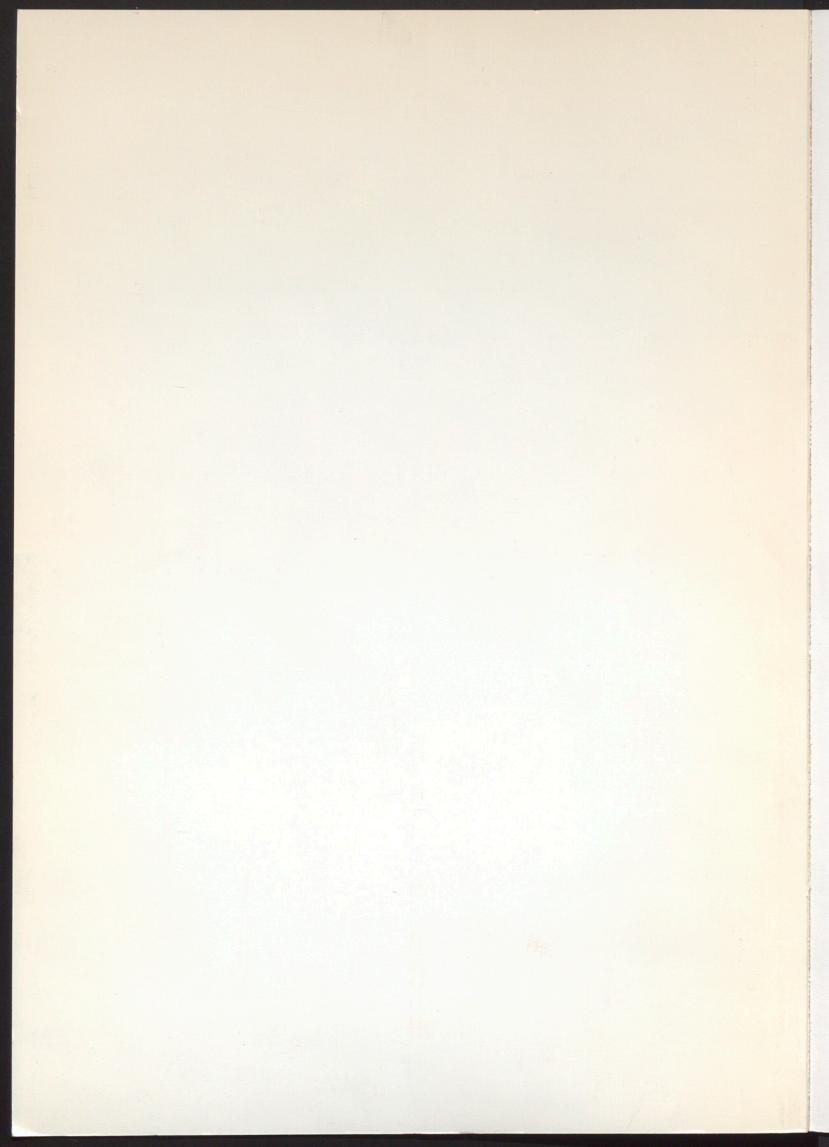
# Acinetobacter 2006

7<sup>th</sup> International Symposium on the Biology of *Acinetobacter* 

PROGRAMME AND ABSTRACT BOOK

er 2006

Sala Prat de la Riba, Institut d'Estudis Catalans Barcelona, 8 - 10 November 2006



# Acinetobacter 2006

7<sup>th</sup> International Symposium on the Biology of *Acinetobacter* 

8-10 November 2006

# **Programme and Abstract Book**

Venue Institut d'Estudis Catalans, Barcelona, Spain





Organising Committee E Neidle, Athens, GA (USA); J Pachon, Sevilla (ES); K Towner, Nottingham (UK); J Vila, Barcelona (ES) Secretariat Sara Marti E-mail: saramarti2@yahoo.es

1986 Manchester; 1990 Paris; 1994 Edinburgh; 1996 Eilat; 2000 Noordwijkerhout; 2004 Dublin; 2006 Barcelona

# Acidetobacter 2006

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On behalf of all the participants, the members of the Organising Committee wish to express their grateful thanks to Wyeth (Spain) for generous financial support of the meeting, to the European Society of Clinical Microbiology and Infectious Disease (ESCMID) for the provision of travel grants to young scientists, and to the Institut d'Estudis Catalans for hosting the meeting in such delightful and historic surroundings.

On behalf of all the participants, the imports of the parents frames is announced to the express their gratched thanks to Wyark (Spring) for generate Relation report of the mother, to the Europeta secret of Christel Victoriticity, and Infections Discase (ESCMED) for the provision of travel grants to young solvation, and to the Institut d'Entation Calabars for housing the meeting in such delighting and hereing a

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# 7<sup>th</sup> International Symposium on the Biology of *Acinetobacter* 8 – 10 November 2006 Institut d'Estudis Catalans, Barcelona, Spain

### Programme

## Wednesday 8 November 2006

1600-1800	Registration
	Destructions

1800-2100 Reception and Get-Together

### Thursday 9 November 2006

Session 1 Chair	J. Vila	Carbapenery resultance in clinical inclutes of demolobor ter- hanmanan OXA-23 and CarO - G. Elisha
0825-0830	Introdu	action and Welcome – J. Vila
0830-0900	Keynot	te Lecture The diversity of the genus Acinetobacter: current state and emerging problems – L. Dijkshoorn
0900-0920	01	Genetic diversity of carbapenem-resistant <i>Acinetobacter</i> isolates in European hospitals: the ARPAC study – K. Towner
0920-0940	02	Genetic backgrounds of carbapenem-resistant and -susceptible clinical isolates of <i>Acinetobacter baumannii</i> from two hospitals in Cape Town, South Africa – H. Segal
0940-1000	03	Evaluation of automated identification systems for identification of <i>Acinetobacter</i> species – H. Seifert
1000 - 1030	Coffee	e / Tea
Session 2 Chair	E. Neid	dle
1030-1100	Keynoi	te Lecture Comparative genomics of Acinetobacter genus; from soil to multidrug resistance – V. Barbe
1100-1120	04	The membrane subproteomes of <i>Acinetobacter baumannii</i> : comparative analysis between a reference strain and a MDR strain – E. Dé

### Acinetobacter 2006

7<sup>10</sup> International Symposium on the Biology of Achaelobacter 8 - 10 November 1996 Institut d'Escudis Catalans, Farcelona, Spain

#### Programme

Repeate Lecture Comparative genatrics of Arthenometer genuel (rate soil to multiding resistance - V. Barbr	

1120-1140	05	The <i>mutS</i> sequence in intermediate and multi-resistant clinical <i>Acinetobacter baumannii</i> is different from the same gene in sensitive bacteria – S. Davies
1140-1200	06	Mini-Tn7 vectors: a new tool for genetic characterisation of Acinetobacter baumannii – H. Schweizer
1200-1400	Poster	r Session – buffet lunch available at 1300
Session 3 Chair	K. To	wner
1400-1430	Kevno	te Lecture
0900-0920	013	Emerging mechanisms of resistance to $\beta$ -lactams in <i>Acinetobacter baumannii</i> – L. Poirel
1430-1450	07	Carbapenem resistance in clinical isolates of <i>Acinetobacter</i> baumannii: OXA-23 and CarO – G. Elisha
1450-1510	08	Clonal outbreak of multidrug-resistant Acinetobacter
0940-1000	015	<i>baumannii</i> in a Lebanon hospital: molecular epidemiology and mechanism of carbapenem resistance – R. Zarrilli
1510-1530	09	Instability of amikacin resistance in a carbapenem-resistant strain of <i>Acinetobacter baumannii</i> isolated during a hospital outbreak – A. Nemec
1530-1600	Kevno	te Lecture
1030-1100	neyno	Tigecycline: a new alternative for the treatment of <i>Acinetobacter</i> infections – R. Zaragoza
1600 - 1630	Coffe	e / Tea
Session 4		
Chair	U. Ge	rischer
1630-1700	Keyno	<i>te Lecture</i> Structural studies of two LysR-type transcriptional activators, BenM and CatM, that explain synergistic response and transcriptional control
		– C. Momany
1700-1720	O10	Functional comparison of two transcriptional regulators, BenM and CatM, in <i>Acinetobacter baylyi</i> ADP1 – E. Neidle
1720-1740	011	Analysis of carbon catabolite repression in <i>Acinetobacter</i> baylyi strain ADP1 – R. Fischer

1740-1800 O12 *ddrR* and SOS-like genes in *Acinetobacter baylyi* strain ADP1 – L. Gregg-Jolly

### 2000 Buses leave for Conference Dinner

## Friday 10 November 2006

Session 5 Chair	L. Dijkshoorn
0830-0900	Keynote Lecture Acinetobacter: a successful nosocomial opportunistic pathogen – J. Rodriguez-Baño
0900-0920	O13 Comparison of <i>ompA</i> , <i>csuE</i> and <i>bla</i> <sub>OXA-51-like</sub> sequences in isolates of <i>Acinetobacter baumannii</i> from the United Kingdom reveals two highly distinct groups of outbreak strains – J. Turton
0920-0940	O14 Identification of a widespread <i>Acinetobacter baumannii</i> strain in Portugal as belonging to European Clone II – G. Da Silva
0940-1000	O15 Epidemiological surveillance of <i>Acinetobacter baumannii</i> colonisation and infection in ICU patients – A. Agodi
1000-1030	Coffee / Tea
Session 6 Chair	B. Averhoff
1030-1100	Keynote Lecture The multiple-level regulation of aromatic catabolic genes includes Crc- dependent RNA stability – U. Gerischer
1100-1120	O16 A unique domain in esterase from <i>Acinetobacter venetianus</i> RAG1 mediates emulsification activity of a variety of polysaccharides – R. Furman
1120-1140	O17 Auxiliary proteins for aromatic tolerance and processing, detected in an <i>A. radioresistens</i> S13 proteome – E. Pessione
1140-1200	O18 A major outer membrane protein Omp38 of <i>Acinetobacter</i> <i>baumannii</i> is imported into the nucleus by a signal-dependent pathway and degrades deoxyribonucleic acids – J. Lee
1200-1400	Poster Session – buffet lunch available at 1300

1740-1300 OI2 add and aCC like gaues in Scindonessee have amon ADE1 - L. Graug-Jolfy

1200-1400 ... Peacer Seadob - bullet bude mailable at 1300

Session 7 Chair	K. Towner
1400-1430	Keynote Lecture The natural transformation system of Acinetobacter baylyi: unique features and environmental impact – B. Averhoff
1430-1450	O19 Loss of heterologous flanking DNA during introgression of an antibiotic resistance gene in mutator and non-mutator populations of <i>Acinetobacter</i> sp. – J. Ray
1450-1510	O20 Investigation into biofilm formation and interaction with human cells to explain the clinical role of <i>Acinetobacter baumannii</i> versus other <i>Acinetobacter</i> species – A. de Breij
1510-1530	O21 Gene expression patterns of respiratory epithelial cells by a major outer membrane protein Omp38 of <i>Acinetobacter baumannii</i> – J. Lee
1530-1600	Coffee / Tea
Session 8 Chair	J. Pachon
1600-1630	Keynote Lecture Treatment of nosocomial infections due to multidrug-resistant Acinetobacter baumannii – J. M. Cisneros
1630-1650	O22 Molecular analysis of <i>Acinetobacter baumannii</i> -induced inflammatory response – C. March
1650-1710	O23 The use of colistin to treat multi-resistant <i>Acinetobacter</i> infection in a regional burns intensive care unit – H Ganapathy
1710-1730	O24 Cecropin A-mellittin peptides are active against clinical colistin-resistant strains of <i>Acinetobacter baumannii</i> – L. Rivas
1730	Final Remarks – J. Vila

End of Symposium

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#### **Poster Presentations**

P1 The diversity of the genus Acinetobacter: current state and emerging problems

L. Dijkshoorn, M. Vaneechoutte, T. DeBaere, T. van der Reijden and A. Nemec

P2 A rapid PCR-based method to differentiate between *Acinetobacter baumannii* and genospecies 13

P. G. Higgins, H. Wisplinghoff and H. Seifert

P3 Evaluation of VITEK for detection of clinical isolates of *Acinetobacter* baumannii

A. A. Alsultan, A. Hamouda and S. G. B. Amyes

P4 What unites *Acinetobacter baumannii* strains isolated in intensive care units in Russia?

A. Solomennyi and A. Goncharov

P5 Do large chromosomally-integrated genomic islands contribute to resistance dissemination in *Acinetobacter* spp?

F. Shaikh, F. Almathen, Hong-Yu Ou, K. Levi, K. J. Towner, M. Barer and K. Rajakumar

P6 A collection of gene replacement mutants of *Acinetobacter baylyi* ADP1 : exploring gene functions

V. de Berardinis, M. Salanoubat, D. Vallenet, V. Castelli, S. Samair, A. Kreimeyer and J. Weissenbach

P7 ISCR2-mediated acquisition of the *bla*<sub>VEB-1A</sub> expanded-spectrum β-lactamase gene in *Acinetobacter baumannii* from Argentina

L. Poirel, S. Corvec, M. Radoport, F. Pasteran, D. Faccone, M. Galas, T. R. Walsh and P. Nordmann

P8 Differential protein expression in wild-type and colistin-resistant Acinetobacter baumannii probed by DIGE proteomic analysis

M. Rodríguez, M. Fernández-Reyes, J. Pachón, C. Chiva, L. Rivas and D. Andreu

#### **Poster Presentations**

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P9 Relationship between the AdeABC efflux system gene content, netilmicin susceptibility and multidrug resistance in a genotypically diverse population of *Acinetobacter baumannii* 

A. Nemec, M. Maixnerová, T. J. K. van der Reijden, P.J. van den Broek and L. Dijkshoorn

P10 Resistance to florfenicol due to *floR* gene in two clinical strains of *Acinetobacter baumannii* 

L. Ramos, C. Fernández, J. Villanueva, M. Domínguez, H. Bello and G. González

P11 Antimicrobial characteristics of hospital urinary isolates of *Acinetobacter* spp. – a 5-year surveillance period

> I. Hadzi-Petruseva Meloska, B. Kurcik Trajkovska, G. Jankoska, A. Hadzi-Petruseva Jankijevic and M. Petrovska

P12 The association of IS1133 with an aminoglycoside resistance gene, *aac(3)-IIa*, in *Acinetobacter baumannii* isolates

R. Jacobson, B. G. Elisha and H. Segal

P13 Differences in antimicrobial susceptibility and carbapenem resistance mechanism between *Acinetobacter baumannii* and *Acinetobacter* 13TU species

J. C. Lee, K. M. Jeong, C. H. Choi, J. Y. Lee and Y. C. Lee

P14 AdeAB multidrug efflux pump is associated with decreased susceptibility to tigecycline in *Acinetobacter calcoaceticus/baumannii* 

A. Ruzin, D. Keeney and P. A. Bradford

P15 Evolution of resistance to antibiotics and detection of carbapenemases since 1999 to 2005 in clinical isolates of *Acinetobacter baumannii* 

C. Valderrey, E. Sevillano, M. Canduela, I. Rosales, F. Calvo and L. Gallego

P16 Prevalence and clinical impact of Acinetobacter baumannii in 13 Italian hospitals

E. Carretto, C. Farina, P. Nicoletti, D. Barbarini, A. Grosini and the APSI Acinetobacter Study Group

- P0. Relationship between us AdoARC efflux system gene contents neutrating asserptibility and multidive resistance in a printipically diversa population of downloador baseannit.
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  - PIO Resistance to Borfention due to floit gene in two clinical straigs of Accastolacter boundary
  - L Ramae C. Ferninska, J. Filipparen, M. Domingurz, H. Balto and G. Gorzáliz
  - P11 Antimicrobial characterizates of prepiral voltary isolates of Arminobacter spin. - a 5-year surveilinner partial
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- P15 Differences in antimicrobiol encogrability and car baperson restances methanism between acetactobacer downward and differentiation 13TD species.
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  - P14 AdaAB audiding efflux point is accorted with accorded subactifielity of reproteine in Actorobucter converses for an instrument
    - A Farm G. Kenny and P. A. Dunfard
  - P15 Elizabilities of resistance to antibiotics and detection of ontheorem elevations, 1999 to 2005 in diluted isolates of accorrommer transmith
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    - P16 Prevalence and eligibel inspect of Accession on the management in 12 itelian, breaksie
  - E. Corrello, C. Pariso, F. Mondon, D. Kanaparist, J. Greater and log. 1991 Actuationates Stress Greats

P17 Emergence and spread of carbapenem-resistant strains of *Acinetobacter* baumannii in a tertiary care hospital in Warsaw, Poland

M. M. Wroblewska, K. J Towner, H. Marchel and M. Luczak

P18 Invasion of *Acinetobacter baumannii* to human epithelial cells via a zipperlike mechanism and the role of outer membrane protein Omp38 as an invasin

C. H. Choi, J. Y. Lee, Y. C. Lee, T. I. Park, and J. C. Lee

- P19 Analysis of adhesion of *Acinetobacter baumannii* to human cells *A. Lübeck, M. Kleinbach and U. Gerischer*
- P20 Rapid pulsed-field gel electrophoresis protocol for typing of *Acinetobacter* species

M. Erdenizmenli and A. Grossato

P21 Metallo-β-lactamases and oxacillinases types in multidrug-resistant *Acinetobacter baumanii* strains isolated from cardiovascular prosthetic deviceassociated infections

R. Cernat, C. Balotescu, V. Lazar, O. Banu, M. Ditu and M. Mitache

P22 In-vivo studies of cecropin A (1-8)-melittin (1-18) hybrid peptide against a pan-resistant *Acinetobacter baumannii* strain

R. López-Rojas, F. Docobo-Pérez, M. E. Pachón-Ibáñez, B. G. de la Torre, J. M. Saugar, L. Rivas, D. Andreu and J. Pachón

P23 Lipase secretion by multi-drug resistant strains of *Acinetobacter baumannii* following exposure to carbapenems

D. W. Wareham and D. C. Bean

P17 Emergence and spend of carbapetent-resistant strains of Activitionactive betweenin in a verticity care incepted in Warsaw, Poland

M. M. Wrothewska, K. J. Therner, R. Marchel and M. Lincard

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B. W. Florenzer and D. C. Budy.

#### GENETIC DIVERSITY OF CARBAPENEM-RESISTANT ACINETOBACTER ISOLATES IN EUROPEAN HOSPITALS: THE ARPAC STUDY

K. J. Towner, K. Levi and M. Vlassiadi, on behalf of the ARPAC Steering Group Department of Clinical Microbiology, Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Nottingham, United Kingdom.

ARPAC ('Antibiotic Resistance, Prevention and Control') was an EU-funded project designed to investigate the measures used to control antibiotic-resistant pathogens in European hospitals. The project was carried out under the auspices of four study groups of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). A specific objective of ARPAC was to determine the genetic diversity of carbapenem-resistant *Acinetobacter baumannii* isolates from European hospitals.

Of 169 European hospitals in 32 European countries that provided data to ARPAC, 130 reported that they had encountered carbapenem-resistant isolates of *Acinetobacter*, ranging from very rare sporadic resistant isolates to an endemic/epidemic situation.

In total, 107 carbapenem-resistant isolates of *A. baumannii* were obtained from 26 hospitals in 17 European countries, plus one hospital in Taiwan. Imipenem MICs ranged from <4 to 128 mg/L on re-testing by Etest. All except one of the carbapenem-resistant clones investigated produced an OXA-51-type enzyme. The one exception (from Germany) produced an OXA-24-type enzyme. In addition, one clone (from Spain) produced OXA-51 + OXA-24-type enzymes; two clones (from Bulgaria and the UK) produced OXA-51 + OXA-23-type enzymes; and six clones (two from Germany, three from Greece, and one from Norway) produced OXA-51 + OXA-58-type enzymes.

Molecular typing was performed by *ApaI* PFGE and DAF4 RAPD analysis. Clonal groupings obtained by both methods were highly similar, with 17 different clones delineated by *ApaI* PFGE at a cut-off similarity level of 85%. In general, multiple isolates from a single hospital belonged to the same clone, but some hospitals provided isolates belonging to more than one clones. Isolates belonging to 'European Clone 1' were identified in hospitals in Bulgaria, Croatia, Poland, Slovenia and the UK, while isolates belonging to 'European Clone II' were identified in hospitals in Czech Republic, Estonia, Germany, Greece, Poland and (remarkably) Taiwan. Nevertheless, 15 other clones were also identified, showing that the problem of carbapenem resistance is not confined solely to the widespread 'European clones I and II'.

This work formed part of a Concerted Action project funded by the European Commission's Research Directorate General within the Fifth Framework Programme (project number QLK2-CT-2001-00915).

#### GENERIC DEVERSITY OF CARBATE NEM-RESETANT AUTOETOMACTER ISOLATES IN EUROPEAN HOSPITAL STEDUARPAC STUDY

K. J. Towner, K. Levi and Mr. Viassian, on bohall of the ARPAC Streng Unset Department of Clinical Microbiology, Machingham University Hospitals XIIS Trust Quest's Medical Centre, Notitingham, United Kleigdom.

ARPAC ("Antibiotic Residuance, Provention and Cumied") was as 15, familed proposidesigned to messigns the measures used to correct antibiodic measures ("attouctes in European bosolide. The project was exercised out under the measures of root ands groups of the European Society of Official Microbiology and faire from Discovers1 5, 54(5), A meeting objective of ARPAC was to determine the genetic diversity of carecordian terretologicater formateries (our fluores propial).

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#### GENETIC BACKGROUNDS OF CARBAPENEM-RESISTANT AND SUSCEPTIBLE CLINICAL ISOLATES OF *ACINETOBACTER BAUMANNII* FROM TWO HOSPITALS IN CAPE TOWN, SOUTH AFRICA

#### H. Segal<sup>a</sup> and B. G. Elisha<sup>a,b</sup>

<sup>a</sup>Division of Microbiology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town & <sup>b</sup>National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

Acinetobacter baumannii is prevalent in hospitals in Cape Town, and the organism has become endemic in many wards at Groote Schuur Hospital (GSH). In the past 5 years, the frequency of isolation of carbapenem-resistant *A. baumannii* has increased. To understand the distribution of the organism, and to determine whether there is an association between the carbapenem resistance phenotype and genotype, pulsed-field gel electrophoresis (PFGE) was performed on a total of 43 *A. baumannii* isolates from GSH and Red Cross War Memorial Children's Hospital (RXH). Twenty-nine isolates were from GSH, of which two (MOS1 and MOS2) were isolated from the same patient in February 2005. MOS1 was susceptible to carbapenems, whereas MOS2 was resistant to these antibiotics. The remaining isolates (27) were obtained in March 2006; 16 were resistant and 11 were susceptible to carbapenems. Fourteen *A. baumannii* isolates (11 resistant and three susceptible) were isolated from children at the RXH during the same period.

All of the isolates were analysed by PFGE. Visual interpretation identified ten clones of *A. baumannii*. One of the clones, designated B, dominated. The B-defining profile was observed in 11 of the carbapenem resistant strains from GSH, including MOS2, and in ten of the isolates from RXH. The six remaining resistant isolates from GSH were assigned to three clones. With the exception of susceptible MOS1, which had a B profile, patterns observed in the carbapenem resistant isolates were not apparent in any of the susceptible isolates.

To understand the acquisition of carbapenem resistance determinants, MOS1 and MOS2 were investigated further. Molecular studies showed that MOS2 contains  $bla_{OXA-23}$ , which encodes resistance to imipenem and meropenem, linked to IS*Aba-1*. This gene was not detected in MOS1. Since plasmid DNA was not observed in either of these isolates, it is assumed that  $bla_{OXA-23}$  has inserted in the chromosome either as part of a composite transposon or plasmid.

