporadic strains. Two major clones, A and B, were identified involving respectively 42.7% and 34.3% of isolates, showing inter-hospital spread in all three ICUs (clone A) and intra-ICU spread (clone B). The impact of *A. baumannii* cross-transmission was estimated to be at least 66.9%, thus defining the exogenous preventable proportion of all cross-transmission episodes. The incidence and spread of *A. baumannii* clones in the ICU setting, remains a concern and merits appropriate and continuous surveillance programmes and infection control measures.

## O9 MOLECULAR EPIDEMIOLOGY OF *ACINETOBACTER BAUMANNII*-GROUP BLOODSTREAM ISOLATES IN THE UNITED STATES, 1995 – 2004

K. Janßen, H. Wisplinghoff, P. G. Higgins & H. Seifert

Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstrasse 19 – 21, 50935 Cologne, Germany

We have investigated the molecular epidemiology of *Acinetobacter* bloodstream isolates taken from patients hospitalized in 28 US hospitals over a 9-year period (1995–2004) during a nationwide surveillance study (Surveillance and Control of Pathogens of Epidemiological Importance [SCOPE]) to compare them to previously identified epidemic clones.

*Acinetobacter* isolates (N=268) were identified to species level by *gyrB* multiplex PCR. Imipenem sensitivity was determined by Etest. *A. baumannii* isolates with an imipenem MIC  $\geq 2$  mg/l (N=37) were investigated for the presence of acquired OXA-type carbapenemase genes and IS*AbaI* upstream of the intrinsic *bla*<sub>OXA-51-like</sub> gene. Molecular epidemiology was investigated by sequence-type multiplex PCR (Turton et al., 2006) for *A. baumannii* and rep-PCR for *Acinetobacter* genomic species (GS) 3 and 13TU using the DiversiLab system. *A. baumannii* multiplex PCR patterns were compared to previously identified worldwide clonal clusters. A rep-PCR cluster is defined as at least 2 isolates with  $\geq 95\%$  similarity.

*Acinetobacter* isolates were identified as *A. baumannii* (N=181), GS3 (N=26) and GS13TU (N=61). *A. baumannii* isolates mostly clustered around the previously defined sequence group (SG) 1 (N=84; corresponding to world-wide (WW) clonal lineage 2), SG2 (N=1; WW 1), SG3 (N=6; WW 3), and variant sequence groups (vSG) vSG4 (N=8), vSG5 (N=19) and vSG7 (N=4). 59 isolates did not cluster with above named ones, but build 8 new variant sequence groups. In contrast, GS3 and GS13TU isolates were more heterogeneous as determined by rep-PCR. GS3 built 5 clusters and GS13 10 clusters. These were small-sized (2-7 isolates per cluster). 14 GS3 and 16 13TU did not cluster.

No acquired OXA-type genes were found. All GS3 and GS13TU isolates were imipenem-sensitive. Thirteen carbapenem non-susceptible and 19 carbapenem-sensitive *A. baumannii* had IS*AbaI* upstream of the *bla*<sub>OXA-51-like</sub> gene, including strains isolated as early as 1996. Five carbapenem non-susceptible isolates have an unknown resistance mechanism. Isolates with IS*AbaI* upstream of *bla*<sub>OXA-51-like</sub> were mainly WW 2 (30/32).

Our study confirms previous data indicating the predominance of a few major clonal *A. baumannii* lineages in the US, particularly WW2. GS 3 and GS13TU, in contrast, appear as much more heterogeneous species. Representatives of WW2 were first isolated in 1984 and termed European clones. However, we have found this lineage to be widespread in the USA in isolates dating from 1995, suggesting global dissemination of *A. baumannii* was an early event. In addition, we have detected IS*AbaI* upstream of the *bla*<sub>OXA-51-like</sub> gene in this lineage, and this is also older than originally thought.

**O10 DO BIOFILM FORMATION AND INTERACTIONS WITH HUMAN CELLS EXPLAIN THE CLINICAL SUCCESS OF *ACINETOBACTER BAUMANNII*?**

**A. de Breij<sup>a</sup>, L. Dijkshoorn<sup>a</sup>, E. L. Lagendijk<sup>b</sup>, J. M. van der Meer<sup>c</sup>, A. J. Koster<sup>c</sup>, G. V. Bloemberg<sup>d</sup>, R. Wolterbeek<sup>e</sup>, P. J. van den Broek<sup>a</sup> & P. H. Nibbering<sup>a</sup>**

**<sup>a</sup>Department of Infectious Diseases, <sup>b</sup>Institute of Biology, <sup>c</sup>Department of Molecular Cell Biology, Section Electron Microscopy, Leiden University Medical Centre, Leiden, The Netherlands; <sup>d</sup>Institute of Medical Microbiology, University of Zurich, Zurich, Switzerland; <sup>e</sup>Department of Medical Statistics, Leiden University Medical Centre, Leiden, The Netherlands**

The dramatic increase in antibiotic resistance and the recent manifestation in war trauma patients underscore the threat of *Acinetobacter baumannii* as a nosocomial pathogen. Despite numerous reports documenting its epidemicity, little is known about the pathogenicity of *A. baumannii*. The aim of this study was to obtain insight into the factors that might explain the clinical success of *A. baumannii*.

We compared biofilm formation, adherence to and pro-inflammatory cytokine induction by human cells for a large panel of well-described strains of *A. baumannii* and compared these features to that of other, clinically less relevant *Acinetobacter* species. Results revealed that biofilm formation and adherence to airway epithelial cells varied widely within the various species, but did not differ among the species. However, airway epithelial cells and cultured human macrophages produced significantly less inflammatory cytokines upon exposure to *A. baumannii* strains than to strains of *A. junii*, a species infrequently causing infection.

The induction of a weak inflammatory response may provide a clue to the persistence of *A. baumannii* in patients.

## O11 SURFACE-ASSOCIATED MOTILITY IS A COMMON TRAIT OF CLINICAL *ACINETOBACTER* ISOLATES

G. Wilharm<sup>a</sup>, P. Morczinek<sup>a</sup>, F. Faber<sup>a</sup>, T. Kerrinnes<sup>a</sup>, D. Lepka<sup>a</sup>, S. Gröbner<sup>b</sup>, Y. Pfeifer<sup>a</sup> & E. Skiebe<sup>a</sup>

<sup>a</sup>Robert Koch-Institute, Wernigerode, Germany; <sup>b</sup>University of Tübingen, Tübingen, Germany

The genus name *Acinetobacter* was coined to indicate that members are akinetic due to the lack of flagella. However, more than forty years ago, certain isolates belonging to the genus have been reported to jerkily move on wet surfaces, a phenomenon later termed ‘twitching motility’. While twitching motility has been intensively studied in other genera like *Neisseria*, *Myxococcus* and *Pseudomonas*, and found to rely on type IV pilus retraction, the genetic and molecular basis of the phenomenon has not been elucidated in *Acinetobacter*.

*A. baumannii*, a nosocomial pathogen appearing increasingly multiresistant, may profit from motility during infection or while persisting in the hospital environment. However, no data is available on the frequency of motility skills among clinical isolates of *A. baumannii*.

We have screened a collection of 50 clinical isolates of different origin and found that 95% of all isolates were motile on wet surfaces. The conditions required to promote motility, however, differed. We found that the colony pattern formed was strain-specific and could be used as a very simple means to judge on a possible clonal relationship of clinical isolates. Microscopic examination of *Acinetobacter* motility suggests the involvement of a surfactant secreted to support motility. Screening a collection of transposon mutants of strain ATCC 17978 for motility defects, we identified several akinetic mutants and sequenced the insertion sites after single primer PCR. We identified a gene cluster likely involved in biosynthesis of the supposed surfactant. Further interesting, many of the tagged genes involved in *Acinetobacter* motility have not been identified in the context of twitching motility in any model organism. Making use of naturally competent clinical isolates of *A. baumannii*, we verified by transformation of the mutants’ DNA that the identified loci were required for motility in different isolates. Taken together, surface-associated motility of clinical *Acinetobacter* isolates is a common trait that deserves further investigation due to its unprecedented features.

## O12 EVALUATION OF CELL SURFACE PROPERTIES OF TWO *ACINETOBACTER BAUMANNII* STRAINS ISOLATED IN A SAME PATIENT

M. Kempf<sup>a,b</sup>, M. Eveillard<sup>a,b</sup>, H. Seifert<sup>c</sup>, C. Lefrançois<sup>b</sup>, F. Kowalczyk<sup>a</sup>, S. Georgeault<sup>d</sup> & M. L. Joly-Guillou<sup>a,b</sup>

<sup>a</sup>University Hospital of Angers, Angers, France; <sup>b</sup>Host-Pathogen Interaction Study Group (GEIHP, UPRES EA 3142), University of Angers, France; <sup>c</sup>Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne Köln, Germany; <sup>d</sup>Service Commun d'Imageries et d'Analyses Microscopiques, University of Angers, Angers, France

*Acinetobacter baumannii* is a nosocomial pathogen of increasing importance, but the pathogenic mechanism of this microorganism has not been fully explored. The ability of *Acinetobacter* strains to adhere to surfaces is an important mechanism in the pathogenicity of these bacteria. Adhesion ability is determined by specific factors such as adhesins, and non-specific factors such as hydrophobicity and cellular surface electrical discharge, which vary among strains. The aim of this study was to evaluate the cell surface properties of 2 strains isolated in a same patient with different virulence characteristics previously determined in the laboratory in a mouse model of pneumonia: a mucoïde strain (AB-M) isolated from CSF (53% of mortality) and a non mucoïde strain (AB-NM) isolated from the ventriculo-peritoneal catheter (19% of mortality).

Method: 1) Bacterial cell surface hydrophobic/hydrophilic characters were determined by partitioning phase in hexadecane (HXD), an apolar solvent. 2) Bacterial cell surface electrical charge was assessed by measuring the electrophoretic mobility of bacteria using a Zetameter (Zeta-sizer 2000, Malvern Instruments), with a salt concentration ranging from 0 to 120 mM. 3) Biofilm formation was measured using crystal violet and XTT methods 4) An *in vitro* model of adherence on silicone catheter sections was performed. The study was focused on the early stage of adherence (3h) and after 24h of incubation. 5) Scanning electron microscopy was performed on the two strains in broth medium.

The AB-NM strain presented the most hydrophobic cell wall surface, with an affinity to HXD of 95%, while the AB-M strain presented an affinity to HXD of 17%. Electrophoretic mobility measurements revealed that AB-M was the most negatively charged, whatever the salt concentration tested. Concerning biofilm formation, the crystal violet technique showed no difference in biofilm formation between the two strains while the XTT technique showed a higher biofilm formation for the AB-M strain. The AB-NM showed a higher adherence on silicone catheter, whatever the time of incubation tested. Finally, scanning electron microscopy showed high differences between the two strains. AB-NM was rough and formed large aggregates while AB-M was smoother and dispersed more homogeneously.

The high variation in the percentage of adhesion to HXD between the two strains revealed a great difference in their hydrophobic character. The AB-NM strain, which was isolated on a catheter, was the most hydrophobic and the less electronegative strain, in comparison with the AB-M strain. Our results confirmed the higher capacity of AB-NM strain to adhere on silicone catheter. The high cell surface hydrophobicity of AB-NM could explain its capacity to form large aggregates in broth medium as well as the contradictory results concerning biofilm formation using staining methods. Moreover, these cell surface differences could explain the differences observed between the two strains in virulence. Adherence, which is the first step to colonize biomaterial surfaces, is strongly dependant on surface properties of the bacteria, especially on hydrophobicity and surface electric properties.

**O13 DISTINCTIVE INFECTION AND HOST DEFENSE PROFILES IN MICE  
INTRANASALLY INFECTED WITH A HYPERVIRULENT CLINICAL ISOLATE OF  
*ACINETOBACTER BAUMANNII***

**Rhonda Kuo Lee<sup>a</sup>, Greg Harris<sup>a</sup>, H. Howard Xu<sup>b</sup> & Wangxue Chen<sup>a</sup>**

**<sup>a</sup>Institute for Biological Sciences, National Research Council Canada, 100 Sussex Drive, Ottawa, Ontario K1A 0R6, Canada, and <sup>b</sup>Department of Biological Sciences, California State University, Los Angeles, Los Angeles, CA 90032, USA**

Despite the clinical significance of *Acinetobacter baumannii* (AB) infection, little is known about the pathogenesis and virulence mechanisms of this pathogen. We have recently compared the virulence and host responses to a panel of clinical AB isolates in a mouse model of intranasal (i.n.) infection. While the virulence of the majority of clinical isolates in mice was similar to the control ATCC strain or other clinical isolates (such as AYE), we identified one hypervirulent clinical isolate, LAC-4. When inoculated with  $5 \times 10^7$  CFU, immunocompetent C57BL/6 (B6) mice infected with LAC-4 had  $>1$  log higher bacterial burdens in the lungs at 24 h than AYE-infected mice. More significantly, LAC-4 appeared to be significantly more capable of extrapulmonary dissemination. Also, B6 mice uniformly succumbed to i.n. inoculation with LAC-4 within 24 to 48 hours after infection. Consistent with the bacterial burdens and dissemination, clinical blood chemistry showed significant increases in levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urea, indicating liver and renal damage, in LAC-4-infected mice as compared to mice infected with AYE or ATCC strain. Moreover, LAC-4 infection induced a distinctive profile of pulmonary and systemic cytokine/chemokine responses. Although both AYE and LAC-4 strains induced comparable influx of macrophages and neutrophils into the lungs, substantially more bacteria were evident in the cytoplasm of LAC-4-infected alveolar macrophages. These results suggest that the hypervirulence of the LAC-4 isolate is partially due to its ability to escape or suppress the anti-bacterial mechanisms of the host innate immunity. Thus, this isolate may be a useful tool in the study of the virulence factors of AB and a mouse model of i.n. infection using this isolate can be further explored for future preclinical development and evaluation of anti-AB therapeutics.

## O14 PROTEOMIC ANALYSIS OF *ACINETOBACTER BAUMANNII* CELL ENVELOPE FROM PELLICLE AND PLANKTONIC GROWTH STATES

S. Martí<sup>a</sup>, Y. Nait Chabane<sup>a</sup>, J. Vila<sup>b</sup>, T. Jouenne<sup>a</sup> & E. Dé<sup>a</sup>

<sup>a</sup>BRICS, UMR 6270 CNRS, Université de Rouen, France; <sup>b</sup>Hospital Clinic, Barcelona, Spain

The clinical importance of *Acinetobacter baumannii* is mainly due to its capacity to survive in the hospital environment. This high persistence may be partly explained by the ability of this microorganism to form biofilm. In a previous study, we have already shown that besides the biofilm at the solid-liquid interface, *A. baumannii* is able to form a pellicle at the air-liquid interface. In this work, we aimed to characterise by 2D electrophoresis, the proteins associated to pellicle formation by comparing protein expression in planktonic and pellicle growth states. *A. baumannii* strain 77 was obtained from the Hospital Clinic in Barcelona and was grown in MH broth at 25°C. For planktonic growth, the strain was incubated with shaking to a final OD<sub>600</sub> of 1.5 (stationary phase); for pellicle recovery, the strain was incubated four days without shaking. The pellicle was recovered from the surface and broken down in a sonicating bath. The cell envelope from planktonic and pellicle cells was recovered after ultracentrifugation of the sonicated samples and proteins were separated by 2D electrophoresis. Protein analysis was performed by duplicate on three different protein extractions. Differences between both conditions were statistically analysed using SameSpots and characterised by nanoLC-MS-MS.

SameSpots analysis reported the presence of 94 spots with a statistically significant difference in expression between both growing states; nevertheless only 60 of them were finally identified by mass spectrometry analysis. From the characterised protein spots, twenty-three (25%) were over-expressed in the planktonic state of growth and thirty-seven (39%) in the pellicle. Most of the proteins showing an increased expression in the planktonic samples were metabolic proteins or Outer Membrane Proteins (OMPs) such as HMP/OmpA. On the other hand, proteins over-expressed in the pellicle were probably more specific from this condition and they were classified in four different groups: a) proteins associated to pili formation; b) proteins associated to iron transport; c) proteins associated to antimicrobial resistance; d) *A. baumannii* hypothetical proteins with a function to be determined. In addition, *ca.* half of the proteins over-expressed in the pellicle samples had a molecular weight  $\geq 70$  KDa; these proteins were mainly associated to pili formation and ferric transport.

The proteomic analysis performed between both growth states shows the importance of pili and iron transport systems in pellicle formation. The presence of pili structures has already been described as a mechanism used by bacterial cells to attach to solid surfaces and we have observed that it is also involved in pellicle formation. On the other hand, iron-uptake systems are also up-regulated within the pellicle and it would be important to perform further studies to establish their real effect on pellicle formation.

## **O15 MECHANISMS OF BIOFILM FORMATION IN *ACINETOBACTER BAUMANNII*-PROTEOMIC PERSPECTIVE**

**N. C. Soares, M. P. Cabral, J. Aranda, C. Rumbo & G. Bou**

**Servicio de Microbiología-Unidad de Investigación Complejo Hospitalario Universitario A Coruña, Spain**

*Acinetobacter baumannii* is a nosocomial pathogen that has been associated with severe infections and outbreaks in hospitals. Biofilms are highly organised communities that are in many aspects different from their counter parts planktonic cells. It has been indicated that adhesion and biofilm formation is the main cause for the persistence of *A. baumannii* in medical devices and play an important role in pathogenesis as well as antimicrobial resistance. However, the mechanism operating during biofilm formation remains yet to be characterised.

Here we have employed a proteomic approach, namely DIGE and iTRAQ to verify whether biofilms of *A. baumannii* ATCC 17879 have a unique phenotype or instead is a combination of exponential and stationary phase planktonic populations. The comparative DIGE based analysis of the 3 conditions planktonic population (exponential and late stationary phase) and biofilms revealed that a total of 371 protein-spots were differentially expressed. From this, comes that 14 % were exclusively up expressed in biofilms and 13.2 % were exclusively down expressed in biofilm cells. Then 33 % of the protein spots increased both in exponential cell as well as in biofilm, 13.2% of spots increased both in biofilm as in late stationary phase. Selected protein spots were then identified by MALDI-TOF/TOF analyses and among those that increased in biofilm, there proteins were previous described with a role in biofilm formation such as CsuA/B, OmpA, but also proteins that so far has not been associated with biofilms such as CarO, putative peptidoglycan-binding LysM, putative RND type efflux pump and others.

Furthermore, recent studies suggest that in many bacterial species, salicylic acid (SA) may act as potential inhibitor biofilm formation. In current study we present evidence that SA (16 mM) inhibits biofilm formation in *A. baumannii* by the down regulation of biofilm associated protein such as CarO, OmpA, etc. Together, the comparative proteomic analyses of planktonic/biofilm populations with the effects of the SA on the *A. baumannii* proteome provide us with a list of target genes with a potential role during biofilm formation. Mutation in genes for selected proteins was made and the respective impact of those mutations was evaluated by crystal violet and by Scanning Electron Microscopy. Taken together our findings, here we propose a mechanism for the role of those biofilm related proteins within the complex processes of biofilm formation.

## O16 DEEP SEQUENCING AND ANALYSIS OF THE TRANSCRIPTOME OF *ACINETOBACTER BAYLYI* ADP1

**B. Segurens<sup>a,b,c</sup>, V. de Berardinis<sup>a,b,c</sup>, K. Labadie<sup>a</sup>, V. Castelli<sup>a,b,c</sup>, M. Durot<sup>a,b,c</sup>, J. Weissenbach<sup>a,b,c</sup>, M. Salanoubat<sup>a,b,c</sup>**

**<sup>a</sup>CEA, DSV, Institut de Génomique, Genoscope; <sup>b</sup>CNRS, UMR8030; <sup>c</sup>Université d'Evry Val d'Essonne, Evry, France**

Our knowledge on the chemical diversity of living organisms remains very patchy, mainly due to an incomplete description of the chemical reactions catalysed by enzymes. In our laboratory, we plan to tackle the experimental functional characterisation of genes, especially those encoding enzymatic activities.

With this in mind, a project to study the aerobic metabolism of *Acinetobacter baylyi* ADP1 has been initiated and a variety of resources developed: (1) a complete collection of single-gene deletion mutants (de Berardinis V. *et al.*, Mol Syst Biol 4:174, (2008)) and (2) an ORFeome focused on metabolic enzymes and proteins with unknown function. To extend our tools for the quest of metabolic knowledge, we have developed a transcriptomics approach.

The biological landscape has been transformed by the sequencing of genomes, and more recently by global gene expression analyses using microarrays. Today, high-throughput sequencing of cDNA has revolutionized transcriptome analysis. Using this technology, we have established an operon map of *Acinetobacter baylyi* ADP1. This map is based on transcription data obtained from multiple growth conditions including response to different perturbations (cold, heat, light, dark, low pH, high pH, carbon sources...). Moreover, analyses of these data have allowed genome-wide discovery of non-coding RNAs and antisense transcription, indicating a higher complexity of regulation than anticipated.

Another goal of this analysis is to identify and characterize genes whose levels of transcription are co-modulated in response to genetic or environmental perturbations. In particular, the comparison of expression profiles obtained on cells grown on succinate versus quinate highlights a differential expression of genes known to be involved in quinate assimilation, but also of genes with no known relationships with this metabolic pathway.

In conclusion, it should be underlined that this transcriptomic approach developed for *A. baylyi* ADP1 can be easily extended to the *Acinetobacter* genus, including human pathogens.

## O17 HOW *ACINETOBACTER* SPECIES REACT TO DNA DAMAGE

T. Elam, G. Howington, S. Wheeler, A. Grice, J. Wilder & J. Hare

Morehead State University, Morehead, USA

Bacteria use both error-free and error-prone DNA damage response mechanisms, such as the inducible SOS response system, in which the *umuDC* operon is required for trans-lesion DNA synthesis after DNA damage. In the naturally competent *Acinetobacter baylyi* strain ADP1, the *umuD* gene possesses an extended 5' region, and a fragmented, incomplete *umuC* gene. In ADP1, *umuD* is required for the full induction of the DNA damage-response gene, *ddrR*. To investigate whether these DNA damage response features were unique to ADP1 or represented novel DNA damage mechanisms in this genus, we surveyed diverse *Acinetobacter* species for their *umuDC* operon presence and configuration as well as phenotypic responses to DNA damage caused by UV exposure and mitomycin C. Surprisingly, these DNA damage-related properties were conserved in *Acinetobacter* along phylogenetic lines, as measured by multiple assays. PCR amplification and cloning of the *umuDC* operon from diverse *Acinetobacter* strains revealed that all *Acinetobacter* strains analyzed had a *umuDAb*-like gene with an extended 5' region. However, two forms of the extra-long *umuD* allele were present in *Acinetobacter*: one form in the closely related Acb (*A. calcoaceticus/A. baumannii*) complex strains, with the other form present in ADP1 and other strains.

While the *umuC* truncation in ADP1 correctly predicted that this strain would not conduct SOS mutagenesis (in a rifampin resistance assay after UV exposure), some *Acinetobacter* species (*A. ursingii* and *A. gyllenbergii*) did conduct SOS mutagenesis, albeit to a lesser degree than *Escherichia coli*. Additional variation was seen in the error-free response to UV exposure: survival of seventeen *Acinetobacter* strains after UV exposure ranged from five orders of magnitude less than ADP1 to 150% of the survival levels seen in ADP1. Acb strains and seven hemolytic strains each had significantly lower survival than non-Acb or non-hemolytic strains.

To further understand the role of the unusual UmuD homolog present in ADP1, we used anti-peptide antibodies directed against UmuDAb in Western blotting experiments to measure its expression and activity in comparison to the *E. coli* model of the SOS response. UmuDAb expression in ADP1 from its native promoter was constitutive, unlike in *E. coli*, but the full-length 23 kD UmuDAb protein disappeared after mitomycin C treatment (half life of ~25 minutes), implying UmuDAb cleavage with similar timing as in the *E. coli* model system. Constitutive expression of UmuDAb from its native promoter in *E. coli* also disappeared with a similar half-life after mitomycin C treatment. This cleavage and/or degradation of UmuDAb required RecA, which suggests responsiveness of UmuDAb to DNA damage. Whether this cleavage is needed to activate previously observed UmuDAb regulation of a DNA-damage-inducible gene is currently under investigation.

Across the genus, *Acinetobacter* exhibit a highly conserved *umuDAb* allele whose product in ADP1 is DNA damage-responsive, yet these species typically do not exhibit SOS mutagenesis, suggesting that observed DNA damage-related features signify novel and varied mechanisms working in the genus.

## O18 ADAPTATION OF *ACINETOBACTER RADIORESISTENS* S13 TO HYDROPHOBIC ENVIRONMENTS: SURFACE MODIFICATION, EMULSIFYING ACTIVITIES AND STRESS RESPONSES REVEALED BY SPECTROSCOPIC AND PROTEOMIC STUDIES

R. Mazzoli<sup>a</sup>, P. Fattori<sup>a</sup>, V. M. Riva Violetta<sup>a</sup>, A. Molinaro<sup>b</sup>, S. Leone<sup>b</sup>, C. Giunta<sup>a</sup> & E. Pessione<sup>a</sup>

<sup>a</sup>Università degli Studi di Torino, Lab. Proteomica e Biochimica dei Microrganismi, DBAU; <sup>b</sup>Università degli Studi di Napoli Federico II, Italy

*Acinetobacter radioresistens* S13 is a strain selected for its ability to degrade aromatic compounds, Phenol and Benzoate, through the  $\beta$ -keto adipate pathway [1]. Spectroscopic investigations revealed a peculiar surface structure where the lipopolysaccharide (LPS) is substituted by lipooligosaccharide (LOS) [2]. Furthermore a surfactant molecule with different cellular localization throughout the growth phases has been detected and characterized. It proved to be a glycoprotein by SYPRO Ruby protein stain gel and the glycosylation site determined (N-linked sugar), with a high degree of homology to both OmpA and Alasan from *A. radioresistens* KA53.

Comparative alkaline proteomics studies on the strain grown either on phenol or benzoate as sole carbon source revealed a different response to stress induced by the two different aromatics. Phenol is a solvent able to solubilize outer membrane lipoproteins, LOS and phosphatidylethanolamine, damaging cell wall [3]. This compound induces an over expression of two proteases (ClpX and Serine protease) and of two RNA polymerase modulator factors (NusA and Rho) suggesting a gene modulation based on  $\sigma^E$  regulation.

Benzoate, on the contrary, induces an alternative phosphorylation-dependent stress-sensing response, maintained by a two-component regulatory system, which consists of a membrane-embedded sensory kinase and a response regulator [4]. The kinase auto-phosphorylates a conserved histidine on the receipt of a stress-signal before transferring the phosphoryl group to an invariant aspartate in its cognate response regulator and in doing so activates its latent biological function [5].

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## **O19 ACINETOBACTER BAUMANNII SERINE PROTEASE IS REQUIRED FOR SERUM RESISTANCE AND BIOFILM DISPERSAL**

**L. S. McDaniel and L. B. King**

**University of Mississippi Medical Center, Jackson, MS, USA**

*Acinetobacter baumannii* has emerged as a relevant multidrug-resistant pathogen responsible for up to 11% of nosocomial infections including pneumonia and bacteremia. These infections are often severe and occur predominantly in immunocompromised patients. The pathogenesis of this bacterium, however, is not well understood and very few virulence factors have been identified. The ability to avoid killing by the innate immune system via inactivation of complement is important to the survival of *A. baumannii* in the host. Additionally, the ability to form biofilms contributes to the overall virulence of this pathogen.

To examine the role of a secreted serine protease in serum resistance, a serum resistant isolate was incubated with either a protease inhibitor cocktail or individual protease inhibitors and then used in a serum survival assay. The gene encoding the serine protease in *A. baumannii* LK41, a serum resistant clinical isolate, was insertionally inactivated using plasmid pCR2.1-TOPO. Serum resistance was then determined for the mutant using a serum survival assay.

The mutant was also used in an assay to detect biofilm production due to an observed change in phenotype. Bacteria incubated with a protease inhibitor cocktail showed up to an 80% decrease in survival following incubation with serum. A serine protease inhibitor (AEBSF) showed the same decrease in survival, whereas other inhibitors showed no decrease. The survival in serum of the *A. baumannii* strain lacking the serine protease decreased by 50% as compared to wildtype, and the production of biofilm was significantly increased. Our data indicate that a secreted serine protease is involved in the serum resistance of *A. baumannii* and that inactivation of this protease results in an increase in biofilm production. Further characterization of this protease should elucidate pathogenic mechanisms in this important nosocomial pathogen.

## **O20 HORIZONTAL TRANSFER OF THE OXA-24 CARBAPENEMASE GENE THROUGH OUTER MEMBRANE VESICLES: A NEW MECHANISM FOR CARBAPENEM RESISTANCE GENE DISSEMINATION IN ACINETOBACTER BAUMANNII**

**C. Rumbo-Lorenzo<sup>a</sup>, E. Fernandez-Moreira<sup>a</sup>, A. Mosquera<sup>a</sup>, M. Merino<sup>a</sup>, M. Poza<sup>a</sup>, F. Chaves<sup>b</sup> & G. Bou<sup>a</sup>**

**<sup>a</sup>Centro Hospitalario Universitario A Coruña, Spain; <sup>b</sup>Hospital 12 de Octubre, Madrid, Spain**

Outer Membrane Vesicles (OMVs) are a means by which bacteria interact with prokaryotic and eukaryotic cells in their environment and previous studies suggest that vesicles may be involved in the transfer of genetic material among similar bacterial species. Two different MDR epidemic strains of *A. baumannii* (AbH12O-A2 and AbH12O-CU3) harbouring the *bla*<sub>OXA-24</sub> gene in two different plasmids (AAC 2010, Vol. 54, N°6) were isolated in a large nosocomial outbreak at the 12 de Octubre Hospital (Madrid, Spain). The aim of this study was to determine whether the carbapenemase gene could be horizontally transferred with OMVs released from the clinical isolates, thus constituting a new mechanism of dissemination of antibiotic resistance genes in *A. baumannii*.