GENETIC BACKGROUNDS OF CARRAPEXEM-RESISTANT AND SUMCEPTIBLE CLINICAL ISOLATES OF ACINETOMACTER BACALINAL MODIFIED OF HOSPITALS IN CAPE TOWN, SOUTH AIRSCA

#### H. Segal and E. G. Eligita

"Division of Microbiology, Institute of Infection's Diverses and Molecular Medicales. University of Cape Town & "Mattenal Health Laboratory Service, Orocae Schuar Heapital, Cape Town, South Africa

Activate/backet Agumentant is prevailed in horizontal to the open town, and the organization as become endemic in using which at Counte Schutt Despiral PoS(D). In the park's yeers, the impactney of isolation of the automation and to conclusion without their increased. To gatherinate the distribution of the automation and to conclusion without their is an automation horizon the carbapenant maintance phenotype and generator, pulsed field get electrophysics: (PCD), was performed on a bished 45 % balancaters isolates from CoH and Hed Coose Wat Alemental Children a Horizon (CND). Preprior and generator was from CoH and Hed Coose Wat Alemental end MOS2) were isolated from the same patient in francase (Doi OSH, of Shich two (MOS2) was obtained in March 2006, was maistant to those automations. The generators (D) was obtained in March 2006, the were reastant to those automations. The generators (D) was obtained in March 2006, the were reastant to those automation for the presentation of routers of the Narch 2006, the were reastant and 11 were encoded to be contained routers at the PCUI during the same patient of the strategeneration of the strategenerators (D) was contained in March 2006, the were reastant and 11 were encoded to be contained to routers at the PCUI during the same patient of the strategenerators of the strategenerators (D) routers at the PCUI during the sense part of the strategenerators are beinged to reastance at the PCUI during the sense part of the strategenerators are beinged to reastance at the PCUI during the sense part of the strategenerators are beinged to reastance at the PCUI during the strategenerators are beinged to be reastance at the PCUI during the sense part of the strategenerators are an and reastance at the PCUI during the sense part of the strategenerators are thoughted from reastance at the PCUI during the strategenerators are strategical to reastance at the strategical during the strategenerators are associated to reastance at the strategical during the strate

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#### EVALUATION OF AUTOMATED IDENTIFICATION SYSTEMS FOR IDENTIFICATION OF ACINETOBACTER SPECIES

#### H. Seifert<sup>a</sup>, M. Horstkotte<sup>b</sup> and H. Geiss<sup>c</sup>

<sup>a</sup>Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, <sup>b</sup>Department of Infectious Diseases, University Medical Center Hamburg-Eppendorf, <sup>c</sup>Institute of Hygiene, University of Heidelberg, Germany

Correct phenotypic identification of acinetobacters to the species level requires the use of a battery of more than 20 physiological and biochemical tests, and is therefore not suited for routine diagnostic microbiology laboratories. A variety of genotypic identification methods have been developed, including ribotyping of the *Acinetobacter calcoaceticus – Acinetobacter baumannii* (*Acb*) complex, tDNA spacer fingerprinting, AFLP, amplified ribosomal DNA restriction analysis (ARDRA), restriction analysis of the 16S-23S rRNA intergenic-spacer sequences, and sequence-based methods such as 16S rDNA analysis. Again, this approach does not meet the demands of a routine diagnostic laboratory. Commercially available identification systems for manual use, such as API 20 NE (bioMérieux), have performed poorly for the identification of *Acinetobacter* spp., and automated identification systems have so far been evaluated only with a small number of ill-defined *Acinetobacter* strains.

The present study compared the performance of four commercially available microbial identifications systems, i.e., VITEK 2 (bioMérieux), Phoenix (Becton Dickinson Diagnostic Systems), MicroScan WalkAway-96 SI (Dade Behring) and BIOLOG MicroLog (Oxoid) with a set of 105 well-characterized Acinetobacter isolates (A. calcoaceticus, n=10; A. baumannii, 25; Acinetobacter DNA group 3, 12; Acinetobacter DNA group 13, 7; A. haemolyticus, 10; A. junii, 11; A. johnsonii, 11; A. lwoffii, 8; A. radioresistens, 11). The isolates had been identified to the species level by the reference phenotypic method and ARDRA. Correct identification to the genus level ranged from 49% (VITEK 2) to 99% of isolates (MicroLog). Correct identification to the genus level was higher (87 - 100%) if only isolates of the Acb complex were considered. Although isolates that belonged to the Acb complex were 'correctly' identified as A. baumannii in most instances by all systems (range of 'correct', i.e., acceptable identification, 78 - 100%), none of the systems permitted correct species identification within the Acb complex. Most identification systems, however, have not included a sufficient number of other named and unnamed *Acinetobacter* spp. in their database and are therefore unable to correctly identify other Acinetobacter spp. with a few exceptions. Apart from A. haemolyticus, only the MicroLog system reliably identified other Acinetobacter spp., i.e., A. junii (100%), A. johnsonii (100%), A. lwoffii (88%), and A. radioresistens (91%). Since the MicroLog system is not available for use with bacterial isolates of human origin, the correct identification of Acinetobacter spp. with automated methods remains an unsolved issue.

#### EVALUATION OF ALTOMATED INENTIFICATION SYSTEMS FOR DENTIFICATION OF ACIAETORACTER SPECIES

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#### THE MEMBRANE SUBPROTEOMES OF ACINETOBACTER BAUMANNII: COMPARATIVE ANALYSIS BETWEEN A REFERENCE AND A MULTIDRUG-RESISTANT (MDR) STRAIN

A. Siroy<sup>a</sup>, C. Lemaître-Guillier<sup>b</sup>, P. Cosette<sup>a</sup>, T. Jouenne<sup>a</sup> and E. Dé<sup>a</sup>

<sup>a</sup>UMR 6522 CNRS, Proteomic Platform, IFRMP23, University of Rouen, F76821 Mt-St-Aignan, France

<sup>b</sup>Proteomic Platform, IBMC, F67084 Strasbourg, France

Acinetobacter baumannii is a ubiquitous Gram-negative coccobacillus which is an important cause of severe infections in hospitals, especially in compromised patients. From 1999 to 2001, multi-drug resistant (MDR) strains were isolated during outbreaks in the emergency unit of the Rouen's hospital (France). At this time, little is known about the mechanisms of multidrug resistance of *A. baumannii* that may imply some alterations of the cell wall permeability. We consequently investigated the membrane protein patterns of a standard strain of *A. baumannii* (ATCC 19606). Then, these patterns were compared with those of a clinical isolate resistant to imipenem.

In a first step, extraction of the bacterial cell wall and separation of the inner from the outermembrane were performed by ultracentrifugation on a sucrose gradient. Fractions enriched in membrane proteins were obtained and analysed by SDS-PAGE and mass spectrometry (nanoLC/MS/MS). A total of 135 membrane proteins were thus identified and this highlighted, for example, the expression of different RND-type efflux systems that could be involved in the membrane resistance mechanisms.

In a second step, using 2D-PAGE and Maldi-ToF mass spectrometry, a comparative analysis of the membrane subproteomes (inner and outer membranes) between the reference strain and the MDR strain was performed. This showed that the MDR strain under-expressed a penicillin-binding protein1b (target of the β-lactams antibiotics), produced a CarO protein (which could be a pathway for antibiotics through the membrane) that differed from CarO in the reference strain in its primary and quaternary structure, and expressed OmpW porin isoforms. Moreover, it was shown that the clinical strain had a high ability to form biofilms, that was correlated with the accumulation of some outer membrane proteins (OMPs) like NlpE or CsuD already described as being involved in bacterial adhesion. These features might partly explain the MDR emergence of the clinical isolate.

THE MEMBRANE SUBPROTEOMES OF ACTAETORIE TAR RAEMANDE COMPARATIVE ANALYSIS BETWEEN A REFERENCE AND A MULTIDRUG-RESISTANT (MDR) STRAIN

A. Siroy, C. Longatte-Cashier, P. Covene, T. Jovenne, and E. D.

UMR 6522 CNRS, Pestsoonic Platform, IFR, MP23; University of Rouer, 176821 No-St-Vignan, France

Proteomic Platform, BMC, F67084 Strasborng, France

A control objective beautiment is a subsequious for all coupled we concorring the which is an important cause of suvero intections in heapithis, especially in compromised patterns, from 1979 to 2001, multi-drog resistant (MDR) strains were constant during outpreaks in the encorrence, and of the Rouse's heapitel (france). At this time, indicit betwee about the mochanisme of multioff the Rouse's heapitel (france). At this time, indicit betwee about the mochanisme of multioff the Rouse is heapitel (france). At this time, indicit betwee about the mochanisme of multioning resistance of A beamagent that must imply some alternitics of the cell wall permeability. We consequently intercepted the multiple some alternitics of a standard strain of A communit (ATCC i Sector, Then, Suste patterns were compared with incise of a strain of A communit (ATCC i Sector, Then, Suste patterns were compared with incise of a strain of A communit (ATCC i Sector, Then, Suste patterns were compared with incise of a strain of A communities the instruction.

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#### THE MUTS SEQUENCE IN INTERMEDIATE AND MULTIRESISTANT CLINICAL ACINETOBACTER BAUMANNII IS DIFFERENT FROM THE SAME GENE IN SENSITIVE BACTERIA

#### S. E. Davies, S. Brown and S. G. B. Amyes

Centre for Infectious Diseases, The University of Edinburgh, Edinburgh, UK.

The apparent ability of *Acinetobacter baumannii* to acquire resistance genes rapidly and become multiresistant (MRAb) has contributed to the threat that these organisms now pose to clinical treatment. Our previous studies have shown that some MRAb possess a novel *mutS* gene, which correlates with increased mutation potential and the development of resistance. This study aimed to investigate origins of the MRAb strains and their ability to develop resistance.

Isolates were obtained from around the world and MICs of a range of antibiotics were determined by the agar dilution method. Representative strains showing sensitive (S), intermediate (I) and resistant (R) phenotypes were selected for further investigation. The *mutS* gene was amplified by PCR using degenerate primers and the amplicons were purified and sequenced. These sequences were compared to each other and non-clinical strains by alignment with hierarchical clustering. Step-wise mutation was performed by challenge with 2 x MIC of ciprofloxacin (CIP), and *gyrA* was analysed for target site mutations by *Hin*fI restriction digestion and sequencing.

Sequence analysis showed a novel amino-acid sequence in the N-terminal region of the *mutS* gene in all the R isolates (n=8) which differs from the S and non-clinical isolates. The sequence of the *mutS* gene in the I isolates (n=7) was identical to the R isolates. Mutation studies of these I isolates demonstrated that they were more likely than the S isolates to develop high levels of CIP resistance (MIC >4 mg/mL) and gyrA target site mutations. The sequences of the *mutS* gene in the S isolates (n=7) were more homologous than the non-clinical isolates, and the strains were less able to develop high CIP resistance upon challenge, and none developed gyrA target site mutations.

The intermediate (I) isolates had a novel *mutS* sequence that was also found in R isolates, which suggests that these genes may have been the precursors of the same gene found in the R isolates, and that they contribute to the development of clinically significant levels of CIP resistance and *gyrA* target site mutations. This suggests that the possession of this *mutS* gene may be important in determining which isolates have the ability to rapidly acquire resistance clinically.

THE AUTS SEQUENCE IN INTERMEDIARD AND MOLTHRESHTANT CLINICAL ACINETOBACTER BAUMANNE IS DIFFERENT FROM THE SAME GENE IN SENSITIVE BACTERIA

#### S. E. Davies, S. Brown and S. G. B. Amyes

Centre for Infections Diseases. The University of Edinburgh, Edinburgh, UK,

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#### MINI-TN7 VECTORS: A NEW TOOL FOR GENETIC CHARACTERISATION OF ACINETOBACTER BAUMANNII

#### A. Kumar and H.P. Schweizer

Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, USA

While plasmid-based vectors are traditionally used for genetic complementation and reporter gene tagging, they suffer from distinct disadvantages such as multiple copy numbers, necessity for continued antibiotic selection and reduced host fitness due to increased DNA replication burden.

To overcome these disadvantages, we recently developed the mini-Tn7 chromosome integration vectors [1,2]. These vectors possess extremely broad host-range and integrate in single-copy and orientation-specifically into the host chromosome downstream of the conserved and essential glmS housekeeping gene. While the majority of bacteria contain single glmS genes and, therefore, single Tn7 insertions (or attTn7) sites, some bacteria possess multiple glmS genes and thus multiple attTn7 sites and, very rarely, bacteria may also possess non-glmS linked attTn7 sites [2]. The utility of mini-Tn7 vectors in A. baumannii was suggested by the recent discovery of Tn7::In2-8 containing a novel class 2 integron which was inserted downstream of glmS in some rare clinical isolates [3]. The present study tested insertion of a mini-Tn7T-Gm-REP element into A. baumannii strain ATCC 19606. Besides a gentamicin resistance (Gm<sup>r</sup>) marker, this Tn7 element contains the R6K origin of replication (REP) on the transposon. Transposition of mini-Tn7 was achieved after conjugal transfer from Escherichia coli in the presence of a helper plasmid encoding only the sitespecific transposition pathway. Chromosomal DNA was extracted from Gm<sup>r</sup>-transformants, digested with XhoI and the resulting DNA fragments were self-ligated. The ligation mixture was used to transform an *E. coli*( $\lambda pir$ ) strain and Gm<sup>r</sup> transformants were selected. All of the transformants examined contained the same plasmid, resulting from re-ligation of DNA fragments containing mini-Tn7 sequences, the Gmr marker and the R6K REP. Sequencing with Tn7-specific primers revealed insertion of the mini-Tn7 element 24 nucleotides downstream of the glmS gene.

These data suggest that *A. baumannii* contains a single *att*Tn7 site, and that the large family of mini-Tn7 elements developed previously for single-copy gene complementation, regulated gene expression and reporter gene tagging will be applicable for *A. baumannii*. The availability of these tools and detailed protocols describing their use will greatly facilitate manipulation of and studies with this important bacterial pathogen in medical and non-medical environments where plasmids systems are impractical, e.g., animal models, biofilms and model microcosms.

- 1. Nat Methods 2:443-448.
- 2. Nat Protocols 1:153-161.
- 3. Antimicrob Agents Chemother 49:5179-5181.

MINETRY VICTORS: A NEW 1001 FOR GENETIC CHARACTERISATION OF ACTIVE TO ACTER BAUM-WAI

A. Kumar and H.P. Schweizer

Department of Manobiology, Immunatogy and Pathology, Colorado State, University, Fort Collins, USA

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#### CARBAPENEM RESISTANCE IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII: OXA-23 AND CarO

B. G. Elisha<sup>a,b</sup>, H. Segal<sup>a</sup>, R. Jacobson<sup>a</sup> and S. Garny<sup>a</sup>

<sup>a</sup>Division of Microbiology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, <sup>b</sup>National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

In 2003, Acinetobacter baumannii strains with high-level imipenem MICs (16 - 256 mg/L) and meropenem MICs (>32 mg/L) emerged in hospitals in Cape Town, South Africa. Molecular studies identified  $bla_{OXA-23}$  in 47 isolates, of which 11 were from Groote Schuur Hospital and 36 were from Tygerberg Hospital. No other carbapenemases were detected in these organisms. As a possible explanation for the variations in imipenem MICs, the presence of an additional resistance mechanism was considered. One mechanism, insertional inactivation of *carO*, which encodes an outer membrane protein, has been shown to reduce accumulation of carbapenems in *A. baumannii*. To determine whether this mechanism contributes to the resistance, PCR assays were carried out using primers specific for *carO* nucleotide sequences. A product larger than the expected size was obtained from two of 47 isolates. Both of these isolates were from Tygerberg Hospital and had impenem MICs of 256 mg/L and 64 mg/L, respectively. One of the amplicons was sequenced; analysis of the data obtained identified IS*Aba1* in the structural *carO* gene. It was concluded that, although insertional activation of *carO* may contribute to resistance in some isolates, it is not the only explanation for the variations in imipenem MICs.

The  $bla_{OXA-23}$  gene has been identified adjacent to ISAba1, and it was suggested that transcription of the gene proceeds from promoters within the IS. To investigate the association of  $bla_{OXA-23}$  with ISAba1 in the 47 isolates, PCR assays were performed using primers directed against ISAba1 and  $bla_{OXA-23}$  nucleotide sequences, respectively. Amplicons were obtained from all 47 *A. baumannii* isolates.

To identify the promoter(s) located upstream of *bla*<sub>OXA-23</sub>, primer extension studies were performed using RNA from one isolate, designated strain RAM. A single cDNA product was obtained and the hexamers, TTAGAA (-35) and TTATTT (-10), separated by 16 nucleotides, are located in IS*Aba-1* upstream of the transcription start site. To test whether transcription signals recognised in *Acinetobacter* are different from their counterparts in *Escherichia coli*, primer extension studies were carried out using *E. coli* JM109 containing a functional *bla*<sub>OXA-23</sub> gene cloned in pGEM-T EASY (pRK001). At least four primer extension products were obtained. These transcripts initiated at a number of promoters within IS*Aba1*, including the hexamers recognised in the transcription of *bla*<sub>OXA-23</sub> in strain RAM. Thus, transcripts observed in *E. coli* were not apparent in *A. baumannii*. Notwithstanding transcription, when compared to the imipenem and meropenem MICs (0.094 mg/L and 0.008mg/L) for *E. coli* JM109, a slight increase only in the meropenem MIC (0.016 mg/L) for *E. coli* (pRK001) was observed.

CARBAPENEM RESISTANCE IN CLINICAL ISOLATES OF ACIMETOMAL TER BAUMANNE: OXA-23 AND CarO

#### B. G. Elitha", H. Segal, R. Jacobaca" and S. Gamy'

Division of Microbiology, Institute of Infectious Discourse and Molecular Modurine. University of Cape Town, "National Health Laboratory Service, Genere Schutt Heapitelt, Cape Town, South Africa

In 2003, Actentiolector banananii statata wile ingh-level interestin bilk (16 - 255 and 1) and meropenen MICs (532 mg/L) errected in heapitate in C are from corots actuate Molecolar studies identified blooks as in \$7 accords, of which 11 were from corots actuate these organisms As a possible explanation for the versions as proposal. MICs the presented in these organisms As a possible explanation for the versions as proposal. MICs the presented in additional resistance mechanism was considered. One the interminest of an additional resistance mechanism was considered. One the interesting machineston of carO, which moodes an outst membrane protein has incertainest accumulation of carO, which moodes an outst membrane protein has incertained mechanisms. Both of these testates were near 1 between the expected size was obtained from the of mechanism Both of these testates were near 1 yerberg Hospital and had interesting of the first and be med. There interview the offer employed and the interview of the isolates. Both of these testates were near 1 yerberg Hospital and had interesting and free that and the additional metation of the offer employed and the interview of the isolates. Both of these testates were near 1 yerberg Hospital and had interesting and free data the additional metation of the offer employed and the addition were of the isolates. Both of these testates were near 1 yerberg Hospital and had interesting and free data additional distribution of early into the offer employed and the addition metational definition of early into the offer employed in the second data which are the data and the data interview of the statement of the metation of the data postated identified the data of the statement of the statement of the data and the data interview of the statement of the statement of the data was postated identified the data of the statement of the statement of the data was and the data interview of the statement of the statement of the statement of the data and the data interview of the statement of the s

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#### CLONAL OUTBREAK OF MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII IN A LEBANON HOSPITAL: MOLECULAR EPIDEMIOLOGY AND MECHANISM OF CARBAPENEM RESISTANCE

A. Di Popolo<sup>a</sup>, A. U. Khan<sup>b</sup>, Z. Daoud<sup>c</sup>, M. Bagattini<sup>a</sup>, C. Afif<sup>c</sup>, M. Triassi<sup>a</sup> and R. Zarrilli<sup>a</sup>

<sup>a</sup>Dipartimento di Scienze Mediche Preventive, Università di Napoli "Federico II", Naples, Italy, <sup>b</sup>Interdisciplinary Biotechnology Unit, A.M.U., Aligarh, India, and <sup>c</sup>University of Balamand and Clinical Microbiology Laboratory of the Saint George University Hospital, Beirut, Lebanon

The molecular epidemiology of a clonal outbreak of multidrug-resistant Acinetobacter baumannii was investigated in Saint George University Hospital, Beirut, Lebanon between November 2004 and October 2005, when A. baumannii was isolated from 17 patients (11 from medical-surgical intensive care unit (ICU) and six from other wards). Eleven patients had ventilator-associated pneumonia, three had wounds or abscesses, one had bacteraemia, one had urinary tract infection, and one had pleural effusion. Genotype analysis of all A. baumannii isolates during the outbreak identified one major PFGE type, B, that differed by more than six bands from one additional isolate from the ICU of the hospital 6 months previously (PFGE type A). All A. baumannii isolates of PFGE type B showed an identical multi-resistant antibiotype, being susceptible to colistin and trimethoprim-sulphamethoxazole, of intermediate susceptibility to ampicillin-sulbactam and meropenem, while resistant to all other antimicrobial agents tested. In these isolates, inhibition of OXA enzymes by 200 mM NaCl reduced the imipenem MIC by up to 8-fold. Molecular analysis of antimicrobial resistance genes showed that all epidemic A. baumannii isolates harboured in their genomic DNA a class 1 integron containing the *aacA4*, orfX, and  $bla_{OXA-20}$  gene cassettes, an ampC gene and a  $bla_{0xa-51-like}$  allele. Moreover, a  $bla_{0xa-58}$  gene surrounded by regulatory insertion sequence elements ISAba1 and ISAba3 was identified in a 21-kb plasmid DNA from A. baumannii strains of PFGE type B, but not PFGE type A. No amplification products were obtained from genomic DNA of epidemic strains of PFGE type B for bla<sub>IMP-type</sub>, bla<sub>VIM-type</sub> or bla<sub>SIM-type</sub> metallo-β-lactamase genes or bla<sub>oxa-23</sub> or bla<sub>oxa-24</sub> carbapenem-hydrolysing oxacillinases. Also, both carbapenem-susceptible A. baumannii strains of PFGE type A and carbapenem-resistant strains of PFGE type B expressed the 26-kDa outer membrane protein CarO. Conjugation experiments demonstrated that resistance to imipenem, along with the blaoxa-58 gene, was transferred from A. baumannii strains of PFGE type B to those of PFGE type A.

The selection and the spread between different wards of a single *A. baumannii* clone producing OXA-58 carbapenem-hydrolysing oxacillinase was responsible for the increase of *A. baumannii* infections that occurred at Saint George University Hospital of Beirut, Lebanon.

CLONAL OUTBREAK OF MELITURED RESISTANT ACTORTOMACDER EAUMANNEIN A LEBANON HOSPITAL, MOLECLEAR FEDERIOLOGY AND MECHANISM OF CARBATENEM RESISTANCE

> A. Di Popolo", A. U. Khan", Z. Davad, M. Begatine, C. Allf, M. Trassi' and R. Zamilu"

"Dipartumento di Scienze Mediatre Preventiva, Polyerstin di Nepel, "Pertonco II", NapleS, Italy, "Interdisciplinary Biotechnology Unit, A MUL, Aligarhi India, and "University of Balannand and Chuical Microbiology Laboratory et the S. Int Cooge Elmonator Hospital, Beirut, Lebana

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# INSTABILITY OF AMIKACIN RESISTANCE IN A CARBAPENEM-RESISTANT STRAIN OF ACINETOBACTER BAUMANNII ISOLATED DURING A HOSPITAL OUTBREAK

A. Nemec<sup>a,b</sup>, M. Maixnerová<sup>b</sup>, T. J. K. van der Reijden<sup>c</sup>, V. Jindrák<sup>d</sup>, J. Smíšek<sup>a</sup> and L. Dijkshoorn<sup>c</sup>

<sup>a</sup>3rd Medical School, Charles University, Prague, Czech Republic, <sup>b</sup>National Institute of Public Health, Prague, Czech Republic, <sup>c</sup>Leiden University Medical Center, Leiden, The Netherlands, <sup>d</sup>Hospital Na Homolce, Prague, Czech Republic.

In January 2000, a carbapenem-resistant *Acinetobacter baumannii* strain was isolated in a Prague hospital. A few months later, additional carbapenem-resistant isolates were recovered from multiple patients in the same hospital. These isolates differed in their susceptibility to amikacin even if they were from the same patient. The present study aimed to assess the relatedness of the carbapenem-resistant isolates and to reveal the genetic basis of their variability in susceptibility to amikacin.

In total, 31 clinical isolates of *A. baumannii* from 11 patients hospitalised in the Hospital Na Homolce in Prague were investigated. The index isolate was recovered from a patient admitted from another hospital in January, the other isolates were collected in June 2000. The MICs of all isolates for both imipenem and meropenem were  $\geq 16$  mg/L. Genotyping was done by *ApaI* macrorestriction analysis and AFLP fingerprinting. Susceptibility to antibiotics was tested by disk-diffusion and agar dilution, while the genes for the aminoglycoside phosphotransferase APH(3')-VI (*aphA6*) and for OXA-type carbapenemases were detected by PCR.

All isolates yielded indistinguishable macrorestriction profiles and were allocated to the European multidrug resistant clone II by AFLP. They were uniformly resistant or intermediately susceptible to all tested antibiotics, except for tobramycin and ampicillinsulbactam. The gene encoding carbapenemase OXA-58 was found in all isolates. Twenty-six isolates were resistant to amikacin (MIC >64 mg/L) and carried the *aphA6* gene, whereas this gene was not detected in any of five amikacin-susceptible isolates (MIC 1 mg/L). In laboratory experiments, isolates originally resistant to amikacin showed spontaneous loss of amikacin resistance with a frequency of 5 x  $10^{-3}$ .

In conclusion, the hospital outbreak was caused by an imported multidrug resistant strain, which harboured the genes for OXA-58 and APH(3')-VI. Susceptibility to amikacin most likely resulted from the loss of *aph6*. Since amikacin-resistant and susceptible variants were present concurrently on the ward, use of amikacin might result in selection of resistant variants and, hence, treatment failure. The study results emphasise the importance of both strain identification and assessment of the genetic basis of resistance in cases of difficult-to-understand treatment failures.

This study was supported by grant IGA MZ ČR 8554-3.

INSTABILITY OF AMIRACTN ELSISTANCE IN A CAPBAPENEM ARSISTANT STRAIN OF ACTVER BACMANNI ISOLATED INHERG A HOSEITAL OUTBREAK

A. Nemeo<sup>16</sup>, M. Maiznerova<sup>6</sup>, T. J. K. van der Reijden<sup>6</sup>, V. Judrák<sup>6</sup>, I. Smišek<sup>6</sup> and L. Dijkshoeru<sup>6</sup>

"Ind Medical School, Chadres University, Prague, Creek Republic, "Sational Institute of Public Health, Prague, Czech Republic, "Leiden Câlversity Medical Center, Leiden, The Netherlands, "Horpital Na Homoles, Prague, Cavis Republic.

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# FUNCTIONAL COMPARISON OF TWO TRANSCRIPTIONAL REGULATORS, BenM AND CatM, IN ACINETOBACTER BAYLYI ADP1

E. L. Neidle, J. Morgan, S. H. Craven and O. C. Ezezika

Department of Microbiology, University of Georgia, Athens, USA.

BenM and CatM, two LysR-type transcriptional regulators of *Acinetobacter baylyi* ADP1, have overlapping function in aromatic compound degradation. Despite a high level of sequence similarity between the two proteins, BenM responds synergistically to two effectors, benzoate and *cis, cis*-muconate, while CatM responds only to muconate. Structural studies of their effector-binding domains revealed that BenM has two distinct effector-binding sites, whereas CatM has only one. BenM and CatM each bind muconate to a primary binding site between two regulatory domains. BenM, but not CatM, additionally binds benzoate to a secondary site. Our regulatory model predicts that benzoate alters BenM conformation to enhance a signal from muconate. Thus, the secondary binding site provides the basis for transcriptional synergism. As described here, mutational approaches were used to test regulatory models based on the structures of effector-bound complexes of BenM and CatM.

Site-directed mutagenesis was used to alter the secondary effector-binding site of BenM and the related region of CatM, which fails to bind benzoate. Key residues in BenM, Tyr293 and Arg160, were altered to create substitutions matching the amino-acids in CatM, Phe293 and His160. These changes in BenM abolished the ability to respond to benzoate and to activate transcription synergistically with both effectors, and muconate responsiveness was retained. Additionally, there is evidence that muconate and benzoate bind competitively to the primary effector-binding site of BenM. Comparable changes were made in CatM to generate a variant that functions more like BenM: Phe293 was changed to Tyr, and His160 was changed to Arg. Although attempts to create a benzoate-responsive CatM have not yet been successful, the two-residue alteration yielded a CatM variant that, like BenM, activates high-level *benA* transcription. Normally, the absence of BenM prevents benzoate from serving as a growth substrate because of relatively poor CatM-mediated expression of *benA*. The CatM variant (F293Y/H160R) enabled BenM-independent growth on benzoate.

Selection for growth on benzoate in parent strains lacking either BenM or CatM yielded spontaneous mutants, including additional mutations that increased the ability of CatM to regulate *benA* or that altered the expression of *catB*, a locus where CatM is the primary regulator. Collectively, these investigations define small changes in protein-effector and protein-promoter interactions that have a large impact on the specificity of the CatM and BenM regulators. A structural framework is now available for interpreting these results. BenM and CatM are the first LysR-type regulators to be structurally characterized while bound to physiologically relevant inducers. Genome sequence analysis indicates that strain ADP1 encodes more than 40 members of this regulatory protein family. The prevalence and diverse metabolic functions of LysR-type regulators, the most common type of transcriptional regulator in proteobacteria, underscore the broader significance of these studies.

## FUNCTIONAL COMPARISON OF TWO-TRANSCRIPTIONAL RECULATORS. Beam AND CAME IN ACLVETOBACTER SATE IS ADET

E.L. Neidle, L. Moyran, S. H. Craven and O. C. Ezerkin

Liepartment of Microbiology, University of General, AGens, USA

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# ANALYSIS OF CARBON CATABOLITE REPRESSION IN ACINETOBACTER BAYLYI STRAIN ADP1

R. Fischer and U. Gerischer

Department of Microbiology and Biotechnology, University of Ulm, Ulm, Germany

Acinetobacter baylyi strain ADP1 is able to modify the diverse structures of many aromatic compounds to common intermediates of central pathway for the *ortho*-cleavage (protocatechuate and catechol). The responsible catabolic genes are induced by the presence of the corresponding aromatic substrates or their intermediates. Using the gene cluster for protocatechate degradation (*pca-qui* operon), we found that the specific regulation of this operon (and two other operons also encoding funneling pathways) is repressed by alternative carbon sources such as acetate and succinate [1]. The molecular mechanism of this global control regulation is unknown. To identify the components involved the genome of *Acinetobacter* was mutagenised using *mariner*-transposon mutagenesis. A screening system based on *pcal,J-lacZ* transcriptional fusions was applied to point towards possible candidates with disrupted gene regions necessary for carbon catabolite repression (CCR).

In this study, we also addressed the question as to which other aromatic degradative operons, besides the *pca-qui* operon, underlie CCR by acetate and succinate, and are therefore probably controlled by the same global regulatory mechanism.

To answer the question, transcriptional fusions were produced between the relevant structural gene promoter and the gene for the *Photinus pyralis* luciferase. Luciferase activity under inducing conditions as well as under CCR conditions was determined. The results indicate that all promoters tested (*ben, hca, dca*) underly catabolite repression control.

1. J Mol Microbiol Biotechnol 4:389-404.

# ANALYSIS OF CARBON CATABOLLIR BEPERSSION IN ACTURIOBACTER BUILT STRAIN ADRI

#### R Fischer and U. Gerischer

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## ddrR AND SOS-LIKE GENES IN ACINETOBACTER BAYLYI STRAIN ADP1

L. A. Gregg-Jolly<sup>a</sup>, B. Cohn<sup>a</sup>, B. Flaherty<sup>a</sup> and J. M. Hare<sup>b</sup>

<sup>a</sup>Department of Biology, Grinnell College, Grinnell IA, USA. <sup>b</sup>Department of Biological & Environmental Sciences, Morehead State University, Morehead, KY, USA.

The response of *Acinetobacter baylyi* strain ADP1 to DNA damage is poorly understood. As in many prokaryotes, the *recA* gene is induced in response to DNA damage. RecA is a key regulator of the SOS response in bacteria. In the canonical SOS response to DNA damage, activated RecA facilitates cleavage of the LexA repressor, yielding increased transcription of >25 genes. Most SOS-regulated genes are involved in DNA repair, mutagenesis and cell division. A survey of SOS genes reveals that some appear to be conserved in ADP1 (including, e.g., *recA*, *ftsK* and *umuD*), whereas others, including *lexA*, are notably absent. Compared to the organisation of SOS genes in *Escherichia coli*, homologues of SOS genes in ADP1 appear clustered.

Thus far, we have found one ADP1 gene, called ddrR, that is expressed at higher levels in response to DNA damage in a RecA-dependent manner. This induction of ddrR is also partially dependent on the adjacent, but transcriptionally divergent, operon encoding UmuDC\* [1]. Saturated mutagenesis and northern analysis of the ddrR locus indicates that the ddrR gene encodes a c.550-base transcript. According to the region mapped for ddrR, the beginning of the gene overlaps about the last 100 bp of the predicted ORF ACIAD2730 [2]. The end of ddrR extends into at least the last 150 bp of the 5'-end of the predicted ORF ACIAD2731 [2], which is transcribed in the opposite direction of ddrR. One possible explanation for this unusual gene arrangement could be that ddrR is not translated and functions as an RNA molecule.

*ddrR* and ACIAD2730 do not share homology with any known sequences. ACIAD2731 was categorised as a homologue to other known sequences of unknown function. ACIAD2731 is the last of ten predicted ORFs transcribed in the same direction. Three of these, *pqiA-1*, *pqiA-2* and *pqiB*, encode homologues of paraquat-inducible proteins. Paraquat (methyl viologen) is known to form superoxide radicals which generate oxidative damage, a major form of DNA damage in all aerobes. The close proximity and divergent transcription patterns of *umuDC*, *ddrR* and the *pqi* genes suggest the possibility of a supra-operonic cluster related to the response of ADP1 to DNA damage. Thus far supra-operonic clustering of genes has only been reported for catabolic genes.

1. Appl Environ Microbiol, in press.

2. Nucleic Acids Res 32:5766-5779.

#### AND SOS-LIKE GENES IN ACTIVITIES OF THE MAIL IN STRAIN ADD

L.A. Gregg July, B. Cohr, B. Fickery and J. M. Hate'

Department of Biology, Granell College, Granell IA, USA Department of Biological & Eaviranmental Sciences, Morehead State Graversly, Morehead KY, USA

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# COMPARISON OF *ompA*, *csuE* AND *bla*<sub>OXA-51-LIKE</sub> SEQUENCES IN ISOLATES OF ACINETOBACTER BAUMANNII FROM THE UNITED KINGDOM REVEALS TWO HIGHLY DISTINCT GROUPS OF OUTBREAK STRAINS

## J. F. Turton, S. N. Gabriel, M. E. Kaufmann and T. L. Pitt

Laboratory of Healthcare Associated Infection, Health Protection Agency, London, UK.

Sequence diversity in the outer membrane protein A (ompA), csuE (part of a pili assembly system involved in biofilm formation) and  $bla_{OXA-51-like}$  (the intrinsic carbapenemase gene in *A. baumannii*) genes in 20 isolates of *Acinetobacter baumannii* was investigated. These included representatives of all the outbreak genotypes (defined by pulsed-field gel electrophoresis (PFGE)) recently found in the United Kingdom, and three sporadic isolates.

Outbreak strains were found to belong to two highly distinct groups, with the widespread genotypes all belonging to a single group (group 1). Isolates within a group all shared the same combination of alleles at these three loci, although 1 - 2 nucleotide differences were observed in some isolates of group 1 in up to two of the loci. Such differences were small compared with the very considerable differences between groups. Over the 686 bp of *ompA* sequence compared, group 1 and group 2 isolates. The *bla*<sub>OXA-51-like</sub> allele of group 1 isolates corresponded to *bla*<sub>OXA-66</sub> (or *bla*<sub>OXA-69</sub>. The *csuE* sequences differed by 5%. Although many of the nucleotide changes were silent, there were at least four amino-acid substitutions between group 1 and group 2 isolates at each of the loci, over the sequences compared. The group 2 isolates investigated included representatives of European clone I, and all the genotypes found to belong to this group are probably representatives of that clone.

Sporadic strains had further highly distinct alleles and each had a unique combination of alleles at the three loci. These data suggest that outbreak strains of *A. baumannii* are highly clonal, and belonged to two distinct lineages. The main *Acinetobacter* problem in the United Kingdom is due to genotypes of group 1 (which include OXA-23 clone 1, the South East clone and the T strain), which appears to be a particularly successful lineage.

PFGE was more discriminatory than the sequence-based typing. It divided isolates into clusters (at 70% similarity) that agreed with those found by the sequence-based typing. Comparison of PFGE profiles and integron cassette arrays, together with detection of additional characteristics (such as other carbapenemase genes) are helpful in outbreak investigations of this highly clonal organism.

CONTARISON OF MEAA, 2005 AND MARA A AND SECTION ENCLES IN INCLATES OF ACINETOBACTER BAUMANNI FROM THE LINITED EDMODOM REVEALS TWO HIGHLY DISTINCT GROUPS OF OUTSRIE AN AUXILIANS

J. F. Turton, S. M. Gabriel, M. E. Kauffaurus and T. L. Pitt

Luboratory of Healthcare Associated Infection, Health Protection Agency, London, UK.

Sequence diversity in the outer membrane provint A (activity could (pur of a phi assembly system involved in biofilm formation) and blackdatera (the invitance carbapersenare good in A community genes in 20 isolates of Activity or activity data was investigated. These included representatives of all the outpress group on a factorie big puscol field get electrophonetic (FFGE) receardy found in the United Singdom, and there groundle isolates

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PPGE was more discriministery than the sequence-based bring. It divides solvers and enoters (a 70% similarity) that agreed with these three by the require orbits (wring Comparison of PFGE profiles and integration casedia arrays, insolver with describes of additional characteristics (such as other contegrations goos) are not full in outgrash investorations of this highly cloud organized.

# IDENTIFICATION OF A WIDESPREAD ACINETOBACTER BAUMANNII STRAIN IN PORTUGAL AS BELONGING TO EUROPEAN CLONE II

G. Da Silva<sup>a</sup>, L. Dijkshoorn<sup>b</sup>, T. van der Reijden<sup>b</sup>, B. van Strijen<sup>b</sup> and A. Duarte<sup>c</sup>

<sup>a</sup>Centre of Pharmaceutical Studies and Laboratory of Microbiology of Faculty of Pharmacy, University of Coimbra, Portugal

<sup>b</sup>Department of Infectious Diseases, Leiden University Medical Center, 2300 RC Leiden, The Netherlands

<sup>c</sup>Laboratory of Microbiology of Faculty of Pharmacy, University of Lisboa, Portugal

In Portugal, the prevalence of *Acinetobacter baumannii* has been increasing since 1998, especially in intensive care units (ICUs) of hospitals from different cities. A previous study identified an endemic multidrug-resistant *A. baumannii* clone in three tertiary care Portuguese hospitals. Unpublished data have indicated that isolates belonging to this clone may have disseminated throughout the country in recent years.

The aim of the present study was to investigate whether these disseminated organisms belonged to the same clone already described, and if so, to assess whether this *A. baumannii* multidrug-resistant clone was genetically related to one of the described European clones I - III.

In total, 264 *A. baumannii* isolates were collected between 1998 and 2004 from different inpatients of eight hospitals in five Portuguese cities, selected on the basis of their multidrug resistance, including imipenem resistance. They were first screened by RAPD and PFGE. Ten representative isolates obtained from epidemic episodes were compared to each other by AFLP analysis and to isolates of the AFLP library of the Leiden University Medical Center database, which includes fingerprints of the European clones I - III. The *bla*-<sub>OXA</sub> gene was detected by PCR with specific primers.

The isolates exhibited a similar multidrug resistance pattern to  $\beta$ -lactams, aminoglycosides and quinolones. AFLP and ARDRA identification of the ten isolates identified them as *A*. *baumannii*. A *bla*-<sub>OXA</sub> gene was detected in the isolates. All isolates clustered in European clone II (considering clone level >80%). Comparison of the ten selected isolates with those of European clones I - III showed that they belonged to European clone II. They were in a subbranch of clone II comprising 18 isolates, including 14 other isolates from the Iberian Peninsula, all linked at 87.4%. Seven isolates clustered at 96.7%, indicating a striking degree of genetic relatedness. Three isolates were a little more separated from the main cluster. It seems that the Iberian strains are not genetically identical, but probably represent a relatively young subgroup within clone II.

The data corroborated the presumption of the inter-hospital spread over the time of the same clone in Portugal. Moreover, Portuguese multidrug-resistant isolates were found as a subcluster of the EU clone II isolates, which suggests that they belong to a recent lineage within clone II.

IDENTIFICATION OF A WIDENPERAD ACTABION ACTER RADMANNA STRAIN IN PORTUGAL AS BELONGING TO EDHOPTAN CLOXE II

O. Da Silva", L. Dijkshoom", T. van der Regiden", B. van Strijen" and A. Dunit:

'Contre of Pharmaceutical Studies and Laboratory of Microbiology of Foculty of Financiary, University of Country, Portugal

Department of Infectious Diseases, Lesien University Medical Cause, 2500 RC Laden, The Netherlands

"Laboratory of Microbiology of Faculty of Planmony University of Laboratory Formigal

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The isolates exhibited a similar multiply resistance pattern to b forchmit, antirophonolates and outpolenes. All D<sup>2</sup> and ARDRA identification of the terminates forchmit different as A boundard A blo-exist gene was detracted in the bootent, all foundus clustered in European close B (considering done to cel - 30%). Comparison of the ten selected indice them as A boundard of the select - 30%). Comparison of the ten selected indice transite, with those of boundard of the select - 30%). Comparison of the ten selected indice transite, with those of bounders of the select - 30%. Comparison of the ten selected indice transite, with those of boundard of close B boundard at a first they belonged to Bubbles does B. They will be ten and beaution of close B boundard B b tensities indicating by each residence from the first an beaution of close B boundard B b tensities with the other solvers from the first an beaution of genetic relations. These isolates were a title more unstanted from the main cluster. It is genetic relations at the first selection with the more unstanted from the main cluster. It is genetic relations at the second selection with the more unstanted from the main cluster. It is genetic relations at the second selection busines the ten selection in the second second tender B bound at a b form second selection with the more unstanted from the main cluster. It

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# EPIDEMIOLOGICAL SURVEILLANCE OF ACINETOBACTER BAUMANNII COLONISATION AND INFECTION IN ICU PATIENTS

A. Agodi<sup>a,b,c</sup>, M. Barchitta<sup>a</sup>, R. Cipresso<sup>a</sup>, L. Giaquinta<sup>d</sup>, M. A. Romeo<sup>d</sup> and C. Denaro<sup>d</sup>

<sup>a</sup>Department of Biomedical Sciences, <sup>b</sup>LAPOSS, Laboratorio di progettazione, sperimentazione e analisi di politiche pubbliche e servizi alle persone, University of Catania, <sup>c</sup>GISIO, Gruppo Italiano Studio Igiene Ospedaliera, <sup>d</sup>Intensive Care Unit, Azienda Ospedaliera "Cannizzaro", Catania, Italy

Multidrug-resistant *Acinetobacter baumannii* has become an important cause of nosocomial infection (NI) worldwide. Studies addressed to clarify the epidemiology of *A. baumannii* colonisation and infection are scarce. In order to identify, assess and apply relevant evidence for better healthcare decision-making, this study evaluated the impact and the routes of acquisition of *A. baumannii* in ICUs, by determining: (i) the occurrence of *A. baumannii* carriage on admission; (ii) ICU-acquired *A. baumannii* infection; and (iii) ICU-acquired colonisation rates, by site.

A 6-month active surveillance survey was performed at the ICU of an Italian Hospital, in accordance with the HELICS protocol. The study focused on three different epidemiological patterns of *A. baumannii* acquisition: (i) carriage on admission; (ii) colonisation of sterile sites; and (iii) infections during ICU stay. Briefly, patients with positive screening cultures in the absence of positive clinical specimens were considered to be carriers. Patients with positive clinical specimens, but without clinical data confirming infection, were considered to be colonised/infected. When both clinical and screening cultures were positive on the same day, the patient was considered as colonised/infected. Standard HELICS definitions of NI were used, including the following NI sites: pneumonia, bloodstream infections (BSIs), central venous catheter-related BSI and urinary tract infections (UTIs).

During the survey period, 123 patients were admitted to the ICU. No episodes of *A. baumannii* carriage on admission were identified. In total, 47 multiresistant *A. baumannii* isolates, all imipenem-susceptible, were identified from 21 different patients, together with *Pseudomonas aeruginosa* in 76.2% (16/21) of cases. The ICU-acquired colonisation rate was 13.8% patients and the incidence density was 7.8% patient-days. The ICU-acquired infection rate was 13.0% patients and the incidence density was 7.4% patient-days. ICU-acquired pneumonia (VAP) was the most frequent *A. baumannii*-sustained infection type (43.0%), followed by local CVC related infections (25.0%), UTI (18.7%) and CVC-related BSIs (12.6%).

*A. baumannii*-sustained infection in ICUs represent the tip of an iceberg, whereas colonisation reflects the submerged part, that would remain hidden in the absence of surveillance. The study confirms the essential role of epidemiological surveillance in providing advanced risk-adjusted comparison of infection rates between ICUs as a measure of quality of care.

## EPIDEMIOLOGICAL SURVEILLANCE OF 4CMETOL4CIER BARRANDE COLONISATION AND INFECTION IN ICU PATIENTS

## A Agodi . M. Barabitta, Jo Cipresso, L. Ausquinta, M. A. Roman, and C. Penaro"

Department of Brannelinal Sciences "Lad (2051, aboratorio di progestazione spectimentatione e malita di postelos perificite e servizi alle pareme, University of Catoma GISIO, Granpa Italiane Studio (piere Parodatiere, "intensive Cara Unit, Aziranta Ospodatiere "Cametazato", Catada, Italy

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# A UNIQUE DOMAIN IN ESTERASE FROM ACINETOBACTER VENETIANUS RAG1 MEDIATES EMULSIFICATION ACTIVITY OF A VARIETY OF POLYSACCHARIDES

## R. Furman, H. Bach and D. Gutnick

Dept. Molecular Microbiology and Biotechnology, Tel Aviv University, Israel

Bioemulsifiers are generally amphipatic biological molecules capable of both forming and stabilising oil/water or water/oil emulsions. One such microbial product which has been produced on an industrial scale is emulsan, a lipohetero- aminopolysaccharide produced by the Gram-negative Acinetobacter venetianus RAG1. When recovered in crude form from the culture broth, emulsan consists of about 10 - 15% protein and 22 - 25% fatty acids (w/w). Recently, it was discovered that among the proteins associated with emulsan is an extracellular esterase which was subsequently cloned and sequenced. The recombinant protein was found to enhance emulsan activity, particularly after initial deproteination of emulsan (apoemulsan). When the C-terminal third of the enzyme was used in place of the intact protein, similar enhancement of emulsification was observed. Surprisingly, esterase can also induce emulsification activity in various polysaccharides which were otherwise inactive. To further characterise the interactions between esterase and different polysaccharides, a system employing cloned esterase fragments fused to the maltose binding protein was used. Different polysaccharides were tested for their affinity both towards intact esterase and towards the esterase fragments. Interestingly, most of the polysaccharides displayed high affinity towards the recombinant esterase. Further observations revealed that within the esterase itself, highest affinity of the polysaccharides was towards the C-terminus, suggesting that this portion of the enzyme is responsible for emulsification enhancement. Using computer-assisted search programs, it was found that the C-terminus of the enzyme contains a unique amphipathic domain which was not detected in any other protein sequence in the database. This domain was shown to be essential for emulsification enhancement. In addition immunological assays showed that this domain is apparently exposed to the protein surface. Moreover, when esterase mediated emulsions were subjected to proteolysis, an 7.5 kDa esterase fragment was detected. This fragment, which was not found when the enzyme was digested in the absence of an emulsion, corresponds in size to the amphipatic site in esterase C-teminus.