OMVs were purified from the two MDR *A. baumannii* clinical isolates by standard protocols, and then filtered (0.22 µm) to exclude any possible bacterial contamination. Purified OMVs were visualized by electron microscopy (TEM). *A. baumannii* ATCC 17978 cells (host) were incubated overnight with OMVs and streaked onto ampicillin plates (500-1000 µg/ml) for selection of OMV-mediated transformants. A biotinylated probe of the *bla*<sub>oxa-24</sub> gene was used as a probe, and the presence of oxa24 gene on OMVs was revealed by Dot blot.

The transmission electron micrography analysis showed OMVs of length between 50 and 200 nm from the clinical isolates. The imipenem and meropenem MICs of *A. baumannii* ATCC17978 incubated with OMVs from the two clinical isolates increased from respectively 0.38 and 0.75, to >32 µg/ml, with both carbapenems. The presence of the respective plasmids harbouring *bla*<sub>oxa-24</sub> was detected in *A. baumannii* ATCC17978 after OMV-transformation. No transformants were obtained when *A. baumannii* ATCC 17978 was incubated with the same amount of free-naked plasmid instead of DNA-packed into OMVs or with the same amount of OMVs previously hydrolyzed with Triton X-100 for 30 min at 37°C. Dot blotting of DNA from OMVs purified from the *A. baumannii* clinical clones as well as OMV-transformed *A. baumannii* ATCC17978 revealed a positive band for *bla*<sub>OXA-24</sub>. No positive signal was obtained with OMVs from mock-transformed ATCC17978.

These experiments show that *A. baumannii* releases OMVs able to carry the *bla*<sub>OXA-24</sub> carbapenem resistance gene, thus constituting a way of disseminating antibiotic resistance genes with a human clinical impact, that was not described so far.

## O21 ANALYSIS OF GENES CODING FOR PENICILLIN-BINDING PROTEINS IN CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* SUSCEPTIBLE OR RESISTANT TO CARBAPENEMS

Rodrigo Cayô<sup>a</sup>, María-Cruz Rodríguez<sup>a</sup>, Paula Espinal<sup>b</sup>, Felipe Fernández-Cuenca<sup>c</sup>, Alain OcampoSosa<sup>a</sup>, Álvaro Pascual<sup>c</sup>, Jordi Vila<sup>b</sup> & Luis Martínez-Martínez<sup>a</sup>

<sup>a</sup> Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Santander, Spain; <sup>b</sup>Servicio de Microbiología, Centre de Diagnòstic Biomèdic, Hospital Clínic, Barcelona, Spain; <sup>c</sup>Servicio de Microbiología, Hospital Universitario Virgen Macarena, Sevilla, Spain

Resistance to carbapenems in *Acinetobacter baumannii* (Acb) is related to several mechanisms, including production of beta-lactamases (particularly oxacillinases), porin loss and efflux pumps. However, there is limited information on the role of penicillin-binding proteins (PBPs) on this phenotype. The objectives of this study were to sequence the genes coding for PBP in Acb and to analyze their allelic variations in isolates susceptible or resistant to carbapenems.

Firstly, we analysed the complete genomes of 6 Acb deposited in the GenBank to identify the PBP genes of this species. The sequences were compared and, according to consensus sequences, primers were designed for all the identified genes. Twenty six Acb clinical isolates (10 susceptible and 16 resistant to carbapenems) representing 12 different clones were evaluated. The DNA and amino acid (aa) sequences of all PBPs obtained were compared with those of the Acb ATCC 17978 strain.

Eight PBP genes were identified in the 6 Acb genomes, coding for 4 high molecular mass (HMM) (2 of class A, 2 of class B) and 4 low molecular mass (LMM; class C) proteins. Class A HMM PBPs were encoded by *ponA* (PBP1a, a class A1 transpeptidase-transglycosylase) and *mrcB* (PBP1b, a class A2 transpeptidase-transglycosylase). Genes for class B HMM PBPs included *pbpA/mrdA* (PBP2, cell division protein) and *ftsI* (PBP3, septum formation peptidoglycan synthetase). The 4 genes for LMM PBPs corresponded to 2 Type-5 carboxypeptidases [*dacC* (PBP5/6) and an unnamed gene homologous to the *dacD* in *E. coli* coding for a PBP6B precursor], 1 Type-7 endopeptidase [*pbpG* (PBP7/8)] and *mtgA* (PBPC, a monofunctional transglycosylase). Every PBP gene presented hotspot mutation regions, unrelated to the clonal patterns of the isolates. Most (> 90%) of the allelic changes observed translated into silent mutations. The aa consensus sequence of the 8 PBPs genes in the Acb genomes deposited in the GenBank and in the Acb clinical isolates were highly conserved.

Considering the sequences of the ATCC 17978 strain, the main changes according to PBP genes were: *ponA* (L117I, A214T, S352N, T606A), *mrcB* (P112S, P764S), *pbpA/mrdA* (E110Q, V509I), *ftsI* (H370Y, G523V), *dacC* (T154V, A264T, N296D, N307S, N329S, V361G, E363K), PBP6B precursor (P28S, T188P, A277T, V350I, S429N), *pbpG* (T26I, T32S, A71T), *mtgA* (F18L, T49P, I54V, Q100E, N179S). The observed changes in aa sequence were associated to concrete clonal patterns, but were not related to susceptibility or resistance to carbapenems. In 2 isolates of an endemic clone resistant to carbapenems an insertion sequence similar to *ISAbal25* disrupting the gene of the carboxypeptidase PBP6B precursor was identified.

We have identified 8 different PBP genes in Acb. Allelic changes were observed in these genes which in few occasions caused aa changes. When the latter were present they were not related to carbapenem resistance. In two carbapenem resistant isolates an IS disrupting the PBP6B precursor gene was identified.

## **O22 STRUCTURE-FUNCTION RELATIONSHIPS OF THE CARBAPENEM RESISTANCE-ASSOCIATED OUTER MEMBRANE PROTEINS CARO FROM *ACINETOBACTER BAUMANNII***

**M. Catel-Ferreira<sup>a</sup>, V. Molle<sup>b</sup>, T. Jouenne<sup>a</sup>, G. Coadou<sup>c</sup> & E. Dé<sup>a</sup>**

**<sup>a</sup>PBS, UMR 6270 CNRS, University of Rouen, France; <sup>b</sup>IBCP, UMR 5086 CNRS, Lyon, France; <sup>c</sup>COBRA, UMR 6014 CNRS, University of Rouen, France**

Multi-drug resistant *Acinetobacter baumannii* strains are regularly isolated during outbreaks in the hospital emergency units and are an important cause of severe infections. It has been shown recently that CarO, the loss of which is associated with the emergence of carbapenem resistance, could participate in the selective uptake of L-ornithine, other basic amino-acids and carbapenems. We have analyzed the different CarO protein sequences in the protein data bank and found two major groups of primary structure that differ essentially in their C-terminal part. The CarO protein from *A. baumannii* ATCC 19606 is the representative of the group 1 whereas the CarO protein from the AYE strain belongs to the group 2. In this study, we have performed a comparative study at the structural and functional levels between the CarO proteins of both groups in order to evaluate their involvement in the carbapenem resistance.

Both CarO proteins have been over-expressed in *Escherichia coli* BL21 and solubilized under denaturant conditions from inclusion bodies. After their purification via affinity chromatography, they have been refolded following a dilution method in 0.1% Triton X-100 solution and their affinity tag have been removed by thrombin cleavage. Refolding has been controlled by circular dichroism. Functional analyses have been performed by reconstitution of these proteins in planar lipid bilayers. They show a similar single-channel conductance (20 pS in KCl 1M) and a cationic selectivity (PK/PCl=2.2) for both CarO proteins. Their specificities towards various substrates, i.e. ornithine, arginine, glutamic acid, imipenem and meropenem have been compared. If both CarO channels do not present any specificity towards glutamic acid and meropenem, the channel from group 1 exhibits the highest specificity for basic amino acids whereas the channel from group 2 shows the highest specificity for imipenem.

Homology models have been developed for both channels using comparison between existing X-Ray structures, sequence alignments of CarO proteins and peptides generated by trypsin digestion of CarO proteins. Generated models formed a 10  $\beta$ -strand barrel with narrow channel that could explain the exclusion of the meropenem. The difference of specificities between the proteins from each group may originate from modifications in charge repartition inside the channels.

Finally, the marked specificity of the CarO channel from group 2 for imipenem may suggest that any decrease in the expression of this protein would have an greater impact on imipenem influx (as compared to a modification of the group 1 channel expression), and could so participate at a higher level to the development of the imipenem resistance.

## O23 THREE NOVEL VARIANTS OF THE ABAR3 RESISTANCE ISLAND IN MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* STRAINS OF EUROPEAN CLONE I

L. Krizova<sup>a</sup>, L. Dijkshoorn<sup>b</sup> & A. Nemeč<sup>a</sup>

<sup>a</sup>National Institute of Public Health, Prague, Czech Republic; <sup>b</sup>Leiden University Medical Center, Leiden, The Netherlands

Multidrug-resistant (MDR) strains of *Acinetobacter baumannii* often belong to one of three international clonal lineages termed European (EU) clones I, II and III. So far, five structurally related genomic resistance islands (AbaR) integrated into the ATPase gene have been described in MDR strains of EU clone I. These islands, i.e. AbaR1, AbaR3, AbaR5, AbaR6 and AbaR7 (sized 20-86 kb), share several conserved regions, e.g. transposon Tn6020 with the *aphA1* gene and class 1 integron with the *aacCI*-(orfP)<sub>1,2</sub>-orfQ-*aadA1* gene cassette array.

In the present study, we describe three novel AbaR variants in EU clone I strains. MDR *A. baumannii* strains RUH 875 (isolated in Dordrecht, The Netherlands, 1984; a reference strain of EU clone I), RUH 3247 (Leuven, Belgium, 1990) and LUH 6125 (Krakow, Poland, 1998) were investigated. These strains were previously allocated to EU clone I and yielded resistance gene patterns different from those associated with the AbaR islands hitherto described in EU clone I. Strain HK302 (*Antimicrob Agents Chemother* 1982; 22:323) harboring AbaR3, was used as a positive control for the detection of the AbaR3-associated genes and PCR mapping.

The disruption of the ATPase gene was detected in all strains using PCR with primers derived from both ends of the gene. Of 20 AbaR3-associated genes tested, 18, 18 and 16 were identified in RUH 875, RUH 3247 and LUH 6125, respectively. PCR mapping followed by RFLP analysis and/or partial sequencing of amplicons revealed that individual strains harbored islands structurally mostly related to AbaR3. However, the three islands differed from each other in the regions identified in all hitherto known AbaR islands of EU clone I. A 53-kb island found in LUH 6125 lacked the Tn6020 transposon with *aphA1* and a part of transposon Tn3. The islands of RUH 3247 and RUH 875, sized 61.2 and 61.1 kb, respectively, differed from AbaR3 only in the structure of class 1 integrons. While RUH 3247 harbored integron with an *aacA4* cassette, RUH 875 carried integron with *dfrA1*.

In conclusion, three novel variants of AbaR3 were found in EU clone I strains, suggesting that structurally related resistance islands are common in strains of this clone. The diversification of AbaR3-like structures may have contributed to the heterogeneity of resistance patterns of EU clone I strains.

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## **O24 MULTIPLE ANTIBIOTIC RESISTANCE IN *ACINETOBACTER BAUMANNII* FROM AN AUSTRALIAN HOSPITAL**

**V. Post, S.J. Nigro & R.M. Hall**

**University of Sydney, Australia**

Multiply antibiotic resistant *A. baumannii* strains isolated at a single Sydney Hospital in 2008 (May-July), 2009 (March-September) and 2010 (January -April) were examined and compared to earlier isolates from 2006. Antibiotic resistance profiles and antibiotic resistance genes, identified by PCR, classification into the global clonal lineages (EC I, EC II and variant types), using multiplex PCR and sequencing of the *recA* and *oxa-Ab* genes, were used to identify related groups. PCR mapping of the AbaR-type genomic island that carries the antibiotic resistance genes was undertaken for the EC I isolates.

Three groups of isolates were identified. Three isolates belonging to the EC I group were recovered in 2008 and carried the same AbaR6 as found in earlier isolates from the same hospital. However, some evolution had occurred and one isolate had acquired the *oxa23* imipenem resistance gene. EC II isolates were rare in 2008 and 2009, but caused a small outbreak in 2010. These carried a specific set of antibiotic resistance genes previously found in EC II isolates from the same hospital and from other Sydney hospitals.

Isolates belonging to a variant type, identified using the allele-specific PCRs and previously designated SG6, appeared sporadically in 2009 and were also found in early 2010. These isolates all came from one of two wards. This variant was shown to be part of the EC I lineage as it had the same *recA* and *oxa-Ab* sequences and carried an AbaR-type island in *comM*. A class 1 integron with the *aacC1*-orfP-orfQ-*aadA1* gene cassette array (*aacC1* confers resistance to gentamicin; *aadA1*, to streptomycin and spectinomycin), and the *sulI* (sulphonamide resistance) and *aphA1b* (kanamycin and neomycin resistance) genes were found in this group. These resistance genes were clustered within an AbaR-type genomic island, which was identified using PCR to detect the external and internal junctions. However, the SG6 isolates all contained a novel AbaR variant form, AbaR8. AbaR8 contains a deletion relative to the longer AbaR3 configuration and has probably also arisen from it or from AbaR5 via an IS26-mediated deletion.

In conclusion, European clone I strains have persisted in this hospital for over a decade, but whether the SG6 type evolved there or was introduced needs further investigation. European clone II isolates also appear to have persisted, but could also have come in from other hospitals because related isolates have been found in other Australian hospitals.

## **O25 INSIGHTS INTO GENE AMPLIFICATION IN *ACINETOBACTER BAYLYI*: VARIATIONS IN THE DUPLICATION FREQUENCY AND GENETIC STABILITY OF DIFFERENT GENOMIC LOCI**

**Kathryn T. Elliott, Sarah H. Craven, Laura E. Cuff & Ellen L. Neidle**

**Department of Microbiology, University of Georgia, Athens, GA, USA**

Gene amplification, the relative increase in copy number of a DNA segment, has significant medical and evolutionary implications. However, due to its inherent reversibility, gene amplification is not usually amenable to comprehensive study. A bacterial system for investigating gene amplification exploits the natural transformability of *Acinetobacter baylyi* ADP1 and takes advantage of the *cat* gene-encoded pathway for catechol degradation. Previous use of this system focused on duplication and amplification events in approximately 10% of the genome. Recently, we expanded this system to enable the study of gene amplification throughout the ADP1 genome. Genome-wide studies are needed to evaluate location-dependent differences in the frequency and nature of gene duplication, which may be affected by the proximity of direct repeats, mobility elements, the origin of replication, and hotspots for DNA break and repair. The original system to study gene amplification in ADP1 was restricted to recombination events near the native chromosomal position of the *cat* genes. To permit genome-wide study, we developed a method to relocate the *cat*-gene cluster to new loci in the genome<sup>1</sup>. After relocation, it is possible to characterize recombination events that occur near the newly positioned *cat* genes. While the data set is now relatively small, the kinds of recombination events involved in gene duplication appear to vary with genomic position. For example, the proportion of events mediated by insertion sequences or homologous recombination differed between distinct genomic regions.

Additionally, an assay was developed to determine naturally occurring gene duplication frequencies in different parts of the chromosome. This assay involves *catA*, which is required for growth on benzoate and is in the cluster moved in the engineered strains described above. A modified *catA* allele (inactivated by an antibiotic resistance marker) was used to transform recipient strains in which a functional *catA* gene resided in the native or relocated position. The phenotype of the resulting transformants could then be used to infer the proportion of cells in an unselected starting population that carried a *catA* duplication. Drug-resistant colonies represent transformants in which allelic replacement had inactivated a chromosomal copy of *catA*. While most of these drug-resistant isolates were unable to use benzoate as a sole carbon source (Ben-), a small portion retained this growth capability (Ben+). The Ben+ drug-resistant isolates derive from a minority portion of cells in the recipient population that originally contained a *catA* duplication. After inactivation of one *catA* copy, these cells retain one functional *catA* gene. Thus, the *catA* duplication frequency is assessed by determining the frequency of Ben+ drug-resistant transformants. By relocating *catA*, the duplication frequency at any non-essential genomic locus can be measured. Our studies found genomic duplication frequencies ranged from  $10^{-4}$  to  $10^{-5}$ , similar to published reports with *Salmonella*. However, the insertion of the *cat*-gene cassette in a region between two large direct repeats (2.5 kb) was highly unstable. When the cassette was inserted in this region, approximately 90% of the population lost the cassette within five hours of growth on non-selective medium. This instability presumably resulted from deletion via homologous recombination. These findings highlight the varied mechanisms and frequency of gene duplication throughout the ADP1 genome.

<sup>1</sup>This new method is detailed in poster abstract P12 of L. Cuff *et al.*, "Insights into Gene Amplification in *Acinetobacter baylyi*: Insertion Sequence-Mediated Genomic Rearrangements".

## O26 THE ACQUISITION OF FOREIGN DNA BY NATURAL TRANSFORMATION OF *ACINETOBACTER BAYLYI* ADP1

K. Harms<sup>a</sup>, P. J. Johnsen<sup>a</sup> & K. M. Nielsen<sup>a,b</sup>

<sup>a</sup>University of Tromsø, Norway; <sup>b</sup>GenØk – Centre for Biosafety, Tromsø, Norway

Natural transformation includes the active uptake of free DNA (donor DNA) from the environment by a prokaryotic cell and its subsequent genomic integration, and is one of the known horizontal gene transfer (HGT) mechanisms in these organisms. HGT is thought to be a driving force of prokaryotic evolution.

The soil bacterium *Acinetobacter baylyi* ADP1 is widely used as model organism to study transformation and is transformed by cognate DNA at high frequencies. Donor DNA homologous to the recipient DNA is recombined with the genome by homologous recombination (HR), which requires the RecA recombinase and integration of the donor at both DNA molecule termini. Transformation by foreign DNA from related species of the *Acinetobacter* genus (homeologous DNA) is decreased at least 100-fold but readily detectable. The transformation frequency by homeologous DNA strongly depends on the chromosomal locus and can vary 10,000-fold over the recipient genome. Recombination with homeologous DNA is RecA-dependent but is suppressed by DNA mismatch repair, indicating that integration occurs by HR but is hampered by DNA sequence divergence. The latter affects transformation efficiency by decreasing the RecA-dependent DNA strand invasion and heteroduplex formation, and by occurrence of mismatches in the heteroduplex which are removed by DNA mismatch repair.

Foreign DNA containing no sequence identity to the ADP1 recipient genome can also be integrated by natural transformation if located adjacent to homologous DNA by homology-facilitated illegitimate recombination (HFIR). This recombination type comprises one homologous, and one illegitimate recombination event, at each DNA terminus and is about 10,000-fold rarer than HR but at least 100,000-fold more frequent than fully illegitimate recombination. Deletion of the RecJ DNA single-strand specific exonuclease strongly increases HFIR but has no detectable effect on HR.

## **O27 LARGE-SCALE EXPERIMENTAL ANNOTATION OF *ACINETOBACTER BAYLYI* ADP1 GENOME BY A BIOCHEMICAL SCREENING FOR LARGE ENZYME FAMILY ACTIVITIES**

**V. de Berardinis<sup>a,b,c</sup>, A. Mariage<sup>a</sup>, JL Petit<sup>a</sup>, A. Perret<sup>a,b,c</sup>, J. Weissenbach<sup>a,b,c</sup> & M. Salanoubat<sup>a,b,c</sup>**

**<sup>a</sup>CEA, DSV, Institut de Génomique, Genoscope; <sup>b</sup>CNRS, UMR8030; <sup>c</sup>Université d'Evry Val d'Essonne, Evry, France**

Since metabolism needs to be further investigated using organisms whose life-styles are different from those of model organisms, *Acinetobacter baylyi* ADP1 had been chosen at Genoscope as an environmental model especially suitable for large-scale genetic manipulation and system biology (de Berardinis V. *et al.*, Curr Opin Microbiol, (2009)). Taking advantage of Genoscope's high-throughput knows how, resources have been constructed in the last few years that can form the basis for diverse metabolic studies: the genome sequence (Barbe V. *et al.*, Nucleic Acids Res, 32(19):5766-79 (2004)), a single gene mutant collection (de Berardinis V. *et al.*, Mol Syst Biol 4:174 (2008)) and a genome-scale metabolic model (Durot *et al.*, BMC Syst Biol, 7:2-85 (2008)).

To complement the available resources to explore ADP1 metabolism, the ADP1 ORFeome focused on metabolic enzymes and proteins with unknown function was done and is being screened for large enzyme family activities for a large-scale functional annotation. This systematic functional analysis of *Acinetobacter baylyi* ADP1 ORFeome is done to confirm or invalidate ADP1 predicted annotations. A special effort is done to determine the function of genes with unknown function.

Taking advantage of the Genoscope cloning platform, 2141 ADP1 non-membrane proteins have been cloned including 1249 (putative) metabolic enzymes and 852 genes with unknown function (689 conserved hypothetical proteins (CHP) and 191 hypothetical proteins (HP)). The systematic enzymatic screening of this ORFeome has been initiated and preliminary results from phosphatase and dehydrogenase assays are reported. The screening is done without *a priori* knowledge on enzyme capabilities and all genes are tested for each activity.

Here, we report the preliminary results obtained from phosphatase assays using *p*NPP as generic chromogenic substrate and deshydrogenase assays using various pools of substrates (amino acid, aldehydes, alcohols, etc.). This screening has revealed ~30 phosphatases including 16 phosphatases previously annotated proteins with unknown function. A substrate profiling with ~80 natural substrates is on going in a secondary screen to determine their accurate function. Preliminary results from deshydrogenase assays are also presented revealing some new deshydrogenases and the substrate promiscuity of many deshydrogenases by a substrate profiling. The ADP1 metabolic and genomic context of all these results is investigated in order to complete the ADP1 metabolic pathway knowledge.

## O28 FITNESS COST AND STABILITY OF CLASS I INTEGRONS

I. Starikova<sup>a</sup>, T. Munthali<sup>a</sup>, K. Harms<sup>b</sup>, P. Johnsen<sup>a</sup>, K. Nielsen<sup>a,b</sup>

<sup>a</sup>University of Tromsø, Tromsø, Norway; <sup>b</sup>GenØk, Center for Biosafety, Norway

The fitness cost of resistance is a key parameter determining the frequency of resistant bacteria in the community. The aim of this project was to measure the relative fitness cost of acquired class I integrons in the model organism *Acinetobacter baylyi* and determine the stability of gene cassettes in the integron in the absence of selective pressure.

The class I integron from the clinical strain *Acinetobacter baumannii* 064 was horizontally transferred to *Acinetobacter baylyi* ADP1 by natural transformation. In order to obtain a defined DNA source and locus, a PCR-amplified integron from the donor strain was ligated into a plasmid containing flanking sequences identical to the selectively neutral locus *lifO-lipB* in *A. baylyi*. The resulting bacterium was called Ab64.T1b. To estimate the cost of the integrase gene, another bacterial strain, Ab64.T1b/int::*cat* that carried a truncated inactive integrase gene was constructed. The presence of class I integrons and resistance in *A. baylyi* was confirmed by PCR and sequencing, as well as by Disc diffusion and E-test. The stability of the acquired gene cassette was tested during a 10 days period of serial transfer and replica plating, and screening of genomic DNA for excised gene cassettes by excision PCR. The fitness cost of these strains was estimated in 24-hours pair-wise competitions with the isogenic strain ADP1. Six competition replicates were performed for each strain. The relative fitness (W) was calculated as the ratio of the Malthusian parameter of each competitor.

Stability tests revealed that both *A. baumannii* 064 and *A. baylyi* Ab64.T1b were able to grow in the absence/presence of the selective agents, thus indicating that they both maintained the gene cassettes. The screening for loss of resistance showed that less than 1 in 1000 colonies lost the resistant phenotype. Cassette excision PCR did not show any positive results for circularised free cassettes, thus supporting gene cassette stability. The mean W was found to be 0.93 ( $\pm$  0.01 S.D) in strain Ab64.T1b that corresponds to a fitness cost of 7%. The mean W in strain Ab64.T1b/int::*cat* was found to be 0.98 ( $\pm$  0.02 S.D) that corresponds to a fitness cost of 2%.

In this study, the observed high fitness cost of 7% measured for strain Ab64.T1b was assumed to be caused by the integron and independent on the exact insertion site in the genome. A competition experiment with strain Ab64.T1b/int::*cat* carrying the inactive integrase imposed only a fitness cost of 2%. These results suggest the integrase is a biologically expensive genetic element.

**O29 INDUCIBLE EXPRESSION PLASMIDS FOR *ACINETOBACTER BAYLYI* ADP1****Charles Daniel Murin<sup>\*</sup>, Kristy Segal<sup>\*</sup>, Anton Bryksin & Ichiro Matsumura****Department of Biochemistry, Center for Fundamental and Applied Molecular Evolution,  
Emory University, Atlanta, Georgia 30322, USA.**

*Acinetobacter baylyi* ADP1 would be an ideal vehicle for directed protein evolution if plasmid-based inducible vectors were available. We have engineered a very broad host range plasmid, pBAV1k, that replicates to high copy numbers in gram-positive and gram-negative bacteria, including *Acinetobacter*. The pBAV1k origin was used to construct a family of inducible expression plasmids. Some promoters developed for use in *E. coli* (PT5, PT7, Ptac, PBAD) also function in *A. baylyi*. The expression of a foreign reporter gene (*E. coli gusA*) from the T5 promoter, for example, was increased >200-fold upon the addition of the synthetic inducer (isothiopropyl-galactopyranoside). These vectors, in combination with the felicitous ability of *A. baylyi* to import and recombine DNA, facilitate directed protein evolution.

<sup>\*</sup>These authors contributed equally to this work

**O30 A NOVEL MECHANISM OF COLISTIN RESISTANCE IN *ACINETOBACTER BAUMANNII***

**J. H. Moffatt<sup>a</sup>, M. Harper<sup>a,b</sup>, P. Harrison<sup>c</sup>, J. D. Hale<sup>d</sup>, E. Vinogradov<sup>e</sup>, T. Seemann<sup>c</sup>, R. Henry<sup>a</sup>, B. Crane<sup>a</sup>, F. St. Michael<sup>e</sup>, A. D. Cox<sup>e</sup>, B. Adler<sup>a,b,c</sup>, R. L. Nation<sup>d</sup>, J. Li<sup>d</sup> & J. D. Boyce<sup>a,b</sup>**

**<sup>a</sup>Department of Microbiology, <sup>b</sup>Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics, <sup>c</sup>Victorian Bioinformatics Consortium, <sup>d</sup>Facility for Anti-infective Drug Development and Innovation, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Victoria, Australia; <sup>e</sup>Institute for Biological Sciences, National Research Council, Ottawa, Ontario, Canada**

Polymyxin antibiotics such as colistin are one of the few remaining antibiotics available for effective treatment of multi-drug resistant *A. baumannii* infections. While the mechanism of antimicrobial action of colistin is poorly defined, it is proposed to require an initial, charge-based interaction between colistin and the lipid A component of lipopolysaccharide (LPS), which forms the outer leaflet of the Gram-negative outer membrane. Here we show that *A. baumannii* can rapidly develop resistance to colistin by complete loss of the initial binding target, lipid A, which is essential for the viability of most Gram-negative bacteria.

Whole-genome sequencing of a paired colistin-sensitive and colistin-resistant derivative of the *A. baumannii* type strain ATCC 19606 revealed that the colistin-resistant strain contained a mutation within *lpxA*, a gene essential for lipid A biosynthesis. Carbohydrate structural analyses and direct measurement of endotoxin activity by *Limulus* amoebocyte lysate assay, showed that the colistin resistant *lpxA* mutant failed to elaborate any LPS. Complementation with an intact *lpxA* gene restored LPS production and sensitivity to colistin. We characterised a further twelve, independent colistin-resistant derivatives of ATCC 19606 and showed that all contained mutations within one of the first three genes of the lipid A biosynthesis pathway, namely *lpxA*, *lpxC* or *lpxD*. All these mutations resulted in the complete loss of LPS production. Furthermore, we showed that spontaneous loss of LPS also occurs in other *A. baumannii* strains, including a colistin-resistant clinical isolate from South Korea. While the loss of LPS did not prevent the stable elaboration of an outer membrane, LPS-deficient cells were more permeable to a hydrophobic probe, and thus susceptible, to a wide range of antibiotics. This is the first study to show that *A. baumannii* is viable without LPS, and the first study to demonstrate loss of LPS as a mechanism of colistin resistance.

### **O31 BIOLOGICAL COST OF EXTREME RESISTANCE TO TIGECYCLINE IN *ACINETOBACTER BAUMANNII***

**S. D'Arezzo<sup>a</sup>, S. Fittipaldi<sup>a</sup>, F. Imperi<sup>b</sup>, S. Sepe<sup>b</sup>, A. Di Giulio<sup>b</sup>, L. Principe<sup>a</sup>, N. Petrosillo<sup>a</sup> & P. Visca<sup>b</sup>**

**<sup>a</sup>Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani I.R.C.C.S. <sup>b</sup>Dipartimento di Biologia, Università Roma Tre, Rome, Italy**

*Acinetobacter baumannii* is an important opportunistic pathogen which has rapidly evolved towards multidrug-resistance (MDR). Tigecycline, a recently developed semi-synthetic tetracycline, provided new hope for the treatment of MDR *A. baumannii* infections, but *in vitro* adaptation to this drug has been documented and clinical isolates showing reduced susceptibility have emerged in many countries.