It was hypothesised that esterase can mediate the interactions of different polysaccharides with oil droplets through this unique amphipathic domain. The results presented here suggest that the esterase protein may be involved in at least two different activities, one enzymatic and the other as a structural mediator. Experiments to further test this hypothesis are currently in progress.

A UNIQUE DOMAIN IN ESTERASE FROM ACTIVITION CERTER FENERANS RAGI MEDIATES EMULSIFICATION ACTIVITY OF A VARIETY OF POLYSACCHARIDES

#### R. Furnan, H. Bana and D. Ounick

Dept. Molecular Microbiology and Bioleconiology. Tel Arth University Israel

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# AUXILIARY PROTEINS FOR AROMATIC TOLERANCE AND PROCESSING, DETECTED IN ACINETOBACTER RADIORESISTENS S13 PROTEOME

E. Pessione<sup>a</sup>, R. Mazzoli<sup>a</sup>, M. G. Giuffrida<sup>b</sup>, P. Fattori<sup>a</sup>, C. Lamberti<sup>a</sup> and C. Giunta<sup>a</sup>.

<sup>a</sup>Dipartimento di Biologia Animale e dell'Uomo, University of Torino, <sup>b</sup>ISPA-C.N.R. c/o Bioindustry Park Canavese, Colleretto Giacosa, Torino, Italy

Several Acinetobacter species have been reported to be able to grow on sites polluted by crude oil (oil spills, oil tankers) due to the production of specific catabolic enzymes able to degrade aliphatic and/or aromatic molecules. Sometimes these substrates are used as the sole carbon and energy source, while otherwise they can be degraded by co-metabolism. The finding of specific catabolic enzymes can supply information about the degradation pathway. Functional proteomics is a useful tool to investigate bacterial responses to environmental stimuli. During aromatic exposure, A. radioresistens S13, a fast phenol-degrading strain isolated in our lab for bioremediation purposes, biosynthesises specific enzymes, absent in acetate cultures, previously detected by both proteomic and transcript analyses. Besides enzymes for either phenol or benzoate catabolism, we found, in aromatic stimulated conditions, de novo production or over-expression of some 'satellite' proteins located both in the cytosol and in the (inner and outer) membranes. The 2-DE gels were performed in both the acidic and the alkaline pH ranges, and proteins have been identified by the ESI Ion trap MS, due to the lack of a known genome in this Acinetobacter species. Proteins and glycoproteins detected when phenol (P) or benzoate (B) were the only carbon source can be divided into six functional groups: (a) biosurfactants; (b) proteins involved in the modification of Acinetobacter surface properties; (c) stress proteins; (d) transport systems; (e) pH homeostasis and phosphate metabolism; and (f) lipid metabolism. Some of them proved to be abundant in certain growth phases. Even if B and P cultures share the majority of protein. nevertheless some proteins (or protein isozymes) are specifically induced by B or P. The present investigation allows a better understanding of A. radioresistens S13 physiological responses to aromatics, demonstrating that the adaptive responses to polluted environments include a sequence of strategies to ameliorate substrate availability and tolerance.

## AUXILIARY PROTTING FOR ABOMATIC TOLERANCE AND PROCESSING. DETECTED IN ACIVETORACTER RADIORISISTENS SIJ PROTEOME

E. Pessione, R. Mazzali, M. G. Gallhals, P. Fanner, C. Lambert, and C. Gunn

Dipartimento di Biologia Aninezie e dell'Uomo, University of Torine, ISPA-C.N.R. e/o Bioindustry Park Canavese, Collectito Oracoste Torine, Italy

Several Linterlobation recents have been manifed to be the to new on state polluted by and coll (oil spills, oil makers) due to the production or excises cale to entrust at the sole excisor and energy source, while collections sometimes that the high several at the sole functional protocomes at reself tool to treetings methal responses to an normalial procurs in protocomes at reself tool to treetings methal responses to an normalial simuli. During atometic encourse, it concrustes at 11 is the protocomes them in solated in our no for interpretation protocomes and the several responses to an normalial solated in our no for interpretation protocomes and the several responses to an normalial solated in our no for interpretation protocomes the theorem experiments simulant accust cultures, previously described by both renormers and theorem experiments and accust cultures, previously described by both renormers and theorem experiments at a solated in our set of interpretation protocomes and theorem and the sector and the events and in the (user and ource) meanmants. The sector protocomes describe the sector and in the (user and ource) meanmants. The sector protocomes at the protocomes describe protocomes in the strength to be tool in the sector describe and in the (user and ource) meanmants. The sector protocomes at the protocomes describe and in the (user and ource) meanmants. The sector protocomes at the protocomes at the sector describes protocomes in the sector protocomes at the protocomes at the sector describes of the strength to be protocomes at a protocome and the sector protocomes and the protocomes at a protocome and the sector protocomes at the protocomes at a strength one protocomes at a protocome and protocomes at the protocomes at a normality. The protocomes at protocomes at the protocomes at a normality of protocomes and the sector protocomes at the protocomes at a normality (a protocomes at protocomes and protocomes at protocomes at anomalies, the protocomes and protocomes ath

# A MAJOR OUTER-MEMBRANE PROTEIN Omp38 OF ACINETOBACTER BAUMANNII IS IMPORTED INTO THE NUCLEUS BY A SIGNAL-DEPENDENT PATHWAY AND DEGRADES DEOXYRIBONUCLEIC ACIDS

C. H. Choi<sup>a</sup>, S. H. Hyun<sup>b</sup>, S. A. Kim<sup>c</sup>, Y. S. Lee<sup>d</sup>, J. Y. Lee<sup>a</sup>, Y. C. Lee<sup>a</sup> and J. C. Lee<sup>a</sup>

<sup>a</sup>Department of Microbiology, Kyungpook National University School of Medicine, Daegu, Korea.

<sup>b</sup>Department of Clinical Pathology and <sup>c</sup>Pharmacology, Eulji University School of Medicine, Daejeon, Korea.

<sup>d</sup>Department of Parasitology, Inje University, College of Medicine, Busan, Korea.

A major outer-membrane protein Omp38 of Acinetobacter baumannii ATCC 19606<sup>T</sup> consisted of 356 amino-acids and showed  $\beta$ -barrel porin. Omp38 induced apoptosis of host cells through mitochondrial targeting in the early time, but induced necrosis in the late time. This study characterised the nuclear translocation of Omp38 and assessed the nuclease activity of Omp38. Recombinant Omp38 proteins targeted the nucleus of the eukarvotic cells. With regard to the nuclear targeting of Omp38, a novel monopartite nuclear localisation signal (NLS), KTKEGRAMNRR, was identified between residues 320 and 330 in the Cterminal region. NLS is predicted to form an  $\alpha$ -helix structure and is exposed to the bacterial surface. The NLS region is conserved in the major OMPs of pathogenic A. baumannii, Acinetobacter sp.3 and sp.13TU, but not in A. radioresistens and Acinetobacter ADP1. Transient expression of Omp38 fused to EGFP at N-terminus or C-terminus caused the nuclear localisation of this fusion protein in cultured cells. Omp38ANLS and a site-directed mutant of NLS region, which substituted lysine with alanine (ATAEGRAMNRR), were exclusively cytoplasmic location of Omp38. Omp38 directly interacted with adaptor protein importin a, as demonstrated by immunoprecipitation. Of interest, recombinant Omp38 proteins degraded eukaryotic DNAs by endonucleolytic and exonucleolytic attack. These results imply that Omp38 of A. baumannii translocates to the nucleus and degrades DNA in the host cells. We propose a new pathogenic mechanism of Acinetobacter species in aspects of host-pathogen interaction.

A MAJOR OUTER-MEMBRIAME PROTEIN Duppe OF ACTIVITOBACTER BAUMANNII IS IMPORTED INTO THE MUCH EUS BY A SICKAL-DEPENDENT PATHWAY AND DECRADES DEOCS REMEMBLICESC ACHIN

C.H. Choi, S.H. Hyan, S.A. Kim, Y.S.Lee, J.Y. Lee, Y. C. Lee and J. C. Lee

Department of Microbiology, Syungpook National University School of Modicine, Dargo Korea.

Department of Cloucal Pathology and Phatmasology, Eulin Liamarcay Science of Medicine, Dacicon, Korea.

Department of Parastology, Inja University, College of Medicine, Husan, Kores

A major outer memiryne preteile Orandi ei el energia perior. Oran 62 m incol mortesis el tres consisted ef 156 amine-auda and aboved o perior presentante or an incol mortesis el tres cells through minochordeni targetag in the energi time bui inductivo consin to an intermentive study characterised the ratisfere maniformics of ourpel's and assessed me ratisfere activity of Ourp38 Recorabinert Ourp38 process and executive targetage and With regard to the makes angeing of Ourp68 is novel recorreging and a 20 m a 20 m a 20 m appal (NLS). El EGURANDER, was identified on the transmission and a 20 m a 20 m a 20 m attransi trepon bill. Sin predicted to form an other structure and a consistent activation attransi trepon bill. Sin predicted to form an other structure and a consistent activation attransi trepon bill. Sin predicted to form an other structure and a consistent activation attransi trepon bill. Sin predicted to form an other structure and a consistent activation attransi trepon bill. Sin predicted to form an other structure and a consistent activation attransi trepon bill. Singen is tomeword in the outpet of the demonstructure and a transmission attransi trepon bill. Singen is tomeword in the outpet of the demonstructure and the attracted expression of Orap38 hard to EGUP a N eminima of C remaines consent the antiast of NLS region, which antisticated trans with attractivation of a structure of the exclusively opticulated expression of Orap38 (struct) interacted with a structure activation definited expression of Orap38 (struct) interacted with a structure attracted expression of Orap38 of A baraneous (structures of the maines and departed points and the tree cells. We propose a tree galoration introduces to the maines and departed DNA in the barbes cells. We propose a tree galoration introduces to the maines and departed DNA in the activation of the structure attractive.

# LOSS OF HETEROLOGOUS FLANKING DNA DURING INTROGRESSION OF AN ANTIBIOTIC RESISTANCE GENE IN MUTATOR AND NON-MUTATOR POPULATIONS OF *ACINETOBACTER* SP.

## J. L. Ray<sup>a</sup>, P. J. Johnsen<sup>a</sup> and K. M. Nielsen<sup>a,b</sup>

<sup>a</sup> Department of Pharmacy, Faculty of Medicine, University of Tromsø, Tromsø, Norway <sup>b</sup> The Norwegian Institute of Gene Ecology, Science Park, Breivika, Tromsø, Norway

This study sought to determine the rate at which heterologous non-selected DNA, flanking a selected genetic marker, is eliminated from a recipient population following a transformation event. To this end, genomic DNA was isolated from transformants of Acinetobacter baylyi strain BD413 (non-mutator) or Acinetobacter sp. strain ADP7021/AmutS6 (mutator) after DNA from nine unique nptII-tagged isolates of Acinetobacter sp. 62A1 were used as donors in filter transformation assays. Acinetobacter sp. 62A1 is approx 24.78% divergent from the recipient strains, as estimated from 3345 bp of the mutS and trpE genes. The first round of transformation using the nine isolates revealed a >800-fold difference in transformation frequencies into the non-mutator recipient (p 0.088) and >500-fold difference into a mutator recipient (p 0.026), demonstrating that transformation frequencies with heterologous DNA vary greatly across the recipient genome. When genomic DNA from transformants for each isolate was used to re-transform the respective recipients, it was observed that restoration of homologous transformation frequencies occurred often after only one backcross, despite high variation in the original transformation frequencies. Introgression of the nptII trait was studied for several generations (backcrosses) of transformants in both recipient strains. Sequencing of the flanking regions on both sides of the nptII marker gene for individual transformants obtained for each transformation and generation (backcross) revealed that heterologous, nonselected flanking DNA often persists for at least four backcrosses in a non-mutator strain, and possibly even longer in a mutator strain. In addition, successive backcrosses gradually, although asymmetrically, reduced the length of heterologous sequencing flanking the nptII marker. These findings provide, to our knowledge, the first empirical evidence for the fate of non-selected heterologous flanking DNA in a sexually recombining population of bacteria. The results suggest competent bacteria rapidly lose sequence heterogeneity in the recombined flanking DNA regions after the acquisition of novel genes from other species, and explain foreign gene introgression in bacteria.

LOSS OF HETEROLOGOUS FLANKING DNA DURING INTROGRESSION OF AN ANTIBIOTIC RESISTANCE GENE IN MUTATOR AND NON-MUTATOR FOPULATIONS OF ACTVETORACTER SP.

#### J. L. Ray, P. J. Jehnson' and K. M. Melseo''

Department of Phannamy, Facury of Medicine, University of Transa, Tromso, Norway, <sup>1</sup> The Norwegian Institute of Gene Ecology, Science Park, Brevika, Tromsa, Norway

# INVESTIGATION INTO BIOFILM FORMATION AND INTERACTION WITH HUMAN CELLS TO EXPLAIN THE CLINICAL ROLE OF ACINETOBACTER BAUMANNII VS. OTHER ACINETOBACTER SPECIES

A. de Breij<sup>a</sup>, L.J.G. van Diemen<sup>a</sup>, M.T. van den Barselaar<sup>a</sup>, G.V. Bloemberg<sup>b</sup>, P.J. van den Broek<sup>a</sup>, P.H. Nibbering<sup>a</sup> and L. Dijkshoorn<sup>a</sup>

<sup>a</sup>Dept. of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands; <sup>b</sup>Institute of Biology, Leiden University, Leiden, The Netherlands

Acinetobacter baumannii is an important clinical species; the clinical or ecological role of most other Acinetobacter species is largely unknown. To obtain insight into the differences in clinical relevance among the various Acinetobacter spp. at the molecular level, this study focused on biofilm formation and adherence of acinetobacters to different human cell types. In addition, cytokine production by human cells in response to the strains was determined.

Adherence of *A. baumannii* and non-*baumannii* species (*A. calcoaceticus*, *A. junii*, and *A. baylyi*) to human cells was assessed by light microscopy. Biofilm formation on plastic was quantitated using a standard microtitre plate assay. The presence and transcription of the *csuE* gene, which has been hypothesised to be associated with biofilm formation, was detected by RT-PCR. Cytokine production by bronchial epithelial cells and blood cells was quantified using ELISAs and multiplex systems.

Both A. baumannii and non-baumannii strains adhered to cultured human bronchial epithelial cells. Using six A. baumannnii strains, the quantitative adherence to bronchial epithelial cells, laryngeal epithelial cells and keratinocytes was compared. Each strain exhibited its intrinsic quantitative adherence regardless of cell type. A wide variation in the amount of biofilm formation among strains was observed, with no difference between A. baumannii and non-baumannii. For a set of 37 A. baumannii, a poor correlation (r 0.34; p < 0.01) between adherence to bronchial epithelial cells and biofilm formation was found. CsuE was detected in 44 of 45 A. baumannii strains, but in none of 13 non-baumannii strains. No correlation between csuE mRNA expression in A. baumannii and adherence to bronchial epithelial cells or biofilm formation was seen. All Acinetobacter strains induced the production of the pro-inflammatory cytokines interleukin (IL)-8 and IL-6 by bronchial epithelial cells. Compared with A. baumannii, it was observed that A. calcoaceticus and A. junii induced a significantly stronger proinflammatory cytokine response in these cells. In contrast, these non-baumannii strains induced less TNF $\alpha$ , a pro-inflammatory cytokine, by blood cells when compared to A. baumannii.

The main finding of this study was that both *A. baumannii* strains and non-*baumannii* strains were able to form biofilms, to adhere to human cells, and to induce cytokine production by human cells. The results do not clarify the differences in clinical relevance between *A. baumannii* and non-*baumannii* strains.

INVESTIGATION INTO PROPIEM PORMATION AND INTERACTION WITH HUMAN CELLS TO EXPLAIN THE CLEVICAL ROLE OF ACINETOBACTER BAUMANNE VS. OTHER ACINETORACTER SPECIES

A. de Breij<sup>\*</sup>, L.J.G. van Dicorep<sup>\*</sup>, M.F. van den Borochan<sup>\*</sup>, G.V. Bloemberg<sup>\*</sup>, P.J. van den Brock<sup>\*</sup>, P.H. Nibberang<sup>\*</sup> and L. Diffeshoom<sup>\*</sup>

Dept. of infectious Diseases, Leiden University Medical Cauter, Leiden, The Metharlands; "institute of Blelogy, Leiden University, Leiden, The Netherlands

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## 021

# GENE EXPRESSION PATTERNS OF RESPIRATORY EPITHELIAL CELLS BY A MAJOR OUTER-MEMBRANE PROTEIN Omp38 OF ACINETOBACTER BAUMANNII

# S. A. Kim<sup>a</sup>, S. M. Yoo<sup>b</sup>, S. H. Hyun<sup>c</sup>, C. H. Choi<sup>d</sup>, Y. C. Lee<sup>d</sup> and J. C. Lee<sup>d</sup>

<sup>a</sup>Department of Pharmacology, <sup>b</sup>Microbiology, and <sup>c</sup>Clinical Pathology, Eulji University School of Medicine, Daejeon, Korea

<sup>d</sup>Department of Microbiology, Kyungpook National University School of Medicine, Daegu, Korea

Pathogen-associated molecular patterns (PAMPs) can modulate the gene expression of host cells during the infection. A major outer-membrane protein, Omp38, is considered to play an important role in the pathogenesis of Acinetobacter baumannii. Using a DNA microarray, gene expression profiles of human laryngeal epithelial HEp-2 cells were analysed following the interaction with Omp38. Among 22 277 gene probe sets, 242 probe sets were found to be differentially expressed at least two-fold. The most abundant functional categories of the differentially expressed genes were transcriptional regulators, signal transduction components, protein phosphorylation, and inflammatory molecules. Omp38 up-regulated the gene expression of toll-like receptor 2 (TLR2). Pro-inflammatory cytokines and inflammatory mediators, such as interleukin-8 (IL-8), IL-1β, IL-6, IL-12, tumour necrosis factor α, phospholipase A2, and arachidonate 5-lipoxygenase-activating protein, were differentially expressed, as demonstrated by real-time PCR. Western blot analysis revealed that phosphorylation of tyrosine residues of total cellular proteins was increased and peaked after incubation for 8 h. Immune responses of HEp-2 cells against Omp38 were mediated by the JNK-ERK mitogen-activated protein kinase (MAPK) signalling pathway, but not by the NFκB pathway. This result indicates that Omp38 is an important PAMP and regulates a relatively small set of genes in epithelial cells. The gene expression of epithelial cells against Omp38 is primarily focused on immune responses at mucosal sites.

CENE EXCRESSION DATTERNS OF RUSTRATORY SECONDAL CELLS BY A MAJOR OUTER MEMBRANE PROTEIN CORP. OF ALEVETOBACIER RAUMONI

### S.A. Kim', S.M. Yoo', S.R. Hynn', C.H. Chei, Y. C. Lee' and J. C. Lee

Department of Pharmacology, "Algnowiology, and "Clinical Pathology, Eulip University School of Medicine, Dasjeets Kotsa

"Department of Microbiology, Kyanapook National University School of Methology, Pacyu, Korea

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## 022

# MOLECULAR ANALYSIS OF ACINETOBACTER BAUMANNII--INDUCED INFLAMMATORY RESPONSE

#### C. March<sup>a</sup>, P. Morey<sup>a</sup> and J. A. Bengoechea<sup>a,b</sup>

<sup>a</sup>Unidad de Investigación and Institut Universitari d'Investigacions en Ciències de la Salut (IUNICS), Hospital Universitario Son Dureta, Palma de Mallorca, Spain <sup>b</sup>Program Infection and Immunity, Fundació Caubet-CIMERA Illes Balears, Bunyola, Spain

Acinetobacter baumannii is a leading cause of important nosocomial infections; however, the mechanisms involved in *A. baumannii* pathogenesis are not yet well-known. The aim of this study was to study the inflammatory response activated by human airway epithelial cells (cell line A549) and human peripheral blood monocytes following infection with *A. baumannii*.

Evidence was obtained showing that *A. baumannii* ATCC19606 was a poor stimulator of inflammatory responses because it induced the secretion of IL-8 by A549 cells and TNF- $\alpha$  and IL-1 $\beta$  by human monocytes only after 8 h of infection. Similar results were obtained with clinical isolates of *A. baumannii* resistant to antibiotics. It was also demonstrated that *A. baumannii* infection of airway epithelial cells resulted in the activation of MAP kinase p38 and NF- $\kappa$ B pathways. Moreover, both pathways were necessary for IL-8 secretion by airway epithelial cells. Finally, it was also found that *A. baumannii* OmpA modulated the inflammatory response induced by *A. baumannii*. An OmpA mutant induced the secretion of inflammatory cytokines by airway epithelial cells and human monocytes at early time points (2 h after infection) in a process dependent on the activation of p38 and NF- $\kappa$ B. These results indicate that *A. baumannii* OmpA may act as a potential virulence factor by limiting the activation of inflammatory responses by eukaryotic cells following infection with *A. baumannii*.

# MOLECULAR ANALYSIS OF ICINETORICTER EXEMPLYDUCED

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# THE USE OF COLISTIN TO TREAT MULTIRESISTNT ACINETOBACTER INFECTION IN A REGIONAL BURNS INTENSIVE CARE UNIT

H. Ganapathy, S. K. Pal, L. Teare and P. Dziewulski

St Andrew's Centre for Burns and Plastics, Chelmsford, UK

A retrospective study was undertaken to evaluate the use of colistin in treating multiresistant *Acinetobacter* infection in the St Andrew's Centre for Burns and Plastics, a regional burns intensive care unit (ICU). Data were analysed from patients admitted over the 2-year period from November 2003 to November 2005. All patients who received colistin, either in the intravenous or nebulised form, or both, were included in the study. Admission data included burn percentage, presence or absence of inhalational injury, other relevant medical history, and blood results at admission (white cell count (WCC), creatinine and C-reactive protein). The following parameters were also recorded: duration of ICU stay, ultimate outcome, time of start of colistin, total dose and repeated doses of colistin, mode of drug delivery, presence or absence of organ support, concomitant antibiotics with colistin, presence of ventilator-associated pneumonia, organisms grown and their resistance. Response to colistin was judged by improvement in clinical status, i.e., reduction in temperature, decrease in WCC, decrease in inflammatory markers and no growth on cultures. The data were subjected to non-parametric Wilcoxon Signed Rank Test using SPSS v.14.

Twenty-nine patients were included in the study, all of whom received colistin in one form or the other. The average total dose of colistin was 69 mU (range 1 - 268 mU). Of the 29 patients, 17 (58.6%) survived and 12 (41.4%) died. However, the results were more meaningful when the number of patients who improved with colistin was analysed. Twenty (69%) patients improved, and nine (31%) did not improve with colistin. Six patients received intravenous colistin only, and four (66.7%) of these patients improved. Six patients received nebulised colistin only, and all of them improved (100%). Seventeen patients received both intravenous and nebulised colistin, and ten (58.8%) of these patients improved. When creatinine levels were compared upon admission and after colistin administration, non-parametric Wilcoxon Signed Rank test showed no difference between the two groups (p 0.38).