Here, two wild type (WT) tigecycline-susceptible MDR *A. baumannii* strains whose complete genome sequence is available, namely ACICU and AYE, were exposed to progressively increasing concentrations of tigecycline, resulting in a rapid rise of the tigecycline MIC from 0.25 and 2 mg/L (in AYE and ACICU, respectively) to 512 mg/L. The tigecycline extreme-resistant (XR) phenotype of adapted cells was characterized by concomitantly increased resistance to other drugs, namely fluoroquinolones, tetracyclines, aminoglycosides and ethidium bromide. The XR phenotype was only partially reversed to tigecycline resistance (R) by serial transfer in drug-free medium, with ACICU and AYE variants retaining a tygecyclin MIC of 32 and 128 mg/L, respectively. Spectrofluorimetric studies of ethidium bromide accumulation, with or without the efflux pump inhibitor carbonyl cyanide m-chlorophenylhydrazone, suggested the involvement of MDR efflux pumps in the adaptation to tigecycline, but also highlighted increased permeability to ethidium bromide of XR cells, compared with WT and R counterparts. Membrane permeability assays with Triton-X100 demonstrated a loss of membrane integrity in XR variants. Electron microscopy examination provided direct evidence for reduction of cell size, alterations of bacterial cell envelope, and massive loss of cell projections in XR cells, compared with WT and R counterparts. The XR cells were also characterized by higher turbidity/cell viability ratios, likely resulting from an increased protein/DNA ratio. Evidence of a different expression of several membrane proteins, including the OmpA porin and the AdeABC efflux pump in the XR cells was evidenced by electrophoresis and MALDI-TOF MS analysis of membrane protein fractions. The XR and R variants of both ACICU and AYE strains were out-competed by the corresponding WT in co-culture assays.

We conclude that tigecycline-XR *A. baumannii* cells undergo profound functional and structural changes resulting in serious deficiencies of cellular functions and loss of fitness *in vitro*.

### O32 CLONING AND CHARACTERIZATION OF *algC* GENE FROM *ACINETOBACTER BAUMANNII*; ESSENTIAL FOR ALGINATE SYNTHESIS IN BIOFILMS

Praveen K Sahu<sup>a</sup>, Karishma R Pardesi<sup>b</sup> & Balu A Chopade<sup>a,b</sup>

<sup>a</sup>Institute of Bioinformatics & Biotechnology, <sup>b</sup>Dept. of Microbiology, University of Pune, India 411007

*Acinetobacter baumannii* is known to form biofilms on abiotic/biotic surfaces, which imparts multiple-level resistance towards antibiotics, often difficult to treat and probably, confers a highly regulated mode of pathogenesis. Genetic studies on factors of biofilms formed by pathogenic microbes have always led not only to understand the process of pathogenesis; but also to develop potential drug targets in future for restricting biofilm associated subsequent infections. *Alginate*, a component of EPS (exo-polymeric substances) matrix of bacterial biofilms, is synthesized through enzymatic catalysis by a bi-functional enzyme phosphoglucomutase/ phosphomannomutase. Previously, *algC* gene from the *alg*-gene cluster of *Pseudomonas aeruginosa* has been characterized, but so far there is no published data on *algC* gene in *Acinetobacter baumannii*.

In the present study, we have experimentally demonstrated the role of *algC* gene in a clinical MDR strain of *Acinetobacter baumannii* strain AIIMS-7, isolated from the neurosurgery ward, All India Institute of Medical Sciences (AIIMS), New Delhi, India. The strain showed substantial biofilm formation on polystyrene and glass surfaces. Using genomic sequences from NCBI and bioinformatics tools, gene specific primers were designed for a calculated 1779bp-sized *algC<sub>A.baumannii</sub>*, an orthologue of *algC<sub>P.aeruginosa</sub>*. After successful *in vitro* PCR-amplification from the genomic DNA, total RNA was purified and transcription of *algC<sub>A.baumannii</sub>* was done using RT-PCR, yielding the same 1779bp amplicon, confirmed that the targeted region is entirely transcribed. Further, for expression of the *algC* gene, it was purified and cloned in to a T-vector, inserted into *E.coli*-DH5 $\alpha$  and the insertion was cross-confirmed by plasmid purification and colony-PCR and DNA sequencing. *in vitro* translation of *algC<sub>A.baumannii</sub>* gene yielded a ~52 kDa protein on SDS-PAGE, analogue to the PGM/PMM protein of *P.aeruginosa*. High resolution scanning electron microscopy (SEM) pictures taken on glass surfaces clearly indicated dense matrix and EPS of biofilm formed by *E.coli* clone containing *algC<sub>A.baumannii</sub>* gene. A quantitative biofilm augmentation assay on 96-well microtitre plate showed a 3.87 fold-increase in biofilm formation compared to wild type *E.coli*-DH5 $\alpha$ , thereby strongly indicating *in vivo* expression and encoding of the bi-functional enzyme by the *algC<sub>A.baumannii</sub>* gene.

This is the first report on cloning and characterization of *algC* gene from *Acinetobacter baumannii*, probably coding the bi-functional enzyme (phosphoglucomutase / phosphomannomutase) required for alginate bio-synthesis pathway; crucial for sustaining a mature biofilm architecture. Further, this study holds tremendous potential for developing futuristic drug target against biofilm-associated multidrug resistant *Acinetobacter baumannii* causing recurrent nosocomial infections in hospitals.

### **O33 VACCINATION AND IMMUNOTHERAPY AGAINST *ACINETOBACTER BAUMANNII* USING BACTERIAL OUTER MEMBRANE PROTEINS**

**Michael J. McConnell, Juan Domínguez-Herrera, Younes Smani, Rafael López-Rojas, Fernando Docobo-Pérez & Jerónimo Pachón**

**Unit of Infectious Disease, Microbiology, and Preventive Medicine, Institute of Biomedicine of Sevilla (IBiS), University Hospital Virgen del Rocío, Sevilla, Spain**

Over the last two decades the incidence of hospital-associated infections caused by multidrug resistant *Acinetobacter baumannii* has increased significantly, requiring the development of novel approaches for preventing and treating these infections. The objectives of the present study were to develop a vaccine for the prevention of *A. baumannii* infection, and to test the use of antibodies generated against bacterial outer membrane proteins for the treatment of *A. baumannii* infection.

Outer membrane proteins (OMPs) were purified from *A. baumannii* strain ATCC 19606, combined with an aluminium adjuvant, and used to vaccinate C57BL/6 mice by intramuscular injection. Levels of anti-OMP antibodies in serum collected at 2 and 4 weeks were quantified by ELISA. Mice were challenged with the ATCC 19606 strain and 2 clinical isolates using a disseminated sepsis model, and the following parameters were measured in vaccinated and control mice: i) bacterial loads in tissues, ii) serum levels of pro-inflammatory cytokines, and iii) mortality. The ability of antibodies generated against OMPs to treat established infections was determined by intravenous administration of antibodies to naïve mice after infection.

Two doses of the vaccine three weeks apart elicited high levels of antigen-specific antibodies. At 12 hours post-challenge, vaccinated mice had fewer bacteria than control mice in spleens (3.78 vs. 9.07 log<sub>10</sub> cfu/g; p<0.001), kidneys (3.50 vs. 8.33 log<sub>10</sub> cfu/g; p<0.001), and lungs (3.73 vs. 8.95 log<sub>10</sub> cfu/g, p<0.001). Vaccinated mice had lower serum levels of the pro-inflammatory cytokines IL-1 beta (20 vs. 396.1 pg/ml, p<0.001), TNF-alpha (59.3 vs. 351.9 pg/ml; p<0.001), and IL-6 (188.6 vs. 51141.3 pg/ml; p<0.001) than control mice 12 hours post-challenge. Vaccinated mice had increased survival over control mice after challenge with the ATCC 19606 strain (100% vs. 0% survival; p<0.001), a multidrug resistant clinical isolate (70% vs. 10% survival; p=0.007), and a panresistant clinical isolate (100% vs. 10% survival; p<0.001). Antibodies against bacterial OMPs could be used to successfully treat mice infected with the ATCC 19606 strain (100% vs. 0% survival of control mice; p<0.01) and a panresistant clinical isolate (71% vs. 0% survival of control mice; p<0.01).

Immunization with an OMP-based vaccine protects against infection with multidrug resistant and panresistant *A. baumannii* in a mouse model of disseminated sepsis. Antibodies against bacterial OMPs can be used to treat established infections in naïve mice. These approaches represent novel strategies for preventing and treating infections caused by *A. baumannii*.

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## **P1 DISTRIBUTION OF PUTATIVE IRON-UTILISATION GENES IN EUROPEAN CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII***

**L. C. S. Antunes<sup>a</sup>, F. Imperi<sup>a</sup>, K. J. Towner<sup>b</sup> & P. Visca<sup>a</sup>**

**<sup>a</sup>University of Roma Tre, Rome, Italy; <sup>b</sup>Nottingham University Hospitals NHS Trust, Nottingham, UK.**

Iron is an essential nutrient for bacteria and therefore bacterial iron-uptake mechanisms are important factors for pathogenesis. In the human opportunistic pathogen *Acinetobacter baumannii*, one gene cluster is responsible for the synthesis and acquisition of the siderophore acinetobactin. Genome inspection of sequenced *A. baumannii* strains enabled us to identify additional gene clusters putatively involved in biosynthesis/transport of other siderophores, heme utilisation and ferrous iron uptake. The aim of this study was to investigate the distribution of these genes in a representative collection of 50 *A. baumannii* clinical isolates belonging to different lineages and isolated over several decades from different worldwide sources.

The *A. baumannii* isolates were initially analysed by randomly amplified polymorphic DNA (RAPD) and allocated to sequence groups (SGs) using the method described by Turton *et al.* (2007), and then screened by PCR to identify specific individual iron-acquisition genes. The presence or absence of genes of interest was also confirmed by dot-blot DNA hybridisation. Two putative siderophore gene clusters, including that coding for acinetobactin, were conserved among the clinical isolates of *A. baumannii*, while a third cluster was present in less than 7% of isolates. Furthermore, a putative heme utilisation gene cluster was conserved in all isolates, while a second heme utilisation cluster was found in approximately 70% of isolates. Lastly, the Feo cluster, putatively coding for genes involved in the uptake of ferrous iron, was conserved in all isolates.

There was no apparent correlation between strain lineage and the presence/absence of specific iron-utilisation genes. When the chromo azurol S (CAS) plate assay was used to detect and quantify iron-chelating compounds, all but one of the isolates was shown to produce iron-chelating compounds. The nature of the compound(s) was further investigated by determining the amount of catechol- and hydroxamate-type groups. A catechol:hydroxamate ratio of approximately 4:5 was calculated. Considering that the siderophore acinetobactin contains one catechol and one hydroxamate group, this indicates the possible presence of at least an additional hydroxamate siderophore.

In conclusion, several iron-acquisition systems appear to be widely distributed in clinical isolates of *A. baumannii*, plausibly giving this species the ability to utilise different iron sources, thereby enhancing the possibility of colonising different habitats, including the human host. Studies are in progress to further characterise the large repertoire of putative iron uptake systems of *A. baumannii* and to verify their contribution to pathogenicity.

**P2 INVESTIGATION OF THE HUMAN PATHOGEN *ACINETOBACTER BAUMANNII* UNDER IRON LIMITING CONDITIONS**

**B. A. Eijkelkamp<sup>a</sup>, K. A. Hassan<sup>b</sup>, I. T. Paulsen<sup>b</sup> & M. H. Brown<sup>a</sup>**

**<sup>a</sup>Flinders University, Adelaide, Australia; <sup>b</sup>Macquarie University, Sydney, Australia**

Iron acquisition systems are important virulence factors in pathogenic bacteria. To identify these systems in *Acinetobacter baumannii* the transcriptomic response of the completely sequenced strain ATCC 17978 under iron limiting conditions was investigated. This was achieved using a genomic microarray that contained probes for all 3367 open reading frames. Transcription levels were more than 2-fold up-regulated for 463 genes, of which 95 genes were up-regulated more than 4-fold, including three heavily overexpressed siderophore gene clusters. Down-regulation under iron limitation was less dramatic as only 202 genes varied more than 2-fold. Motif searches in the promoter regions of up-regulated genes identified a prominent role for the ferric uptake regulator in iron uptake regulation. Siderophore mediated iron-acquisition mechanisms were further investigated by means of comparative genomic analysis of seven sequenced *Acinetobacter* isolates and revealed variation of these siderophore mediated iron-acquisition mechanisms between different *Acinetobacter* strains. Transcriptional profiling also showed significant down-regulation for various genes involved in motility when iron was less readily available, which was confirmed by phenotypic characterisation. Collectively, these studies demonstrate an apparent redundancy of iron uptake systems in *Acinetobacter* spp.

### **P3 EFFECT OF BIOFILM FORMATION ON THE SURVIVAL OF ACINETOBACTER BAUMANNII ON DRY SURFACES**

**P. Espinal, S. Martí & J. Vila**

**Department of Microbiology, Hospital Clínic, School of Medicine, University of Barcelona, Barcelona, Spain**

*Acinetobacter baumannii* is becoming an important nosocomial pathogen in hospitals worldwide. The clinical interest is mainly attributed to its ability to survive for long periods on dry surfaces and the development of multidrug resistance. Biofilm formation by this microorganism could be associated with the high level of resistance to desiccation and disinfection, facilitating its survival in the hospital setting. The aim of this work was to compare the effect of biofilm formation on the survival of *A. baumannii* on dry surfaces.

The study was carried out with four different *A. baumannii* clinical isolates previously identified by ARDRA. Biofilm formation was evaluated by crystal violet staining. The survival experiments were performed by preparing bacterial suspensions of the 4 strains: 2 biofilm-forming strains and 2 non biofilm-forming strains that were inoculated onto glass coverslips and stored under controlled conditions of temperature and relative humidity. Cells washed from coverslips were used to determine viable counts at zero time and every 72 hours until the CFU/ml was <20. Changes in biofilm formation on dry surfaces were compared with samples in liquid medium by scanning and transmission electron microscopy.

The ability to survive on dry surfaces was compared between two biofilm-forming *A. baumannii* clinical isolates and two isolates that lack this ability. The survival times for the biofilm forming isolates were longer than those observed when the isolates were not able to form biofilm (36 days versus 15 days respectively,  $p=0,006$ ). All experiments were performed in triplicate. In addition, the biofilm-forming isolates were further studied by microscopy analysis. When the biofilm structure was observed by scanning and transmission electron microscopy, a biofilm structure and a polysaccharide layer were observed in the biofilm-forming strains which lack in the non biofilm-forming strains.

Our results show that biofilm formation increases the survival rate of *A. baumannii* on dry surfaces. Therefore, biofilm may contribute to the long survival of *A. baumannii* in the hospital setting increasing the occurrence of outbreaks and nosocomial infections.

#### P4 *ACINETOBACTER BAUMANNII* FORMS PELLICLES: STRUCTURE AND CHARACTERIZATION OF THE MATRIX

S. Martí<sup>a</sup>, Y. Nait-Chabane<sup>a</sup>, S. Alexandre<sup>a</sup>, C. Rihouey<sup>a</sup>, J. Vila<sup>b</sup>, T. Jouenne<sup>a</sup> & E. Dé<sup>a</sup>

<sup>a</sup>BRICS, UMR6270 CNRS, Rouen University, France; <sup>b</sup>Hospital Clínic, Barcelona, Spain

*Acinetobacter baumannii* has a natural ability to survive in the hospital environment; this persistence could be partially explained by the capacity of this microorganism to form biofilms. We have recently demonstrated that *A. baumannii* and *Acinetobacter* genospecies 13TU can grow at the air-liquid interface and form pellicles with a higher capacity than other less pathogenic species such as *Acinetobacter johnsonii*, *Acinetobacter lwoffii* and *Acinetobacter radioresistens*. The objective of this work was to characterize the matrix of the pellicles formed by *A. baumannii* to attempt to evaluate their contribution to the spread of this organism.

To understand how more than 35% of the strains of the non-motile *A. baumannii* may be able to form a pellicle at the air-liquid interface, we compared the hydrophobicity of different strains: non-forming, biofilm-forming and pellicle-forming strains by an extraction method in organic solvent. Pellicle-forming strains were markedly more hydrophobe (35%-60% of bacterial extraction by hexadecane) than the biofilm-forming strains (12-17%) or the non-biofilm strains (3-10%). Then the air-liquid interface provides an opportune niche for the hydrophobic and aerobic *A. baumannii*.

From a screening performed by inverse optical microscopy on different *A. baumannii* strains that were positive for the pellicle formation, we have realized a pre-discrimination among pellicles and considered three main groups of morphology: a) egg-shaped; b) ball-shaped; c) channel-containing pellicles. One strain representative of each group was further analysed by Brewster angle microscopy which confirmed the morphological data obtained by optical microscopy. Atomic force microscopy of both air- and water-facing sides of the pellicle show differences on the exopolysaccharide (EPS) secretion.

Finally, pellicles are multicellular assemblages in matrices composed of diverse extracellular polymeric substances. After the matrix extraction of each morphogroup, sugar analysis was performed by gas-chromatography. It shows that exopolysaccharide of two morphogroups are mainly composed glucose units, whereas the morphogroup “channel” present a high percentage of N-acetylglucosamine. After a cellulase treatment of the extracted matrix samples, several proteins, tightly bound to the EPS, were identified by a proteomic study. Different pilins were identified depending on the morphogroup. They may have a role in keeping the pellicle structure but also in enhancing the bacterial adhesion at the interface.

## **P5 DETERMINATION OF MULTIDRUG-RESISTANT *ACINETOBACTER* SPP. BIOFILM FORMATION BY TWO DIFFERENT SCREENING METHODS**

**F. F. Yılmaz Köz<sup>a</sup>, H. Taşlı<sup>a</sup>, A. Büyük<sup>a</sup>, S. Gül-Yurtsever<sup>b</sup> & M. Hoşgör-Limoncu<sup>a</sup>**

**<sup>a</sup>Ege University Faculty of Pharmacy, Dept. of Pharmaceutical Microbiology, İzmir, Turkey; <sup>b</sup>Clinical Microbiol. Lab. of İzmir Atatürk Training and Research Hospital, İzmir, Turkey**

Multidrug-resistant (MDR) *Acinetobacter* spp. strains are responsible several types of nosocomial infections including pneumoniae, urinary tract infections. Recent studies showed a positive correlation between biofilm formation and antibiotic resistance. Biofilm formation is a significant pathogenic mechanism in device related infections in hospitals. In the present study, we tested biofilm formation in clinical MDR *Acinetobacter* spp. isolates by using two different methods.

Between 2009 and 2010, 84 MDR *Acinetobacter* spp. isolates were collected at the Clinical Microbiology Laboratory of İzmir Atatürk Training and Research Hospital. Biofilm formation was determined qualitatively by using the tube method, and quantitatively by using the microtitre plate method. *Enterococcus faecalis* ATCC 29212 was used as a positive control.

In our study, qualitative and quantitative test results showed different rates of biofilm production capacity. The qualitative test revealed that 38 strains (45.2%) were weak/non biofilm producers, 38 strains (45.2%) were moderate producers, and 8 strains (9.5%) were strong biofilm producers. However, the quantitative test indicated that 34 strains (40.5%) were weak/non biofilm producers, 13 strains (15.5%) were moderate producers, and 37 strains (44%) were strong biofilm producers.

Today, treatment of nosocomial infections caused by biofilm producing pathogens is more difficult. It is known that biofilm formation influences the efficacy of antibiotics and the susceptibility of the bacteria. In this study, although the qualitative and quantitative tests showed different results, it was observed that, generally, MDR strains were biofilm producer organisms.

## **P6 PREDICTION OF *ACINETOBACTER BAUMANNII* VIRULENCE DETERMINANTS BY COMBINING COMPARATIVE GENOMICS AND PHENOTYPIC TESTING**

**F. Imperi<sup>a</sup>, L.C.S. Antunes<sup>a</sup>, A. Carattoli<sup>b</sup> & P. Visca<sup>a</sup>**

**<sup>a</sup>University Roma Tre, Rome, Italy; <sup>b</sup>Istituto Superiore di Sanità, Rome, Italy**

Although *Acinetobacter baumannii* has emerged as a major nosocomial pathogen worldwide, its pathogenicity still remains elusive. With the aim of identifying genetic determinants implicated in virulence, the genomes of multidrug resistant strains AYE and ACICU (belonging to International lineages I and II, respectively) were compared with those of the non-clinical strain SDF and the reference strain ATCC 17978. A number of phenotypic assays were performed to tentatively correlate the presence/absence of putative virulence genes with changes in specific virulence-related phenotypes.

The 50% lethal dose (LD<sub>50</sub>) in the *Galleria mellonella* model of infection was comparable for AYE, ACICU and ATCC 17978, but ca. 100-fold higher for SDF. Thus, the SDF genome was used as reference to identify virulence-related traits acquired by human pathogenic strains. Sixty-two clusters of at least 5 adjacent genes were identified in ACICU, AYE and/or ATCC 17978 that were absent in SDF, the majority of which contains putative virulence-related genes encoding (i) drug resistance and efflux systems; (ii) pili and fimbriae; (iii) hemolysin- and hemagglutinin-related proteins and (iv) functions involved in iron uptake. Phenotypic testing revealed that SDF is unable to produce iron-chelating compounds, in agreement with the absence in its genome of clusters for siderophore synthesis. Accordingly, growth of SDF was strongly impaired under severe iron deficiency. All strains showed comparable hemolytic activity on horse blood, suggesting that the additional hemolysin-related genes exclusively present in clinical strains hardly contribute to this phenotype. The SDF strain was impaired in twitching motility but demonstrated strong ability to form biofilms, indicating that the clusters for pilus assembly missing in the SDF genome may be crucial for motility rather than surface attachment and/or biofilm formation. Notably, SDF also showed reduced metabolic versatility, inability to grow at temperatures > 37°C and poor resistance to desiccation, but these general properties can hardly be associated to any specific gene cluster.

This work provides further insight into the pathogenic potential of *A. baumannii* and opens the way for future studies aimed at deciphering the role of specific virulence-related gene clusters in *A. baumannii* pathogenicity.

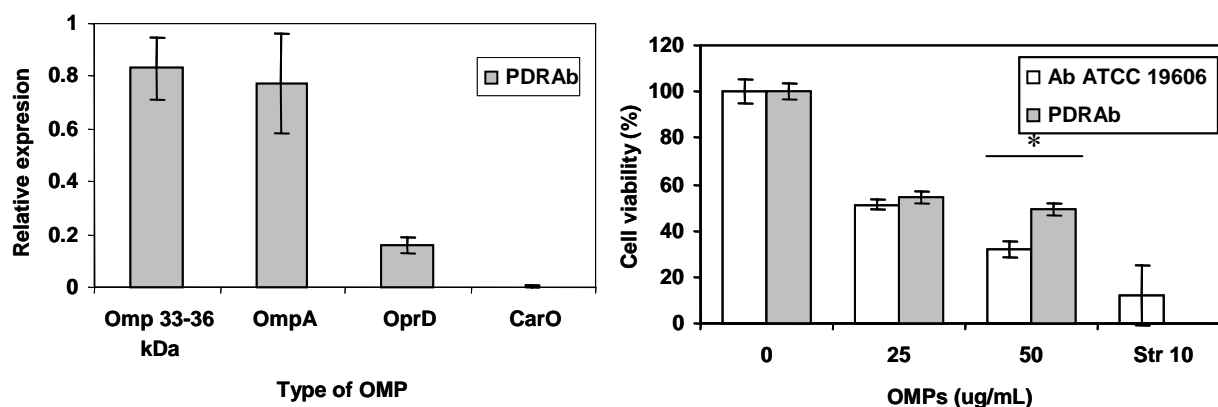
**P7 ATTENUATED VIRULENCE OF A PAN-DRUG RESISTANT CLONE OF ACINETOBACTER BAUMANNII (PDRAb) IS ASSOCIATED WITH DECREASED EXPRESSION OF THE OUTER MEMBRANE PROTEINS (OMPs) CarO AND OprD-LIKE**

Y. Smani<sup>a</sup>, F. Fernández-Cuenca<sup>b</sup>, F. Docobo-Pérez<sup>a</sup>, M.C. Gómez-Sánchez<sup>b</sup>, J. Domínguez-Herrera<sup>a</sup>, F. Caballero<sup>b</sup>, J. Pachón<sup>a</sup> & A. Pascual<sup>b</sup>

<sup>a</sup>Service of Infectious Diseases, Institute of Biomedicine of Sevilla (IBiS), University Hospital Virgen del Rocío/CSIC/University of Sevilla, Seville, Spain; <sup>b</sup>Service of Microbiology, University Hospital Virgen Macarena, Seville, Spain

*In vitro* interaction of OMPs of gram-negative bacteria with host cells has been recently shown. We compared the virulence and expression of OMPs of a PDRAb clone to that of the susceptible reference strain Ab ATCC 19606. A PDRAb isolate representative of an outbreak at the Univ. Hosp. Virgen del Rocío of Sevilla (Spain) was studied. Virulence was determined by a cytotoxicity assay with MTT using crude extracts of OMPs and the A549 cell line. Expression of OMPs was determined by real-time RT-PCR using the 16S rDNA as endogenous control and the  $2^{-\Delta\Delta C_t}$  method. Experiments were performed in triplicate. Means were compared using the Student's t-test.

The OMPs of the PDRAb were less cytotoxic than those of the ATCC 19606 strain, particularly at 50  $\mu\text{g/mL}$  ( $p=0.027$ ). Relative expression of genes coding for OmpA and the Omp 33-36 kDa was not significantly different compared to that of the ATCC strain (expression=1), whereas expression of CarO and OprD-like was significantly reduced ( $p<0.001$ ).



The PDRAb clone is less virulent than susceptible ATCC 19606 strain. Attenuated virulence in this PDRAb is associated with reduced expression of the genes coding for the porins CarO and OprD.

## **P8 STUDYING *ACINETOBACTER BAUMANNII* PATHOGENICITY IN A MOUSE MODEL OF PNEUMONIA BY DETERMINING MORTALITY AND THE LEVEL OF DISSEMINATION IN DIFFERENT ORGANS**

**M. Eveillard<sup>a</sup>, M. Kempf<sup>a</sup>, P. Legras<sup>b</sup> & M-L. Joly-Guillou<sup>a</sup>**

**<sup>a</sup>Groupe d'étude des interactions hôte – pathogène (GEIHP, UPRES EA 3142); <sup>b</sup>Service commun d'animagerie hospitalo-universitaire, UFR Sciences médicales, Université d'Angers, France**

*Acinetobacter baumannii* (AB) can be responsible for various pathologies like ventilated-associated pneumonia or soft tissue infections, but few data exist about the variability of virulence among different clinical strains. We aimed to study this variability by assessing mortality and AB dissemination in different organs with a mouse model of pneumonia.

For the study of mortality, 8 AB strains were used (70.34, CIP-53.77, NM, M, AYE, 107 292, 12, SAN). All experiments were performed with 6-week old female C3H/HeN mice, and according to European regulations. The mice were rendered transiently neutropenic by injecting cyclophosphamid on days 4 (D-4) and 3 (D-3) before intra-tracheal inoculation of AB ( $5.10^6$  CFU/mL) (D0). Each strain was inoculated to 20 mice and mortality was followed-up at D+1, D+2, D+3, D+4, and D+7. The dissemination from lungs (Lu) to different organs was studied for 2 strains (AYE and CIP 53.77). Following inoculation, Lu, kidneys (Ki), liver (Li), brain (Br) and spleen (Sp) were extracted and dissected for 4 mice at D+1, D+2, D+3, and D+4. Lu were extracted from one mouse and dissected at D0 (positive control). A control to evaluate the influence of blood on bacterial counts in organs was performed by infusing mice in deep anaesthesia by 40mL of saline with a circulation entering by the heart and going out through the femoral artery before organ extraction. After weighing, organs were homogenised by grinding and quantitative cultures were performed onto blood agar media.

The mortality varied from 7% (strain 70.34) to 78.9% (strain SAN), (means = 34.8%, median = 30%). All mice died within the 4 days following inoculation. The study of AB dissemination was performed with strains presenting a weak or moderate mortality (13% for CIP 53.77 and 24% for AYE). Whereas the maximal concentration in Lu was obtained at D+2, AB concentrations were similar between D+1 and D+2 (Br and Li) or began to drop from D+1 to D+2 (Sp and Ki). There was not any difference concerning the organ diffusion between the 2 strains tested and the dissemination in the different organs was homogeneous. Indeed, the ratios of mean log concentrations between Lu and other organs within the 4 days following inoculation varied from 2.5 to 4 ([Lu] / [Ki] = 4.08 (AYE) and 3.31 (CIP 53.77), [Lu] / [Br] = 3.70 (AYE) and 3.25 (CIP 53.77), [Lu] / [Sp] = 3.38 (AYE) and 3.46 (CIP 53.77), and [Lu] / [Sp] = 3.39 (AYE) and 2.51 (CIP 53.77). Finally, the evolution of bacterial concentrations in organs was not different if mice underwent infusion or not.

We demonstrated *in vivo* the strong variability of the mortality induced by different AB strains. Our model is compartmentalized (ratios [Lu] / [organs] > 2.5). The preliminary results concerning the dissemination of AB in organs will be completed by observing the compartment of highly-virulent strains and by assessing if the bacterial dissemination is responsible for colonization or infection (dosage of pro-inflammatory mediators in organs). Like mortality, results about dissemination will be compared between strains.

**P9 SECRETION OF VIRULENCE FACTORS VIA *ACINETOBACTER BAUMANNII* OUTER MEMBRANE VESICLES AND THEIR PATHOGENIC POTENTIALS ON HOST CELLS**

**J. S. Jin<sup>a</sup>, S. O. Kwon<sup>b</sup>, D. C. Moon<sup>a</sup>, M. Grunga<sup>a</sup>, J. H. Lee<sup>a</sup>, S. I. Kim<sup>b</sup> & J. C. Lee<sup>a</sup>**

**<sup>a</sup>Kyungpook National University School of Medicine, Daegu, Korea; <sup>b</sup>Korea Basic Science Institute, Daejeon, Korea**

Gram-negative bacteria secreted outer membrane vesicles (OMVs). *Acinetobacter baumannii* outer membrane protein A (AbOmpA) is a potential virulence factor to induce host cell death, but its secretion and subsequent delivery into host cells have not been determined.