It was concluded that the use of colistin to treat multiresistant *Acinetobacter* infections in burns patients is safe and effective, and no statistically significant impairment of renal function was observed.

# THE USE OF COLLETIN TO TREAT MULTIRESISTNT ACLASTOBACTER INFECTION IN A REGIONAL BURNS INTENSIVE CARE UNIT

R. Ganapathy, S. K. Pal, L. Taus and P. D'ziev uista

St Andrew's Centre for Burns and Plantics. Chelmatord, UK

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It was concluded that the use of collecting to one weathresselling (climerobacter infections in parties partonts is safe and effectives, and no variation(), significant impartment of renal matching was observed.

# **CECROPIN A-MELITTIN PEPTIDES ARE ACTIVE AGAINST CLINICAL COLISTIN-RESISTANT STRAINS OF** *ACINETOBACTER BAUMANNII*

M. Fernández-Reyes<sup>a</sup>, J. M. Saugar<sup>a</sup>, B.G. de la Torre<sup>c</sup>, R. López-Rojas<sup>b</sup>, F. Docobo-Pérez<sup>b</sup>, J. Pachón<sup>b</sup>, D. Andreu<sup>c</sup> and L. Rivas<sup>a</sup>

<sup>a</sup>Center for Biological Research-CSIC, Madrid, Spain,

<sup>b</sup>Service of Infectious Diseases, University Hospital Virgen del Rocío, Sevilla, Spain, <sup>c</sup>Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona, Spain.

Acinetobacter baumannii possesses an outstanding capacity to develop resistance against antibiotics. This has progressively led to an exhaustion of the antibiotic armamentarium available in clinics. This trend has included energence of resistance against colistin, the last drug universally active against the pathogen [1]. Synthetic hybrid cecropin A-melittin peptides combine the N-terminal sequences of the two parental peptides [2]. This results in a broader specificity with respect to cecropin A and a much lower cytotoxicity than melittin. Even though these peptides share common traits with polymyxin, such as a strong cationic character and amphipathic structure, the differences in the mechanism of bactericidal action have been previously demonstrated with the colistin-sensitive reference strain ATCC 19606 [3].

The present study tested four cecropin A-melittin peptides (CA(1-8)M(1-18) (KWKLFKKIGIGAVLKVLTTGLPALIS-NH<sub>2</sub>), CA(1-7)M(2-9) (KWKLFKK-IGAVLKVL-NH<sub>2</sub>), its N- $\alpha$ -octanoyl derivative (Oct-KWKLFKKIGAVLKVL-NH<sub>2</sub>), and CA(1-7)M(5-9) (KWKLLKKIGAVLKVL-NH<sub>2</sub>) against clinical *A. baumannii* isolates with variable levels of colistin resistance, ranging from 4 to 64 mg/L. The four hybrid peptides were active against all isolates, regardless of their level of colistin resistance, with MICs in the 1.1 - 2.5  $\mu$ M range. This activity was attributed to a higher affinity for the LPS of resistant strains, relative to colistin, as demonstrated by dansyl-polymyxin displacement, as spheroplasts were lysed equally by peptides [4]. In order to improve unexpected levels of toxicity of the cecropin A-melittin hybrid peptides in mouse models, a new generation of peptides was designed, with an increase in overall cationicity achieved by the incorporation of two additional L-lysine residues at the N-terminus KK-CA(1-7)M(2-9): KKKWKLFKKIGAVLKVL-NH<sub>2</sub>. The improvement in therapeutic index was mainly caused by a reduction in haemolytic activity, rather than an increase in antibacterial activity.

Thus, this study demonstrated the viability of cecropin A-melittin hybrid peptides as an alternative for the treatment of patients with colistin-resistant isolates, as well as an improvement over previous efforts in the search for effective chemotherapeutic agents against *Acinetobacter*.

1. Emerg Infect Dis 9:1025.

2. In: Pore-forming peptides and protein toxins, p.215-259. Harwood: Reading, UK.

3. Antimicrob Agents Chemother 46:875.

4. Antimicrob Agents Chemother 50:1251.

## CECROPIN A-MELITIAN PEPTIDES ARE ACTIVE AGAINST CLINICAL COLISTIN-RESISTANT STRAINS OF ACLVETØRACTER RAUMLANT

M. Feminder Reyes", J. M. Saugar', B.G. de la Tone", R. Lopez-Kojas", F. Docobo-Perez", J. Pachón", D. Andreu' and L. Rivas'

#### Center for Stological Research CSIC, Medrid, Spain,

Service of infectious Diseases. University Hospital Virgen del Modo, Sevillo, Spala, "Department of Experimental and Health Sciences, Fompeu Faira University, Barralona, Spain.

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3. Antinderah Agalar Chalaothar 46:515.

4. Antimitista Avenus Committee 98:1251

# THE DIVERSITY OF THE GENUS ACINETOBACTER, CURRENT STATE AND EMERGING PROBLEMS

L. Dijkshoorn<sup>a</sup>, M. Vaneechoutte<sup>b</sup>, T. DeBaere<sup>b</sup>, T. van der Reijden<sup>a</sup>, A. Nemec<sup>c</sup>

<sup>a</sup>Department of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands; <sup>b</sup>Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium; <sup>c</sup>Centre of Epidemiology and Microbiology, National Institute of Public Health, Prague, Czech Republic

The genus *Acinetobacter* comprises a total of 33 genomic species. Delineation of all 33 (named and unnamed) species has been based on or corroborated by DNA-DNA hybridisation studies. Only 17 species have valid names, while an additional one - '*Acinetobacter venetianus*'- fulfils all criteria for validation. Of the unnamed species, genomic sp. 9 is congruent with *Acinetobacter lwoffii*, while haemolytic genomic sp. 13 described by Tjernberg & Ursing (APMIS, 1989) corresponds to genomic sp. 14 described by Bouvet & Jeanjean (Res Microbiol, 1989).

Despite the increase in knowledge of the diversity of the genus *Acinetobacter*, there are still many problems to solve. First, identification of most species by phenotypic tests including commercial systems is problematic. Identification by 16S rDNA analysis is also problematic since several unrelated species have high similarity values. Next, the description of most unnamed species and of six of seven named, environmental species was based on only one or a few strains per species. Because of this low number, the character divergence of the species is unknown, which makes identification of new isolates to these species cumbersome. Another problem is that most unnamed species have not been described in the official journal of the International Committee of Systematic Bacteriology, the International Journal of Systematic and Evolutionary Microbiology (IJSEM). From the taxonomic point of view, this is logical since no names were proposed, but the journals in which the groups with potential species status have been described may not be widely read. Altogether, due to the complexity of the taxonomy and the lack of practical identification methods, relatively little is known about the ecology and clinical significance of most *Acinetobacter* species.

Comparison of fingerprints generated by selective restriction fragment amplification with AFLP<sup>™</sup> and amplified 16S rDNA restriction analysis (ARDRA) to those of libraries of reference strains appear to be powerful tools for delineation of (novel) *Acinetobacter* spp. and for species identification. These methods have been used to analyse >1500 isolates of *Acinetobacter*, obtained over the past two decades and including isolates from different ecological and geographical origin. In addition to the existing 33 species, it was possible to delineate 28 groups containing multiple strains and 21 single strains. It is conceivable that these groups and strains represent additional species in the genus *Acinetobacter*. In the short term, it will be difficult, if not impossible, to confirm the existence of so many species by DNA-DNA hybridisation and to develop identification schemes. These findings raise the question about the sense of extensive speciation within the genus and the way to go ahead.

## THE DIVERSITY OF THE GENES ACTMETORICIER, CONDENT STATE AND ENDREING PROBLEMS

#### D. Dijkaboort, M. Vanechoute, T. Dellane, T. van der Rahden, A. Vener,

"Department of Infections Diserses, Louise University Medical Carrier, Louise, The Netherlands: "Department of Circleal Chertoons, Muschindogs and Invantology, Chant University Hospital, Ghent, Belging, Cardover Epifermatogy and Muschindogy, Mattonal Institute of Fushic, Prague, Creek Republic

The genus Armetedoctor comprises a total of 35 generals sectors indication of all 33 (named and unterned) spectre has been based on or contracted of EDLA-INCA hytridisenon studies. Only 13 spectre have valid names, while en additioned one - 'article-'inch hytridisenon renerators' faiths all criteria for valid names, while en additioned one - 'article-'inch hytridisenon comprised with Antoneonoder heght, while harmely do generate point and the faith of the lighthering & Using (AFMIS, 1989) concepted to generate print described of lighthering (Fee Merchick, 1989) concepted to generate print described of beingen (Res Merchick, 1989).

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Comparison of insperjence, gas model by associve restriction fragment amplification with AFEF\*\* and amplitude 165 films A creationan analysis (AED\*\*\*) to those of libraries of reference strates appear to be presented to be formeating of (norsh) storeroburnet app, and for species identification. These provides have sent used to analyse > 1000 moleties of aconomication obtained over the part (we consists and to analyse > 1000 moleties of aconomication obtained over the part (we consists and including sociates from different evolutioned and geographical origin. In addition to the subscript 17 species it was persible to define as 25 project continues multiple scales and molecular from the reaction was defined to the obtained restriction and the subscript fill and to the provide to define and geographical origin. In addition to the subscript fill provide it is contributed to define a provide and the imposed and the induce to the species of the defined to will be difficult or an imposed by the obtaining the strategies to the define and the strategies and the imposed by the contribution of the set of the difficult of the difficult of the obtained the strategies of the negative to define a strategies and the strategies and the set of the set of the strategies to define a strategies and the strategies of the strategies of the set of the strategies to define a strategies and the strategies and the set of the set of the strategies to define a strategies and the strategies and the set of the set of the strategies and define a strategies and the strategies and the set of the set of the strategies and define a strategies and the strategies and the set of the set of the set of the define a strategies and the strategies and the set of the set of the set of the define a strategies and the strategies and the set of the set of the set of the set of the define and set of the strategies and the set of the set of the set of the set of the define as the set of the set

# A RAPID PCR-BASED METHOD TO DIFFERENTIATE BETWEEN ACINETOBACTER BAUMANNII AND GENOSPECIES 3

P. G. Higgins, H. Wisplinghoff and H. Seifert

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

PCR-based methods to differentiate between *Acinetobacter baumannii* and genospecies 13 at present are laborious and time-consuming, e.g., ARDRA, or do not differentiate at all, e.g., tRNA spacer regions. A simple and quick method is therefore desired.

Alignment of the *A. baumannii* and sp.13 *gyrB* nucleotide sequences revealed species-specific polymorphic regions that enabled the design of a primer pair, one of which would anneal specifically to *A. baumannii*. PCR was initially performed using a temperature gradient to determine the optimum annealing temperature that yielded a PCR product for *A. baumannii* but not sp.13. A third universal-primer was included as a control. PCR was performed on both purified DNA and crude cell lysate. Standard strains representing all the major clinical acinetobacters were also included in the study. tRNA spacer fingerprinting showed no difference between the *A. baumannii* and sp.13 strains used in the study.

Using the three-primer mix, both *A. baumannii* (*n*=30) and sp.13 (*n*=12) amplified a 300-bp PCR product. This PCR product served as a control and was not found with the other *Acinetobacter* spp. In addition, *A. baumannii* had a second PCR product of 490 bp that was not found with sp.13. Using a very short PCR cycle, the PCR was completed in 1 h. PCR products were analysed by gel electrophoresis.

In conclusion, it was possible to demonstrate a rapid and simple PCR-based method to differentiate between *A. baumannii* and sp.13. It is estimated that a result is possible from a culture plate to species determination in 2 h, and the test could thus be of use in a clinical laboratory.

## A RAPID PER-BASED METHOD TO DIFFERENTIATE BETWEEN ACINETOBACTER RAUMANNIFAND CEMOSPECIES 3

#### P. G. Higgan, H. Waninghoff and H. Soileit

Institute for Medical Microbiology, Immunology and Hygions, University of Colegos, Goldenfelsstessa 19-21, 50935 Gologne, Germany

PCR-based methods to differentiate between Acimetebacter baranannii and genorpeoies 13 nt present are laborious and time constituting, e.g., ARDRA, or do not differentiate et all, e.g., tRNA spacer regions. A simple and quick method is therefore desired.

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# EVALUATION OF VITEK FOR DETECTION OF CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

A. A. Alsultan, A. Hamouda and S. G. B. Amyes

Centre for Infectious Diseases, Molecular Chemotherapy, University of Edinburgh 49 Little France Crescent, Edinburgh EH16 4SB, Scotland, United Kingdom

Acinetobacter baumannii genospecies 2 is an opportunistic pathogen found in hospitals that causes serious infections, especially in intensive care units (ICUs). It is reported to be involved in healthcare-associated infection with an increasing frequency all over the world. Many hospitals use VITEK for identification of Acinetobacter spp., including A. baumannii, and clinicians determine treatment for infections caused by this bacterium based on results of this test. The aim of the work described here was to evaluate the sensitivity of VITEK for detection of A. baumannii.

Ten isolates of *Acinetobacter* spp. were isolated from clinical specimens in Scotland, between January and March 2006, were identified by VITEK as *A. baumannii*. Growth at 44 °C was used to differentiate between *A. baumannii* genospecies 2, 13TU and other *Acinetobacter* spp. Identification of *A. baumannii* was done by restriction analysis of the 16S-23S rRNA intergenic spacer sequences.

Of the ten isolates identified as *A. baumannii* by VITEK, only two could be confirmed as *A. baumannii* genospecies 2 from the restriction pattern obtained after digestion of the 16S-23S rRNA intergenic spacer sequences amplicon with *Nde*II. This genospecies is clearly identified by the production of 50-bp and 360-bp fragments. The restriction site between these two fragments was lost in the eight remaining isolates. Two isolates had a pattern compatible with sp.13 or N1, one had a pattern compatible with sp.3 or N2, two had a pattern most similar with sp. 13, and three had previously unreported patterns.

The results show that VITEK classifies a diverse group of *Acinetobacter* spp. as *A. baumannii*, and that further genotypic analysis is required to classify them reliably.

## EVALUATION OF VITER FOR DETECTION OF CLINICAL ISOLATES OF ACINETORACTER RADMANN

#### A. A. Alsulian, A. Hammuda and S. G. B. Amyees

Centre for Infections Discosse, Molecular Chametharapy, University of Editibuted 49 Little France Creatent: Edinburgh EH16 18B: Scotland, United Kingdom

Activated causer bournament genotypectes 2 is an apportunitive periodent to use in haspitals that causes serious infections, expectality in intensive care initia [10] if is reported to by involved in healthcare associated infection with the increasing frequency all over the world. Many hospitals can VITEK for identification of *formatobares* up, multiding A. baumanni, and clinicians determine treatment for intention cares to evaluate the semainity of VITEK for this test. The aim of the work described increasing to evaluate the semainity of VITEK for detection of A baumanni.

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The results show that VITER classifies a diverse group of Active sector app, as A between it, and thus further activity analysis is required to classes when reliably

# WHAT UNITES ACINETOBACTER BAUMANNII STRAINS ISOLATED IN INTENSIVE CARE UNITS IN RUSSIA?

A. Solomennyi<sup>a</sup> and A. Goncharov<sup>b</sup>

<sup>a</sup>Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, Perm, Russia.

<sup>b</sup>Department of Epidemiology, I.I. Mechnikov State Medical Academy, St. Petersburg, Russia.

The Russian nosocomial infection surveillance program has monitored antimicrobial resistance profiles for hospital-acquired *Acinetobacter baumannii*. With rare exceptions, no significant differences are observed in antibiograms as compared with the European outbreak strains. Antimicrobial resistance appears to be increasing both in Russia and across the Europe in the same manner.

There is little (or nothing) known of the genetic determinants characterising the population of *Acinetobacter* isolates in Russia. This study documents, for the first time, a class 1 integron with a variable segment of 2.5 kb in a long-persisting strain in a St.Petersburg burns ICU. Restriction analysis has revealed its similarity to an integron which has been investigated in the European epidemic clones of *A. baumannii* during 1989 - 2004. The same gene cassette content was found in a local ICU strain in Krasnodar (South Russia). However, in a set of outbreak strains isolated in St.Petersburg and Krasnoyarsk (Siberia) ICUs during 1998 - 2004, the nucleotide sequences specific for class 1 integrons were not detected. In contrast, all outbreak strains studied for class 2 integron by integrase PCR demonstrated an amplified fragment of 288 bp in size [1]. Strains evaluated as *int11+int12*-positive gave a product of the expected size (approx. 250 bp) by PCR for ATPase gene insertion [2]. The strains studied appear to have this hotspot site intact.

The public health risk of integron-positive multidrug-resistant *A. baumannii* strains is no longer in question. However, the analysis of integrons and/or other insertion events in the genome of outbreak *Acinetobacter* is accessible only to an extremely limited number of laboratories and probably not many strains are really investigated.

1. *J Clin Microbiol* **39**: 8-13. 2. *PLoS Gen* **2**(1): e7.

### WHAT UNITES ACTIVETORACTER RADIMANNIESTRAINS ISOLATED IN INTENSIVE CARE UNITS IN RUSSIA?

#### A. Solomennyi' and A. Conolarov'

"institute of Ecology and Genetics of Microorganiana, Ural Branch of the Kussian Academy of Sciences, Perm, Russia

Department of Epidemiology, II. Mechaikov State Medical Academy, St. Petersjang, Russia.

The Russian nosoconial infration surveillance program has memored antimicrobial resistance profiles for hospital-acquired *Activationacter basmatran*. With rare exceptions, no, significant differences are charrened to antibiograms as compared with the European outbreak status. Antipicrobial test status repeare to be increasing both in Russia and across the Furope in the same manuer.

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## DO LARGE CHROMOSOMALLY-INTEGRATED GENOMIC ISLANDS CONTRIBUTE TO ESISTANCE DISSEMINATION IN ACINETOBACTER SPP.?

F. Shaikh<sup>a</sup>, F. Almathen<sup>a</sup>, H-Y. Ou<sup>a,c</sup>, K. Levi<sup>b</sup>, K. J. Towner<sup>b</sup>, M. Barer<sup>a</sup> and K. Rajakumar<sup>a</sup>

<sup>a</sup>Dept of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK; <sup>b</sup>Dept of Microbiology, University Hospital, Nottingham, UK; <sup>c</sup>School of Life Science & Biotechnology, Shanghai Jiaotong University, Shanghai, China

Despite considerable research into plasmid- and chromosome-borne resistance determinants in Acinetobacter spp., little is known about the larger genomic context within which these resistance determinants lie. The presence of a partially characterised Acinetobacter chromosomal antibiotic resistance locus that resembled an equivalent region in the Shigella Resistance Locus island and the prevalence and predominant chromosomal location of class 1 integrons in Acinetobacter spp. led us to hypothesise that a proportion of chromosomal antibiotic resistance-encoding loci in A. baumannii and other related Acinetobacter spp are borne on large, chromosomally integrated resistance islands that themselves contain integron or transposon structures. The recent identification of an 86-kb resistance island in A. baumannii strain AYE that harbours three class I integron structures supports this hypothesis. To investigate the prevalence of resistance islands in multidrug-resistant clinical A. baumannii isolates, the chromosomal antibiotic resistance loci were cloned, associated integron structures were detected and analysed, and the sequences of flanking regions were determined. Separate genomic libraries were generated for A. baumannii strains CW1, CW14 and CW20 using restriction enzymes BamHI, EcoRI, HindIII, PstI and SalI, and the low-copy number vector pWSK29 (Ap<sup>R</sup>). The genomic ligations were transformed into E. coli DH5a by electroporation. Bacterial clones with desired target genes were selected on media supplemented with the appropriate antibiotic. We obtained marker rescue clones from CW14 genomic libraries bearing the following fragments: a 3.8-kb BamHI fragment encoding gentamicin<sup>R</sup> (pGent14), a 23-kb PstI fragment encoding streptomycin<sup>R</sup> (pStr14), and a 9-kb Sall fragment encoding amikacin<sup>R</sup> (pAmi14). Similarly a 6-kb BamHI fragment from CW1 encoding gentamicin<sup>R</sup> (pGentA1), and a 3.8-kb BamHI fragment from CW20 encoding gentamicin<sup>R</sup> (pGentA20) were obtained. These clones were then further characterised by endsequencing, restriction mapping, PCR analyses and shot-gun subcloning to facilitate 2ndround end-sequencing. In addition to universal vector primers, primers for the 5'-conserved segment (CS) and 3'-CS of class 1 integrons and forward and reverse primers for the aminoglycoside-resistance genes aac(3)-Ia (aacC1) and ant(3")-Ia (aadA1) were used. Identification of several integron structures revealed the presence of multiple distinct class I integrons within a single strain, and identified a class 1 integron containing both aacC1 and *aadA1* that has several features in common with a multiple-cassette-bearing integron present in the A. baumannii strain AYE resistance island.

This study was funded by a British Society for Antimicrobial Chemotherapy grant to KR, KT and KL.

### DO LARGE CHROMOSOMALLY-INTEGRATED GENOMIC ISLANDS CONTRIBUTE TO ESISTANCE DISSEMINATION IN ACINETOBACTER SPR.1

### F Shaikh", F. Almahan", H-Y. Ou", K. Levi", K. L. Towner", M. Barer' and K. Raiskrumer"

"Dept of Infection, Immunity and Inflammation, University of Leiceauer, Leiceater, UK; "Dept of Microbiology, University Hospital, Nottingham, UK; "School of Life Science & Biotechnology, Shanghai Jicotong University, Shanghai, China

Despite consistentials research mini plasmade and chroninautre borne restance determinant in *Actacologicae* app, italia is known acou the trear genome context within which these restances determinant its. The presence of a pairing characterized determinant determinant indicate restances (context and presence of a pairing) restances documentation and the presence of a pairing characterized determinant of the similar determinant in the presence of a pairing characterized determinant determinant in the presence of a pairing characterized determinant of the minibility restances exceeding fort main annumant and other stand determinant income of transpoon structures. The presence of control of the structure approximation of transpoon structures in the previous of the structure approximate determinant stans of C that hadrons there cases i isoperal structures approximate a determinant stans of C that hadrons there cases i isoperal structures approximate and C W 20 mang extinction reservings for the second structure of the day and C W 20 mang extinction reservings for the second structure of the determinant isolates, the chromosonial indicates and the day of mining approximation isoperation and stand the structure of the day approximate the day of the second determinant isoperation of the day of determinant (C W 20 mang extinction restance in the second structure (W 20 marks vector pW SK20 (Ap<sup>2</sup>). The genome transport exting the day of marks vector pW SK20 (Ap<sup>2</sup>). The genome transport exting the day of promoting genome the support of the structure of the day of the day of the structure in the support of the structure of the day of the day of the structure in the support of the structure of the day of the day of the structure in the support of the structure of the day of the day of the structure in the support of the structure of the day of the day of the structure in the support of the structure of the day of the day of the structure in the support of the structure of the day of the structure of the structure in the

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## A COLLECTION OF GENE REPLACEMENT MUTANTS OF ACINETOBACTER BAYLYI ADP1: EXPLORING GENE FUNCTIONS

V. de Berardinis, M. Salanoubat, D. Vallenet, V. Castelli, S. Samair, A. Kreimeyer and J. Weissenbach

UMR 8030 CNRS, Genoscope – Centre national de séquençage et Université d'Evry, 2 rue Gaston Crémieux, 91000 Evry, France

Acinetobacter baylyi ADP1 is highly competent for natural transformation and strictly aerobic. Its strong natural tendency towards homology-directed recombination affords extraordinary convenience for genetic manipulation. Several metabolic features, as well as its simplified sugar metabolism, make it a versatile organism suitable for biotechnological applications. Of a total of 3207 CDSs predicted by the sequence analysis of ADP1, 36% were assigned to known functions, 29% encoded proteins with putative functions, 28% conserved hypothetical proteins (CHP), and 7% hypothetical proteins (HP). This analysis also showed that Acinetobacter ADP1, similarly to other soil bacteria, has oriented its metabolism towards the degradation of organic compounds found in their natural habitat. To confirm these metabolic features and others, and to gain additional knowledge on the putative functions encoded by ADP1 genes, we have realised a collection of gene replacement mutant consisting of an excision of the target gene via homologous recombination and insertion of a kanamycin resistance marker. Replacement recombinants were selected for kanamycin resistance on a mineral medium supplemented with succinate as the carbon source. To date, 2452 knock-out mutants have been obtained, including 780 CHPs, 188 HPs, 785 putative proteins and 690 known proteins. The number of essential genes is estimated at about 500 genes. As already described in other knockout collections of Escherichia coli and Pseudomonas aeruginosa, the majority of essential genes are known proteins. The set of essential genes estimated on minimal medium allowed the essentiality to be examined of genes with known and unknown functions involved in biosynthetic pathways as amino-acid or co-factor biosynthetic pathways. The collection of mutants has been tested for its ability to grow on various carbon sources in liquid and solid conditions (acetate, 2-oxoglutarate, asparagines, glucose, lactate, malonate, citrate, lactate, quinate, pyruvate, succinate and 2,3 butanediol). Some unexpected phenotypes were observed. A general scheme to collect experimental evidence and explore enzyme function is proposed.