In this study, we investigated the secretion of virulence factors via OMVs, vesicle entry into host cells and subsequent cellular damage. *A. baumannii* secreted OMVs during both *in vitro* culture and *in vivo* infection, and the OMVs secreted from bacteria were interacted with adjacent host cells in mouse pneumonia model.

The potential virulence factors, including AbOmpA, AmpC  $\beta$ -lactamase and putative hemolysin, were found in the *A. baumannii* OMVs. The OMVs entered host cells as early as 30 min via a cholesterol-rich membrane microdomain (lipid raft). The OMVs from *A. baumannii* 19606<sup>T</sup> induced host cell death in a dose-dependent manner, whereas the OMVs from its isogenic  $\Delta$ AbOmpA did not induce cell death at all. The N-terminus of AbOmpA was directly responsible for host cell death via mitochondrial targeting.

In conclusion, the OMVs are an important vehicle to deliver virulence factors of *A. baumannii* to host cells and contribute to *A. baumannii* pathogenesis in infected hosts.

**P10 ACINETOBACTER BAUMANNII OUTER MEMBRANE PROTEIN A INDUCES DENDRITIC CELL DEATH THROUGH MITOCHONDRIAL TARGETING**

**J. S. Lee, C. H. Choi, J. W. Kim & J. C. Lee**

**Kyungpook National University School of Medicine, Daegu, Korea**

*Acinetobacter baumannii* outer membrane protein A (AbOmpA) is a potential virulence factor that induces epithelial cell death, but its pathologic effects on the immune system have yet to be determined.

The present study investigated the pathologic events occurring in dendritic cells (DCs) exposed to cytotoxic concentrations of AbOmpA. Murine bone marrow-derived DCs were treated with AbOmpA. Mitochondrial targeting of AbOmpA and subsequent pathological events, including the production of reactive oxygen species (ROS), caspase activation and DC death were determined. AbOmpA induced early-onset apoptosis and delayed-onset necrosis in DCs. AbOmpA targeted the mitochondria and induced the production of ROS. ROS were directly responsible for both apoptosis and necrosis of AbOmpA-treated DCs.

These results demonstrate that the AbOmpA secreted from *A. baumannii* induces DC death, which may impair T cell biology to induce adaptive immune responses against *A. baumannii*.

**P11 MACROPHAGE-MEDIATED HOST DEFENSE AGAINST INTRANASAL ACINETOBACTER BAUMANNII INFECTION**

**Hongyu Qiu, Rhonda Kuo Lee & Wangxue Chen**

**Institute for Biological Sciences, National Research Council Canada, 100 Sussex Drive, Ottawa, Ontario K1A 0R6, Canada**

*Acinetobacter baumannii* (AB) is a major cause of both community-associated and nosocomial pneumonia. Although previous studies have shown that innate immunity (TLR-4 signaling, neutrophils and ROS) is crucial in the host defense against respiratory AB infection, the role of other innate immune components remains to be defined. Here, we studied the *in vitro* and *in vivo* role of macrophages in host resistance to AB infection.

We found that mouse macrophages were able to phagocytose (within 10 min) and efficiently kill AB *in vitro*. Moreover, pharmaceutical disruption of the cellular actin microfilament or microtubule inhibited the rate of AB phagocytosis by macrophages. The bactericidal ability of mouse macrophages was significantly suppressed by treatment with iNOS inhibitors, but not with NADPH oxidase inhibitors. Infection of mouse macrophages also led to the production of several key proinflammatory cytokines and chemokines. More importantly, *in vivo* depletion of pulmonary macrophages in mice significantly enhanced their susceptibility to respiratory AB challenge.

These results indicate that macrophages efficiently uptake and kill AB through an NADPH oxidase independent pathway and play important roles in host defense against the infection by secreting proinflammatory cytokines and chemokines.

## **P12 INSIGHTS INTO GENE AMPLIFICATION IN *ACINETOBACTER BAYLYI*: INSERTION SEQUENCE-MEDIATED GENOMIC REARRANGEMENTS**

**Laura E. Cuff, Sarah H. Craven, Kathryn T. Elliott & Ellen L. Neidle**

**Department of Microbiology, University of Georgia, Athens, GA, USA**

A system for studying chromosomal gene amplification was previously developed in the soil bacterium *Acinetobacter baylyi* ADP1. Gene amplification has many important consequences, including drug resistance, increased virulence, adaptation, and evolution. Despite the frequent occurrence of gene amplification in all organisms, this phenomenon remains poorly understood since it is difficult to establish good experimental systems. Initial studies in *A. baylyi* exploited the genetic malleability of ADP1 to characterize many independently occurring duplication events. However, the original methodology only allowed study of one region of the chromosome, representing approx. 10% of the genome. This study expanded the experimental system to investigate rearrangements that occur in different chromosomal regions.

In the original and modified experimental systems, amplification mutants are readily selected from a parent strain that fails to grow on benzoate due to the absence of two transcriptional activators. Mutants will grow on benzoate if gene amplification compensates for loss of the transcriptional activators. Growth depends on increased dosage of weakly expressed *cat*-genes to enhance the consumption of catechol, a metabolite of benzoate. The amplification process is initiated by recombination events that occur between chromosomal DNA on either side of a cluster of *cat* genes to create a duplicated DNA segment. Therefore, the recombination events that can be isolated from selected mutants must be in relatively close proximity to the *cat*-gene cluster. To expand the system, we used metabolic engineering to move the entire 10-kbp *cat* cluster as a genetic cassette to other genomic positions after deletion from the native site. With this cassette, gene amplification mutants are selected on solid medium by demanding growth on benzoate. Following the isolation of independent mutants, a relatively simple transformation assay is used to identify DNA sequences involved in the underlying genomic rearrangement. These DNA sequences reveal the types of recombination events that are involved in gene duplications.

We demonstrated the success of this approach for systematic investigations of recombination events throughout the genome. After the cassette was moved to five new locations in the genome, individual duplication events were characterized in which DNA sequences on either side of the cassette had recombined. In some regions of the chromosome, duplications frequently involved one of the inverted repeats of IS1236, an IS that resides in six copies in the ADP1 genome. This IS, an apparent member of the IS3 family, is predicted to transpose via a copy-out, paste-in type of excision mechanism. However, sequence analysis of rearrangements in three different regions of the chromosome indicated that IS1236 can generate duplications without excision of the IS from the chromosome. One end remained anchored in its native locus, while the other end was joined to distant DNA. The apparently frequent involvement of IS1236 in generating duplications without complete excision was surprising. While there are a few reports of one-ended genetic rearrangements mediated by IS, this type of mechanism has not been widely cited or completely characterized. Ongoing studies with ADP1 will reveal the full spectrum of mechanisms underlying gene amplification throughout the genome.

### **P13 DISSEMINATION OF INTEGRONS CARRYING ANTIBIOTIC RESISTANCE GENES BY NATURAL TRANSFORMATION**

**S. Domingues<sup>a</sup>, K. M. Nielsen<sup>b,c</sup> & G. J. da Silva<sup>a</sup>**

**<sup>a</sup>Centre of Pharmaceutical Sciences, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; <sup>b</sup>Department of Pharmacy, Faculty of Medicine, University of Tromsø, Tromsø, Norway; <sup>c</sup>Genøk-Centre for Biosafety, Norway**

Identical class 1 integrons, genetic units associated with multiple antibiotic resistance genes, have been found in the chromosome of unrelated bacteria, suggesting the presence of mechanisms facilitating horizontal transfer of these elements beyond plasmid conjugation or phages. The potential role of natural transformation in integron recombination and dissemination remains to be determined. This study seek to test the hypothesis that natural transformation can facilitate horizontal transfer of integrons, and to identify and describe the DNA sequences involved in the recombination events.

The natural competent *Acinetobacter baylyi* BD413 was used as recipient bacterium and integron-carrying bacteria *Acinetobacter baumannii* 65FFC and 064, *Pseudomonas aeruginosa* SM, *Salmonella rissen* 486 and *Salmonella typhimurium* 490 were the donors in transformation assays. Integron transfer were screened by antimicrobial susceptibility profiles and confirmed by PCR with specific primers for classic class 1 integrons. Some transformants that acquired an integron were also used as donor cells in further experiments. In order to identify the completed transferred DNA, outward primers were designed and integron flanking regions were determined by primer walking.

Transformation of strain BD413 with DNA of wild-type donor bacteria resulted in a few transformants with integron (from *A. baumannii* 064 and *S. typhimurium* 490); subsequent transformation of strain BD413 with these integron-carrying transformants produced much higher numbers of transformants with integrons. The increase in transformation frequency suggested that the acquired integron was incorporated in the genome of the recipient. Sequencing of integron flanking regions showed that it was not only the integron that was transferred, but also some adjacent DNA. Preliminary sequencing results show that the transferred fragments can be longer than 17 Kb. The integrons were found incorporated in the BD413 chromosome. As a part of an ongoing sequencing effort, junction sequences between the transferred DNA and the *A. baylyi* genome have been determined for 3 transformants obtained with the species-divergent donor bacteria and in 6 of the subsequent transformants obtained with DNA isolated from the initial transformants. The sequencing of the inserted DNA in the 3 transformants obtained by interspecies gene transfer showed that the acquired DNA had integrated in different regions of the *A. baylyi* genome.

Our study shows for the first time that integrons can be transferred among bacteria genomes by natural transformation. Moreover, the different genetic location of the acquired integrons results in the generation of genome variability, which contributes to the evolution of genomes through the time.

**P14 DISPERSION AND FUNCTIONAL CHARACTERIZATION OF THE UNCOMMON ARRANGEMENT OF CLASS 2 INTEGRON, IN2-8, IN ACINETOBACTER BAUMANNII CLINICAL ISOLATES**

**María Soledad Ramírez<sup>a</sup>, María Paula Quiroga<sup>a</sup>, Helia Bello<sup>b</sup>, Carolina Márquez<sup>c</sup> & Daniela Centrón<sup>a</sup>**

**<sup>a</sup>Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina UBA, CABA, Argentina; <sup>b</sup>Facultad de Ciencias Biológicas, Departamento de Microbiología, Laboratorio de Antibióticos, Universidad de Concepción, Concepción, Chile; <sup>c</sup>Cátedra de Microbiología, Instituto de Química Biológica, Facultad de Ciencias- UDELAR, Montevideo, Uruguay**

*Acinetobacter baumannii* (Ab) is considered an important pathogen in our hospital environment, and is well known that this species has the capacity to acquire different mechanisms of antibiotic resistance. Previous studies of our laboratory had exposed the high dispersion of class 2 integron in this species, also describing the presence of the integron called In2-8, in non-epidemiologically-related Ab multiresistant clinical isolates from Argentina. This integron contains an unusual class 2 integron array consisting in *sat2-aadB-catB2(attI2)-dfrA1-sat2-aadA1-orfX-ybfA-ybfB-ybgA* with the particularity that downstream the stop codon of the *catB2* gene, the expected *attC* site has been replaced by a region with 100% identity to the *attI2* class 2 integron recombination site. This feature converts *catB2* cassette in an unusual cassette, indicated as *attC-catB2-(attI2)*.

In the present study, we analyze the dispersion of the of this array among 30 *intI2* positive Ab clinical isolates from Argentina, Chile and Uruguay and also define, from a functional perspective, which cassettes and/or unusual cassettes of the class 2 integron array In2-8 could be mobilized by the type 1 integrase.

The In2-8 array was found in 6 isolates (20%), all of them corresponding to Chilean isolates belonging to different clones. This result demonstrates an unusual high distribution of In2-8 array among different clones of Ab from Chile, suggesting a particular behavior of these elements at a geographical level.

Different plasmids containing the cassettes and/or the unusual cassettes found in the In2-8 array were constructed and used as substrates, with the plasmid harboring the type 1 integrase (pLQ369), in the *in vivo* recombination assays. The four gene cassettes found in In2-8, *attI2-dfrA1-attC*, *attC-aadB-attC*, *attC-aadA1-attC*, and *attI2-sat2-attC* were excised in our system. We also found that the unusual cassette, *attC-catB2-(attI2)*, was a good substrate for IntI1 recombinase. When we examined the precise site of the recombination event occurred with the unusual cassette, we noticed that at least IntI1 could mediate the two different cuts by recognizing two different sites/sequences in the called *attI2*, showing the plasticity of the integrase to mediate excision.

On one hand, this works evidences the unusual high distribution of the In2-8 array among Ab isolates from Chile, and on the other hand shows that all the cassettes and unusual cassettes localized in its variable region could be recognized and excided by the IntI1 integrase.

**P15 DISSEMINATION OF CLASS 1 AND 2 INTEGRONS IN MULTIDRUG-RESISTANT CLONES OF *ACINETOBACTER BAUMANNII***

**María Soledad Ramírez<sup>a</sup>, María Silvana Stietz<sup>a</sup>, Paola Jeric<sup>a</sup>, Adriana S. Limansky<sup>b</sup>, Mariana Catalano<sup>a</sup> & Daniela Centrón<sup>a</sup>**

**<sup>a</sup>Departamento de Microbiología, Parasitología e Inmunología, Facultad de Medicina, Universidad de Buenos Aires, Argentina; <sup>b</sup>Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET), Departamento de Microbiología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina**

Ab is considered a serious threat in the hospital environment over the world. Furthermore, some strains of Ab have become resistant to almost all currently available antibacterial agents through multifactorial combinatory antimicrobial resistance mechanisms. Class 1 and 2 integrons are both usually associated with antimicrobial resistance gene cassettes in clinical samples. Prior reports from our laboratory revealed a particular dispersion of class 2 integrons in Ab comparing to the dispersion of class 1 integrons in this species. All the clinical isolates used in the previous study were from Buenos Aires City.

In order to contribute to the epidemiology and clinical impact of class 1 and 2 integrons in Ab isolates, we performed an integron survey analyzing 20 isolates of non-epidemiologically related clinical samples from Rosario city collected during 2005-2009. All isolates were multidrug resistant being resistant to at least 4 different antibiotic family.

The *intI1* gene was recovered in 11 isolates and the *intI2* gene was found in 14 Ab isolates. Both integrons were present in 7 isolates. This data was different to the epidemiology previously observed in Ab isolates from Buenos Aires, where we did not find many isolates harboring both genes. PFGE analysis identified 4 different clones (I, III, VII, IX), showing a policlonal dispersion of integrons. Clone III was the predominant clone, which is another distinctive feature from the Buenos Aires isolates where clones IV and I are more widespread. Furthermore, with the aim of seeing if the ATPase gene was disrupted by the well known AbaR1-like resistant island, which is associated to class 1 integrons, we used the AbaR1-based PCR mapping described by Shaikh *et al*, in all *intI1* positive isolates. We obtained positive results for the 3' and the 5' junction in all of them (n=11) suggesting the presence of potential genomic islands in our isolates.

In conclusion, we found a high dispersion of class 1 and 2 integrons in this specie, showing that several isolates (7) harbored the two types of integrase genes. The obtained results show a distinctive epidemiology in Rosario's isolates comparing to our previous reports. We also found a policlonal dispersion of integrons and the presence of AbR1-like elements suggesting a possible localization of the class 1 integrons. The difference observed to the previous integron survey reveals the necessity of a continuous molecular surveillance in each region. As almost all isolates from our study harbored integrons which are usually associated to horizontal gene transfer, a molecular surveillance of integrons and resistance genes involved in multidrug resistance should be considered an important target for infections in this species.

## **P16 CHARACTERIZATION OF *ACINETOBACTER BAUMANNII* A118: COMPARATIVE GENOMICS USING OPTICAL MAPS**

**M. S. Ramirez<sup>a,b</sup>, D. Centrón<sup>a</sup> & M. E. Tolmasky<sup>b</sup>**

**<sup>a</sup>University of Buenos Aires, Buenos Aires, Argentina; <sup>b</sup>California State University Fullerton, Fullerton, CA, USA**

*Acinetobacter baumannii* is an emerging opportunistic human pathogen responsible for a growing number of community and nosocomial infections including bacteremia, urinary tract infection, wound infection, meningitis, and pneumonia. The high incidence of *A. baumannii* infections may be due to a combination of factors such as its ability to survive for a prolonged length of time in different environments and a rise in the number of susceptible individuals as a result of advancements in medical support of critically ill patients. The multiresistance nature of most *A. baumannii* strains makes them difficult to manipulate for genetic studies. The clinical *A. baumannii* A118 isolate is rather exceptional for its susceptibility to several antibiotics. This characteristic makes it an ideal model for genetic manipulations. We characterized this strain by determining its competence, ability to support stable replication of the most used plasmid replicons, and by comparative genomics using optical mapping.

Antibiotic susceptibility was assessed by the agar dilution method. Plasmid stability was determined by successive subculturing followed by determination of plasmid content. Uptake of oligonucleotides was tested using laser scanning confocal microscopy. Optical mapping was carried out at OpGen Technologies, Inc. Data analysis was performed using the MapSolver software 2.1.1. When necessary confirmation of results was carried out by PCR amplification using primers designed based on known *A. baumannii* genomes.

*A. baumannii* A118 is susceptible to ceftazidime, cefepime, piperacillin, minocycline, amikacin, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin. Untreated cells could be transformed using pJHCMW1, pMET1, pAADA1KN, pAADB, and pVK102 DNA. At least four of them were stably maintained after 40 generations. A fluorophore-labeled 10-mer phosphorothioate oligodeoxynucleotide analog was readily taken up by a low percentage of cells from an *A. baumannii* A118 culture. The predicted size of the *A. baumannii* A118 chromosome is 3.841.807 bp. Therefore the genome is smaller than those of AB0057, AYE, ACICU and ATCC 17978 and larger than those of strains SDF and AB307-0294. A comparison of the *A. baumannii* A118 optical map with those of the strains AYE, AB307-0294, AB0057, ACICU, ATCC 17978 and SDF identified insertions, deletions, inversions, and other genetic modifications.

The property of *A. baumannii* A118 to take up DNA and support stable inheritance of numerous replicons, together with its susceptible phenotype make this strain a useful model for genetic manipulations. Accordingly, utilization of strain A118 as model for genetic studies of molecular mechanisms of *A. baumannii* virulence, requires a better understanding of the particular characteristics of this strain. The information acquired by genomic analysis of its optical map will facilitate interpretation of experimental results using *A. baumannii* A118 as model.

## **P17 AMINOGLYCOSIDE RESISTANCE GENES IN *ACINETOBACTER BAUMANNII* FROM AUSTRALIAN HOSPITALS**

**S. J. Nigro, V. Post & R. M. Hall**

**University of Sydney, Australia**

A set of 47 multiply antibiotic resistant *Acinetobacter baumannii* strains isolated in 6 different Australian hospitals over the period 2000-2010 were studied. These isolates were all resistant to sulphonamides, third generation cephalosporins, carbapenems (imipenem and meropenem) and fluoroquinolones. The isolates were shown to belong to the European clonal lineage II (EC II) using allele-specific PCR and this was confirmed by determining the sequence of the *recA* gene and of the intrinsic *A. baumannii* oxacillinase gene, *oxa-Ab*, from representative isolates. In addition, these isolates all carry a set of shared antibiotic resistance genes, *bla*TEM, *tet*(B) *strAB* and *sul2* and *oxa-23* and they all have an ISAb1 upstream of the chromosomal *ampC* gene. Based on these features, they appear to represent a specific sub-line of the EC II clonal complex.

Some isolates, coming from 2 of the hospitals, were susceptible to all of the aminoglycosides tested, but others, coming from four hospitals, were additionally resistant to specific combinations of the aminoglycosides amikacin, gentamicin, tobramycin, kanamycin and neomycin. The isolates were screened for the presence of several aminoglycoside resistance genes. Thirteen isolates from four hospitals were resistant to gentamicin and tobramycin, did not contain a class 1 integron, and carried the *aadB* gene cassette. The *aadB* cassette was found to be integrated at a secondary site on a small plasmid closely related or identical to a plasmid pRAY that was previously reported in *A. baumannii* isolates from South Africa.

A search of a broader set of *A. baumannii* isolates revealed that pRAY is also present in one of the early isolates from the Netherlands that are used as type strains for the EC I clonal cluster. Hence, pRAY has been contributing to gentamicin and tobramycin resistance in *A. baumannii* since the early 1980s and has become globally distributed.

**P18 A 63–KB GENOMIC RESISTANCE ISLAND FOUND IN A MULTIDRUG–RESISTANT *ACINETOBACTER BAUMANNII* ISOLATE OF EUROPEAN CLONE I FROM 1977**

**Lenka Krizova & Alexandr Nemeč**

**Laboratory of Bacterial Genetics, National Institute of Public Health, Prague, Czech Republic**

Multidrug-resistant *Acinetobacter* strain HK302 was isolated from an outbreak of nosocomial infections in Switzerland in 1977. The aim of the present study was to assess whether this archive strain belongs to one of the known international clonal lineages of *Acinetobacter baumannii* and whether it harbours a genomic structure related to the AbaR1-like resistance islands.

Multilocus sequence typing (MLST) and *Hind*III-ribotyping were used to determine the taxonomic position of HK302 at the species and subspecies (clonal) levels. The position and structure of the putative resistance island were investigated by AbaR1-based PCR mapping followed by restriction analysis and partial sequencing of amplicons. *A. baumannii* AYE harbouring AbaR1 was used as a positive control for PCR mapping.

The MLST allelic profile (1-1-1-1-5-1-1) and *Hind*III ribotype of HK302 were typical of *A. baumannii* European (EU) clone I. In addition, an AbaR1-related region inserted into the ATPase gene at the same position as AbaR1 was found in HK302. PCR mapping and partial sequencing revealed that this region is structurally congruent with AbaR3, a 63.4 kb island described in an *A. baumannii* isolate from 2004.

*A. baumannii* HK302 belongs to EU clone I and harbours a large genomic island related to resistance islands (AbaR1, AbaR3 and AbaR5) described in EU I clone strains. Our findings suggest that variants of these sophisticated genomic structures existed in *A. baumannii* already in the late 1970s.

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**P19 INVESTIGATION OF THE PREVALENCE AND ROLE OF MOBILE GENETIC ELEMENTS ASSOCIATED WITH AN AMINOGLYCOSIDE RESISTANCE GENE, *aacC2a*, IN *ACINETOBACTER BAUMANNII***

**T. I. Jongwe & H. Segal**

**University of Cape Town, Cape Town, South Africa**

Mobile genetic elements contribute significantly to antibiotic resistance in *A. baumannii* isolates worldwide. In our local hospitals, the genetic arrangement IS1133<sub>L</sub>-IS*Aba-1*-IS1133<sub>R</sub>-*aacC2a* has been characterised in strains of *A. baumannii* spanning more than 23 years.

Sequence analysis of a 1 586 bp fragment from an *EcoRI-HindIII* genomic DNA library of *A. baumannii* strain MOS1 identified a portion of IS*Kpn12* upstream of IS1133<sub>L</sub>. Using primers complementary to IS*Kpn12* sequence in a PCR assay, a full copy of IS*Kpn12* was detected in *A. baumannii* MOS1. Southern hybridisation studies showed that MOS1 contains a single copy of IS*Kpn12*. A reverse transcriptase PCR assay indicated that the *tnpA* of IS*Kpn12* is expressed during both stationary and logarithmic growth phases in *A. baumannii*. Interestingly, all *A. baumannii* isolates identified as carrying a copy of IS*Kpn12* also contain the IS1133<sub>L</sub>-IS*Aba-1*-IS1133<sub>R</sub>-*aacC2a* conserved genetic arrangement, whilst strains that do not carry this conserved region do not contain IS*Kpn12*. The IS*Kpn12* element, as seen with IS*Aba-1*, was not present in any other nosocomial isolates screened, including representative isolates of *E. coli*, *P. aeruginosa*, and *K. pneumoniae*. These findings suggest that IS*Kpn12* and IS*Aba-1* are part of the conserved genetic arrangement that has been identified in *A. baumannii* strains since 1983 and may be confined to this species in our setting.

The association of IS*Aba-1* and IS*Kpn12* with this genetic arrangement suggests a role for these elements in *A. baumannii*. The role of IS*Aba-1* in transcriptional regulation of associated antibiotic resistance genes including *bla*<sub>OXA-23</sub> and *sulIII* is well established. It may be that IS*Aba-1* provides promoters for expression of *aacC2* in this genetic arrangement. Furthermore, that the *tnpA* of IS*Aba-1* is expressed in both stationary and logarithmic growth phases of carbapenem susceptible and carbapenem resistant isolates suggests that the element is mobile and is therefore able to transpose upstream of newly acquired resistance genes to regulate transcription of these genes.

**P20 THE GENOME OF THE HYDROCARBON-DEGRADING BACTERIUM  
*ACINETOBACTER VENETIANUS* VE-C3**

**M. Fondi<sup>a</sup>, G. Emiliani<sup>b</sup>, E. Rizzi<sup>c</sup>, G. Corti<sup>c</sup>, M.C. Papaleo<sup>a</sup>, E. Perrin<sup>a</sup>, I. Maida<sup>a</sup>, F. Baldi<sup>d</sup>, M. Vaneechoutte<sup>e</sup>, L. Dijkshoorn<sup>f</sup>, G. De Bellis<sup>c</sup> & R. Fani<sup>a</sup>**

<sup>a</sup>Laboratory of Microbial and Molecular Evolution, Dept, of Evolutionary Biology, Via Romana 17-19, University of Florence, I-50125 Florence, Italy; <sup>b</sup>Tree and Timber Institute, National Research Council, Via Biasi 75, 38010 San Michele all'Adige, Trento, Italy; <sup>c</sup> Istituto di Tecnologie Biomediche, Consiglio Nazionale delle Ricerche (ITB-CNR), Segrate (MI), Italy; <sup>d</sup>Department of Environmental Sciences, Cà Foscari University, Calle Larga S. Marta, Dorsoduro 2137, 30121 Venice, Italy; <sup>e</sup>Laboratory Bacteriology Research, Faculty Medicine & Health Sciences, University of Ghent, Belgium; <sup>f</sup>Department of Infectious Diseases, Leiden University Medical Center, PO Box 9600, 2300 RC, Leiden, The Netherlands

Some strains of the genus *Acinetobacter* were demonstrated to be able to degrade alkanes of various chain lengths and were shown to possess genomic distinctness from the other strains belonging to the genus. Accordingly, these strains were designated as members of the species *Acinetobacter venetianus*. In particular, one of them, isolated from a bacterial oil-degrading consortium from the Venice lagoon (Italy), namely *A. venetianus* VE-C3, was shown to be able to grow on C10, C14 and C20. Moreover fragmentation of diesel fuel at the cell wall was observed, thus suggesting strong bioemulsifying activity by *A. venetianus* VE-C3. These physiological properties led us to choose this strain as a good candidate for the determination of the complete genome sequence in order to gain more insights on the molecular mechanisms responsible for the degradation of n-alkanes and on the genetic features of a representative of the species *A. venetianus*.

Preliminary analysis of genome assembly allowed us to identify putative orthologous and xenologous regions of *A. venetianus* VE-C3 compared to the available *Acinetobacter* species genomes. In particular, this analysis pointed out a strong genomic distinctness from the strains belonging to other species of this genus. Moreover, gene prediction resulted in the identification of about 3600 putative genes. The predicted genes were clustered in COG categories allowing the identification of putative niche-adaptation specific gene sets.

In addition to its interest for comparative genomics with closely related bacteria this affords an opportunity for analysis and manipulation of genes and gene products, and paves the way for a future analysis of metabolic transformations in the environment.

**P21 TAXONOMY OF THE *ACINETOBACTER CALCOACETICUS* –  
*ACINETOBACTER BAUMANNII* COMPLEX WITH THE PROPOSALS OF  
*ACINETOBACTER PITTII* SP. NOV. (FORMERLY GENOMIC SPECIES 3) AND  
*ACINETOBACTER NOSOCOMIALIS* SP. NOV. (FORMELY GENOMIC SPECIES 13TU)**

**A. Nemeč<sup>a</sup>, L. Krizova<sup>a</sup>, M. Maixnerova<sup>a</sup>, T. J. K. van den Reijden<sup>b</sup>, P. Deschaght<sup>c</sup>, V. Passet<sup>d</sup>, M. Vaneechoutte<sup>c</sup>, S. Brisse<sup>d</sup> & L. Dijkshoorn<sup>b</sup>**

**<sup>a</sup>National Institute of Public Health, Prague, Czech Republic; <sup>b</sup>Leiden University Medical Center, Leiden, The Netherlands; <sup>c</sup>University of Ghent, Ghent, Belgium; <sup>d</sup>Institut Pasteur, Paris, France**

The *A. calcoaceticus*-*A. baumannii* (ACB) complex includes *A. calcoaceticus*, *A. baumannii*, and four genomic species with provisional designations, i.e. gen. sp. 3, gen. sp. 13TU, gen. sp. ‘close to 13TU’ and gen. sp. ‘between 1 and 3’. Studies have shown that gen. sp. 3 and 13TU are medically important groups commonly isolated from hospitalized patients. The present study investigated intra- and inter-species diversity of the ACB complex with the aim to propose formal species names for gen. sp. 3 and 13TU. 78 strains of the ACB complex were studied, which were allocated to *A. baumannii* (n=25), *A. calcoaceticus* (n=11), gen. sp. 3 (n=18), gen. sp. 13TU (n=20), gen. sp. ‘close to 3TU’ (n=2) or gen. sp. ‘between 1 and 3’ (n=2). Strains were selected to cover the breadth of the known intra-species diversity and were investigated by AFLP, amplified rDNA restriction analysis and by 45 nutritional or physiological tests. Subsets were studied by 16S rRNA gene (n=21) and *rpoB* sequence analyses (n=47), multilocus sequence analysis (MLSA) (n=39) or had been classified previously by DNA–DNA reassociation (n=35). 190 strains representing other *Acinetobacter* species were also included for comparative analysis.