## A COLLECTION OF GENE REPLACEMENT MUTANTS OF ACINETOBACTER BATLY ADPT: EXPLORING GENE FUNCTIONS

V. de Berardinis, M. Salanoubat, D. Vallenat, V. Castelli, S. Samair, A. Kielmey is and J. Weinsenbach

CMR 8010 CNRS, GEROMMER - Centre trational de requiraçage al Université d'Evry, 2 me Gaston Crémienz, 91006 Barg, France

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## ISCR2-MEDIATED ACQUISITION OF THE blaveb-1A EXPANDED-SPECTRUM B-LACTAMASE GENE IN ACINETOBACTER BAUMANNII FROM ARGENTINA

L. Poirel<sup>a</sup>, S. Corvec<sup>a,b</sup>, M. Rapoport<sup>c</sup>, F. Pasteran<sup>c</sup>, D. Faccone<sup>c</sup>, M. Galas<sup>c</sup>, T. R. Walsh<sup>d</sup> and P. Nordmann<sup>a</sup>

<sup>a</sup>Service de Bactériologie-Virologie, Hospital Bicêtre, South-Paris Medical School, 94275 K.-Bicêtre; <sup>b</sup>Lab. de Microbiologie, Centre Hospitalier de Nantes, France; <sup>c</sup>Serv. Antimicrobianos INEI-ANLIS "Malbran", Buenos Aires, Argentina; <sup>d</sup>Dept. of Pathology and Microbiology, Univ. Walk, Bristol, UK

The expanded-spectrum  $\beta$ -lactamase gene  $bla_{VEB-1}$  has been identified mostly in South Asia in Enterobacteriaceae and *Pseudomonas aeruginosa*, and also in France in *Acinetobacter baumannii*. In addition, *P. aeruginosa* isolates producing the VEB-1a variant have been reported from Kuwait and India. The  $bla_{VEB-1/VEB-1a}$  genes have been identified mostly as part of class 1 integrons, but also with peculiar genetic structures called Re repeated elements (Re). Recently, several *A. baumannii* isolates producing the VEB-1a variant have been recovered in Argentina. Preliminary experiments showed that the  $bla_{VEB-1a}$  gene was not identified inside a class 1 integron and was not associated with Re elements.

PCR with primers specific for the  $bla_{VEB-1}$  gene and for class 1 integrons were tested. Cloning and expression in *Escherichia coli* was performed and the genetic background was evaluated by the I-CeuI technique. The  $bla_{VEB-1}$  gene was identified with the so-called insertion sequence ISCR2 (formerly CR2 element). This IS91-like genetic element was identified upstream of the  $bla_{VEB-1a}$  gene, likely providing promoter sequences for the expression of that  $\beta$ -lactamase gene. A truncated copy of the ISCR2 element was present downstream of  $bla_{VEB-1a}$ , likely suggesting that a recombination between two ISCR2 copies had occurred and was at the origin of  $bla_{VEB-1a}$  acquisition. Analysis of the  $bla_{VEB-1a}$  genetic location showed that it was very likely chromosome-encoded.

This study constitutes the second identification of the  $bla_{VEB-1}$  ESBL gene in *A. baumannii* after that observed in France, and the first identification of an IS*CR2* element in *A. baumannii*. This study further demonstrates that  $bla_{VEB-1a}$  acquisition may be linked to a variety of genetic elements. In addition, this finding represents the first evidence of an IS*CR2*-mediated acquisition of a  $\beta$ -lactamase gene after that of the SXT sulphonamide resistance gene and *sul2* sulphonamide resistance gene.

### ISCRI-MEDIATED ACQUISITION OF THE MARTHE EXCANDED-SPECTROM B-LACTAMASE GENE IN ACINETORACTER BACHANNE FROM ARGENTINA

L. Poirel', S. Corste<sup>w</sup>, M. Rapoport, F. Pastenas, D. Facouzs, M. Galas', T. R. Walth' and P. Nordmann<sup>\*</sup>

"Service de Bactériologie Viralogie, Hospital Bieture, South-Paris Medical School, 94275 K. Bicêtre: "Lab. de Microbiologie, Centre Hospitalier de Mantes, Frances: "Serv. Antimicrobianos DNEI-ANLIS Mathem", Buenos Aires, Argentins; "Dept. of Pathology and Microbiology, Univ. Walk, Bristol, UK

In expanded spectrum k-lactumes gene of average has been ubcelled mostly in South A an in Enterobacteriaceae and Panadorocaus nerugatora, and also in France en Actificationer teamorant in addition. P are agained isolates producing the VEB-1a variant have been reported from K-twein and hadin. The biotecomora genes have been identified mostly as part of class 1 integrant, but also with pertainer genetic screetenes called Re repeated demons (Re). Recently, several A dominance isolates producing the VEB-1a variant have been reported in Activity, several A dominance isolates producing the VEB-1a repeated demons (Re). Recently, several A dominance isolates producing the VEB-1a remain have been repovered in Augenting Pretingents isolates producing the VEB-1a researed the integrant is for the pretingent isolates and the secret of the table of the reported repovered in Augenting Pretingents and was not associated with Re researces

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## DIFFERENTIIAL PROTEIN EXPRESION IN WILD-TYPE AND COLISTIN-RESISTANT ACINETOBACTER BAUMANNII PROBED BY DIGE PROTEOMIC ANALYSIS

M. Rodríguez<sup>a</sup>, M. Fernández-Reyes<sup>b</sup>, J. Pachón<sup>c</sup>, C. Chiva<sup>a</sup>, L. Rivas<sup>b</sup> and D. Andreu<sup>a</sup>

<sup>a</sup>Department of Experimental and Health Science, Pompeu Fabra University, Barcelona, Spain; <sup>b</sup>Eukaryotic Antibiotic Lab, Centre of Biological Research-CSIC, Madrid, Spain; <sup>c</sup>Infectious Disease Service, Virgen del Rocío University Hospital, Sevilla, Spain

Acinetobacter baumannii is a non-motile coccobacillus that acts as an opportunistic pathogen causing severe nosocomial infections with high mortality rates. A. baumannii can be a causal agent of diseases like pneumonia, bacteraemia, meningitis, soft-tissue and urinary tract infections. Multidrug resistance is common among A. baumannii isolates and has left polymyxins as the only alternative treatment. However, recent reports of sporadic outbreaks of colistin-resistant A. baumannii have prompted the search for resistance targets and new antimicrobial strategies.

Colistin resistance was induced in the reference strain of *A. baumannii* ATCC 19606 and differential protein expression was studied in the wild-type and resistant strains by 2D electrophoresis and mass spectrometry. As a first step, cytosolic protein extracts from wild type *A. baumannii* were analysed. A total of 136 protein spots were used for normalisation of the average intensity, of which 122 were successfully identified by either MALDI-TOF/MS PMF or LC-MS/MS, using MASCOT search engines in either case. Most proteins were identified by homology with *A. baylyi* ADP1, some with other species of *Acinetobacter*, and some by homology with other bacterial genera. Their cellular localisations were mostly cytoplasmic although some were from the cell membrane or had multiple cellular localizations. Most identifications (78%) were associated with metabolic processes, 12% with transport events including membrane, intracellular and electron-chain transport, 7% had unknown functionalities, 3% were related to cell organisation and biogenesis processes and with cellular division, 2% were involved in cell communication and regulation of biological processes, and 1% with sensory perception and development.

2D fluorescence difference gel electrophoresis (DIGE) analysis was chosen to study differential protein expression between wild-type (or colistin-sensitive) and colistin-resistant *A. baumannii*. In total, 42 differentially expressed proteins were found, of which 23 were identified successfully by either of the above MS methods. Again, most localisation was cytoplasmic although a few were membrane-related and some had unspecified localisations. Among the functionalities found, 90% were related to metabolism, 5% were transport-related and 9% were involved in other cellular events.

DIFFERENTIAL PROTEIN EXPRESSION IN WILD-TYPE AND COLISTIN-RESISTANT ACINETORACTER BAUMANNE PROBED BY DIGE PROTEOMIC ANALYSIS

M. Rodniguez, M. Fernaldez-Reyes, J. Pacifor, C. Chivar, L. Rivar, and D. Andrey

"Department of Experimental and Health Science, Pompeu Fabra University, Barcelona, Spain: "Eukaryone Antibiotic Lab, Centre of Hological Research-CSIC, Madrid, Spain; "Infectious Disease Service, Virgen del Scole University Hospital, Sevilla, Spain

Activetobacter baumanuli is a non-notile encodoacilius that acts as an opportunistic pathogen eausing severe nonocomial indections with high mortality rates. A baumanui can be a causal agent of diseases like procuration bacteriaema, meningins, soft-tissue and urfnery tract infections. Multiding resistance is contrate among 4 baumanui isolates and has left polymyxins as the only alternative treatment. Forwave, recent reports of sporadic outbreaks of colistin-resistant 4 baumanuf have prompted the search for resistance angets and new automotopid grategies.

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## **RELATIONSHIP BETWEEN THE AdeABC EFFLUX SYSTEM GENE CONTENT, NETILMICIN SUSCEPTIBILITY AND MULTIDRUG RESISTANCE IN A GENOTYPICALLY DIVERSE POPULATION OF** *ACINETOBACTER BAUMANNII*

A. Nemec<sup>a,b</sup>, M. Maixnerová<sup>b</sup>, T. J. K. van der Reijden<sup>c</sup>, P. J. van den Broek<sup>c</sup> and L. Dijkshoorn<sup>c</sup>

<sup>a</sup>3rd Medical School, Charles University, Prague, Czech Republic; <sup>b</sup>National Institute of Public Health, Prague, Czech Republic; <sup>c</sup>Leiden University Medical Center, Leiden, The Netherlands.

The AdeABC efflux system, encoded by three structural genes (*adeA*, *adeB*, *adeC*), has been associated with decreased susceptibility to aminoglycosides and other antibiotics in clinical strains of *Acinetobacter baumannii*. Up-regulation of this system has been ascribed to mutation in the regulatory genes *adeR* or *adeS*. This study assessed the occurrence of the AdeABC efflux system, and its association with antibiotic resistance, in a well-documented set of *A. baumannii* strains.

A genotypically and epidemiologically heterogeneous set of 120 *A. baumannii* strains was investigated. The strains were allocated to six multidrug resistant (MDR) clonal groups (n=71) or to unique genotypes (n=49) by AFLP analysis. PCR detection of the *adeA*, *adeB*, *adeR* and *adeS* genes was performed. Susceptibility to 11 antibiotics was tested by diskdiffusion. In addition, MICs to netilmicin, a possible phenotypical marker for up-regulation of the AdeABC system, was determined. Finally, PCR was used to detect two genes (*aacC2* and *aacA4*) which encode netilmicin-modifying enzymes and are commonly found in *A. baumannii*.

Ninety-nine (83%) strains, including all except one of the strains belonging to EU clones I -III, were positive for all four genes, while ten strains were positive for one to three genes. None of the genes were found in 11 strains, eight of which were fully susceptible (FS). Strains positive for all genes were MDR (n=75) or FS (n=23). Seven MDR strains were positive for aacC2 or aacA4. All A. baumannii strains (n=61) with netilmicin MICs  $\geq 4$  mg/L, but without the netilmicin resistance genes, were both MDR and positive for all four genes, while strains with netilmicin MICs  $\leq 2$  mg/L (n=52) included all FS strains and those that tested negative for one or more efflux genes. The FS strains were genotypically highly heterogeneous as indicated by their AFLP profiles.

In conclusion, AdeABC is common in both MDR and FS *A. baumannii*, but may be absent in some strains. Strains with AdeABC genes present but not expressed, as indicated by susceptibility to netilmicin, are not uncommon and are usually susceptible to many other antibiotics. It is postulated that multidrug resistance in *A. baumannii* is generally associated with the presence of upregulated AdeABC.

The study was supported by grant 8554-3 of the Internal Grant Agency of the Ministry of Health of the Czech Republic and NWO fellowship (B93-483).

RELATIONSHIP BETWEEN THE ADAINC REFLUX SYSTEM GENE CONTENT. NETHANCIN SUSCEPTIBILITY AND MULTIDRUG RESISTANCE IN A GENOTYPICALLY DIVERSE FOR ULATION OF ACIVETOBACTER BAUMANNI

A. Nemeca-D, M. Maintenevale, T. J. K. van der Reijder<sup>e</sup>, P. J. van den Brock<sup>e</sup> and I. Dijkshoom<sup>e</sup>

<sup>6</sup>3rd Medical School, Chattes University, Prague, Czech Republic, Phational Institute of Public Health, Prague, Czech Republic, I eiden University Medical Center, Leiden, The Netherlanda

The AddABC efflux system, snooded by three supprised genes (add, add<u>al, edeC)</u>, has been associated with decreased susceptibility to anathogized des and other autibiories in clinical attains of Activitoriacian betweennul. Up regulation of this avaitment has been ascribed to mutation in the regulatory genes add/f of add5. This muty meeters the occurrence of the AddABC efflux system, and its association with antibiotic assistence, in a well-documented and of A. brawarnal Strains.

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The study was supported by great \$329-3 or the Internal Great Agrees of the Manary of Houles at the Castle Resulting and NWD followsking \$387-4355

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# RESISTANCE TO FLORFENICOL DUE TO *flor* GENE IN TWO CLINICAL STRAINS OF *ACINETOBACTER BAUMANNII*

L. Ramos, C. Fernández, J. Villanueva, M. Domínguez, H. Bello and G. González

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

Florfenicol is a broad-spectrum, primarily bacteriostatic, antibiotic with a range of activity similar to chloramphenicol, including many Gram-negative and Gram-positive organisms, and has been developed specifically for veterinary use to treat respiratory pathogens in bovines. It was the first antibiotic approved in the USA for use in fish farms, and is widely used in Chile and other countries. Resistance to this antibiotic is mainly caused by a specific efflux pump system, encoded by the *flo* gene. Florfenicol is not approved for human use; however, it is related to chloramphenicol and can select for cross-resistance among bacterial pathogens. For this reason, the aim of this work was to study the prevalence of the *floR* gene among 30 clinical isolates of *Acinetobacter baumannii*, resistant to chloramphenicol, isolated from various Chilean hospitals during 2000 and 2001.

Resistance to florfenicol was determined by the NCCLS agar diffusion method (2000). The presence of *floR* was investigated by PCR using specific primers, giving an expected amplicon size of 399 bp. PCR products were confirmed by restriction with *Ava*II, yielding two fragments of 287 and 112 bp which were later sequenced and compared with sequences in GeneBank. Matings and curing experiments were carried out in order to investigate the possible transferability of florfenicol resistance.

Three (10%) isolates yielded the expected PCR product, and these isolates had very similar antibiotic patterns. The nucleotide sequence analysis showed that PCR products amplified from two isolates of *A. baumannii* had 99% similarity to *flo* gene sequences from other Gramnegative bacteria included in GenBank. Florfenicol resistance was not transferred to the recipient strain *Escherichia coli* K-12 Rif<sup>R</sup> and no cured strains were obtained.

We believe that this is the first report of the presence of floR gene in isolates of A. baumannii and, more importantly, in clinical isolates of this non-fermenting Gram-negative bacterium.

Supported by FONDECYT-CHILE Grants 1000352 and 1040924

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## ANTIMICROBIAL CHARACTERISTICS OF HOSPITAL URINARY ISOLATES OF ACINETOBACTER SPP. - A 5-YEAR SURVELLANCE PERIOD

I. Hadzi-Petruseva Meloska<sup>a</sup>, B. Kurcik Trajkovska<sup>a</sup>, G. Jankoska<sup>a</sup>, A. Hadzi-Petruseva Jankijevic<sup>b</sup>, M. Petrovska<sup>a</sup>

<sup>a</sup> Institute of Microbiology and Parasitology, Medical Faculty, <sup>b</sup>Secondary Medical School, Skopje, Macedonia

Acinetobacter belongs in the top ten most frequent nosocomial isolates from lower respiratory tract, wounds, drains, chemocultures and the hospital environment. Isolation from urinocultures is less frequent, and is often associated with other sites of colonisation and infection. The mode of infection is usually ascendant, and the strains isolated show high resistance and are difficult to treat. The aim of this retrospective study was to analyse the antimicrobial susceptibilities of hospital urinary isolates of *Acinetobacter* spp. in a 5-year surveillance period (1 January 2000 - 31 December 2005).

In total, 2327 isolates of *Acinetobacter* spp. were obtained from in-patients and the hospital environment of the Clinical Center, Skopje. Standard microbiological techniques, API 20E and VITEK GNI/GNS cards were used for isolation, identification and susceptibility testing to amoxycillin-clavulanic acid (AMC), piperacillin (PIP), ceftazidime (CAZ), ceftriaxone (CRO), cefotaxime (CXM), cefipime (CPM), amikacin (AN), gentamicin (GM), ciprofloxacin (CIP), ofloxacin (OF), norfloxacin (NOR), imipenem (IMP), meropenem MEM), pipemidic acid (PI) and nitrofurantoin (NF), using NCCLS recommendations for MIC breakpoints.

Of the 2327 *Acinetobacter* isolates, 972 (41.7%) originated from lower respiratory tract samples, 488 (21.0%) from wounds and drains, 396 (17.1%) from the hospital environment, 253 (10.9%) from upper respiratory tract samples, 143 (6.1%) from chemocultures, and 75 (3.2%) from urine. The number of viable bacteria in 50 (66.7%) urines was higher than 100 000 CFU/mL. All urinary *Acinetobacter* spp. isolates were resistant to the third-generation cephalosporins CRO and CXM, and also to AMC, PI and NF. The susceptibility was very low to GM, PIP and quinolones (five, ten and 14 susceptible isolates, respectively); the greatest susceptibility was to AN and IMP (46 and 56 isolates, respectively). High levels of resistance to the third-generation cephalosporins and quinolones was present in isolates from other sites.

The increasing pathogenic importance and frequency of resistant hospital *Acinetobacter* infections has made periodic patient and environmental surveillance essential, combined with further typing of *Acinetobacter* spp. isolates.

## ANTIMIC ROBIAL CHARACTERISTICS OF HOSPITAL URINARY ISOLATES OF ACENEFORACTER SPR. - A 5-YEAR SURVEILANCE PERIOD

 Hadzi-Petruscve McIoska", B. Kunsik Trijkovska", G. Jankoska", A. Hadzi-Petruseva Jankijevie", M. Petrovska"

"Institute of Microbiology and Perturbiogy, Medical Faculty, "Secondary Medical School, Skople, Macedonia

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# THE ASSOCIATION OF IS1133 WITH AN AMINOGLYCOSIDE RESISTANCE GENE, *aac(3)-IIa*, IN ACINETOBACTER BAUMANNII ISOLATES

R. Jacobson<sup>a</sup> B. G. Elisha<sup>a,b</sup> and H. Segal<sup>a</sup>

<sup>a</sup>Division of Microbiology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town; <sup>b</sup>National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

Previous studies identified a portion of an insertion sequence, IS1133 sandwiched between an aminoglycoside resistance gene, aac(3)-IIa, and ISAba1 in A. baumannii strain PAU. DNA sequence analysis indicated a T to A transversion in the right inverted repeat (IR<sub>R</sub>) of the IS1133 fragment. The identical transversion was observed in a portion of IS1133 upstream of aac(3)-IIa in A. baumannii strain SAK isolated 9 years earlier. It was suggested that this mutation may limit recognition of the IR<sub>R</sub> by IS1133 transposase, inhibiting transposition of this element, and may therefore play a role in the stabilisation of aac(3)-IIa in clinical isolates of A. baumannii.

In total, 45 A. baumannii isolates, collected from patients at Groote Schuur Hospital and Red Cross War Memorial Children's Hospital from 1983 to 2006, were included in this study. The aac(3)-IIa gene was detected in 12 isolates by PCR, and similar assays indicated that this gene was linked to the right end of IS1133 in all of these isolates. Sequencing analysis of the IR<sub>R</sub> of IS1133 from one isolate obtained in 2001, 9 years after strain PAU, also contained the transversion in the IR<sub>R</sub>. To determine whether the aac(3)-IIa genes are linked to complete copies of IS1133 rather than fragments, PCR assays using primers directed against aac(3)-IIa and left-end IS1133 sequences were performed. Products of the expected size were not obtained; instead, 2.3-kb amplicons were obtained from the 12 isolates. A similar-sized product was obtained from strain PAU and strain SAK, suggesting an insertion into IS1133 in all 14 isolates. An amplicon from strain SAK was purified and sequenced. Analysis of the sequencing data indicated that IS1133 is disrupted by insertion of ISAba1, and that the insertion site is identical to that identified previously in strain PAU. It may be that disruption of IS1133 tnpA by ISAba1, combined with the T to A transversion in the IR<sub>R</sub>, has stabilised IS1133 and, in turn, aac(3)-IIa, in A. baumannii strains, and that strains with this genetic organisation have been maintained in the hospitals studied.