Using AFLP, the species of the ACB complex formed well-separated clusters at the species cut-off level of 50 % similarity. The results of 16S rDNA and *rpoB* analyses, and MLSA supported the monophyly and genomic distinctness of the ACB complex and each of its individual species. The only exception was *A. baumannii* which clustered separately from the other ACB species using 16S rRNA sequence analysis. Phenotypic analysis corroborated both the metabolic versatility of the ACB complex and its phenotypic distinctness from the other *Acinetobacter* species. Although no phenotypic features were identified that could unambiguously differentiate between the ACB complex species, uneven distribution of some properties or their combinations among the species was found. While the growth at 44°C and inability to assimilate malonate, L-tartrate and citraconate were distinguishing features for most gen. sp. 13TU strains, the vast majority of the gen. sp. 3 strains could be separated from the other species by the ability to assimilate L-tartrate and to grow at 41°C but not at 44°C.

Considering the genomic distinctness and medical relevance of gen. sp. 3 and 13TU, the names *Acinetobacter pittii* sp. nov. and *Acinetobacter nosocomialis* sp. nov. are proposed for these genomic species, respectively. The type strain of *A. pittii* sp. nov. is LMG 1035<sup>T</sup> (= ATCC 19004<sup>T</sup>) and that of *A. nosocomialis* sp. nov. is LMG 10619<sup>T</sup> (= CCM 7791<sup>T</sup>).

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## **P22 DEVELOPMENT OF A PCR-BASED METHOD FOR THE *ACINETOBACTER* GENUS AND *A. BAUMANNII* IDENTIFICATION**

**Shiao-Ee Chan<sup>a</sup>, Asma Ismail<sup>a</sup> & Kirnpal-Kaur Banga Singh<sup>b</sup>**

**<sup>a</sup>Institute for Research in Molecular Medicine, <sup>b</sup>Department of Medical Microbiology and Parasitology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, Kelantan, Malaysia**

*Acinetobacter baumannii* has emerged as an important opportunistic pathogen in nosocomial infections in hospitals all over the world. A rapid and accurate identification of this pathogen is required in diagnostic microbiology laboratories due to its importance in ensuring a proper clinical treatment and patients' management. The objective of this study was to design specific primers for the detection of *Acinetobacter* genus and *A. baumannii*, and to evaluate the specificity and sensitivity of the designed primers.

A specific and antigenic outer membrane protein (omp) had been identified in a previous study and was identified through N-terminal amino acid sequencing. The obtained amino acid sequence was confirmed with MALDI-ToF analysis. Its complete nucleic acid sequence was obtained by translating the identified amino acid sequence. Conventional PCR assay targeting two genes was developed. Specific primers were designed for 16S rRNA and *omp* genes to identify the *Acinetobacter* genus and *A. baumannii* respectively. Specificity and sensitivity of the assay for both genes was evaluated with known reference strains and clinical isolates. The obtained PCR results were compared with conventional identification methods.

In total, 12 different reference strains of *Acinetobacter* spp. were tested against the 16S rRNA and *omp* genes, indicating their analytical specificity was 100% respectively. Subsequently, the specificity and sensitivity of the assay was evaluated using 41 known other bacteria and 116 *Acinetobacter* spp. clinical isolates. The specificity using known negative strains was 100% for both genes. All the *Acinetobacter* spp. isolates (116/116) were positive for 16S rRNA gene, while the assay only revealed 115 isolates (115/116) as *A. baumannii* by amplifying the *omp* gene. The PCR results for each targeted gene were comparable with conventional methods, showing 100% sensitivity.

These specific primers are important in developing a multiplex PCR which able to enhance the efficiency and speed of *Acinetobacter* genus and *A. baumannii* identification.

**P23 EMERGENCE OF OXA-CARBAPENEMASE- AND *armA*-PRODUCING ACINETOBACTER BAUMANNII ISOLATES OF CLONAL COMPLEX CC92/EUROPEAN CLONE II IN NORWAY**

**Nabil Karah<sup>a,b</sup>, Bjørg Haldorsen<sup>a</sup>, Nils O. Hermansen<sup>c</sup>, Yngvar Tveten<sup>d</sup>, Eivind Ragnhildstveit<sup>e</sup>, Dag H. Skutlaberg<sup>f</sup>, Ståle Tofteland<sup>g</sup>, Arnfinn Sundsfjord<sup>a,b</sup> & Ørjan Samuelsen<sup>a</sup>**

<sup>a</sup>Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway; <sup>b</sup>Research Group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway; <sup>c</sup>Department of Microbiology, Ullevaal University Hospital, Oslo, Norway; <sup>d</sup>Department of Microbiology, Vestfold Hospital, Tønsberg, Norway; <sup>e</sup>Department of Microbiology, Østfold Hospital, Fredrikstad, Norway; <sup>f</sup>Department of Microbiology, Haukeland University Hospital, Bergen, Norway; <sup>g</sup>Department of Microbiology, Sørlandet Hospital, Kristiansand, Norway

The aim of this project was to characterise eleven carbapenem-resistant isolates of *A. baumannii* submitted to the Reference Centre for Detection of Antimicrobial Resistance in Norway. The isolates were collected between 2004-09 from various clinical specimens by six different laboratories. Isolates were typed by MLST and epidemic clonal lineage multiplex PCRs. Susceptibility testing was performed by Etest. PCR assays and sequencing were performed to detect the occurrence of OXA-carbapenemase genes, 16S rRNA methylase genes, *ISAbal*, *ISAbal2*, *ISAbal3*, *intI1* and integron associated gene cassettes. The full-length sequences of *bla*<sub>OXA-51-like</sub> genes, class 1 integron gene cassettes and mutations in the quinolone resistance determining regions (QRDRs) were investigated by sequencing.

Epidemiological data showed that all isolates were associated with hospitalization abroad; Greece, Thailand (*n*=2), China, India (*n*=3), Cyprus, Italy and Pakistan (*n*=2). Seven isolates belonged to clonal complex CC92, contained the *bla*<sub>OXA-66</sub> variant and belonged to European clone II. The remaining four isolates contained the *bla*<sub>OXA-69</sub> (*n*=3) and *bla*<sub>OXA-51</sub> variants with one of them belonging to European clone I. All eleven isolates expressed multidrug resistance (MDR), only consistently susceptible to colistin. Carbapenem resistance was explained by the presence of OXA-carbapenemases; *bla*<sub>OXA-23-like</sub> (*n*=9), *bla*<sub>OXA-58-like</sub> (*n*=1) and *bla*<sub>OXA-24-like</sub> (*n*=1). *ISAbal1* was detected upstream of *bla*<sub>OXA-23-like</sub> genes in all isolates and downstream in five isolates. *ISAbal3-like* and *ISAbal3* were detected upstream and downstream of the *bla*<sub>OXA-58-like</sub> gene, respectively. The 16S rRNA methylase gene *armA* was detected in the four isolates with high level aminoglycoside resistance to all tested aminoglycosides. All eleven isolates were resistant to ciprofloxacin due to mutations in the QRDR region (Leu83Ser in GyrA and Leu80Ser in ParC). *intI1* was detected in 7/11 isolates and a variety of class 1 integron gene cassette arrays was detected among the isolates.

MDR *A. baumannii* isolates belonging to European clones I and European clones II (clonal complex CC92) have emerged in Norway, all associated with hospitalization abroad. Resistance mechanisms included OXA-carbapenemases, 16S rRNA methylases and mutations in the QRDRs. Three isolates of European clone II co-produced *bla*<sub>OXA-23-like</sub> and *armA*.

**P24 MOLECULAR EPIDEMIOLOGY OF A NOVEL MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII EPIDEMIC CLONE IN NAPLES, ITALY**

**M. Giannouli<sup>a</sup>, M. Bernardo<sup>b</sup>, S. Cuccurullo<sup>b</sup>, A. Di Popolo<sup>a</sup>, V. Crivaro<sup>a,b</sup>, E. Durante Mangoni<sup>b</sup>, G. Amato<sup>c</sup>, S. Brisse<sup>d</sup>, M. Triassi<sup>a</sup>, R. Utili<sup>b,e</sup> & R. Zarrilli<sup>a,f</sup>**

<sup>a</sup>Dipartimento di Scienze Mediche Preventive, Università di Napoli Federico II, Naples, Italy; <sup>b</sup>Azienda Ospedaliera V. Monaldi, Naples, Italy; <sup>c</sup>Azienda Ospedaliera A. Cardarelli, Naples, Italy; <sup>d</sup>Institut Pasteur, Genotyping of Pathogens and Public Health, Paris, France; <sup>e</sup>Dipartimento di Scienze Cardiorespiratorie, Seconda Università degli Studi di Napoli, Naples, Italy; <sup>f</sup>CEINGE Biotecnologie Avanzate, Naples, Italy

The molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* was investigated in two intensive care units of the V. Monaldi university hospital in Naples, Italy, from May 2006 to December 2007. The aim of this study was to study the genetic characteristics of *A. baumannii* isolates responsible for the epidemic, and analyze the antimicrobial susceptibilities of the *A. baumannii* isolates and their mechanisms of resistance.

Genotype analysis by pulsed-field gel electrophoresis (PFGE), trilocus sequence-based typing (3LST), and multilocus sequence typing (MLST) of *A. baumannii* isolates from 71 patients identified two distinct genotypes, one assigned to PFGE group A, 3LST group 1, and ST2 in 14 patients and the other to PFGE group B, 3LST group 6, and ST78 in 71 patients, that we named ST2/A and ST78/B, respectively. Of these, ST2/A corresponded to European clone II identified in the same hospital during 2003 and 2004; ST78/B was a novel genotype that was isolated for the first time in May 2006 but became prevalent during 2007. The ST78/B profile was also identified in five patients from two additional hospitals in Naples during 2007. The ST2/A and ST78/B isolates were resistant to all antimicrobials tested, including carbapenems, but were susceptible to colistin. Both ST2/A and ST78/B isolates possessed a plasmid-borne carbapenem-hydrolyzing oxacillinase gene, *bla*OXA-58, flanked by *IS**Aba*2 and *IS**Aba*3 elements at the 5' and 3' ends, respectively. The selection of the novel ST78/B *A. baumannii* clone might have been favored by the acquisition of the *bla*OXA-58 gene.

**P25 INVESTIGATION OF *A. BAUMANII* INFECTIONS IN THREE FRENCH HOSPITALS USING A NOVEL MULTIPLE LOCUS VNTR ANALYSIS (MLVA) TYPING SCHEME**

**Y. Hauck<sup>a</sup>, C. Falco<sup>b</sup>, C. Soler<sup>b</sup>, C. MacNab<sup>b</sup>, P. Gérome<sup>c</sup>, A. Mérens<sup>d</sup> & C. Pourcel<sup>a</sup>**

<sup>a</sup>Université Paris-Sud, Institut de Génétique et Microbiologie, CNRS, Orsay F-91405, France; <sup>b</sup>HIA PERCY, Clamart 92141, France; <sup>c</sup>HIA Desgenettes, Lyon 69275, France; <sup>d</sup>HIA Bégin, Saint-Mandé 94160, France

*Acinetobacter baumannii* represents a serious threat in intensive care units particularly for severely burnt and other immunocompromised patients. Although nosocomial infection is a reality, in many instances the patient enters the hospital with the germ already present in the wounds as a result of contact with soil or other environmental sources. It is therefore of importance to be able to rapidly genotype the bacteria and take measures to prevent spreading of infections inside the hospital.

In the present study, a total of 113 isolates were recovered over a period of 3 years from patients in three French military hospitals. The patients were mostly civilians and came from France and from several countries of Middle East, Maghreb and Africa. They were treated in different hospital units including intensive care units, and some suffered from severe burns. In some case the bacteria were repeatedly isolated from the same patient over a long period of time. *A. baumannii*, a strictly aerobic non fermentative Gram-negative bacillus, oxidase test-negative, was easily identified using a commercial standardized system (biochemical strips api 20E, bioMérieux®) with possible growth at 44°C. The antibiotic resistance profile was determined.

Genotyping was performed using a newly developed MLVA scheme (see abstract O2 by Pourcel et al.) which investigates 10 VNTR loci. Both agarose gel and capillary electrophoresis were used to evaluate the VNTR allele size. For each isolate a code was produced and used for clustering analyses with the BioNumerics software. For comparison the MLVA profile of 6 strains which genome has been sequenced were included in the study.

The results showed that more than 50% of strains were distributed into four main clusters (clusters encompass isolates which differ by a maximum of three alleles out of 10), three of which corresponding respectively to the reference strains ACICU, AYE and ATCC 17978. The fourth larger cluster includes 15 isolates from all three hospitals and is not related to any sequenced strain. The rest of the isolates form small clusters or possess a unique genotype.

Some of the strains were analyzed by pulsed field gel electrophoresis following *SmaI* digestion and the results correlated with those of MLVA. Multidrug resistant strains were mostly found in the ACICU and AYE clusters

The MLVA method used in the present study was highly efficient for epidemiological studies, allowing the follow-up of infections and identification of new clonal complexes.

**P26 MOLECULAR EPIDEMIOLOGY AND CLINICAL IMPACT OF COLONIZATION/INFECTION OF CARBAPENEM-RESISTANT CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* COLLECTED IN A SOUTH PORTUGUESE HOSPITAL**

**Generosa Batista<sup>a</sup>, Aida Duarte<sup>b</sup> & Gabriela J. Da Silva<sup>c</sup>**

**<sup>a</sup>Service of Clinical Pathology, Hospital Espírito Santo, Évora, Portugal; <sup>b</sup>Research Institute for Medicines and Pharmaceutical Sciences and Laboratory of Microbiology, Faculty of Pharmacy, University of Lisboa Portugal; <sup>c</sup>Center of Pharmaceutical Sciences and Laboratory of Microbiology, Faculty of Pharmacy, University of Coimbra, Portugal**

*Acinetobacter baumannii* is a major nosocomial pathogen in Portugal, but no studies were performed in the South of the country. The main goal of the study was to study the epidemiology and clinical impact of colonization/infection by *A. baumannii* isolates in the Hospital of Évora-EPE, a hospital with 356 beds.

Forty *Acinetobacter* isolates were collected between October 2008 and March 2009. Susceptibility tests were performed with the VITEK 2 AST system, using GN-022 card, and the method of Kirby-Bauer for amikacin, gentamicin and netilmicin. Phenotypic detection of metallo- $\beta$ -lactamases was done by E-test. Twenty-four representative isolates were selected on basis of susceptibility profile, ward and date of isolation for M13 fingerprinting. PCR was used for detection of IMP and VIM-type lactamases (MBLs) and oxacillinases, identified by further sequencing.

Analysis of susceptibility patterns showed 3 groups: one susceptible to all antimicrobials (not typed); another with multiresistant (MDR) isolates only susceptible to colistin, tobramycin and/or amikacin; a third, susceptible to colistin and all aminoglycosides tested or to three of them. The number of infections was higher than the colonizations (74% versus 53%). Five patients were infected and colonized simultaneously. Eight infected patient died with respiratory infections, one with cellulitis and one with cystitis and bacteremia. The later has symptomatic AIDS. All patients were over 70 years old, except the one that develop cellulitis (63 years old). M13 fingerprinting showed four patterns differing by few bands, indicating that the isolates are genetically related or in the case of identical profiles belonged to a single clone. The predominant pattern was identical to the representative strain of European clone II (40HSFX). E-tests and PCR revealed that the isolates did not produce MBLs. However, the gene bla<sub>OXA24-like</sub> was identified in 16 isolates and the gene bla<sub>OXA-40</sub> in 13 isolates.

The study demonstrates the emergence and spread *A. baumannii* belonging to European clone II, whose resistance may contribute to the epidemiological success of this strain. As shown, this strain may be quite virulent, and colonization is a risk factor for some patients. Improved methods of surveillance for MDR *A. baumannii* carriers are necessary.

**P27 MULTIDRUG-RESISTANT CLONE OF *ACINETOBACTER BAUMANNII* PRODUCING THE OXA-58 CARBAPENEMASE OBTAINED FROM HOSPITALS OF COCHABAMBA, BOLIVIA**

**E. Fernández<sup>a</sup>, E. Sevillano<sup>a</sup>, I. Rosales<sup>a</sup>, Z. Bustamante<sup>b</sup>, S. Zabalaga<sup>b</sup>, A. Umaran<sup>a</sup>, R. Cisterna<sup>a</sup> & L. Gallego<sup>a</sup>**

<sup>a</sup>Facultad de Medicina y Odontología, Universidad del País Vasco, Bilbao, Spain;

<sup>b</sup>Facultad de Bioquímica y Farmacia, Universidad Mayor de San Simón, Cochabamba, Bolivia

The aim of this work was to study the presence of carbapenemases and their related genetic structures in clinical isolates of *A. baumannii* isolates from hospitals of the city of Cochabamba, Bolivia.

The study included 49 *A. baumannii* isolates obtained in the Hospital Gastroenterológico Boliviano-Japonés and Clínica Los Olivos (Cochabamba, Bolivia) from April 2008 to July 2009. Susceptibility to antimicrobial agents was determined by the disk diffusion and MIC (Minimal Inhibitory Concentration) methods following the CLSI recommendations. Antibiotics tested were amikacin, cotrimoxazole, cloranphenicol, amoxicillin/clavulanic acid, ampicillin/sulbactam, imipenem, meropenem, ceftazidime, cefepime, ceftriaxone and ciprofloxacin. Clonal relatedness was performed by PCR-*fingerprinting* using primer M13 and PFGE with *Apa*I. Class 1 integrons were determined with PCR experiments with the corresponding primers. Multiplex PCR was used to detect OXA-type carbapenemases (-23, -40, -51 and -58) followed by sequencing experiments when OXA-58 gene was amplified. Plasmid DNA was obtained by a commercial kit and the corresponding profiles were used to locate the *bla*OXA-58 gene by Southern transfer and hybridization with a specific probe labeled with digoxigenin.

The level of resistance was high with the majority of isolates resistant to at least 8 drugs, pointing out that 18 were resistant to imipenem. Typing experiments showed 13 different clones from which a multiresistant clone was predominant, named A, with 18 isolates (15 imipenem-resistant isolates). Clone A isolates showed *bla*OXA-51 (18 isolates) and *bla*OXA-58 (13 isolates) carbapenemase genes. Sequencing experiments of the coding region showed total homology with the sequence previously described in other countries. All clone A isolates showed plasmids ranging sizes from 2 to 60 kb. Hybridization with an OXA-58 probe located the gene on the chromosome but also on plasmids of 40 and 60 kb.

Detection of a multiresistant clone, named A, and the presence of the *bla*OXA-58 gene on 40kb and 60kb plasmids is of great concern as it means the possibility of spreading carbapenem resistance amongst the hospitals of the country.

**P28 CLONAL SPREAD OF AN IMPORTED STRAIN OF CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* CARRYING GENES FOR OXA-90, OXA-72 AND A TRUNCATED VIM METALLO- $\beta$ -LACTAMASE GENE TO SPLIT UNIVERSITY HOSPITAL, CROATIA**

**I. Goic-Barisic<sup>a</sup>, K. J. Towner<sup>b</sup>, A. Kovacic<sup>c</sup>, K. Sisko-Kraljevic<sup>c</sup>, M. Tonkic<sup>a</sup>, A. Novak<sup>a</sup> & V. Punda-Polic<sup>a</sup>**

<sup>a</sup>Split University Hospital and University of Split, School of Medicine, Split, Croatia;

<sup>b</sup>Nottingham University Hospitals NHS Trust, Nottingham, UK; <sup>c</sup>Teaching Public Health Institute of Split and Dalmatia County, Split, Croatia

*Acinetobacter baumannii* is a well-recognised opportunistic pathogen that gives rise to nosocomial infections and outbreaks, particularly in the intensive care unit setting. The first carbapenem-resistant strain (meropenem MIC 16  $\mu\text{g/ml}$ ) at Split University Hospital, Croatia, was isolated in 2002. Between 2002 and 2008, more than 100 clonally-related isolates belonging to the sequence group 2 (European/worldwide clone 1) lineage were collected, with a high percentage of these isolates carrying a *bla*<sub>OXA-107</sub> gene associated with IS*Aba1*. Thus, isolates belonging to the European/worldwide clone 1 lineage and carrying a *bla*<sub>OXA-107</sub> gene can be considered to be the domestic endemic clone.

On January 5, 2009, a 51 year-old female was transferred from the General Hospital, Mostar, Bosnia and Herzegovina, to the Intensive Care Unit of Split University Hospital following brain surgery for glioblastoma. According to the transfer letter, multiresistant *Acinetobacter* spp. was isolated from a bronchial aspirate during hospitalisation in Mostar. The susceptibility pattern of the isolate at this stage showed susceptibility only to colistin and ampicillin/sulbactam. During the next 6 months (January – July 2009), 32 similar consecutive isolates were collected from different patients hospitalised in two ICUs and three different departments at Split University Hospital. All isolates displayed the same resistance pattern, with no inhibition zone around imipenem and meropenem disks, and were collected from blood cultures, urine samples, catheter tip specimens, cerebrospinal fluid, throat and nasal swabs, and bronchial secretions.

Pulsed-field gel electrophoresis performed following macrorestriction with *ApaI* confirmed that all isolates belonged to a single new clone, shown by multiplex PCR to belong to the sequence group 1 lineage (European/worldwide clone 2). Multiplex PCR also revealed that isolates belonging to the new clone possessed *bla*<sub>OXA-51</sub>-like and *bla*<sub>OXA-40</sub>-like genes. No other class D oxacillinase genes were detected by PCR. Sequencing of the *bla*<sub>OXA-51</sub>-like gene (both strands) revealed the presence of a gene encoding OXA-90 (a variant of OXA-66). Sequencing of the *bla*<sub>OXA-40</sub>-like gene revealed the presence of a gene encoding OXA-72. PCRs to detect the presence of integrons and MBL genes revealed three different integron structures (530, 750 and 1130 bp, respectively) with the 750 bp and 1130 bp structures containing a truncated (at the 5'-end) VIM-type gene cassette in the distal position.

The new clone of *A. baumannii* belonging to the European/worldwide clone 2 lineage has become dominant in Split University Hospital hospital during the last year and is a major causative agent of nosocomial infection. These findings highlight the importance of international transfer of patients in the spread of novel antimicrobial-resistant pathogens, and emphasise the need for hospitals to isolate and screen patients admitted to hospitals from foreign countries.

**P29 CLONAL SPREAD OF CARBAPENEM-RESISTANT OXA-40 POSITIVE ACINETOBACTER BAUMANNII IN A CROATIAN UNIVERSITY HOSPITAL**

**Branka Bedenić<sup>a,b</sup>, Jasmina Vraneš<sup>c</sup>, Ana Budimir<sup>a,b</sup>, Zoran Herljević<sup>b</sup>, Smilja Kalenić<sup>a,b</sup>, Ivana Goić-Barišić<sup>d</sup>, Mirna Ladavac<sup>e</sup> & Paul G. Higgins<sup>f</sup>**

<sup>a</sup>School of Medicine, University of Zagreb, Zagreb, Croatia; <sup>b</sup>Clinical Hospital Centre Zagreb, Zagreb, Croatia; <sup>c</sup>Zagreb Institute of Public Health „A. Štampar“, Zagreb, Croatia; <sup>d</sup>Department of Microbiology, University Hospital Split, Croatia; <sup>e</sup>County of Istria Public Health Institute, Croatia; <sup>f</sup>Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany

Carbapenems have a potent activity against and are often used as last resort for the treatment of infections due to multiresistant *Acinetobacter baumannii* isolates. Recently an increase in the prevalence of carbapenem resistant *A. baumannii* isolates has been observed at the Clinical Hospital Center Zagreb. The aim of the study was to characterize the mechanisms of carbapenem resistance in our *A. baumannii* isolates.

Antibiotic susceptibilities were determined by broth microdilution. Oxacillinase genes were detected by multiplex PCR. Metallo  $\beta$ -lactamases were detected by E-test and PCR with primers specific for VIM, IMP and SIM  $\beta$ -lactamases. Genotyping of the strains was performed by pulsed-field gel electrophoresis (PFGE), rep-PCR, random amplification of polymorphic DNA (RAPD), and determination of sequence groups according by multiplex PCR.

Thirty three clonally related strains were positive for *bla*<sub>OXA-40</sub>. One unrelated isolate was positive for *bla*<sub>OXA-58</sub>. The nine remaining isolates possessed only the naturally occurring OXA-51  $\beta$ -lactamase which was associated with *ISAbal* insertion sequence in five strains. No metallo  $\beta$ -lactamase genes were found. The *bla*<sub>OXA-40</sub> positive isolates were shown to be clonally related by RAPD rep-PCR and PFGE. Other isolates showed distinct PFGE and RAPD patterns. The isolates producing only OXA-51  $\beta$ -lactamase were found to belong to EU clone 1 (sequence group II) whereas the OXA-40 and OXA-58 producers belonged to EU clone 2 (sequence group I).

On the basis of susceptibility testing,  $\beta$ -lactamase characterization and genotyping of the strains we can conclude that spread of endemic isolates was responsible for high frequency of detection of OXA-40- like positive multidrug-resistant *A. baumannii* in this setting. Most of the strains originated from the ICU indicating local dissemination within the hospital and pointing to the potential source of isolates. Colistin is the only therapeutic option in most cases. High rate of resistance to ampicillin/sulbactam is worrying and is probably due to the production of TEM-1  $\beta$ -lactamase.

**P30 A MULTICENTER STUDY INVESTIGATING THE OCCURRENCE OF ACINETOBACTER CALCOACETICUS-BAUMANNII COMPLEX SPECIES IN CLINICAL SPECIMENS IN SINGAPORE**

**T. H. Koh<sup>a</sup>, T. T. Tan<sup>a</sup>, C. T. Khoo<sup>a</sup>, L. Ng<sup>b</sup>, L-Y. Hsu<sup>c</sup>, E. E. Ooi<sup>d</sup>, T. J. van der Reijden<sup>e</sup> & L. Dijkshoorn<sup>e</sup>**

**<sup>a</sup>Singapore General Hospital, Singapore; <sup>b</sup>Changi General Hospital, Singapore; <sup>c</sup>National University Hospital, Singapore; <sup>d</sup>Duke-National University of Singapore Graduate Medical School, Singapore; <sup>e</sup>Leiden University Medical Centre, Leiden, The Netherlands**

A total of 192 non-replicate *Acinetobacter calcoaceticus-baumannii* species complex clinical isolates were collected from 6 hospitals in Singapore over a 1-month period in 2006. Identification of *Acinetobacter baumannii* was carried out by a one tube multiplex PCR targeting an internal 208-bp fragment from the intergenic spacer (ITS) region of *A. baumannii* and a highly conserved 425-bp region of the *recA* gene of *Acinetobacter* spp (Chen TL, Clin Microbiol Infect. 2007, 13(8):801-6). Other members of the *Acinetobacter calcoaceticus-baumannii* species complex were identified by ITS sequencing (Chang HC, J Clin Microbiol. 2005, 43(4):1632-9) and amplified fragment length polymorphism analysis (van den Broek PJ, J Clin Microbiol. 2009, 47(11):3593-9). Minimal inhibitory concentrations to piperacillin-tazobactam, ceftazidime, cefepime ciprofloxacin, imipenem, meropenem, gentamicin, amikacin, and tigecycline were carried out by microbroth dilution using custom Sensititre plates.

There were 152 *A. baumannii* (79%), 17 *Acinetobacter* genomic species (gen. sp.) 3 (9%), and 23 *Acinetobacter* gen. sp. 13TU (12%). 72% of *A. baumannii*, 41% of *A. gen. sp. 3*, and 35% of *A. gen. sp. 13TU*, were nonsusceptible to imipenem. 79% of *A. baumannii*, 41% of *A. gen. sp. 3*, and 39% of *A. gen. sp. 13TU*, were nonsusceptible to ciprofloxacin. 74% of *A. baumannii*, 53% of *A. gen. sp. 3*, and 52% of *A. gen. sp. 13TU*, were nonsusceptible to gentamicin. Eleven *A. baumannii*, 5 *A. gen. sp. 13TU*, and 2 *A. gen. sp. 3* were isolated from blood indicating their clinical significance.

Almost 80% of *Acinetobacter calcoaceticus-baumannii* species complex clinical isolates in Singapore were *A. baumannii* of which the majority were resistant to multiple antimicrobials. It is of note that *A. gen. sp. 3* and 13TU also appeared to be associated with clinically significant infections and these species can also be multiply-resistant.

**P31 TRANSFER OF MULTI-DRUG RESISTANT CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII BETWEEN OLD AND NEW FACILITIES OF LAC+USC MEDICAL CENTER GENERAL HOSPITAL**

**Y. Chu<sup>a</sup>, M. Osby<sup>b</sup> & H. H. Xu<sup>a</sup>**

<sup>a</sup>**Department of Biological Sciences, California State University, Los Angeles, USA;**

<sup>b</sup>**Department of Pathology, LAC+USC Medical Center, Los Angeles, USA**

*Acinetobacter baumannii* has emerged as a problematic opportunistic human pathogen due to its increasingly multi-drug resistance nature. It is a frequent cause of nosocomial outbreaks world wide.

We have obtained nosocomial clinical isolates of *A. baumannii* collected both before and after a major hospital move in November, 2008. This presents a rare opportunity to examine whether clonally related clinical isolates were transferred along with the hospital move.

To answer this question, we determined antimicrobial susceptibility patterns and genetic profiles of 98 clinical isolates and 17 environmental isolates of *A. baumannii* from LAC+USC Medical Center General Hospital in Los Angeles, California. Our results indicate that among the 98 clinical isolates, 93% of the isolates are multi-drug resistant. In contrast, only 18% (3 out of 17) environmental isolates from the new hospital are multi-drug resistant.

Twenty three groups of isolates were identified based on pulsed-field gel electrophoresis (PFGE) analysis of genomic DNA following *ApaI* restriction digest. Two groups of clinical isolates collected after the hospital move were found to be clonally identical or closely related to two groups of clinical isolates obtained before the move and these isolates can be traced to two environmental isolates. Additionally, isolates of the same groups were found to have highly similar antimicrobial susceptibility profiles.

**P32 THE EMERGENCE OF THE MAJOR EUROPEAN CLONES OF CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* IN KUWAIT**

**A .R. Al-Hasan<sup>a</sup>, A. Hamouda<sup>a</sup>, A. A. Dashti<sup>b</sup> & S. G. B. Amyes<sup>a</sup>**

<sup>a</sup>Centre for Infectious Diseases, University of Edinburgh, UK; <sup>b</sup>Kuwait University, Kuwait

*Acinetobacter baumannii* is an increasingly important nosocomial pathogen because of its ease of transmission and ability to become multidrug resistant. It has become a particular problem in Kuwaiti hospitals and this study aimed to examine the genotypic changes in the organism as it spread through one hospital.

A total of 88 *Acinetobacter baumannii* samples were collected from the Mubarak Al-Kabeer Hospital, over a three year period, 2006-2008, and they were identified phenotypically, by Vitek-2 systems, and then genotypically, by PCR amplification of *bla*<sub>OXA-51-like</sub> gene. The resistance to the carbapenems, imipenem and meropenem, was identified by use of the Minimal Inhibitory Concentration (MIC) test. Pulsed Field Gel Electrophoresis (PFGE) was used to type the strains and classify them into clonal groups. With this information, a dendrogram showing the resistance profile and clonal relationship of all the isolates was constructed. Gene sequencing was used to identify the *bla*<sub>OXA-51-like</sub> gene types of each of the isolates.

All 88 isolates were identified as *Acinetobacter baumannii* by Vitek-2 system and were shown to carry a *bla*<sub>OXA-51-like</sub> gene. Resistance to imipenem was found in 30% of the isolates, whereas resistance to meropenem was found in 24% of the isolates. Overall carbapenem resistance was observed in 32% of the total isolates, with a slight increase in resistance of isolated over the 3 years of collection. In all, there were 10 different *bla*<sub>OXA-51-like</sub> genes identified. The sequences of these genes suggested there was some degree of real-time evolution of the *bla*<sub>OXA-51-like</sub> genes during the study period. There were four main clonal clusters. There were three main European clones (*bla*<sub>OXA-66</sub>, *bla*<sub>OXA-69</sub>, and *bla*<sub>OXA-71</sub>) plus a new clone-with *bla*<sub>OXA-51-like</sub> genes with sequences clustered around the *bla*<sub>OXA-98</sub> gene,

This study has shown four major clones were found in the hospital during the study period, three closely associated with those found in Europe and elsewhere and one new clone, containing a *bla*<sub>OXA-98-like</sub> gene that appears to be more prevalent in this part of Asia.

### P33 THE EPIDEMIOLOGY OF *ACINETOBACTER BAUMANNII* OF ANIMAL ORIGIN

A. Hamouda, L. Al Hassan & S. G. B. Amyes

Centre for Infectious Diseases, University of Edinburgh, UK

The aim of this study was to determine whether *A. baumannii* strains collected from animals slaughtered for human consumption from Scottish abattoirs possessed major epidemiological characteristics similar to strains isolated from clinical patients.

Sixteen *A. baumannii* isolates (8 from cattle and 8 from pig) obtained from 1381 animal samples, collected between the period February 2006 to August 2007, were used in this study. For species identification, *bla*<sub>OXA-51-like</sub> genes were amplified by the polymerase chain reaction (PCR) and confirmed by sequencing. Minimum inhibitory concentration (MIC) of antibiotics was performed according to the BSAC guidelines. PCR was also used to amplify sections of DNA that harboured resistance islands (RIs), *bla*<sub>ampC</sub> and the insertion sequence *IS*<sub>Aba1</sub>. All strains were genotyped by pulsed-field gel electrophoresis (PFGE) using *Xba*I restriction endonuclease.

All isolates were confirmed as *A. baumannii* using *bla*<sub>OXA-51-like</sub> genes and, interestingly, they all harboured the *bla*<sub>OXA-51</sub> gene itself. The PFGE profile of the animal isolates showed some genotypic diversity and a very different profile from *A. baumannii* European clone I, II, and III. All isolates lacked any evidence of resistance islands and *IS*<sub>Aba1</sub> but every strain harboured *bla*<sub>ampC</sub> genes. All 16 isolates were sensitive to imipenem (MIC  $\leq$ 4mg/L), meropenem (MIC  $\leq$ 4mg/L), ciprofloxacin (MIC  $\leq$ 0.5mg/L) and piperacillin/tazobactam (MIC  $\leq$ 16mg/L) but they were all (100%) resistant to ceftazidime (MIC  $>$ 2mg/L). When animal isolates were compared with the European clones I, II, and III they belonged to a different species pool.

Amongst these animal isolates of diverse source, the indigenous OXA-51-like  $\beta$ -lactamase was OXA-51 itself. Thus there was no variation within *bla*<sub>OXA-51-like</sub> genes as is normally found in human isolates. One possible explanation is that these isolates have not yet undergone any major antibiotic selective pressure. They were all sensitive to major antibiotics, with the exception of basal resistance to ceftazidime, encoded by the *bla*<sub>ampC</sub> gene. In addition, the absence of resistance islands and the lack of *IS*<sub>Aba1</sub> elements is further evidence that these isolates have not yet progressed to a multidrug resistance genotype. These results coupled with the different PFGE profile of *A. baumannii* animal isolates from human strains suggest that they are not the precursors of *A. baumannii* strains found to cause hospital-acquired infections.

**P34 PATTERNS OF ANTIMICROBIAL RESISTANCE OF *ACINETOBACTER BAUMANNII* STRAINS ISOLATED FROM CLINICAL SPECIMENS IN AN INFECTIOUS DISEASES HOSPITAL IN BUCHAREST, ROMANIA**

**A. Rafila<sup>a,b</sup>, O. Dorobat<sup>a</sup>, I. Badicut<sup>a</sup>, V. Arama<sup>a,b</sup>, D. Talapan<sup>a</sup>, I. D. Olaru<sup>a</sup>, D. Iovanescu<sup>a,b</sup> & R. Mihailescu<sup>a</sup>**

<sup>a</sup>**“Prof. Dr. Matei Bals” National Institute for Infectious Diseases, Bucharest, Romania;**

<sup>b</sup>**“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania**

*Acinetobacter baumannii* is an increasingly common nosocomial pathogen which frequently causes therapeutic problems in clinical practice due to high levels of antimicrobial resistance. To date there are few data concerning resistance rates from Eastern and Central European countries. This study aimed to evaluate the resistance patterns of *Acinetobacter baumannii* isolates from the Intensive Care Unit (ICU) and non-ICU departments of “Prof. Dr. Matei Bals” National Institute for Infectious Diseases (INBI-MB).

*Acinetobacter baumannii* strains from patients admitted at the INBI-MB during 2009 were identified and tested for antimicrobial resistance. The isolates were grouped as follows considering their origin: lower respiratory tract (sputum, bronchoalveolar lavage, tracheal aspirate), from patients with systemic infection (blood, cerebral spinal fluid, catheter), and other sites (wounds, urine). Identification procedures were performed on *Vitek 2C* and *MicroScan* systems and antimicrobial susceptibility testing used the same systems as well as the *E-test*. Interpretation was made in conformity with CLSI 2009.

A total of 116 non-duplicated strains of *Acinetobacter baumannii* were identified: 57 from patients admitted to the ICU and 59 from non-ICU patients. Of all isolates, 66 were from the lower respiratory tract, 16 from patients with systemic infection and 34 from other sites. Resistance rates to the following antimicrobial drugs were as follows ranking highest to lowest: ciprofloxacin (94%), piperacillin-tazobactam (90.6%), cefepime (87.8%), ampicillin-sulbactam (85.5%), ceftazidime (84.1%), levofloxacin (82.1%), meropenem (79.8%), imipenem (72.5%), gentamicin (72.5%), amikacin (63.2%), tobramycin (38.7%). Colistin was found to be effective in 100% of the tested strains (n=66). Strains isolated from the ICU were significantly more resistant to carbapenems than isolates from non-ICU (87.3% vs 71.4%, p=0.039). There was no statistical significant difference between the resistance rates for quinolones and aminoglycosides for the two groups. There were no significant differences between the patterns of resistance from isolates originating from different sites.

In our study, and probably at country-level, *Acinetobacter baumannii* had high rates of resistance to almost all antimicrobial drugs tested, making treatment options limited. There is significant concern due to the increased resistance rates to carbapenems, considerably higher than those described in literature. Colistin maintains excellent antimicrobial activity making it the drug of choice when treating this infection. Reconsidering infection control protocols that could limit the spread of multi-drug resistant *Acinetobacter baumannii* in Romania is advisable.

### **P35 OUTBREAK OF MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* IN A TERTIARY HOSPITAL**

**P. Marin-Casanova, F. Galán-Sanchez, F. Gil de Sola, J. Cañadas, P. Aznar-Marín & M. Rodríguez-Iglesias**

**Clinical Microbiology Lab, Puerta del Mar Univ. Hosp., Cádiz, Spain**

The role of multidrug resistant *Acinetobacter baumannii* and its clinical relevance have been recently appreciated as a ubiquitous opportunistic nosocomial pathogen. Risk factors associated with *A. baumannii* infection include severe underlying diseases, previous surgery, invasive procedures, treatment with broad-spectrum antibiotics, length of hospital stay and admission to intensive care units (ICU). Carbapenem-multidrug resistant *A. baumannii* infections are probably associated to greater severity and more complications. This study describes an outbreak due to multidrug-resistant (MDR) *A. baumannii* in a tertiary hospital.

From January to March 2010, *A. baumannii* was isolated from 25 patients in a total of 43 clinical samples, including tracheal aspirate (21), blood (6), catheter (5), abscess (5), urine (5) and CSF (1). All *Acinetobacter* strains were identified by standard microbiological methods (gram staining, colony and cell morphology, and biochemical test). Susceptibility characterization was established by Clinical and Laboratory Standards Institute (CLSI) recommendations, including detection of carbapenemases (Hodge's test). Molecular typing was performed by rep-PCR. Epidemiological and clinical data, including demographics data, hospitalization area, underlying diseases and other risks factors, were collected from all patients.

All strains were resistant to almost all the antimicrobials tested, including aminoglycosides,  $\beta$ -lactams and quinolones, and were susceptible only to colistin. Carbapenemase production was detected in fourteen strains. The clonality study, performed by rep-PCR, showed one predominant clade; however, four patients were colonized/infected by two different strains of *A. baumannii*. Patients were hospitalized in different hospital areas (rehabilitation, hematology, internal medicine and others), but almost all were previously hospitalized in the ICU.

We describe an epidemic outbreak of MDR *A. baumannii*. Patients were probably colonized/infected during hospitalization in the ICU, spreading the bacteria to other clinical units of the hospital. Almost all strains showed an identical antimicrobial susceptibility pattern, and we demonstrated by molecular methods a common genetic profile. We underline the interest of surveillance mechanisms for detection and prevention of MDR microorganisms.

**P36 MULTIPLE OUTBREAKS CAUSED BY AN EPIDEMIC MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII* CLONE IN CENTRAL ITALY**

**S. D'Arezzo<sup>a</sup>, L. Principe<sup>a</sup>, A. Capone<sup>a</sup>, N. Petrosillo<sup>a</sup> & P. Visca<sup>b</sup>**

**<sup>a</sup>Istituto Nazionale per le Malattie Infettive Lazzaro Spallanzani IRCCS; <sup>b</sup>Dipartimento di Biologia, Università Roma Tre, Rome, Italy**

Infections sustained by multidrug-resistant (MDR) and pan-resistant *Acinetobacter baumannii* have become a challenging problem in Intensive Care Units (ICUs). Ubiquity and propensity to develop stable antibiotic resistance make *A. baumannii* a common yet difficult-to-treat pathogen.

Here, the molecular epidemiology and the genetic basis of antibiotic resistance in 114 MDR *A. baumannii* strains isolated from June 2005 to March 2009 from infected patients in ten ICUs in Central Italy were investigated. Genotype analysis by random amplified polymorphic DNA (RAPD), multilocus sequence typing (MLST) and macrorestriction analysis identified one predominant clonal type, related to the International *A. baumannii* lineage II, accounting for 95.6% of *A. baumannii* ICU isolates. The dominant clone was isolated from all ICUs under survey and was characterized by resistance to all classes of antimicrobials including carbapenems. Exceptions to the overall trend of reduced susceptibility to antibiotics during the study period were tigecycline and colistin (97.4% and 100% of susceptibility, respectively). Of note, a high percentage of carbapenem resistant *A. baumannii* isolates were very high-level resistant to imipenem showing a MIC value of 128-256 mg/L (60.5% isolates) with a MIC<sub>50</sub> value of 128 mg/L. Multiplex polymerase chain reaction of *bla*<sub>OXA</sub> genes showed that carbapenem resistance was associated with the presence of two carbapenem-hydrolyzing oxacillinase genes, namely *bla*<sub>OXA-23</sub> (75.7%) or *bla*<sub>OXA-58</sub> (24.3%). Comparative typing of *A. baumannii* strains showed that the emerging *bla*<sub>OXA-23</sub>-positive clone was genetically related to *bla*<sub>OXA-58</sub>-positive clone previously isolated from ICU outbreaks in the Rome urban area (D'Arezzo *et al.*, Clin Microbiol Infect. 2009;15:347-57), raising serious concern as to its persistence and adaptation in the ICU environment.

This study demonstrates the worrying spread of a carbapenem-resistant MDR *A. baumannii* clone in Central Italy, and suggests that tigecycline and colistin still remain effective therapeutic options for the management of this difficult-to-treat pathogen. It also highlights that continuous surveillance of MDR *A. baumannii* and elucidation of its antibiotic-resistant mechanisms can be of guidance in definition of therapy regimens and prevention strategies.

**P37 OUTBREAK OF MULTIRESISTANT *ACINETOBACTER BAUMANNII* IN A INTENSIVE CARE UNIT: PATIENTS AND ENVIRONMENTAL**

**C. Pascolini<sup>a</sup>, G. Prignano<sup>a</sup>, L. Pelagalli<sup>b</sup>, P. Basili<sup>b</sup>, A. Di Giacobbe<sup>c</sup>, S. Pecetta<sup>c</sup>, A. Silvestri<sup>c</sup>, M.T. Gallo<sup>a</sup>, L. Cilli<sup>a</sup>, A. De Santis<sup>a</sup>, M. Vespaziani<sup>a</sup>, A. Di Maio<sup>a</sup> & C. Passariello<sup>c</sup>**

**<sup>a</sup>Laboratory of Clinical Pathology and Microbiology, San Gallicano Dermatologic Institute IRCCS; Rome, Italy; <sup>b</sup>Intensive Care Unit, Regina Elena Cancer Institute IRCCS; Rome, Italy; <sup>c</sup>Department of Public Health Sciences, University of Rome La Sapienza; Rome, Italy**

*Acinetobacter baumannii* colonization and infection, frequent in Intensive Care Unit (ICU) patients, is commonly associated with high morbimortality. An outbreak of multidrug-resistant *Acinetobacter baumannii* (MRAB) occurred over the course of a 10 days period in intensive care unit (ICU) of our Institute. Four patients were found positives to infection-colonization by *A. baumannii* resistant to all antibiotics except for colistin. We describe the investigation to discover if an environment can be an infection reservoir because patients were hospitalized in the same room of the intensive care unit.

For this purpose samples were taken from surfaces and objects within the room before and after disinfection protocol. MRAB was collected from bronchial aspirate samples and from swabs cultures of environmental samples taken from surfaces and various objects and cultivated by standard microbiological methods and isolated by culture with McConkey agar plates. Thirteen isolated strains were tested for antimicrobial susceptibility and for PFGE typing.

Among environmental samples, we found that 7/18 and 2/18, respectively pre and post disinfection protocol, were positive for *A. baumannii*. The isolated strains were resistant to all antibiotics except for colistin. A single PFGE type was identified from all patients and all environmental samples. This suggests that the environmental contamination was an important reservoir of the epidemic strains. In addition it is necessary to optimize the disinfection protocol and training of health workers, in active collaboration with the CIO (Committee Nosocomial Infections) to prevented recurrence of the pathogen.

### P38 MOLECULAR ANALYSIS OF *ACINETOBACTER BAUMANNII* ISOLATES FROM AN INTENSIVE CARE UNIT

H. M. Sharon Goh<sup>a</sup>, S. A. Beatson<sup>a</sup>, J. Szubert<sup>a</sup>, N. Runnegar<sup>b</sup>, H. E. Sidjabat<sup>c</sup>, D. L. Paterson<sup>b,c,d</sup>, G. R. Nimmo<sup>b,c</sup>, J. Lipman<sup>d,e</sup> & M. A. Schembri<sup>a</sup>

<sup>a</sup>School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Australia; <sup>b</sup>Pathology Queensland Central Laboratory, Brisbane, Australia; <sup>c</sup>University of Queensland Centre for Clinical Research, Brisbane, Australia; <sup>d</sup>Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia; <sup>e</sup>Burns, Trauma and Critical Care Research Centre, University of Queensland, Brisbane, Australia

*Acinetobacter baumannii* is a non-fermenting Gram-negative bacillus that causes serious infections such as ventilator-associated pneumonia, urinary tract infection and hospital-acquired bloodstream infection. In the hospital setting, *A. baumannii* acquires resistance to multiple classes of antibiotics. For example, approximately 20% of isolates worldwide are now resistant to carbapenems, a class typically regarded as the "last resort" in intensive care units (ICUs). Carbapenem resistant *A. baumannii* are typically resistant to all other  $\beta$ -lactam antibiotics, fluoroquinolones and most aminoglycosides. Numerous outbreaks of infection with multi-drug resistant *A. baumannii* have now been described leading to huge expense and operational difficulties involved in the containment of spread of this organism.

Two major outbreaks of carbapenem-resistant *A. baumannii* have occurred in the ICU of the Royal Brisbane and Women's Hospital (RBWH), one in 2001 and the other in 2006. We have determined a draft genome sequence of the index case strain from each outbreak (MS1968 and MS1984, respectively). The two strains are closely related with the exception of 10 single nucleotide polymorphisms (SNPs) and a large plasmid that is absent in MS1968. We also performed multi-locus sequence typing (MLST) for these two strains, as well as for several *A. baumannii* strains isolated from individual cases prior to and in-between the outbreaks, by sequencing 7 housekeeping genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, *rpoD*).

MLST data on our library of 41 *A. baumannii* isolates suggests that 60% of the strains belonged to sequence type 92, indicating a large proportion of isolates were closely related. Bioinformatic analysis of the genome sequence of MS1968 and MS1984 identified the presence of a gene encoding the biofilm-associated protein (Bap) that has been previously characterised from *Staphylococcus aureus* (239 kDa), *Enterococcus faecalis* (202 kDa), *Pseudomonas fluorescens* (888 kDa), *Salmonella typhimurium* (386 kDa) and recently in an *A. baumannii* bloodstream isolate (854 kDa). We analysed MS1968 whole-cell protein samples by Western blot using an antibody generated against a conserved (~ 400 amino acid) Bap fragment and detected a hybridising band of ~ 250 kDa. The exact size and function of Bap from MS1968 is currently under investigation, as well as cloning of the *bap* gene and examination of its role in biofilm formation by *A. baumannii*.

### **P39 FIRST DETECTION OF *BLA*<sub>OXA-24-LIKE</sub> GENE IN *ACINETOBACTER BAUMANNII* ISOLATED FROM A CHILEAN HOSPITAL**

**A. Opazo-Capurro, H. Bello, M. Domínguez & G. González-Rocha**

**Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile**

In the last decades *Acinetobacter baumannii* has emerged as a major relevant world nosocomial pathogen and in Chile is one of the most important Gram-negative bacilli causing infections in hospitalized patient, mainly in intensive care units.

Carbapenems are considered a last-line agent for the treatment of serious infectious diseases caused by multi-resistant Gram-negative bacilli, such as *A. baumannii*. However, various studies have reported the incidence of resistance to imipenem or/and meropenem among strains of *A. baumannii* in hospitals, and at some locations pandrug-resistant strains have been identified.

The most prevalent mechanism of resistance to  $\beta$ -lactams in this species is the enzymatic degradation of antibiotic by different kinds of  $\beta$ -lactamases. Among these enzymes are the carbapenemases belonging to molecular class D (OXA enzymes) and these have globally emerged as the main mechanism responsible for this resistance, although metallo-enzymes are locally prevalent, especially in East Asia.

The OXA carbapenemases of *Acinetobacter* spp. are classified into four phylogenetic subgroups: OXA-23-like; OXA-24-like; OXA-51-like; and OXA-58. In South America, enzymes of the subgroups OXA-23-like, OXA-58 and OXA-51-like have been mostly reported, with OXA-51-like enzymes considered as intrinsic enzymes of *A. baumannii*.

The aim of this work was to study the origin of carbapenem resistance in two clonally unrelated strains of *A. baumannii* resistant to imipenem, isolated in a Chilean Hospital in 2008. Resistance was investigated according to recommendations of CLSI and the study of clonality was carried out by DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE). The presence of *bla*<sub>OXA</sub> genes was determined through a multiplex-PCR.

Both strains were isolated from an intensive-care unit in a hospital of Santiago (the Capitol of Chile). The strain Ab1 was isolated from a wound and the strain Ab2 from urine. The minimum inhibitory concentration (MIC) of imipenem and meropenem for strain Ab1 were 16  $\mu$ g/ml and 32  $\mu$ g/ml, respectively, upon strain Ab2 MICs were slightly higher (32  $\mu$ g/ml and 64  $\mu$ g/ml of imipenem and meropenem, respectively). Strains were also resistant to gentamicin, cefepime, piperacillin/tazobactam and ciprofloxacin, but susceptible to tigecycline and tetracycline. Respect to *bla*<sub>OXA</sub> genes, in both strains the multiplex-PCR yield an amplicon of 353 bp and another of 246 bp, which correspond to similar size of positive control for *bla*<sub>OXA-51-like</sub> and *bla*<sub>OXA-24-like</sub> genes.

This is the first report of the presence of OXA-24-like carbapenemases in South America in carbapenem-resistant *A. baumannii*.

## **P40 FIRST IDENTIFICATION AND CHARACTERIZATION OF AN AdeABC-TYPE EFFLUX PUMP IN ACINETOBACTER GENOMOSPECIES 13TU**

**I. Roca, P. Espinal, S. Marti & J. Vila**

**Microbiology Department, Hospital Clínic, Faculty of Medicine, University of Barcelona, Barcelona, Spain**

The main mechanism of multidrug resistance in *Acinetobacter baumannii* relies on the overexpression of active efflux pumps that extrude multiple antimicrobial agents outside the bacterial cell wall. The AdeABC system was the first efflux pump to be described in *A. baumannii* and it mainly confers resistance to aminoglycosides,  $\beta$ -lactams, chloramphenicol, erythromycin and tetracycline although it has never been identified in other *Acinetobacter* spp. The aim of this study was to investigate the presence of the AdeABC pump in non-*A. baumannii* spp., such as *Acinetobacter* genomospecies 13TU, and to characterize its substrate specificity.

The *Acinetobacter* genomospecies 13TU clinical isolate used in this study was identified by ARDRA, and antibiotic susceptibility testing showed resistance to ceftriaxone, erythromycin, piperacillin, tetracycline and ceftazidime. An AdeABC-type system was identified by PCR using primers matching the sequence of the *A. baumannii* counterpart. Subsequent chromosome walking allowed us to obtain the complete DNA sequence of a 7780 bp fragment containing the genes encoding an AdeABC-type pump plus the *adeRS* genes encoding a two-component system presumably controlling *adeABC* expression (GeneBank accession number: GU319112). Analysis of the translated sequence indicated 93-98% similarity to the *A. baumannii* AdeABC system and the use of a *knock-out* mutant allowed us to characterize its range of substrate specificity.

Non-*A. baumannii* spp. are recently emerging among clinical isolates causing nosocomial infections and some of them (such as genomospecies 13TU) appear to be multidrug resistant as well. It is likely that the prevalence of non-*A. baumannii* spp. in the hospital setting is understated due to poor identification techniques. In this work we report the first identification of an AdeABC-type efflux pump in an *Acinetobacter* genomospecies 13TU clinical isolate as well as its contribution to multidrug resistance.

**P41 THE MOVEMENT OF INSERTION SEQUENCE ELEMENTS RESULTS IN LOSS OF LPS AND COLISTIN RESISTANCE IN *ACINETOBACTER BAUMANNII***

**J. H. Moffatt<sup>a</sup>, M. Harper<sup>a,b</sup>, B. Adler<sup>a,b</sup> & J. D. Boyce<sup>a,b</sup>**

**<sup>a</sup>Department of Microbiology, <sup>b</sup>Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics, Monash University, Victoria, Australia**

To date, ten different insertion sequence (IS) elements have been described in *Acinetobacter baumannii*. The mobilisation of many of these elements has been shown to be associated with the development of antibiotic resistance. For example insertion of IS*Aba1* upstream of the native *bla*<sub>OXA-51/69</sub>-like genes in *A. baumannii* results in increased levels of carbapenem resistance. In this study we show that the insertion of a novel IS element (designated IS*Aba11*) into either of the LPS biosynthesis genes *lpxA* or *lpxC* of the *A. baumannii* type strain ATCC 19606 results in the complete loss of lipopolysaccharide (LPS) production and a concomitant increase in resistance to the polymyxin antibiotic colistin. Bioinformatic analysis indicated that IS*Aba11* is also present in several environmental species of *Acinetobacter*, including *A. lwoffii* and *A. johnsonii*. PCR screening of *A. baumannii* clinical isolates revealed that IS*Aba11* is present in several strains, including isolates from both North America and Australia. Furthermore, analysis of a colistin-resistant *A. baumannii* clinical isolate from South Korea identified the presence of another novel IS element (designated IS*Aba12*) in the LPS biosynthesis gene *lpxD*. This study is the first to show that IS element insertion can play an important role in the generation of resistance to colistin, one of the few remaining antibiotics effective in the treatment of multi-drug resistant *A. baumannii*.

**P42 COMPARATIVE PROTEOMIC CHARACTERISATION OF LIPOPOLYSACCHARIDE-DEFICIENT COLISTIN RESISTANT *ACINETOBACTER BAUMANNII***

**R. Henry<sup>a</sup>, N. Vithanagie<sup>a</sup>, J. Moffatt<sup>a</sup>, B. Adler<sup>a,b</sup>, M. Harper<sup>b</sup> & J. Boyce<sup>a</sup>**

**<sup>a</sup>Department of Microbiology, <sup>b</sup>Australian Research Council Centre of Excellence in Structural and Functional Microbial Genomics, Monash University, Clayton, Australia**

Pan-drug resistant strains of *Acinetobacter baumannii* have recently been reported from within clinical settings. Furthermore, the incidence of resistance to colistin, a last-line treatment option, is increasing and is of significant clinical concern. Colistin is a polymyxin antibiotic and its mode of action, while not fully defined, is reported to require a direct charge-based interaction between colistin and lipopolysaccharide (LPS), the major surface component of Gram-negative bacteria.

We have recently shown that colistin resistance in *A. baumannii* can result from mutation of genes required for LPS synthesis, including *lpxA*. These colistin-resistant strains express no LPS on their surface. We hypothesised that loss of the important surface component LPS may result in significant changes to the outer membrane (OM) protein profile of the cell. To investigate the consequences of colistin resistance resulting from loss of LPS expression, we compared the OM protein profiles of the colistin sensitive *A. baumannii* type strain ATCC19606 and an LPS-deficient colistin-resistant *lpxA* mutant (designated ATCC19606R).

A proteomic approach was applied to identify protein expression differences between the strains utilising TX-114 and sarkosyl membrane fractionation procedures, two-dimensional gel electrophoresis (2DGE) and matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). The colistin-resistant LPS-deficient *lpxA* mutant displayed significantly altered OM protein expression. Moreover, protein expression differences were not limited to the OM fraction, suggesting that LPS loss and/or colistin resistance have a global affect on cellular protein expression.

**P43 THE IN-VITRO FITNESS COST OF AdeABC-MEDIATED TIGECYCLINE RESISTANCE ASSOCIATED WITH TIGECYCLINE THERAPY IN AN EPIDEMIC UK CLONE OF *ACINETOBACTER BAUMANNII***

**M. A. Hornsey<sup>a</sup>, M. J. Ellington<sup>b</sup>, M. Doumith<sup>c</sup>, C. P. Thomas<sup>d</sup>, D. M. Livermore<sup>c</sup>, N. Woodford<sup>c</sup> & D. W. Wareham<sup>a</sup>**

**<sup>a</sup>Barts and The London School of Medicine and Dentistry, London, UK; <sup>b</sup>Health Protection Agency, Addenbrooke's Hospital, Cambridge, UK; <sup>c</sup>Health Protection Agency, Centre for Infections, London, UK; <sup>d</sup>Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, UK**

*Acinetobacter baumannii* is an important nosocomial pathogen that is of increasing concern owing to the many isolates that are resistant to nearly all available antimicrobials. Tigecycline is one of the few agents usually to remain active against otherwise multiresistant *A. baumannii* although resistance has been described; associated with up-regulation of the RND efflux transporter AdeABC. We used a pre- and post-tigecycline treatment pair of clinical isolates and a laboratory-selected mutant to investigate the emergence of tigecycline resistance in the widespread, multidrug-resistant UK lineage OXA-23 clone 1. The resistant clinical isolate had been selected during on-label usage for an abdominal drain infection. We further assessed the fitness cost of the resistance under normal and stressed laboratory growth conditions.