## THE ASSOCIATION OF ISFIES WITH AN AMINOGLYCOSIDE RESISTANCE. GENE, 49(3)-104, IN ACINETORACTER RACMANYII ISOLATES

#### R. Jacobson B. G. Elisina" and H. Segal

"Division of Microbiology, Institute of Infectious Diseases and Molecular Medicine, University of Cape Town; "National Health I aboratory Service, Groote Schutt Hespital, Cape Town, South Africa

Frevrous studies identified a partition of an macrition sequence, [S]f(t) is stud withed between an autooplycoside resistance gene, eac(3) flat and [S.abel] in A banazanii strate PAU, DNA sequences analysis indicated a T to A transversion in the right involved repeat (Rx) of the 181133 fragment. The identical transversion was observed in a portion of [S7733] upstream of acc(3)-flat in A banazanii strate (Rx) of the acc(3)-flat in A banazanii strate (Rx) of the identical transversion was observed in a portion of [S7733] upstream of acc(3)-flat in A banazanii strate (Rx) by (S1733) fragment. The identical transversion was observed in a portion of [S7733] upstream of acc(3)-flat in A banazanii strate (Rx) by (S1733) transposes (Rx) by (Rx)

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## DIFFERENCES IN ANTIMICROBIAL SUSCEPTIBILITY AND CARBAPENEM RESISTANCE MECHANISM BETWEEN ACINETOBACTER BAUMANNII AND ACINETOBACTER SP. 13TU

### J. C. Lee, K. M. Jeong, C. H. Choi, J. Y. Lee and Y. C. Lee

Department of Microbiology, Kyungpook National University School of Medicine, Daegu, Korea

The emergence and spread of antimicrobial resistance among Acinetobacter spp. is of great concern in the clinical setting. This study aimed to characterise the antimicrobial susceptibility of 232 non-duplicate Acinetobacter isolates from two Korean hospitals and to investigate the mechanisms responsible for resistance to carbapenems. Acinetobacter baumannii and Acinetobacter 13TU were the most prevalent species, together accounting for 87% of isolates. A. baumannii infections were associated significantly with wounds, while Acinetobacter sp. 13TU was isolated frequently from the respiratory tract (p < 0.05). Resistance rates for A. baumannii against penicillins, cephalosporins, monobactam, aminoglycosides, fluoroquinolones and trimethoprim-sulphamethoxazole were significantly higher than those for Acinetobacter sp. 13TU (p <0.05), while resistance rates against imipenem were significantly higher for Acinetobacter sp. 13TU than for A. baumannii (p <0.05). No pan-drug resistant Acinetobacter isolates were found. Almost half (48.2%) of A. baumannii isolates were resistant to more than five classes of anti-pseudomonal agents, while all Acinetobacter sp. 13TU isolates were resistant to one to four classes of anti-pseudomonal agents. Of the 32 imipenem-resistant acinetobacters, 14 Acinetobacter sp. 13TU isolates, belonging to three different clones, produced VIM-2 metallo-\beta-lactamase. However, 17 imipenem-resistant A. baumannii isolates, derived from five different clones, did not produce metallo-\beta-lactamases. In conclusion, there were significant differences in infection site, antimicrobial susceptibility and resistance mechanisms against carbapenems between A. baumannii and Acinetobacter sp. 13TU. The results of this study will be of use in epidemiological studies of antimicrobial resistance in Acinetobacter based on genomic species.

DUTTERENCES IN ANTIMICROBIAL SUSCEPTIBILITY AND CARBAPENEM RESISTANCE MECHANISM BETWEEN ACINETOBACTER RAUMANNIFAND ACINETOBACTER SP. 13TU

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#### P14

## AdeAB MULTIDRUG EFFLUX PUMP IS ASSOCIATED WITH DECREASED SUSCEPTIBILITY TO TIGECYCLINE IN ACINETOBACTER CALCOACETICUS/BAUMANNII

A. Ruzin, D. Keeney and P. A. Bradford

Wyeth Research, Infectious Disease, Pearl River, USA

It was previously shown that reduced susceptibility to tigecycline is associated with broadspecificity efflux pumps such as AcrAB and MexXY in several bacterial pathogens, such as *Proteus mirabilis, Morganella morganii, Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae* and *Pseudomonas aeruginosa*. This study investigated the role of the multidrug efflux pump AdeAB in decreased susceptibility to tigecycline in clinical isolates of *Acinetobacter calcoaceticus/ baumannii*. As most *A. calcoaceticus/baumannii* isolates are susceptible to tigecycline, cases of acquired decreased susceptibility to tigecycline in these species are unusual.

Gene expression was analysed by northern blot hybridization. Insertional inactivation of the *adeB* gene was achieved by a single crossover with a suicide plasmid construct carrying an *adeB* PCR fragment. Analysis of the *adeRS* locus was performed by PCR and sequencing. The MICs were determined by E-test.

Expression analysis revealed constitutive over-expression of *adeAB* in less-susceptible clinical isolates G5139 and 5140 (tigecycline MIC 4 mg/L) when compared with the isogenic clinical isolates G4904 and G5141 (MIC 1.5 mg/L). The expression of *adeAB* is regulated by the two-component signalling system encoded by the *adeR* and *adeS* genes. PCR and sequencing analyses suggested that up-regulation of AdeAB pump production in G5139 and G5140 is likely to result from an insertion of the IS*Aba1* element in the *adeS* gene. Insertional mutants GC7945 (*adeB* knockout in G5139) and GC7951 (*adeB* knockout in G5140) were obtained that resulted in tigecycline MICs of 0.5 mg/L.

In conclusion, this study demonstrated that decreased susceptibility to tigecycline in *A*. *calcoaceticus/baumannii* is associated with over-expression of the AdeAB multidrug efflux pump.

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A. Ruzin, D. Keeney and P. A. Bradford

Wyeth Research, infectious Disease, Pearl River, USA

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In conclusion, this study demonstrated the decreased subserptibility to tigethe in A calconecticut barmanat is associated with over-expression of the AdeAD multidrug offree pump.

## EVOLUTION OF RESISTANCE TO ANTIBIOTICS AND DETECTION OF CARBAPENEMASES SINCE 1999 TO 2005 IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII

C. Valderrey<sup>a</sup>, E. Sevillano<sup>a</sup>, M. Canduela<sup>a</sup>, I. Rosales<sup>a</sup>, F. Calvo<sup>b</sup> and L. Gallego<sup>a</sup>

<sup>a</sup>Dpto Inmunología, Microbiología y Parasitología, Facultad de Medicina. Universidad País Vasco; <sup>b</sup>Servicio de Microbiología del Hospital de Santa Marina, Bilbao, Spain

This study analysed the evolution in 1999 - 2005 of antibiotic resistance, mainly to carbapenems, in clinical isolates of *Acinetobacter baumannii* and its relationship with the presence of carbapenemases. The study included all isolates of *A. baumannii* collected during 1999 - 2005 (102 and 30, respectively) in a hospital in northern Spain. Susceptibilities were determined by the NCCLS disk-diffusion method to amikacin, ampicillim/sulbactam, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, chloramphenicol, co-trimoxazole, gentamicin, imipenem, meropenem, ofloxacin, piperacillin/tazobactam, tetracycline and tobramicin. Fingerprinting experiments were by PCR with M13 primer and by *ApaI* PFGE. Phenotypic detection of carbapenemases was with the Hodge and double-disk synergy tests. PCR was used to detect the *bla*<sub>OXA-40</sub>, *bla*<sub>VIM</sub> *bla*<sub>IMP-1</sub>, *bla*<sub>SPM-1</sub>, *bla*<sub>GIM-1</sub> genes and class 1 integrons, and selected isolates were analysed for plasmid content.

**RESISTANCE (%)** Year AMK SAM ATM FEP CTX CAZ CRO CIP CT CHL SXT GEN IPM MEM OFX TZP TET 1999 (n=102) 32.3 34.5 83.3 74.6 88.2 75.5 NT NT 0 NT NT 88.2 64.7 60.8 95.2 60.9 NT 2005 100 34.5 100 0 93.1 93.1 41.4 3.4 100 96.5 100 92.6 89.6 89.6 100 96.5 89.6 (n=30)

Overall resistance results are shown in the following table:

NT, not tested

Eleven different genetic patterns were detected during 1999, although the majority belonged to clone I (27.45%) and clone II (49%). Isolates from 2005 comprised only two genotypes with different prevalences: clone I (82.1%) and clone II (17.8%). Results for the presence of carbapenemases and integrons are detailed below:

Year	OXA-40		Integron	is (nº/%)	Integrons (size)		
	1999	2005	1999	2005	1999	2005	
Clon I	7 (25%)	23 (99.8%)	22 (78.6%)	23 (100%)	760 bp	760, 1500 bp	
Clon II	50 (99.8%)	5 (99.8%)	50 (100%)	5 (100%)	550 bp	550 bp	

No metallo-carbapenemases were detected. Plasmid analysis showed the presence of plasmids of 112, 82, 32 and 8 kb.

In conclusion, a gradual increasement in resistance to antibiotics was detected, mainly to some  $\beta$ -lactams, such as imipenem, meropenem and piperacillin/tazobactam. The clonal prevalence has changed with time, agreeing with the dissemination of the  $bla_{OXA-40}$  gene in clone I and the detection of another class 1 integron. More studies are needed to clarify the relevance of the plasmids detected.

EVOLUTION OF RESISTANCE TO ANTIBIOTICS AND DETECTION OF CARBAPENEMASES SINCE 1999 TO 2005 IN CLINICAL ISOLATES OF ACTIVETOBACTER INCREMENT

C. Valderrey", E. Scyllinno", M. Canduela", I. Rosales", F. Calvo" and L. Gallego"

"Dpio Immunologia, Microbiologia y Parasitologia, Facoltad de Medicina. Universidad Pais Vasco: "Servicio de Microbiologia del Hozrital de Sama Murina, Bilhuo, Spain

This study analyses (are evolution as 1979 - 2005 of antihietto resistance, mainly to carbapenents, in citairal isotares of fetuerolectory havalanti and its relationship with the presence of carbapenenteses. Fire there isotary instituted all isotates of A harmannii collected dama 1993 - 2005 (102 and 20, respendively in a respital in nombern Static Susceptibilities were determined by the NCC LS dask diffusion method to analogith in nombern Static Susceptibilities were actroname, or inplicing constraints, diffusion method to analogith, ampirithmysubactan, actroname, or inplication of the diffusion method to analogith, ampirithmysubactan, presented by the NCC LS dask diffusion method to analogith, ampirithmysubactan, actroname, or inplication, continuation of the diffusion method to analogith, ampirithmysubactan, provide the determined by the NCC LS dask diffusion and the analogith in more than a the sector and the data and the data data and the analogithmy and the sector and the provide the determined by the PCR were M11 primer and by the LTGE. Physical data and the strategies are the the Hodge and houthle dask stating research to a sector data and the stating were by the Hodge and houthle dask stating research to a static data and the stating and the sector and the stating of the the Hodge and houthle dask stating research to a static data the static were by the Hodge and houthle dask stating research to the static data the physical the Hodge and houthle dask stating research to the static data and research to the static data and the physical the static data and physical the static data to the static data the data and the static data and th

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# PREVALENCE AND CLINICAL IMPACT OF ACINETOBACTER BAUMANNII IN 13 ITALIAN HOSPITALS.

E. Carretto<sup>a</sup>, C. Farina<sup>b</sup>, P. Nicoletti<sup>c</sup>, D. Barbarini<sup>a</sup>, A. Grosini<sup>a</sup> and the APSI "Acinetobacter Study Group"\*

<sup>a</sup>Laboratori Sperimentali di Ricerca, Area Infettivologica, Fondazione IRCCS Policlinico "San Matteo", Pavia; <sup>b</sup>U.O. Microbiologia, Azienda Ospedaliera "Ospedale San Carlo Borromeo", Milano; <sup>c</sup>U.O. Microbiologia, Azienda Ospedaliera "Careggi", Firenze; and <sup>4</sup>Associazione Prevenzione Studio Infezioni (APSI), Italy\*

Recent reports suggest that *Acinetobacter* spp. account for 9% of nosocomial infections in Europe, particularly in ICUs. From a clinical point of view, it is an opportunistic pathogen, causing mainly bacteraemias, lower respiratory tract (LRTIs) and wound infections (WIs). However, data on the prevalence of this microorganism in different countries are not reported frequently.

The APSI "Acinetobacter Study Group" collected data about Acinetobacter baumannii between 15 May and 11 June 2006 from 13 northern and central Italian hospitals of different size (range 300 - 3500 acute-care beds; total study population >14 000 beds) and classification. During the study period, >22 000 clinical specimens from different patients were processed by standard microbiological procedures; a single isolate from each patient was considered, irrespective of the site of isolation. A. baumannii identification was performed mainly by automated instruments, and was then confirmed in the Reference Lab (Laboratori Sperimentali di Ricerca, Pavia).Susceptibilities to ceftazidime, ceftriaxone, imipenem, meropenem, amikacin, co-trimoxazole, ciprofloxacin, piperacillin/tazobactam and tetracyclines was evaluated by the CLSI disk-diffusion method (2006) on Mueller Hinton agar. MICs for ampicillin/sulbactam and colistin were determined by Etest.

Twenty-eight A. *baumannii* isolates were obtained from seven different hospitals. More than 50% of cases (15/28) were in two big tertiary University hospitals (known to have had *A. baumannii* outbreaks in the recent past), while the other five hospitals had four (one centre), three (one centre) and two (three centres) isolates, respectively. *A. baumannii* was considered to be the cause of infection in 13/28 cases (46.4%); 15 isolates were considered to be colonisers. Five cases of bacteraemia, 3 WI, 3 LRTI and 2 UTI were observed. The multi-drug resistance phenomenon was documented frequently in different centres; this involved resistance to carbapenems (no MBLs) in two hospitals. Colistin and ampicillin/sulbactam showed the best efficacy; all isolates had a colistin MIC of 0.38 - 0.75 mg/L.

All the isolates have been stored at  $-20^{\circ}$ C at the reference laboratory. In the near future they will be analysed using a molecular method (rep-PCR with commercial kits) to evaluate their similarity.

\*Institutions involved: Ancona, 'Ospedali Riuniti' (E. Manso; coordinator); Bergamo, AO 'Ospedali Riuniti di Bergamo' (A. Goglio); Como 'Ospedale Valduce' (R. Terramocci); Firenze, AO 'Careggi' (P. Nicoletti); Lecco, AO 'Ospedale A. Manzoni' (R. Vaiani); Milano, AO 'Ospedale San Carlo Borromeo' (C. Farina); Modena, 'Policlinico' (F. Rumpianesi); Monza, AO 'Ospedale San Gerardo' (S. Bramati); Negrar, 'Ospedale Sacro Cuore don Calabria' (C. Savio); Pavia IRCCS 'San Matteo' (E. Carretto); Pescara, PO 'Santo Spirito' (P. Fazii); Roma Università Biocampus (G. Di Cuonzo); Vicenza, AO 'Ospedale San Bortolo' (C. Scarparo).

## PREVALENCE AND CLINICAL INFACT OF ACIMETORACTER FAUMANTIN

E. Carretto", C. Farina", P. Nicoletti", D. Barbarini", A. Grosbu" and the APSI "Acutatobacter. Study Group"?

\*Laboratori Sperimentali di Ricerca, Area Infettivologica, Foudazione IRCCS Policinico "Sca Matteo", Pavia: <sup>1</sup>U.O. Microbiologia, Astaria Ospedalicia "Ospedale dan Carlo Borromeo", Milano: 'ELO, Microbiologia, Azienda Ospedalica: "Careggi", Firenze, and 'Associazione Prevenzione Studie Infeziorei (APSI), Italy"

Recent reports suggest that Advances and top, account for V's of noncommutiniteness in Europer particularly in IGUs. From a clinical point of view, it is an opportuniatic pathogen, extering mainly basteraccoust, have respiratory trace (ERTIs) and wound introdices (WIs). However, data on the prevatence of sign meanorgenism in different countries are not reported frequently.

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## P17

## EMERGENCE AND SPREAD OF CARBAPENEM-RESISTANT STRAINS OF ACINETOBACTER BAUMANNII IN A TERTIARY CARE HOSPITAL IN WARSAW, POLAND

## M. M. Wroblewska<sup>a</sup>, K. J Towner<sup>b</sup>, H. Marchel<sup>c</sup> and M. Luczak<sup>a,c</sup>

<sup>a</sup>Department of Medical Microbiology, Medical University of Warsaw, Poland; <sup>b</sup>Department of Clinical Microbiology, Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Nottingham, United Kingdom; <sup>c</sup>Microbiology Laboratory, Central Clinical Hospital, Medical University of Warsaw, Poland.

Acinetobacter baumannii continues to emerge as an important nosocomial pathogen. A recent increase in the number of carbapenem-resistant strains, particularly in eastern Europe, is currently of great concern, Epidemiological and molecular surveillance is necessary to prevent their spread. This study investigated the emergence of carbapenem resistance in *Acinetobacter* isolates from patients hospitalised in a tertiary care hospital in Warsaw, Poland, by analysing the molecular epidemiology and resistance mechanisms of these strains and the risk-factors for their acquisition and possible nosocomial spread.

The medical charts of 21 patients with *Acinetobacter* infection or colonisation were reviewed. Isolation of *A. baumannii* strains was recorded most often in the ICU and from surgical patients (particularly transplantation surgery). The mean time following admission to first isolation was 21 days (range 5 - 45 days). Infection with *Acinetobacter* contributed directly to the death of seven patients. Several patients were infected with more than one strain of *A. baumannii*. Strains of *A. baumannii* were isolated repeatedly during the period of the study, stressing the need for continuous observation of procedures for prevention of nosocomial infections.

Molecular typing by *Apa*I pulsed-field gel electrophoresis (PFGE) and DAF4 randomly amplified polymorphic DNA (RAPD) analysis revealed the co-circulation of three clones (types 1 – 3). Type 1 appeared to belong to 'European Clone I', and type 3 belonged to 'European Clone II'. Carbapenem resistance of *A. baumannii* strains was not associated with any particular molecular type, and isolates susceptible or resistant to both imipenem and meropenem were found. All three types were shown in multiplex PCRs to carry a gene encoding an OXA-51 family enzyme, but were negative for genes encoding enzymes belonging to the OXA-23, OXA-24 and OXA-58 families. The OXA-51 enzyme did not appear to be associated directly with carbapenem resistance, and was found in both resistant and susceptible isolates. Etests with imipenem and imipenem plus EDTA indicated the presence of a metallo- $\beta$ -lactamase (MBL) in resistant isolates. PCRs for IMP-type MBLs were negative, but PCR using consensus primers for VIM-type MBLs were positive. VIMtype MBLs in *Acinetobacter* have recently been described in Korea, Germany and Greece, but this is their first description in Poland. The occurrence of VIM-type MBLs in association with the epidemic *A. baumannii* 'European Clones' is a cause of great concern. EMERGENCE AND SPREAD OF CARBAPENEM-RESISTANT STRAINS OF ACEVETOBACTER BAUMANNERY A TERTIARY CARE HOSPITAL IN WARSAW. POLAND

#### M.M. Wroblewska, K. J. Towner, H. Marchell and M. Luczak

Department of Medical Microbiology, Medical University of Warsaw, Poland, "Department of Clinical Microbiology, Notifughers University Hospitals MHS Trust, Queen's Medical Centre, Nottingham, United Eingdom; "Microbiology Laboratory, Central Clinical Hospital, Medical University of Warsaw, Poland.

Activelobactor houseoned backness to emerge as an important noncocontal pathogen. A recent increase in the mainler of an importen-resistant strains, particularly in eastern Europe, is sumently of great concern. Epidemiological and molecular surveillance is necessary to prevent their spread. This study averdigated the emergence of carbapenets reastance in Accessionactor isolates from partents hospitalised to a terbary care hospital in Warnaw, Poland, by analysing the molecular epidemiology and resistance mechanisms of these strains and the risk-factors for their accelent and possible noncoments spread.

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Molecular typing by And related triad gel electrophonesis (PFOE) and DAF4 randoms) amplified polymorphic DMA (BAPO) and wait revealed the co-monitation of three clones (types 1 – 3). Type trappeared to before to "innocent Clone I", and type 3 bolocged to "European Clone II". Cathageneral to before to "innocent Clone I", and type 3 bolocged to any puriorlar molecular type, and review successfore or maintain was not associated with meropase an were found. All three types are access to methode a PCRs to carry a gent meropase at 0X A.23. DAA.34 and DAA or mathins. The OXA-31 carrying data theorem to be associated directly with carrons are access to mathing. The OXA-31 carrying data bolonging to the OXA-23. DAA.34 and DAA or mathing. The OXA-31 carrying data appear to be associated directly with carrons are access to and sear found in both resistant and associated thready with carrons are access to a mathing. The OXA-31 carrying data appear to be associated directly with carrons are accessing the appear and the total and associated thready and compares and the presence of a methode the presence of a metabole factarates (1001) in temperative transmiss. If Cls for this type MBLs were negative, but FCR using conservation from the transmiss. If Cls for this type MBLs were negative, but FCR using conservation from the transmiss. If Cls for this type MBLs is to the transmission of the transmission of the type MBLs in association with the type MBLs in Atomation in Foliand. The conservation of the type MBLs in association with the type MBLs in the transmission from the type of the type MBLs in the type type the type type of the type of the type type type type type.

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## INVASION OF ACINETOBACTER BAUMANNII TO HUMAN EPITHELIAL CELLS VIA A ZIPPER-LIKE MECHANISM AND THE ROLE OF OUTER-MEMBRANE PROTEIN Omp38 AS AN INVASIN

## C. H. Choi<sup>a</sup>, J. Y. Lee<sup>a</sup>, Y. C. Lee<sup>a</sup>, T. I. Park<sup>b</sup> and J. C. Lee<sup>a</sup>

<sup>a</sup>Department of Microbiology and <sup>b</sup>Pathology, Kyungpook National University School of Medicine, Daegu, Korea

Acinetobacter baumannii is an important opportunistic pathogen that is responsible for hospital acquired-infections, but the interaction of A. baumannii with epithelial cells in the early stage of infection remains unclear. This study characterised the ability of A. baumannii to invade epithelial cells and determined the role of major outer-membrane protein Omp38 in cell invasion. Invasion of epithelial cells depends on A. baumannii strain and host-cell type. NCI-H292 and HEp-2 cells derived from the respiratory tract were more susceptible to invasion than non-respiratory tract-derived HeLa cells. A. baumannii attached loosely to the surface of epithelial cells through fimbrial-like projections and invaded the epithelial cells via a zipper-like mechanism. The internalised A. baumannii were located separately in the cytoplasmic membrane-bound vacuoles. The specific cytoskeletal inhibitors cytochalasin D and vinblastine inhibited A. baumannii invasion of epithelial cells significantly. Invasion of epithelial cells by an Omp38 knock-out mutant was significantly decreased compared with the wild-type A. baumannii strain. Recombinant Omp38 protein bound directly to the surface of epithelial cells. Pre-treatment with recombinant Omp38 attenuated the interaction of A. baumannii with the surface of epithelial cells and inhibited invasion of epithelial cells significantly. The major outer-membrane protein Omp38 plays an essential role in the invasion of host cells by A. baumannii. Invasion of epithelial cells provides a novel insight into the pathogenesis of A. baumannii in the early stage of infection.

INVASION OF ACTIVETOBACTER BAUMANNII TO RUMAN EFITHELIAE CELLS VIA A ZIPPEH-LIKE MECHANISM AND THE ROLE OF OUTER-MEMIRANE PROTEIN Omp38 AS AN EXVASIN

C.H. Choi, J.Y. Lee, Y.C. Lee, T. L. Park' and J.C. Lee

Department of Microbiology and Tathology, Kenagpook National University School of Medicine, Daegu, Korea

Activation of the second is an important orperformance pathogen that is responsible for hospital acquired infections, but the interaction of 4 downards with epithelial cells in the early stage of infection crisemi enciest. For their chiracterised the ability of 4 mamman to invade epithelial cells and datamined the rais of many membrane potein Omp36 in cell invasion invasion invation of epithelian cells topends on a better many membrane potein. Omp36 in NCL-H292 and H1p-2 cells datamined the rais of many mount membrane potein. Omp36 in the association that non-respondery and derived them is responded to a state invasion that non-respondery and derived them is cells 4 formation interaction of the states of quite tial cells dataway indicated them is cells 4 formation interaction of the states of quite tial cells through first the provide to a based the epithelial cells via a states of quite tial cells through first the provide to the state data data and collopismic membrane bound vectories the epicate provide at the state of topset, to the and viniblestine inhibited 4 formation investor of quite tail of the epicated of topiasmic membrane bound vectories. The epicate provide at the transfer of the and viniblestine inhibited 4 formation investor of quite tail of the epicated of topiasmic membrane bound vectories the epicate provide and therefore of a still-type 4, bount with the strateget of the state and therefore in the strateget of a still-type 4, bount with the strateget of the state and therefore of the state at the off a splite tial cells by an Omo 38 kmock on maken was supplicated to the there at the off a still-type 4, bount with the strateget of the state and therefore of the state of a splite tial cells by an Omo 38 kmock on maken was supplied with the strateget of a state of host cells by 4. Annotation to the state at the state at the state of the stategets of 4, warearmane to the state acces at the state of a state of the stategets of 4, warearmane to the state at a state of a state at the state at the s

# ANALYSIS OF ADHESION OF ACINETOBACTER BAUMANNII TO HUMAN CELLS

A. Lübeck, M. Kleinbach and U. Gerischer

Department of Microbiology and Biotechnology, University of Ulm, Ulm, Germany

Acinetobacter baumannii causes severe nosocomial infections such as pneumonia, meningitis and sepsis with high mortality rates (up to 70%). This organism represents an increasing danger for immunocompromised adults, especially since there are an increasing number of resistances against antibiotics. Until now, scientific investigation was mainly focused on taxonomy and antibiotic resistance mechanisms. The goal of this project was to analyse the interaction between clinical strains of Acinetobacter baumannii and human cells in order to address the molecular mechanisms causing pathogenicity.