Isolates were identified by API20NE and species-specific PCR. MICs were determined by BSAC agar dilution and Etest on IsoSensitest agar. PFGE was used to confirm relatedness. Expression of *adeABC* was examined by real-time RT-PCR using primers specific for *adeB*, and quantified relative to that of the RNA polymerase beta subunit gene, *rpoB*. AdeABC was inactivated using a suicide plasmid containing an internal fragment of *adeB*. The fitness cost of tigecycline resistance was determined in LB broth using a microtitre plate-based growth kinetics assay. The influence of stress was investigated by growing the isolates in (i) LB at pH 4.5, (ii) in LB supplemented with 200 mM NaCl or (iii) in one-third strength LB diluted in saline.

Real time RT-PCR identified mean 50- and 407-fold increases in *adeABC* transcript in the post-therapy isolate (tigecycline MIC, 16 mg/L) and laboratory mutant (MIC, 64 mg/L), respectively, compared with the pre-therapy isolate (MIC, 0.5 mg/L). Inactivation of *adeB* in the post-therapy isolate restored full susceptibility to tigecycline. The post-therapy isolate was less fit than its pre-therapy counterpart under all conditions tested including unstressed growth in full strength LB. The laboratory mutant was even less fit than the post-therapy clinical isolate, again under all conditions whilst the *adeB* knockout mutant was less fit under all conditions than its parent clinical isolate.

AdeABC-mediated tigecycline resistance can be selected during tigecycline therapy in the important UK lineage OXA-23 clone 1, even with the drug used in a licensed indication. There is an *in vitro* fitness cost associated with both up-regulation and loss of AdeABC in *A. baumannii*. The *in vivo* fitness cost is now being assessed in an animal model of infection.

#### **P44 BACTERICIDAL ACTIVITY OF DORIPENEM AGAINST CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII***

**M. L. Mezzatesta, F. Gona, D. Sciortino, V. Petrolito, R. Scollo, C. Caio & S. Stefani**

**Department of Microbiology, University of Catania, Italy**

Doripenem, a  $\beta$ -methylocarbapenem, is active against non-fermentative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* that often occur in severely debilitated patients and may be associated with poor clinical outcomes; moreover, these organisms have a significant capacity to develop resistance. Doripenem seems less prone to select spontaneous resistant mutants of *P. aeruginosa* than other carbapenems do. The current study examines the activity of doripenem and selected comparator compounds against 82 carbapenem-resistant *A. baumannii* isolated from severe infections. Antibiotic susceptibility was determined by standard broth microdilution method following CLSI guidelines against the following antibiotics: doripenem, imipenem, meropenem, ceftazidime, cefepime, aztreonam, piperacillin/tazobactam, and ciprofloxacin. MBC for doripenem was performed on all strains following standard methods. Quality controls were performed using *E. coli* ATCC 25922. The carbapenem-resistant *A. baumannii* were characterized for the presence of genes encoding MBLs (*bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub>) and oxacillinases (*bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-51-like</sub> and *bla*<sub>OXA-58-like</sub>) using PCR.

The vast majority of strains of *A. baumannii* showed a multi-drug resistance phenotype (MDR) and more than 90% of strains were simultaneously resistant to ceftazidime, cefepime, aztreonam and ciprofloxacin, around 70% of strains are also resistant to gentamicin, amikacin and piperacillin/tazobactam, while tigecycline remains very active (4%). Carbapenem-resistance, in these strains is due to the presence of different oxacillinases, 58 and 23: the OXA-58 carried in all strains and the OXA-23 was associated in 18 strains. The activity of doripenem was superior to imipenem and meropenem for all strains tested with MIC<sub>90</sub> 16, 128 and >128 mg/l respectively. In particular, we obtained different MIC<sub>90</sub> values of doripenem between strains expressing the *bla*<sub>OXA-58</sub> (4 mg/l) and *bla*<sub>OXA-23</sub> (16 mg/l). The results of the bactericidal activity studies demonstrated that doripenem is an antibiotic with MBC values corresponding to MIC values in most strains, and in 4 cases it was superior by only 1 dilution.

Based on the activity profile presented in this study, doripenem appears to be a promising new agent for the treatment of severe infections caused by carbapenem-resistant *A. baumannii* and it is important to continue to monitor the activity of this antibiotic throughout its clinical development and after its introduction into clinical use.

**P45 THE ROLE OF NON- $\beta$ -LACTAMASE MECHANISMS OF CARBAPENEM RESISTANCE IN *ACINETOBACTER* SPECIES ISOLATED FROM AN IRISH UNIVERSITY HOSPITAL**

**M. P. McCusker<sup>a</sup>, T. W. Boo<sup>b</sup>, M. Martins<sup>a</sup>, B. Crowley<sup>c</sup> & S. Fanning<sup>a</sup>**

**<sup>a</sup>Centre for Food Safety and Food-borne Zoonomics, UCD Veterinary Sciences Centre, University College Dublin, Belfield, Dublin 4, Ireland; <sup>b</sup>National Salmonella Reference Laboratory (NSRL), Clinical Science Institute, National University of Ireland, Galway, Ireland; <sup>c</sup>Department of Clinical Microbiology, Trinity College, University of Dublin, James's Street, Dublin 8, Ireland**

Carbapenem resistance in *Acinetobacter* has been increasingly reported worldwide, attributable mainly to Ambler class D carbapenemases and class B metallo- $\beta$ -lactamases (MBLs). In a previous study to investigate the prevalence of carbapenem resistance in *Acinetobacter* species, encountered in St James's Hospital, Dublin, Ireland, carbapenem resistance was shown to be mediated by Ambler class D  $\beta$ -lactamase OXA-23 in all resistant isolates. Additional mechanisms implicated in reduced meropenem susceptibility among *A. baumannii* include efflux pump (EP) activity. Here we investigate the potential role of efflux in carbapenem resistance in *A. baumannii* and *Acinetobacter* genomic species 3 (AGS3).

Imipenem and meropenem susceptibility testing was performed using E-test gradient and the microbroth dilution method in the absence and presence of the efflux pump inhibitor (EPI) Phe-Arg-beta-naphthylamide (PAN). To assess EP activity, the Ethidium Bromide (EB)-agar cartwheel method was used. Gene expression analysis was determined for the two known RND efflux pumps in each species as well as for the *bla*<sub>OXA-23</sub> gene by RT-qPCR analysis. This was done in the absence and presence of sub-MIC concentrations of meropenem. Outer membrane protein (OMP) profiles and sequencing of the *A. baumannii carO* gene was also carried out.

The resistant isolates showed a high level resistance (>32 mg/L) to both imipenem and meropenem. PAN lowered the MICs of meropenem by 8-fold but not imipenem. Both sensitive and resistant strains accumulated EB indicating a lack of EP activity. Real-time PCR experiments showed that expression levels of the EP genes was a maximum of 3-fold higher in resistant strains in the presence of meropenem. Expression of the *bla*<sub>OXA-23</sub> gene did not change in the presence of meropenem. In carbapenem resistant *A. baumannii*, expression of a 29 kDa OMP consistent with CarO was absent, relative to ATCC 19606. No other differences in the *A. baumannii* OMP profiles were observed. PCR and sequencing of *carO* gene showed no mutations or insertion sequences within the *carO* gene. No significant differences in OMP profiles of the AGS3 isolates were identified.

These findings indicate that overexpression of the EP does not play a significant role in carbapenem resistance in our *Acinetobacter* strains. It also suggests that the chromosome-borne OXA-23 is the main resistance mechanism to carbapenems.

**P46 ACINETOBACTER BAUMANNII: AN EVALUATION OF FIVE SUSCEPTIBILITY TEST METHODS TO DETECT TOBRAMYCIN RESISTANCE IN AN EPIDEMIOLOGICALLY RELATED CLUSTER**

**V. Mischka Moodley<sup>a,b</sup>, Stephen Oliver<sup>a,b</sup>, Iva Shankland<sup>b</sup> & B. Gay Elisha<sup>a,b</sup>**

**<sup>a</sup>Department of Medical Microbiology, University of Cape Town, South Africa; <sup>b</sup>National Health Laboratory Services, Western Cape, South Africa**

*Acinetobacter baumannii* is a major pathogen causing nosocomial infections, particularly in critically ill patients. Further, this organism has acquired the propensity to rapidly develop resistance to most antibiotics. At several academic hospitals within Cape Town, tobramycin and colistin remain frequently the only therapeutic options. The Vitek2 automated susceptibility testing (AST) is used in the clinical laboratory to determine selected susceptibility profiles. The suspicion of a possible AST-related technical error when testing for susceptibility to tobramycin in *A. baumannii* precipitated this study.

Forty *A. baumannii* strains obtained from clinical specimens (June-December 2006) were included in this prospective study. The strains selected exhibited MICs close to the tobramycin breakpoints as outlined by the CLSI. The clinical isolates were predominately from intensive care unit patients (33/40) obtained from various sites of infection. All duplicate specimens were excluded. AST was compared to disk diffusion, Epsilometer test and agar dilution using broth microdilution as the reference standard. Additionally, PCR was performed to detect *aac(3)-II'* gene which encodes an aminoglycoside modifying enzyme with activity against tobramycin. Molecular tools were utilised to study the relatedness of the isolates.

The identification of all isolates was confirmed twice by the Vitek2 system. The tobramycin susceptibility results revealed errors in 25/39 isolates (10 very major and 15 minor errors) when AST was compared to broth microdilution (reference standard), 12/39 (1 very major and 11 minor errors) when Etest was compared to the reference standard, and 15 errors (3 very major and 12 minor errors) when disc diffusion was compared to the reference standard. The result for AST vs. the reference standard was statistically significant ( $p < 0.001$ ). Additionally, the tobramycin resistance gene, *aac(3)-II'*, was detected in 21/25 of the discrepant isolates, confirming the resistant phenotype detected by the reference standard. It is possible that the catalytic activity of the enzyme is too slow to allow accurate detection by the Vitek2 system. Molecular typing showed that these isolates were genetically related.

In conclusion, the Vitek2 automated tobramycin susceptibility testing should not be recommended for *Acinetobacter baumannii*.

## P47 CARBAPENEM HETERO-RESISTANCE IN *ACINETOBACTER BAUMANNII*: A COMPARISON BETWEEN ETEST AND VITEK 2

P. G. Higgins & H. Seifert

Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Goldenfelsstrasse 19 – 21, 50935 Cologne, Germany

Carbapenem hetero-resistance is a phenomenon that is best described as a resistant subpopulation within a susceptible population. By Etest or disc diffusion, this is seen as a clear ellipse or zone diameter, within which there are individual resistant colonies. Thus although the majority of the culture can be considered susceptible, the isolate is recorded as resistant. We have previously observed failure of Vitek 2 to identify *Acinetobacter baumannii* carbapenem-resistance in isolates that by Etest were hetero-resistant. In the present study, we have investigated this phenomenon against 94 non-duplicate carbapenem-resistant *A. baumannii* isolates with an acquired OXA-carbapenemase or IS*Aba1* associated with *bla*<sub>OXA-51</sub>, comparing susceptibility to imipenem and meropenem by Etest and Vitek 2, and recording hetero-resistance. Carbapenem-resistance was defined as an MIC  $\geq$ 16 mg/L for imipenem and/or meropenem.

Results are summarised in the table. Imipenem and/or meropenem hetero-resistance was observed for the majority of isolates (82 and 86, respectively). Hetero-resistance was not associated with a particular mechanism conferring resistance to carbapenems. Over 50% of isolates were falsely recorded as susceptible to imipenem, meropenem or both by Vitek 2, potentially leading to inappropriate therapy. All these isolates were hetero-resistant. Two isolates were imipenem-resistant by Vitek 2 but imipenem-susceptible by Etest. Given that over half the isolates were falsely recorded as susceptible by Vitek 2, these data suggest that to prevent inappropriate use of carbapenems, alternative methods may be considered to perform carbapenem-sensitivity testing with *A. baumannii*.

No. Isolates	Imipenem		Meropenem	
	Etest	Vitek 2	Etest	Vitek 2
24	R	S	R	S
7	R	S	R	R
2	R	S	S	S
7	S	S	R	S
10	R	R	R	S
2	S	S	R	R
40	R	R	R	R
1	S	R	R	S
1	S	R	R	R

**P48 INTEGRON-ENCODED AND CARBAPENEM-HYDROLYSING GES-TYPE EXTENDED SPECTRUM  $\beta$ -LACTAMASE IN *ACINETOBACTER BAUMANNII***

**P. D. Mugnier<sup>a</sup>, L. Poirel<sup>a</sup>, R. Bonnin<sup>a</sup>, H. Lecuyer<sup>b</sup>, J. R. Zahar<sup>b</sup> & P. Nordmann<sup>a</sup>**

<sup>a</sup>Service de Bactériologie-Virologie, INSERM U914 "Emerging Resistance to Antibiotics", Faculté de médecine and Université Paris-Sud, Hôpital de Bicêtre, 94275 Kremlin-Bicêtre;

<sup>b</sup>Service de Bactériologie, Hôpital Necker, 75015 Paris, France

The aim of this study was to characterize the resistance mechanism(s) of a carbapenem-resistant *A. baumannii* isolate. MICs were determined by agar dilution and E-test techniques. PCR and sequencing were used to characterize the  $\beta$ -lactamase. The  $\beta$ -lactamase gene was cloned in pTOPO vector and expressed in *E. coli* TOP10. The  $\beta$ -lactamase was purified and its  $K_m$  and  $k_{cat}$  values for  $\beta$ -lactams were determined by UV spectrophotometry.

A carbapenem-resistant *A. baumannii* isolate (strain AP) was recovered in January 2010 from a broncheal sample of a patient hospitalized in an intensive care unit. *A. baumannii* strain AP was resistant to broad-spectrum cephalosporins and double disk synergy test revealed the possibilities of expression of an extended-spectrum  $\beta$ -lactamase (ESBL). PCR and sequencing identified ESBL GES-14 which differs from GES-1 by two amino acid substitutions, gly-170-ser and gly-243-ala. The  $bla_{GES-14}$  gene was located inside a class 1 integron structure located on a 95-kb self-transferable plasmid. Cloning of the  $bla_{GES-14}$  gene followed by expression into *E. coli* TOP10 showed that GES-14 hydrolysed cephalosporins, aztreonam and imipenem. Kinetic measurements using a purified GES-14 showed increased catalytic efficiency against cephalosporins and imipenem compared to the other GES variants (GES-1). The catalytic efficiencies ( $k_{cat}/K_m$ ) of this GES variant were increased for cephalosporins and imipenem.

The class A carbapenemase GES-14 is the first ESBL that include cephalosporins, cephamycins, monobactams and carbapenems in its hydrolytic spectrum that is identified in *A. baumannii*. It may contribute to carbapenem resistance in that species.

**P49 PLASMID-ENCODED RESISTANCE TO PENICILLINS IN *ACINETOBACTER BAUMANNII* BL54 MEDIATED BY CARBENICILLIN-HYDROLYSING (CARB) TYPE OF  $\beta$ -LACTAMASE**

**M. R. Shakibaie**

**Department of Microbiology, Kerman University of Medical Sciences, Kerman, Iran**

*Acinetobacter baumannii* strains occupy an increasingly important position as opportunistic pathogens in the hospital environment, and one particular attribute of these strains is resistance to multiple antibiotics including  $\beta$ -lactams. The aim of this study was to find out the mechanism of resistance of *A. baumannii* BL54 to different penicillins.

*A. baumannii* BL54 was isolated from hospital. The identification of the isolate was confirmed by chromosomal transformation assay and by biochemical tests. Preliminary sensitivity test was carried out by disk diffusion break point assay and MIC to different antibiotics was performed by agar dilution method.  $\beta$ -lactamase test for the above strain was carried out by Macro-Iodometric test. The enzyme was isolated and observed on polyacrylamide gel electrophoresis. Substrate hydrolysis in presence of inhibitors; sulbactam, para-chloromercuribenzoate (p-CMB) clavulanic acid, cloxacillin and NaCl was carried out by rapid fixed time method. Plasmid was isolated from the BL54 strain by the alkaline lysis technique and observed in 0.75% agarose gel.

*A. baumannii* BL54 was resistant to various penicillins. The plasmid isolation revealed a 32kb plasmid pUPI 276 carrying genes for Ag resistance as well as different antibiotics.  $\beta$ -lactamase test was positive for this bacterium while negative for plasmid-cured derivatives. The rate of substrate hydrolysis in the presence and absence of inhibitors revealed that the highest rate of hydrolysis was when carbenicillin was used as a substrate (116  $\mu$ mol), while the lowest rate of hydrolysis was achieved when cloxacillin was used as a substrate (50.2  $\mu$ mol). The rate of substrate hydrolysis was adversely affected in presence of sulbactam and NaCl. However, the hydrolysis was not affected by p-CMB or cloxacillin.

From the above results it can be concluded that *A. baumannii* BL54 produced an extended spectrum CARB type  $\beta$ -lactamase. The gene responsible for production of the enzyme was present on plasmid pUPI276.

**P50 CROSS-REGULATION IN THE  $\beta$ -KETOADIPATE PATHWAY OF ACINETOBACTER BAYLYI IS MEDIATED BY TWO Lys-R-TYPE TRANSCRIPTIONAL REGULATORS, BenM AND CatM**

**F. S. Bleichrodt<sup>a</sup>, E. L. Neidle<sup>b</sup> & U. C. Gerischer<sup>c</sup>**

**<sup>a</sup>Institute of Microbiology & Biotechnology, University of Ulm, Germany; <sup>b</sup>Microbiology Department, University of Georgia, Athens, GA, USA; <sup>c</sup>Max Planck Institute for Biophysical Chemistry, Theoretical and Computational Biophysics Department, Göttingen, Germany**

The soil bacterium *Acinetobacter baylyi* degrades aromatic compounds presented in a carbon source mixture in a chronological order. This regulated hierarchy occurs in metabolic pathways being part of the  $\beta$ -ketoadipate pathway or leading to it (funneling pathways). In this branched pathway, diverse aromatic compounds are converted to catechol or protocatechuate, substrates of ring-cleaving enzymes. Then, parallel routes feed the metabolites into the tricarboxylic acid cycle. Here we studied the degradation of compounds by the protocatechuate branch of the pathway in the presence of benzoate, a growth substrate of the catechol branch known to repress numerous parts of the pathway at the transcriptional level.

Fusions to the *Photinus pyralis luc* gene were used to assess the expression of genes needed to degrade vanillate (*van*), hydroxycinnamates (*hca*), and dicarboxylates (*dca*). Whereas vanillate and hydroxycinnamates (such as caffeate, chlorogenate, coumarate and ferulate) are degraded via protocatechuate, the dicarboxylates are degraded through  $\beta$ -ketoadipyl-CoA but do not form catechol or protocatechuate as intermediates. Benzoate was found to prevent the *van*, *hca*, and *dca* genes from being induced by their cognate transcriptional regulators and inducers. These observations suggested possible roles for LysR-type regulators known to activate genes for benzoate degradation. To explore this possibility, their genes, *benM* and *catM*, were inactivated in strains with *luc*-gene reporter fusions. Without BenM and CatM, benzoate failed to prevent induction of the *van* or *hca* genes. In contrast, inhibition of the *dca* genes remained. The direct repression by BenM and CatM of the *hca* and *van* genes, but not the *dca* genes, was confirmed by studying protein-DNA interactions. Electrophoretic mobility shift assays demonstrated that purified BenM and CatM bind to sites that appear to control the expression of *van* and *hca* genes. The respective DNA regions contained potential LysR-type binding sites which are therefore suggested as a part of the cross-regulation system between the branches of the  $\beta$ -ketoadipate pathway in *A. baylyi*.

## **P51 ADAPTATION OF *ACINETOBACTER* TO SALINE AND DRY ENVIRONMENTS**

**Miriam Sand, Beate Averhoff & Volker Müller**

**Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt/Main, Germany**

*Acinetobacter* is known for its metabolic versatility that includes the ability to use different carbon and energy sources for growth. However, little is known about the adaptation of *Acinetobacter* to dry environments like the human skin which is of particular importance for the pathogen *A. baumannii*. To test whether or not *Acinetobacter* is able to adapt to dry environments, growth experiments were performed using the non-pathogen *Acinetobacter baylyi* ADP1, which exhibits a growth phase dependent natural transformation phenotype, as a model. When grown in complex media, *A. baylyi* was able to adapt to increasing salinities (NaCl) up to an upper limit of 900 mM NaCl. The increase in salinity leads to an increase of the lag phase and a decrease in growth rate as well as cell yield. Interestingly, KCl was tolerated much better, indicating that cellular Na<sup>+</sup> homeostasis is involved in adaptation to high NaCl concentrations. To analyze the adaptation to low water activities, growth in the presence of sugars was monitored. Again, cells were able to adapt to 900 mM sucrose indicating the general capability of ADP1 to cope with low water activities. Analyses of the natural transformation phenotype during growth in the presence of high salt concentrations revealed 100fold increased natural transformation frequencies throughout the prolonged lag phase in the presence of 900 mM NaCl.

The molecular basis of adaptation to low water activities was first evaluated by genome analyses. ADP1 has the ability to take up glycine betaine from the medium and has a pathway to take up choline from the medium and oxidize it to glycine betaine. In the absence of exogenous glycine betaine or choline, ADP1 is also able to adapt to high salinity, but the maximal concentrations tolerated are much less. Currently, we are trying to stimulate growth under these conditions by addition of glycine betaine or choline. Furthermore, mutant studies will give clues to which enzymes/pathways are involved in the adaptation process.

## **P52 IN VIVO LOCALIZATION OF THE DNA TRANSLOCATOR IN ACINETOBACTER BAYLYI ADP1**

**Miriam Sand, Anna Desch & Beate Averhoff**

**Molecular Microbiology & Bioenergetics, Institute of Molecular Biosciences, Goethe University Frankfurt/Main, Germany**

*Acinetobacter baylyi* ADP1 is a Gram-negative soil bacterium which is ubiquitous in nature and notable for its high competence of natural transformation. Gene disruption and mutant studies led to the identification of 16 distinct genes essential for natural transformation in *Acinetobacter baylyi* ADP1 (1). The conserved proteins of the *Acinetobacter* DNA translocator can be assigned to two distinct groups: I. type IV pili (tfp)- and type II protein transport-related proteins (pseudopilins, prepilin-processing leader peptidases, secretins, biogenesis factors) and II. widely conserved proteins in DNA transport systems (polytopic inner membrane proteins, DNA-binding proteins). Despite the similarities tfp and natural transformation were found to be functionally unrelated in *A. baylyi*.

To get insights into the function of the conserved competence protein ComEA the subcellular localization and DNA binding of ComEA was analyzed. These studies revealed that ComEA is an outer membrane protein with DNA binding activity. Furthermore we have addressed the *in vivo* localization of ComEA by generating functional ComEA-mCherry fusions. Expression of these fusions in *A. baylyi* under the control of the *comEA* promoter led to the detection of ComEA in the cell periphery. Further studies of the ComEA *in vivo* localization revealed that ComEA is present in nonpolar foci or equally distributed in the cell periphery in dependence of growth phase, growth conditions and natural transformation frequencies.

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**P53 ALKANES ACTIVATION BY *ACINETOBACTER RADIORESISTENS* S13 HYDROXYLASE: FIRST STEPS FOR OMEGA-OXIDATION OF C9-C18 FATTY ACIDS TO BE CONVERTED BIODEGRADABLE POLYMERS.**

**M. Zapponi, P. Fattori, C. Nogarol, M. Riva Violetta, A. Nastro, E. Pessione & C. Giunta**

**Università degli Studi di Torino, Lab. Proteomica e Biochimica dei Microrganismi, DBAU, Torino, Italy**

Biodegradable polymers can find applications in several commercial products, so-called “environmental friendly”. Different bacterial species can synthesize, as storage material, bio-compatible plastics, for instance poly-hydroxyalkanoates (PHA), or be the source of lactic acid (LAB) for the organic synthesis of PLA (polylactides).

In the present research we try to obtain biodegradable polymers from vegetable oils, low-cost industrial by-products that constitute a renewable source of fatty acids (FA). The limiting step of FA polycondensation is the lack of a second carboxyl or hydroxyl function in a terminal (omega) or subterminal (-1) position. We employed an aromatic-degrading *Acinetobacter radioresistens* S13 strain whose hydroxylating ability, via both mono- and di- oxygenases, has been previously proved (Pessione *et al.*, Eur. J. Bioch., 2003). The growth of *A. radioresistens* S13, that is also a good surfactant producer, was firstly tested on several aliphatic hydrocarbons (nonane, decane, dodecane, hexadecane, octadecane) as the sole carbon source with and without detergent supplementation. The strain proved to be able to metabolize them, with growth different kinetics, suggesting the presence of an omega-oxidative enzyme system. We also tested the toxicity of several C6-C18 fatty acids. Experiments are underway to inhibit catalitically or by gene deletion omega-oxidative enzymes in order to obtain omega-hydroxy and omega-carboxy fatty acid by co-metabolism with a second low-cost carbon source.

## **P54 CO-CULTURE OF TWO MODEL ORGANISMS – TOWARDS UNDERSTANDING MICROBIAL CONSORTIA *IN SILICO***

**S. Myllyntausta, A. Larjo, T. Aho, M. Karp & V. Santala**

**Tampere University of Technology, Tampere, Finland**

*Acinetobacter baylyi* is a Gram-negative, non-pathogenic, mesophilic, strictly aerobic and widely spread soil bacterium. The strain ADP1 has awakened a lot of interest among scientists due to its high potential for metabolic studies and biotechnological applications, mainly for its compact easily-transformable genome and an interesting metabolic network. The genome of ADP1 has been sequenced, revealing several similarities to the genome of *Escherichia coli*, thus providing possibility to exploit the knowledge applied to *E. coli* so far. As well as for *E. coli*, the genome sequence of ADP1 has provided bioinformatic backbone for construction of a constraint-based metabolic model and a single gene knock-out mutant library [1, 2].

In a microbial community bacteria cooperate producing metabolites which serve as substrates to others. Metabolic pathways and reactions of a single model organism can be predicted by *in silico* models, but there is still a long way towards modeling a whole microbial community by computational means. However, the simplest microbial community – co-culture of two cooperative model organisms – can be simulated. In this study, the metabolic networks of two well-known model organisms are connected and integrated, providing interesting aspects to bacteria co-culturing.

The wet-lab experiments exploit the knowledge of glucose metabolism of *E. coli* and ADP1; the knock-out mutants which are unable to grow on glucose or alternatively on acetate serve as an excellent controls for data handling. As the data is collected from few different culture combinations at different time points, the carbon flux of the two strains in the same culture can be calculated. Reporter constructs expressed in the strains are used for monitoring the culture dynamics, and metabolic end-product analysis is carried out to get qualitative and quantitative data of the catabolic reactions in the co-culture. The possibility to model more than one metabolic network at the same time would have a great significance in any biotechnological application exploiting microbial co-cultures.

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## **P55 REGULATION OF AN *ACINETOBACTER BAYLYI* DNA DAMAGE RESPONSE GENE**

**Zhaoying Xu, Nathan Arnone, Laura Cuff, Ruth Emrick & Leslie A. Gregg-Jolly**

**Department of Biology, Grinnell College, Grinnell, Iowa, USA**

The *Acinetobacter* response to DNA damage has not been well studied. An unusual DNA damage response gene (*ddrR*) has been identified in *Acinetobacter baylyi* that apparently only shares homology with sequences in *Acinetobacter baumannii*. Compared to wild type, *A. baylyi ddrR* mutants are sensitive to DNA damage and impaired for growth. The structure of *ddrR* is unusual and suggests the possibility that RNA is fully or partially the functional product of the gene. Previous work using a strain carrying a *ddrR::lacZ* reporter fusion (AGC14) demonstrated that *ddrR* is upregulated in response to DNA damage in a manner that is fully dependent on RecA and partially dependent on UmuD (Hare, Perkins and Gregg-Jolly. 2006. Appl. Environ. Microbiol. **72**: 4036-4043). Usually RecA transcriptional regulation of DNA damage response genes occurs indirectly through cleavage of the LexA repressor in response to DNA damage. However, LexA is not found in *A. baylyi* so we must look further to understand the mechanism of *ddrR* regulation.

In order to identify other genes involved in regulation of *ddrR*, *A. baylyi* strain AGC14 was mutagenized with EMS. Using media containing the color indicator X-gal, survivors were screened for aberrant patterns of *ddrR* expression in the presence and absence of the DNA damaging agent MMC. A strain designated AGC28 was isolated that exhibits relatively high levels of *ddrR* expression in the absence of DNA damage. Genetic crosses indicate that the AGC28 mutation is not closely linked to *ddrR* nor *umuD*. Transformation of AGC28 with *A. baylyi* fragments ligated to a gene fragment conferring ampicillin resistance yielded recombinants (two out of 600 screened) that restored the AGC14 phenotype. This indicates that the AGC28 mutation is recessive.

Levels of *ddrR* expression were measured using qPCR. In wild type cells, *ddrR* was expressed at a level 6.25 times greater in the presence of DNA damage. In cells grown in the absence of MMC, the expression of *ddrR* in AGC28 was higher than in wild type cells and in the AGC14 parent. Nonetheless, *ddrR* expression was further increased in AGC28 in the presence of DNA damage. The increased levels of *ddrR* expression in the AGC28 background do not appear to reflect increased levels of gene expression generally since expression of *benA*, a gene unrelated to the response to DNA damage, was not increased. These results indicate that the AGC28 mutation could affect a gene that downregulates *ddrR*. Furthermore, since *ddrR* is still upregulated in response to DNA damage, the AGC28 mutation does not seem to function as a key regulator responsive to DNA damage, as *lexA* does in other organisms. Further study of the nature of the AGC28 mutation and *ddrR* regulation will increase our understanding of the unusual mechanism underlying the *A. baylyi* response to DNA damage.

**P56 SEXUAL ISOLATION IN MUTATOR AND NON-MUTATOR POPULATIONS OF ACINETOBACTER BAYLYI ADP1**

**M. Al-Haroni<sup>a</sup> & K. M. Nielsen<sup>a,b</sup>**

<sup>a</sup>Department of Pharmacy, University of Tromsø, N-9037 Tromsø, Norway; <sup>b</sup>GenØk-Centre for Biosafety, Science Park, N-9294 Tromsø, Norway

It has been shown that sexual isolation in *Acinetobacter baylyi* is locus specific and varies 10,000-fold over the genome in a wild type population of *A. baylyi* ADP1. In the present study, the sexual isolation of mutator (*mutS*) and non-mutator populations of *A. baylyi* ADP1 were compared.

A kanamycin resistance marker, i.e. *nptI*, was inserted at random chromosome locations in *Acinetobacter baumannii* SDF strains. DNA from 6-tagged *A. baumannii* strains was used to transform the recipient mutator and non-mutator populations of *A. baylyi* ADP1 using liquid transformation assay.

Transformation frequencies ranged from  $6 \times 10^{-9}$  to  $3 \times 10^{-7}$  in a non-mutator population depending on *nptI* insertion site, i.e. locus specific, whereas those of the mutator population the transformation frequencies were not locus specific and were 7-480 folds higher than that of non-mutator population.

Our data suggests that the mismatch repair system has a profound effect on heterogamic recombination in *A. baylyi*.

## **P57 ACINETOBACTER BAYLYI GENES INDUCED BY STARVATION DURING LONG-TERM STATIONARY PHASE**

**C. P. Lostroh<sup>a</sup> & B. A. Voyles<sup>b</sup>**

**<sup>a</sup>Colorado College, Colorado Springs, USA; <sup>b</sup>Grinnell College, Grinnell, USA**

As soil organisms, *Acinetobacter* encounter cycles of feast and famine in nature. Starvation during long-term stationary phase (LTSP) can serve as a laboratory model for natural competitive conditions such as those found in soils. We have reported the isolation of 30 *lacZ* fusion strains of *A. baylyi* ADP1 that are highly expressed during starvation compared with much lower expression during exponential growth. Six of these strains (20%) have *lacZ* fusions to genes annotated as conserved hypothetical proteins (CHPs).

We are now examining the reporter strains and their corresponding knock-out strains, with a focus on strains AGCV8 (*CHP0615::lacZ*, now known as *AsiB::lacZ*), AGCV21 (*lacZ* fusion inserted backwards in *CHP2746*), AGCV24 (*almA::lacZ*), and AGCV28 (*gshA::lacZ*). We determined the percent survival at 24 hours (before the onset of starvation) and at 7 days (after 6 days of starvation) of knock-out strains *asiB::kan*, *ACIAD2746-R::kan*, *almA::kan*, and *gshA::kan* compared with controls. We have also exposed these strains to UV and heat shock stresses and determined their survival compared with control strains.

We conclude that the four *asi* genes (*asiB*, *ACIAD2746-R*, *almA*, and *gshA*) are required for persistence under starvation conditions, and are also important for surviving other stressors.

**P58 RIFAMPICIN IN COMBINED THERAPY IN THE TREATMENT OF NOSOCOMIAL INFECTIONS DUE TO CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII**

**E. García-Cabrera<sup>a</sup>, A. Martín-Peña<sup>a</sup>, A. Gutiérrez-Pizarra<sup>b</sup>, M. E. Jimenez-Mejías<sup>a,b</sup>, J. Palomino-Nicas<sup>a, b</sup>, P. Irarurgi Alcarazo<sup>b</sup>, M. V. Gil-Navarro<sup>c</sup>, J. M. Cisneros<sup>a, b</sup> & J. Pachón<sup>a,b</sup>**

**<sup>a</sup>Spanish Network for Research in Infectious Disease (REIPI); <sup>b</sup>Clinical Unit of Infectious Diseases, Microbiology and Preventive Medicine, <sup>c</sup>Pharmacy Service, University Hospital Virgen del Rocío, Seville, Spain**

Carbapenem-resistant *Acinetobacter baumannii* is resistant to multiple antimicrobial agents and may be considered as a paradigm for nosocomial multiresistant bacteria. Because of it, in many cases, optimal treatment for *A. baumannii* nosocomial infections is not available. This is the reason why new therapeutic strategies are needed. The aim of this study is add knowledge about the clinical and microbiological effectiveness, monitoring of side effects and development of resistance to rifampin in combination, with tigecycline and minocycline, combinations that in our knowledge have not been previously assessed, and bring out our experience in combination with colistin.

Interventional and uncontrolled case series conducted as a pilot study in a comparative fashion performed in a tertiary university hospital (1250 beds) since October 2008 to October 2009. Patients suffering from serious nosocomial infections due to carbapenem-resistant *Acinetobacter baumannii* isolates. Patients were treated with rifampin in combination with tigecycline, minocycline or colistin.

Forty-seven patients that received combined therapy with rifampin were identified and 18 of them (38%) were excluded for different reasons. The overall clinical cure/improvement or rifampin combination treatment was 19 patients (65%), rifampin plus tigecycline combination show best results with 7/9 (77%) of global rate of cure/improvement, the second more effective was rifampin plus minocycline with a global rate of cure/improvement 6/9 (66%) followed by rifampin plus colistin with 6/11 (55%). No side effects led to discontinue the combined treatments. The global microbiological eradication and resistance development rates were 69% and 14% respectively. The overall mortality rate was 10%, three patients, and only one death was considered related to carbapenem-resistant *A. baumannii* infection.

New alternatives of combined antimicrobial therapy with rifampin should be take in account for the treatment of severe carbapenem-resistant *Acinetobacter baumannii* nosocomial infections.

**P59 INFLUENCE OF THE PRESCRIBED ANTIBIOTIC IN THE MORTALITY AND REINFECTION IN *ACINETOBACTER BAUMANNII* INFECTED PATIENTS.****F. García-Colchero, A. Martín-Aspas, F. Guerrero-Sánchez, S. Rodríguez-Roca & J. A. Girón-González****Internal Medicine Department, University Hospital Puerta del Mar, Cádiz, Spain**

The aim of this study was to analyze the antibiotic use, adverse events and rate of re-infection and mortality in patients treated for a first episode of *A. baumannii*. We performed a retrospective analysis of nosocomial isolates during a six month period in a tertiary hospital. Cases of infection were defined by CDC criteria. Co-morbidities, risk factors, antibiotic used and side effects, rate of re-infection and mortality were analysed.

Twenty nine (29) cases (82.8% male, mean age  $56.3 \pm 18.9$  years) of *A. baumannii* infection were analyzed. Underlying conditions were detected in 69.0% of them. McCabe' score no fatal was observed in 79.3%, ultimately fatal in 20.7% and rapidly fatal in 0% of individuals. The median Charlson's index was 1.14. All had received previous antibiotics (carbapenems in 48.3% of cases). Microbiological samples proceeded from tracheal aspirate 79.3%, sputum 13.8%, blood and surgical wound 3.4%. Pneumonia was diagnosed in 51.7% of patients, purulent bronchitis in 41.4%, primary bacteremia and wound infection each in a 3.4%. 48.3% were on mechanical ventilation and 34.5% were tracheotomised. Sepsis was present in 86.2%, severe sepsis in 3.4% and septic shock in 10.3%. Most isolates were multidrug-resistant: resistance to aztreonam in 88.9% of isolates, to ceftazidime in 100.0%, to cefepime in 82.8%, to piperacilin-tazobactam in 96.4%, to carbapenem in 20.7%, to gentamicin in 87.7%; no isolate was resistant to colistin or tigecycline. There was no difference in the susceptibility pattern in function of the previous use of carbapenems. The prescribed antibiotics were: colistin in 23 (79,3 %) patients; carbapenem in 17 (58.6 %); tigecycline in 7 (24.1%); tobramycin in 2 (6.9%); amikacin and cefepime in 1 (3.5%) patient. Antibiotic monotherapy was prescribed in 10 patients (34.4%), two antibiotics were used in 14 patients (48.3%) and three antibiotics in 5 patients (17.2%). Only one patient presented hepatotoxicity related to tigecycline; none of the patients developed renal impairment. Six patients were reinfected: three of them by a *A. baumannii* isolate with a different susceptibility pattern, two by other pathogens and one patient by *A. baumannii* plus other pathogen. Six (20.7%) patients died, none of them with secondary bacteremia; all of them suffered from underlying conditions. There was no statistical difference in reinfection or mortality when compared antibiotic used or number of them.

Mortality or reinfection after treatment of a respiratory tract infection episode by *A. baumannii* is not influenced by the antibiotic used or the combination of them. The prescribed antibiotics show a low number of adverse effects even when used in combination.

**P60 CLINICAL, MICROBIOLOGICAL AND PROGNOSTIC DIFFERENCES BETWEEN PATIENTS WITH INFECTION OR COLONIZATION BY *ACINETOBACTER BAUMANNII***

**A. Martín-Aspas, F. Guerrero-Sánchez, F. García-Colchero, D. Gutierrez-Saborido & J. A. Girón-González**

**Internal Medicine Department, University Hospital Puerta del Mar, Cádiz, Spain**

When *Acinetobacter baumannii* infection is endemic in the hospital setting, clinical infection or colonization may depend on patient characteristics and the area of hospitalization where *Acinetobacter* is acquired: intensive care unit (ICU) or hospital ward (HW). The aim of the study is to know if these differences in patients could help to differentiate colonization from clinical infections as well as the evolution of infected or colonized individuals. We therefore performed a retrospective analysis of isolates during a six month period in a tertiary hospital. Cases of infection were defined by CDC criteria; otherwise were considered colonization. Hospital area of attendance, comorbidities, risk factors and mortality were analysed.

105 isolates from 54 patients were analyzed. Patients were considered to be infected (n = 29) or colonized (n = 25) attending to CDC criteria. Acquisition was nosocomial in all cases. There was no difference between both groups when age (56.3 vs. 65.7 years), presence of underlying conditions (69.0% vs. 90.9%) or McCabe score (no fatal: 79.3% vs. 50.0%; ultimately fatal 20.7 vs. 45.0%, rapidly fatal 0% vs. 5%) were considered. However, Charlson index was significantly lower in infected patients (1.14 vs. 2.77, p = 0.013). Only 2 out of 12 (16.6%) who had never been in ICU were infected (both died). The other infected patients were in ICU (62.5%) or had been previously admitted in ICU (31.0%) (p = 0.021), with no differences from colonized patients in both settings (40% vs. 20%). Most infected patients had received antibiotics (93.1%); a 48.3% was on mechanical ventilation and a 34.5% had been tracheotomised.

The majority of microbiological samples in infected and colonized patients were from sputum (13.8 and 20.0% respectively) and tracheal aspirates (79.3% and 44%); samples from catheter, urine and press ulcer or surgical wound were from colonized patients. Colonization isolates tended to be less resistant than infectious ones: resistance to aztreonam, 69.6 vs. 88.9%; to ceftazidime 88.0% vs. 100%; to cefepime 54.2% vs. 82.8%; to piperacilin-tazobactam 88.0% vs. 96.4%; to carbapenem 24.0% vs. 20.7%; to gentamicin 80.0% vs. 87.7%; 100% had susceptibility to colistin and tigecycline. However, no significant differences (p > 0.05 in each case) were obtained among isolates from colonized or infected patients. No differences in mortality were detected between colonized (32.0%) and infected patients (20.7%, 6 patients: 3 because of infection and 3 because of underlying disease) (p > 0.05).

The isolation of *A. baumannii* in patients attended in HW (especially if they had not previously attended at ICU), with an increased number of underlying diseases (Charlson index), as well as those samples not obtained from respiratory tract, probably represent colonization. Infection must be suspected in patients in ICU and in those whose isolates proceed from tracheal. No increase of mortality as a consequence of *Acinetobacter* infection is observed.

**P61 EFFECT OF THE INOCULUM SIZE ON THE ACTIVITY OF IMIPENEM (IMP) AND MEROPENEM (MPM) AGAINST *ACINETOBACTER BAUMANNII* (Ab) WITH PHENOTYPE OF HETEROGENEOUS RESISTANCE (PHR) TO CARBAPENEMS (CP)**

**Felipe Fernández Cuenca<sup>a</sup>, M<sup>a</sup> del Carmen Gómez Sánchez<sup>a</sup>, Francisco Javier Caballero<sup>a</sup>, Jordi Vila<sup>b</sup>, Luis Martínez-Martínez<sup>c</sup>, Germán Bou<sup>d</sup>, Jesús Rodríguez Baño<sup>a</sup> & Alvaro Pascual<sup>a</sup>**

**<sup>a</sup>Hospital Virgen Macarena, Sevilla, Spain; <sup>b</sup>Hospital Clinic, Barcelona, Spain; <sup>c</sup>Hospital Marqués de Valdecilla, Santander, Spain; <sup>d</sup>Hospital La Coruña, La Coruña, Spain**

This study aimed to evaluate the effect of the inoculum size in Ab with PHR to CP: i) on the activity of IMP and MPM and ii) on the visualization of growing colonies inside the zone of inhibition of discs with IMP or MPM (see below).

20 unrelated clones of Ab selected from the Ab GEIH-2000 project and the type strain Ab ATCC 19606 were included. The clones of Ab were selected accordingly to: i) the presence or not of PHR to CP, ii) the MICs of IMP, and iii) the types of oxacillinases (*bla<sub>OXA</sub>*). The PHR to CP was determined by disc diffusion (DD) in Mueller-Hinton agar using paper discs with IMP or MPM. The PHR to CP was defined by the presence of slow-growing colonies inside the inhibition halos. The presence of oxacillinases was determined by PCR using primers specific of the *bla<sub>OXA-51-like</sub>*, *bla<sub>OXA-58-like</sub>*, *bla<sub>OXA-23-like</sub>* and *bla<sub>OXA-24-like</sub>* subgroups. The antimicrobial activity of IMP and MPM was assayed by broth microdilution (MD) and by DD following the CLSI recommendations. The inocula evaluated were 10<sup>6</sup> cfu/mL (low inoculum) and 10<sup>8</sup> cfu/mL (high inoculum).

The clones of Ab were classified into 4 groups (I to IV). In comparison with the low inoculum, the high inoculum increased the MICs of IMP 4-8 folds (group I), 4 folds (group II), 2-8 folds (group III) and 2 folds (group IV). For MPM, the MICs increased 2-4 folds (group I), 4 folds (group II), 4-16 folds (group III) and 2-4 folds (group IV) using the high inoculum. By DD, the inhibition zones were reduced 0-3 (group I), 0-1 (group II), 0-2 (group III) and 0 (group IV) millimeters for IMP, using the high inoculum in comparison with the low inoculum. For MPM the inhibition zones were reduced 0-2 (group I), 0-4 (group II), 1-2 (group III) and 0 (group IV) millimeters using the high inoculum. The growth of colonies were not visualised inside the inhibition zones in 10 out of the 12 clones of Ab with PHR to CP (5 clones of group II and III, respectively) when the low inoculum was used instead of the high inoculum.

It was concluded that the inoculum size affects the antimicrobial activities of imipenem and meropenem, particularly when using microdilution, against clones of Ab with PHR to CP and clones without PHR to CP. The type of oxacillinase seems to be related with this inoculum effect: *bla<sub>OXA-51-like</sub>* was detected in all the clones of Ab and the type strain Ab ATCC 19606, whereas among clones PHR to CP (groups II and III) the inoculum effect was more evident in clones of group III which produced *bla<sub>OXA-58-like</sub>*. Visualization of colonies growing inside the halos of inhibition depends on the size of the inoculum used. The mechanism(s) implicated in this phenomenon need further investigation.

**P62 MASTOPARAN: A POTENTIAL NEW ANTIMICROBIAL AGENT AGAINST COLISTIN-SUSCEPTIBLE AND -RESISTANT *ACINETOBACTER BAUMANNII*****X. Vila-Farres<sup>a</sup>, C. García-De la Mária<sup>a</sup>, E. Giralt<sup>b</sup> & J. Vila<sup>a</sup>****<sup>a</sup>Hospital Clinic and University Barcelona Spain; <sup>b</sup>IRB (Institute for Research in Biomedicine) and University Barcelona Spain**

Multidrug-resistant *A.baumannii* isolates are commonly found worldwide. There is a need for new therapeutic strategies since isolates resistant to most agents, including colistin have been reported. The aim of this study was to investigate a peptide (mastoparan) as a possible antibacterial agent against colistin-susceptible and resistant *A.baumannii*.

Different strains of both susceptible and colistin-resistant *A.baumannii* were used. Colistin-resistant mutants were selected by serial passages under subinhibitory concentrations of colistin. The MIC of mastoparan for colistin-susceptible and resistant *A.baumannii* were determined by microdilution in Muller-Hinton broth. Time-killing curves were performed using mastoparan (8x, 4x, 2x and MIC) and colistin (32x, 16x, 8x, 4x and MIC) against colistin-susceptible and resistant strains. Moreover, TEM (Transmission Electron Microscope) was applied to both resistant and susceptible-colistin *A.baumannii* strains in the presence and absence of mastoparan.

Good activity of mastoparan against both colistin-susceptible and resistant *A.baumannii* strains, MIC<sub>50</sub> (4 mg/ml) and MIC<sub>90</sub> (8 mg/ml) was shown. MIC values of colistin were 0.5 mg/ml for colistin-susceptible and 256 mg/ml for colistin-resistant *A.baumannii* isolates. Time-killing curves showed bactericidal activity of mastoparan 8x against colistin-susceptible and resistant *A.baumannii* strains and 4x only against colistin-resistant along the curve. Colistin was not bactericidal at any concentration or time. TEM experiments showed how mastoparan acts versus both strains by destroying the cell wall of the bacteria.

Mastoparan showed good activity for both colistin-susceptible and resistant *A.baumannii* suggesting that the mechanism of action of this AMP may be different from that used by colistin. TEM analysis showed how mastoparan affects bacteria and supports the different mechanism of action of mastoparan compared to colistin. We also observed a higher bactericidal activity of mastoparan compared to colistin at lower concentrations of the AMP suggesting its role as a good potential antimicrobial agent to treat infections generated by this organism.

### **P63 EPIDEMIOLOGY AND CLINICAL SIGNIFICANCE OF *ACINETOBACTER BAUMANNII* INFECTIONS IN AGED PATIENTS WITH PNEUMONIA**

**A. V. Martynova, A. A. Sheparyov & O. A. Chulakova**

**Epidemiology Department, State Vladivostok Medical University, Ostryakova 2, Vladivostok, Russia**

Despite the detailed study of *Acinetobacter baumannii* infection, the epidemiology aspects of this infection remain underestimated both in epidemiology and clinical practice. Being opportunistic pathogen, it is often isolated in immunocompromised hosts in different forms of hospital-acquired infections, but more often it was recognized as the main pathogen agent of hospital-acquired pneumonia. The aim of our research was to establish the clinical significance of *A. baumannii* in development of hospital-acquired pneumonia, to define its epidemiology and to characterize antimicrobial agents resistance pattern.

We made 1-year surveillance of all hospital-acquired pneumonias (HAP) in adult patients in the main clinics of Vladivostok (Hospital №1, №2), defined etiology with standard microbiology methods. All isolates of *A. baumannii* were tested for antimicrobial resistance according to NCCLS to meropenem, gentamicin, amikacin, cefepime, ciprofloxacin, cefoperazone/sulbactam and ampicillin/sulbactam. The strains with the same antimicrobial resistance pattern were checked to clonality by pulsed-field gel electrophoresis (PFGE).

During 2008, we studied all cases of HAP in 450 adult patients (<60 years) admitted to ICU and revealed that *A. baumannii* has taken the second place in etiology structure (19.1%, 94 cultures from 86 patients). The first place was in *Pseudomonas aeruginosa* (29.3%, 132 strains) and the third one was in *Stenotrophomonas maltophilia* (11.7%, 53 strains). Mostly (58 strains, 61.7%), *A. baumannii* was isolated as mono-infection, but in other cases it was isolated in association with other strains of *A. baumannii*, or *P. aeruginosa*, *S. maltophilia*, *S. aureus*, *E. faecalis* or *E. cloacae*.

Epidemiology of *A. baumannii* confirmed our suggestion about seasonality of this infection, though it was not revealed in *P. aeruginosa*. On antimicrobial agents resistance study we revealed that 8.51% were non-susceptible to meropenem, 36.17% to gentamicin, 38.2% to cefepime, 44.6% to ciprofloxacin, and to cefoperazone/sulbactam were resistant 15.96%. About 30% of all isolates could be considered multi-drug resistant. The 42 non-susceptible isolates to ciprofloxacin were characterized by PFGE and here were defined 6 cluster groups with the same pattern of clonality (4 groups in one clinic and 2 groups in another one), which confirms the possibility of contamination of ICU by this pathogen and forming of the hospital isolate of *A. baumannii*.

In conclusion, the clinical significance of *A. baumannii* in the development of hospital-acquired infections is obvious though epidemiology need to be checked more precisely with molecular methods to establish the exact mechanism of spread of this pathogen.

**P64 ACINETOBACTER BAUMANNII BLOODSTREAM ISOLATES  
SUSCEPTIBILITY TO TIGECYCLINE AND 8 OTHER ANTIBIOTICS**

**A. Liskova<sup>a</sup>, J. Sokolova<sup>b,c</sup> & V. Krcmery<sup>c</sup>**

**<sup>a</sup>National Reference Laboratory of Antimicrobial Resistance, Nitra, Slovakia; <sup>b</sup>Trnava University, Trnava, Slovakia; <sup>c</sup>St. Elizabeth University College, Bratislava, Slovakia**

Antimicrobial activity of 274 *Acinetobacter* bloodstream isolates from ICU patients has been tested to 8 antimicrobials in National Reference Laboratory of Antibiotics of the Ministry of Health of Slovak Republic in Nitra. Susceptibility to tigecycline was 87%, to AMP/sulbactam 72%, meropenem 79%, amikacin 64%, ceftazidime 72%, piperacillin/tazobactam 73%, netilmicin 66% and cefepime 75%. Tigecycline showed in vitro activity to *Acinetobacter baumannii* that was better or comparable to other antibiotics. 13% of stains resistant to tigecycline were susceptible to either meropenem or other  $\beta$ -lactam antibiotics tested.

**P65 IN VITRO ACTIVITY OF 8 BIOCIDES AGAINST PREDOMINANT CLONES OF ACINETOBACTER BAUMANNII AND ACINETOBACTER GENOSPECIES 3**

**M. C. Gómez Sánchez, F. Fernández Cuenca, S. Ballesta Mudarra, J. Vila, L. Martínez-Martínez, G. Bou, J. Rodríguez-Baño & A. Pascual**

**Clinical Microbiology and Infectious diseases Department of 'Virgen Macarena' University Hospital, Sevilla, Spain**

Decreased susceptibility to biocides can facilitate the selection or dissemination of predominant clones of *Acinetobacter spp.* in the nosocomial environment. The aim of this study was to investigate if there are differences in the susceptibilities of predominant clones in Spain of *Acinetobacter baumannii* (Abm) and *Acinetobacter genospecies 3* (Ac-G3) to biocides.

Forty-nine clones of *Acinetobacter spp.* (30 Abm and 19 Ac-G3) and the type strain Ab ATCC 19606 were included. The following biocides were assayed: sodium hypochlorite (domestic bleach) (LD), Sterillium® (ST) (an alcoholic solution containing propan-2-ol, propan-1-ol and mecetronium ethyl sulphate), ethanol (ET), clorhexidine digluconate (CHD), mercurochrome (MM), benzalkonium chloride (CB), povidone-iodine (BD) and benzethonium chloride (HY). The antimicrobial activity of these biocides was determined by microdilution in Mueller-Hinton broth (Difco) using two-fold dilutions of the biocides. The maximum dilution of biocide at which there is no visible growth (MID, microdilution) was determined for clones of Abm and Ac-G3, and was compared with that for the type strain Ab ATCC 19606. Differences in  $\pm 1$  two-fold dilutions were considered not significant.

No significant differences were observed for domestic bleach, benzalkonium chloride and povidone-iodine when Abm clones were compared with the ATCC strain ( $\pm 1$  two-fold dilutions). The Abm clones were more susceptible to mercurochrome (93.3%), ethanol (30%), benzethonium chloride (16.7%) and Sterillium® (16.7%) than Ab ATCC 19606. The 23.4% of the Abm clones were less susceptible to clorhexidine digluconate than Ab ATCC 19606, whereas the 40% were more susceptible than Ab ATCC 19606. The percentage of Ac-G3 clones that showed no significant differences in their susceptibility to biocides when were compared with the ATCC strains were: 100% (Sterillium®, domestic bleach, ethanol, povidone-iodine), 26.7% (clorhexidine digluconate), 0% (mercurochrome), 93.3% (benzalkonium chloride) and 60% (benzethonium chloride). The percentage of Ac-G3 clones that were more susceptible to biocides than ATCC strains were: 73.3% (clorhexidine digluconate), 100% (mercurochrome), 6.7% (benzalkonium chloride) and 40% (benzethonium chloride). All Ac-G3 clones were more susceptible to clorhexidine digluconate than Ab ATCC 19606.

In conclusion: (i) Susceptibility of Abm clones to biocides was more variable for clorhexidine digluconate, and to a lesser extent ethanol, than for the other biocides evaluated; (ii) The Abm clones were as susceptible or were more susceptible to biocides than the ATCC strain, except for clorhexidine digluconate, for which there was a great variability in the MIDs.

The Ac-G3 clones presented similar susceptibilities to biocides than Ab ATCC 19606, except for CHX and MM, for which they were more susceptible.

**P66 ISOLATION AND CHARACTERIZATION OF THREE NOVEL BACTERIOPHAGES AGAINST *ACINETOBACTER BAUMANNII***

**M. Elbreki<sup>a</sup>, H. Neve<sup>b</sup>, J. O'Mahony<sup>a</sup>, O. McAuliffe<sup>c</sup>, R. P. Ross<sup>c</sup> & A. Coffey<sup>a</sup>**

**<sup>a</sup>Department of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork, Ireland; <sup>b</sup>Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Department of Microbiology and Biotechnology, Hermann-Weigmann-Strasse 1, Kiel, Germany; <sup>c</sup>Biotechnology Department, Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland**

*Acinetobacter baumannii* is an emerging nosocomial pathogen, which has an increasing prevalence of multi-drug resistance. *A. baumannii* exists widely in natural environment, and frequently in health-care settings where it has proven difficult to eradicate using antibiotic therapy. A possible alternative to conventional antibiotics is the use of antibacterial viruses or bacteriophages (phages) as antibacterials. Here we present the isolation and characterization of phages against this pathogen. A mixture of three distinct *A. baumannii* strains was used as a host to screen for phages from sewage and soil samples from different locations in County Cork in Ireland.

After multiple screenings and elimination of similar phages, three distinct phages were successfully isolated. These were designated  $\Phi_{me1}$ ,  $\Phi_{me2}$ ,  $\Phi_{me3}$ . The phages had plaque sizes ranging from pinpoint ( $\Phi_{me2}$  and  $\Phi_{me3}$ ) to 0.5 mm in diameter ( $\Phi_{me1}$ ). In all cases, plaques were surrounded by a zone of clear lysin activity. Their host ranges differed from each other, indicating that they should be used as a cocktail if employed as an antibacterial strategy. Phage DNAs were isolated and digested with a variety of restriction endonucleases indicating distinct genomes. Electron microscopy studies clearly showed contractile tails and distinct tail fibres in all cases. It was ascertained that all three phages had icosahedral heads in the region of 80 nm in diameter. All three belonged to the Myoviridae family.

**P67 ISOLATION OF BACTERIOPHAGES ACTIVE AGAINST MULTI-DRUG RESISTANT CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII***

**Maya Merabishvili<sup>a,b,c</sup>, Gilbert Verbeken<sup>b</sup>, Daniel De Vos<sup>b</sup>, Serge Jennes<sup>d</sup>, Mario Vaneechoutte<sup>c</sup> & Jean-Paul Pirnay<sup>b</sup>**

<sup>a</sup>Eliava Institute of Bacteriophage, Microbiology and Virology (EIBMV), Tbilisi, Georgia; <sup>b</sup>Laboratory for Molecular and Cellular Technology (LabMCT), Burn Centre, Queen Astrid Military Hospital, Brussels, Belgium; <sup>c</sup>Laboratory of Bacteriology Research (LBR), Ghent University, Ghent, Belgium; <sup>d</sup>Burn Centre, Queen Astrid Military Hospital, Brussels, Belgium

Among newly emerging nosocomial infections, multidrug-resistant (MDR) strains of *Acinetobacter baumannii* appear to be one of the most important causative agents. MDR and even pan-resistant strains of *A. baumannii* are responsible for up to 50% of outbreaks of infections in ICUs worldwide. It has been suggested that we are closer to the end of the antibiotic era with *Acinetobacter* than with methicillin-resistant *Staphylococcus aureus*. Bacteriophages active against *A. baumannii* may serve as a possibility to circumvent the high level antibiotic resistance of this species.

Different samples of sewage and wastewater obtained from several hospitals in Belgium were used for the isolation of bacteriophages active against various clinical strains of *A. baumannii*. In total, 38 clinical strains of different genotypes, including reference strains and the notorious '*Iraqibacter*' strains were used. Twenty different clones of bacteriophages were isolated.

According to electron microscopic studies the newly isolated bacteriophages belong to two different morphological families, i.e. the *Siphoviridae* and the *Podoviridae*. The range of lytic activity of the different clones of phages against the whole set of bacterial strains varies from 5 up to 92%. The overall activity of all clones is up to 97%. Genetic comparison of phages was performed by genomic RFLP and several groups of phages were identified.

Further investigations will imply more thorough genetic analysis of the newly isolated phages (full genome sequencing) and assessment of the characteristics essential for potential therapeutic application.

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