Adherence is the first step in colonisation of human tissue, and is therefore a key event in pathogenesis. To demonstrate the adhesion of bacteria to human cells, a colony counting assay is being established. These experiments used the type strain of *A. baumannii* ATCC19606, as well as clinical isolates from the collection of Prof. Seifert, University of Cologne. All *A. baumannii* strains investigated showed adhesion to the lung epithelial cells A549, but the adhesion capacity was variable for the different strains. Surprisingly, the type strain of *A. baumannii* was less adherent than several clinical isolates.

Furthermore, the topic of gene inactivation in *A. baumannii* is being investigated by evaluating the capability for uptake of DNA by conjugation, transformation and electroporation using a number of different plasmids.

In addition, transposon mutagenesis of *A. baumannii* is being established in order to be able to search for components that are involved in adherence. A highly efficient Tn5 derivative was used for transposon mutagenesis of several species of *Acinetobacter*, as well as of *Pseudomonas fluorescens* as a control [1].

1. Arch Microbiol 178:193-201.

ANALYSIS OF ADRESION OF ACTVETURACTER EAUMANNITTO HUMAN

A. Lübech, M. Kleinbach and G. Gerische

Department of Microbiology and Biotexinology, University of Ulm, Ulm, Germany

Activitation of the parameteria causes prevent cosponential infections such as pneumonia, meningrits and sensis with high mostality rates up to WeQ. This organism represents an increasing danger for fumitinocompremised adults expectably since there are an increasing number of resistances against antibiotic remission. Unch new, taterning investigation was mainly focused on thomorry and antibiotic remissions of restantisms. The goal of this project way to analyze the increaction between clinical states of remissions of remaining the goal of this project way to analyze the address life molecular memory and antibiotic remissions of remacharities of an analyze the address life molecular memory and ensions of remacharities and presences and busines can be address to a address life molecular memory and states and an address and presences of the states and busines can be addressed and the states of the states of the states of the states and busines and busines and the states of the states of the states of the states and busines and busines and busines and busines and an address the molecular memory and antibility of the states of the states of the states and busines and busines and busines and busines and busines are addressed and address and busines and an address and address and addressed and address and busines and busines and busines and address and addressed address and addressed and addressed addres

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1. Arch Marabial 178199 Act

## **RAPID PULSED-FIELD GEL ELECTROPHORESIS PROTOCOL FOR TYPING OF** *ACINETOBACTER* SPECIES

M. Erdenizmenli and A. Grossato

Department of Histology, Microbiology and Medical Biotechnology, University of Padua, Padova, Italy

Among molecular typing methods, pulsed-field gel electrophoresis (PFGE) of genomic DNA is generally considered to be the reference standard because of the high discriminating power of the DNA fragments generated, and it has been used successfully for epidemiological purposes. The main problem with this method is that the preparation of DNA is a time-consuming procedure. Moreover, the use of different procedures for preparation of DNA, restriction digestion and electrophoretic separation of DNA fragments generally makes it difficult to interpret and compare the data reported in the literature.

This study established a relatively brief and simple method for typing of *Acinetobacter* spp., implementing some features described previously for typing *Escherichia coli* [1] and various Gram-positive and Gram-negative microorganisms other than *Acinetobacter* [2]. In brief, a shortened incubation time for cell lysis and proteinase K treatment was used with bacterial cell suspensions prepared directly from colonies grown on the agar plates, and shortened washing times were applied using pre-heated sterile purified water and TE buffer. Using this procedure, intact genomic DNA was prepared within 9 h for restriction enzyme digestion, depending on the number of the isolates being tested simultaneously. After restriction enzyme digestion with *Apa*I for 4 h, electrophoresis occurred in the CHEF-DRIII apparatus for 23 h.

Using this protocol, results can be obtained within 36 - 48 h, allowing timely evaluation of clinical *Acinetobacter* isolates.

1. J Clin Microbiol **35**: 2977-2980. 2. J Clin Microbiol **34**: 2598-2600. RAPID PULSED-FIELD GEL ELECTROPHOIDSIS PROTOCOL FOR TYPING OF ACINETORACIER SPECIES

M. Entenizmenth and A. Crossela

Department of Histology, Microbiology and Medical Frotechnology, University of Padua, Padova, Italy

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Paña tine protocol, readits can be obtained within 15 -46 h allowing turnely chalcation of thereal demoletance isolates.

L.J.C.M. Merrobid 35: 2975-2980.

## METALLO-B-LACTAMASES AND OXACILLINASE TYPES IN MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII STRAINS ISOLATED FROM CARDIOVASCULAR PROSTHETIC DEVICE-ASSOCIATED INFECTIONS

## R.Cernat<sup>a</sup>, C.Balotescu<sup>a</sup>, V.Lazar<sup>a</sup>, O.Banu<sup>b</sup>, M.Ditu<sup>a</sup> and M. Mitache<sup>a</sup>

<sup>a</sup>Depart. Microbiology, Faculty of Biology, University of Bucharest, Bucharest, Romania; <sup>b</sup>Institute for Cardiovascular Diseases "C.C. ILIESCU", Bucharest, Romania

The purpose of this work was to determine the type of acquired metallo- $\beta$ -lactamases (MBLs) and oxacillinases with carbapenem-hydrolyzing activity in multidrug-resistant *Acinetobacter baumanii* strains isolated from cardiovascular devices associated infections.

This study was carried out at the Institute for Cardiovascular Diseases "C.C. Iliescu" during 2003 -2005. The susceptibility testing data were provided by a VITEK II automatic system. MBL production was screened using imipenem (IPM) Hodge disk tests and IPM-EDTA+SMA double-disk synergy tests. Double-disk tests in Mueller Hinton agar plus cloxacillin (250 mg/L) was used for phenotypic detection of oxacillinases producers. MICs of  $\beta$ -lactams and colistin were determined by a microdilution test, as recommended by CLSI. PCR amplification with primers specific for  $bla_{IPM-1}$ ,  $bla_{VIM-2}$ ,  $bla_{OXA-23}$ ,  $bla_{OXA-24}$ ,  $bla_{OXA-40}$  and  $bla_{OXA-58}$  was performed to identify the MBL- and OXA-type determinants. The clonal relationship between the isolates was evaluated by PFGE and Rep-PCR.

Of the total number of 676 isolates from different clinical specimens, 3% were *A. baumanii*, isolated predominantly from surgical wounds and blood cultures. The susceptibility testing data showed that the *A. baumanii* isolates exhibited high resistance rates to all  $\beta$ -lactams and all aminoglycosides, with >50% resistance to amikacin, colistin, fluoroquinolones and IPM (MICs 16 - 64 mg/L). Among the IPM-resistant isolates tested, 24% were MBL-producers carrying the *bla*<sub>IPM-1</sub> and *bla*<sub>VIM-2</sub> alleles, respectively. Moreover, a PCR product was obtained with *bla*<sub>OXA 23</sub>-specific primers for 75% of carbapenem-resistant strains. Genotyping showed a relatively low level of clonal relationship among strains.

In conclusion, the present study demonstrated that the *A. baumanii* strains implicated in the aetiology of cardiovascular prosthetic device-associated infections exhibit high levels of antibiotic resistance and multiresistance. This is also the first report of the prevalence of *A. baumanii* producing *bla*<sub>IPM-1</sub>, *bla*<sub>VIM-2</sub> and *bla*<sub>OXA 23</sub> in Romania. Taking into account the very narrow antibiotherapy choices for these infections, and also the increasing resistance to colistin, the spreading of carbapenem-hydrolysing oxacillinases is of a considerable concern for antimicrobial chemotherapy.

### METALLO-B-LACTAMASES AND OXACILLINASE TYPES IN MULTIDRUG RESISTANT AGINETOBACTER BAUMANNI STRAINS ISOLATED FROM CARDIOVASCULAR PROSTHETIC DE VICE-ASSOCIATED INFECTIONS

R.Cernal", C.Balotescu", V.Lazar", O.Banu", M.Ditu" and M. Mitache"

Depart, Microbiology, Faculty of Biology, University of Bucharest, Bocharest, Romaniat, Institute for Cardiovascular Diseases "C.C. ILIESCU", Bucharest, Romania

The purpose of tims work was in determine the (ype of acquired metallo-[i-laciamates (MBJ,a) and oxaci[jinases with carbapenem-hydrolyzing activity in multiding resistant Acinetobacter brannati strains isolated from certileverspular devices associated infections

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Of the ford number of 500 moders from different clinical spectrates, 5% were 4, hoursed, solated predominantly from servical workets and blood calaary. The unsceptibility renting data showed that the 4 browers isolanes calificited to be restricted rate to all fractions and all similogic cosines with 250% restrictive to anticacity, column, fluctoquity, bactant and (MICs 10 - 64 mg 1). Among the life restrictive to failed to be restricted rate to all fractions and carrying the shaped primer in 25% of carrying to barries thered. Here were MBL producers with blooks trespective primers for 25% of carrying to be set to be an MCB producers and all blood relatively fow break of clonal volutions are primer to be and the set of the restriction of the set of the s

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## **IN-VIVO STUDIES OF CECROPIN A (1-8)-MELITTIN (1-18) HYBRID PEPTIDE** AGAINST A PAN-RESISTANT ACINETOBACTER BAUMANNII STRAIN

R. López-Rojas<sup>a</sup>, F. Docobo-Pérez<sup>a</sup>, M. E. Pachón-Ibáñez<sup>a</sup>, B. G. de la Torre<sup>b</sup>, J. M. Saugar<sup>c</sup>, L. Rivas<sup>c</sup>, D. Andreu<sup>b</sup> and J. Pachón<sup>a</sup>.

<sup>a</sup>Service of Infectious Diseases, University Hospitals Virgen del Rocío, Sevilla; <sup>b</sup>Department of Experimental and Health Sciences, Pompeu Fabra University, Barcelona; <sup>c</sup>Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

Objectives: To evaluate the toxicity of the cecropin A(1-8)-melittin(1-18) hybrid peptide [CA(1-8)M(1-18)] in C57BL/6 immunocompetent mice, the inflammatory response triggered by CA(1-8)M(1-18), and the efficacy of the peptide in a murine model of peritoneal sepsis caused by a pan-resistant Acinetobacter baumannii strain.

Methods: The clinical pan-resistant, including to colistin, A. baumannii Ab208628 was used. To evaluate the toxicity of the peptide, lethal dose parameters (LD<sub>0</sub>, LD<sub>50</sub>, and LD<sub>100</sub> - Reed and Muench method) were calculated. Groups of 6 mice were treated with Log<sub>2</sub> increasing doses of CA(1-8)M(1-18), from 0.5 mg/kg until a 100 % mortality rate was reached. To study the inflammatory response due to CA(1-8)M(1-18), serum TNF- $\alpha$  levels (ELISA, BioSource, Nivelles, Belgium - pg/ml) were determined in 2 groups: control and CA(1-8)M(1-18) (16 mg/kg of peptide intraperitoneally). To ascertain the in vivo activity of the peptide against Ab208628, the PD<sub>50</sub> was determined. First, the MLD (bacterial minimal lethal dose) was calculated. Groups of 10 mice were inoculated intraperitoneally with Log<sub>10</sub> decreasing concentrations of Ab208628, from 8 Log<sub>10</sub> cfu/ml to the minimum inoculum causing 100% of mortality (MLD). Next, PD<sub>50</sub> was calculated using mice inoculated with the MLD and treated with doses of the peptide ranging from 0.5 mg/kg to LD<sub>0</sub>. Furthermore, the efficacy of the peptide against Ab208628 was assessed in a murine model of peritoneal sepsis, using two groups of 30 mice: control (non-treated) and CA(1-8)M(1-18) (16 mg/kg of peptide intraperitoneally). An intraperitoneal inoculum of 7 Log<sub>10</sub> cfu/ml was used. After 4 hours, a single dose of peptide was administered. Sequential sacrifices were done at 1, 3, 5, 7 h after treatment. Bacterial concentration in spleen (Log<sub>10</sub> cfu/g), peritoneal fluid (Log<sub>10</sub> cfu/ml), and serum TNF- $\alpha$  levels (pg/ml) were determined.

**Results:** The parameters of toxicity of the peptide were:  $LD_0 = 32 \text{ mg/kg}$ ,  $LD_{50} = 64 \text{ mg/kg}$ , and  $LD_{100} = 128 \text{ mg/kg}$ . Adverse effects were observed from 8 mg/kg. The administration of peptide in non-infected mice did not increase serum TNF-a levels. PD<sub>50</sub> was not achieved with non-toxic doses: maximal survival 30% with 4 and 16 mg/kg. The results in the model of peritoneal sepsis were the following:

Group	T	12420 62	Control	(non-treat	ed)	CA(1-8)M(1-18)						
Time (Hours)	0	4	5	7	9	11	0	4	5	7	9	11
Spleen (Log <sub>10</sub> cfu/g)	0	8.61	9.05	9.27	9.48	9.56	0	8.61	8.40	8.42	8.55	8.59
Peritoneal fluid (Log <sub>10</sub> cfu/ml)	0	7.53	7.12	8.20	7.99	8.23	0	7.53	4.15	5.12	6.01	6.63
TNF-α levels (pg/ml)	0	1011,4	971,4	1271,4	1016,4	1731,4	0	1011,4	1461,4	1101,4	951,4	1106,4

Conclusions: The cecropin A(1-8)-melittin(1-18) hybrid peptide showed a short-length and local bactericidal activity in an experimental model of peritoneal sepsis caused by a panresistant Acinetobacter baumannii strain.

### IN-VIVO STUDIES OF CECKOPIN A (I-8)-MELITIN (I-18) ILVIGID PETIDE AGAINST A PAN-RESISTANT ACIVETOBACTER BAUMANYII STRAIN

R. López-Rojas", F. Docobo-Perez', M. E. Pechon-Ibádez", B. O. de la Forre", J. M. Saugur", L. Rivas", D. Andreu" and I. Pachon",

"Service of Infectious Diseases, University Hospitals Virgen del Rocio, Sevilla; "Department of Experimental and Health Sciences, Pompeu Feixa University, Barcelona; "Centro de Investigaciones Biologicus (CSIC), Madrid, Spain

Objectives: To evaluate the toxicity of the eccopin A(1-8)-meltitud(1-13) injurid peptide [CA(1-8)M(1-13)] in C57BL 6 immunocompetent mice, the inflatomatory response triggered by CA(1-8)M(1-18), and the efficacy of the peptide in a murine model of peritoneal sensis caused by a pan-resistant *keinetowater banacunit* strain.

Methods: The clinical pain resistant, isoluding to colutin A brawning Ab208023 was used. To evaluate the taxiety of the poptide, label does parameters (LDs, LDs, and LDse, Reed and Miertsh mathod) were calculated. Groups of 6 mice were treated with Legy increasing doese of CA(1-8)(Mi + 8), from 0.5 mayag until a 100 % mertality rate was reached. To study the inflammatory response due to CA(1-8)(Mi + 18), serum TMP-o levels (ELISA, BroSourec, Mirethes, Beiguine opend) were determined in 2 groups control and CA(1-8)(Mi + 18) (16 Mirethes, Beiguine opend) were determined in 2 groups control and CA(1-8)(Mi - 18) (16 Ab208023; the TDs was determined. First, the MiD (noticetal minemal lettal does) was reached to coups of 10 mice were insortiate interperimentally with Logic decreasing extended to coups of 10 mice were insortiated interperimentally with Logic decreasing reactioned (MLD), Next, TDs was calculated interperimentally with Logic decreasing reactions of ab208033. And S 10 mice were insortiated interment increasing 100% of generality (MLD), Next, TDs was calculated interperimentally with Logic decreasing region general to the equate the to the minimum increasing 100% of generality (MLD), Next, TDs was managed as a minimum increasing 100% of region region and the equate transmitted of the minimum increasing 100% of anotality (MLD), Next, TDs was managed in a minimum increasing to 10 mice of the openregion and the equation equate to a set of the minimum increasing 100% of anotality (MLD), Next, TDs was managed in a minimum increasing to 10 mice anotality (MLD) in the open to anotal the minimum increasing to 10 mice the open of the open of the open open of the endowing the efficiency of the provide minimum base and contrast to anotal the dominance of the efficience of the interperiode of the open of the open open of the endowing is another to open of the efficience of the open open of the open open of the open open of the efficience of the efficience of the open open of the open open of the open open of the ef

Results: The parameters of forther of the popular verse. Use = 32 mode,  $1.5_{0} = 64$  mp/kg, and  $1.5_{100} = 123$  angleg. Adverse efficits were discreted from 5 mg/kg. The administration of peptide in non-ministed mine did not increase section Tables levels. File was not adjusted with ministerse descet maximal superical 34% with 4 and 15 mg/kg. The results in the model of mentioned secure were the following:

Conclusions: The occuppin A(1-8)-melium(1-13) hybrid peptide showed a short-length and local bacterizidat activity in an experimental model of periorical separa caused by a pairresistant Actual observe brandsmit strain.

## LIPASE SECRETION BY MULTI-DRUG-RESISTANT STRAINS OF ACINETOBACTER BAUMANNII FOLLOWING EXPOSURE TO CARBAPENEMS

D. W. Wareham<sup>a,b</sup> and D. C. Bean<sup>a</sup>

<sup>a</sup>Centre for Infectious Disease, Institute of Cell and Molecular Science, Barts and The London, Queen Mary's School of Medicine and Dentistry, London, UK <sup>b</sup>Department of Medical Microbiology, Division of Infection, Barts and The London NHS Trust, London, UK

Acinetobacter baumannii has emerged as an important nosocomial pathogen causing ventilator-associated pneumonia, bacteraemia and sepsis in the immunosuppressed and critically-ill. In the UK, multidrug-resistant (MDR) strains producing OXA carbapenemases have been particularly problematic in intensive care units, where they have been associated with ongoing outbreaks of infection. Most of these strains belong to three distinct clones and exhibit resistance to all  $\beta$ -lactams, fluoroquinolones and aminoglycosides, remaining consistently susceptible only to polymyxin. Although considerable attention has been paid to the mechanisms of antibiotic resistance in these strains, virtually nothing is known concerning their ability to produce any specific virulence factors or to elaborate toxins. Following a clinical observation that burn wounds infected with *A. baumannii* exhibited marked loss of subcutaneous fat, the present study investigated the lipase activity of representative MDR strains of *A. baumannii*. As carbapenems are used widely for the empirical treatment of sepsis in critical care and burns units, the study also investigated the action of sub-therapeutic concentrations of both imipenem and ertapenem on the production of *A. baumannii* lipases.

A spectrophotometric bioassay for lipase production was developed, based on the cleavage of Tween-20 substrate in the presence of calcium. Representative isolates of epidemic UK *A. baumannii* clones, OXA-23 clones 1 and 2, and the South East clone, were tested for lipase production. Each strain was grown in both Luria-Bertani (LB) and minimal (MM) broths, with and without imipenem or ertapenem added at 1/8 x the MIC. Ertapenem induced 33.3 (range 2.2 - 95.0)-fold and 1.2 (0.8 - 1.5)-fold mean increases in lipase production among the three strains (LB and MM, respectively). Likewise, imipenem induced a 3.3 (0.8 - 7.6)-fold and 4.2 (1.3 - 7.7)-fold mean increase in lipase production in LB and MM, respectively. Proteins present in supernatants were precipitated with trichloroacetic acid, quantified by BCA, and separated on 4 - 12% SDS-PAGE. Antibiotic-induced cultures showed increased production of a 32-kD protein, corresponding to the size of a previously described *Acinetobacter* lipase enzyme.

These results indicate that lipase production by *A. baumannii* is induced *in vitro* by treatment with carbapenems. Induction occurs regardless of the organism's susceptibility to antibiotics, which is a particular consideration when treating multiply-resistant *A. baumannii* infections.

#### P23

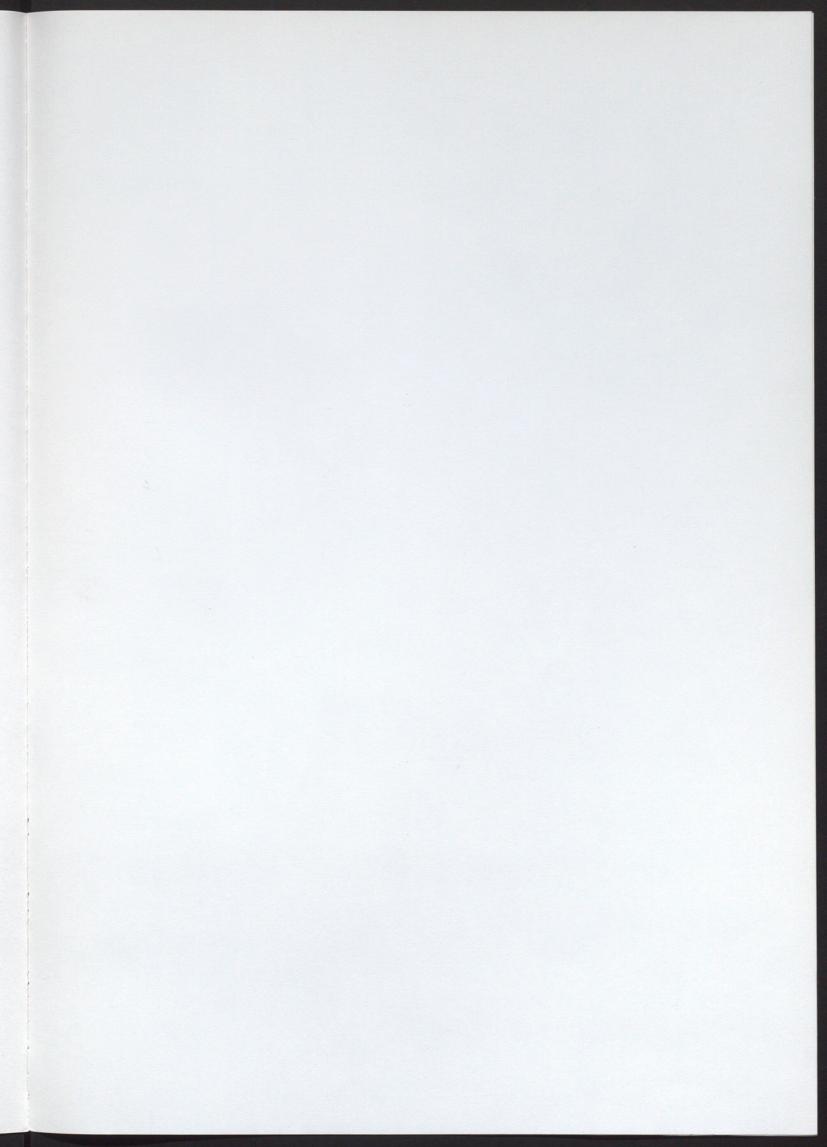
### LIPASE SECRETION BY MULTI-DRUG-RESISTANT STRAINS OF ACTVETORACTER RAUMANNEEFOLLOWING EXPOSURE TO CARBAPENEMS

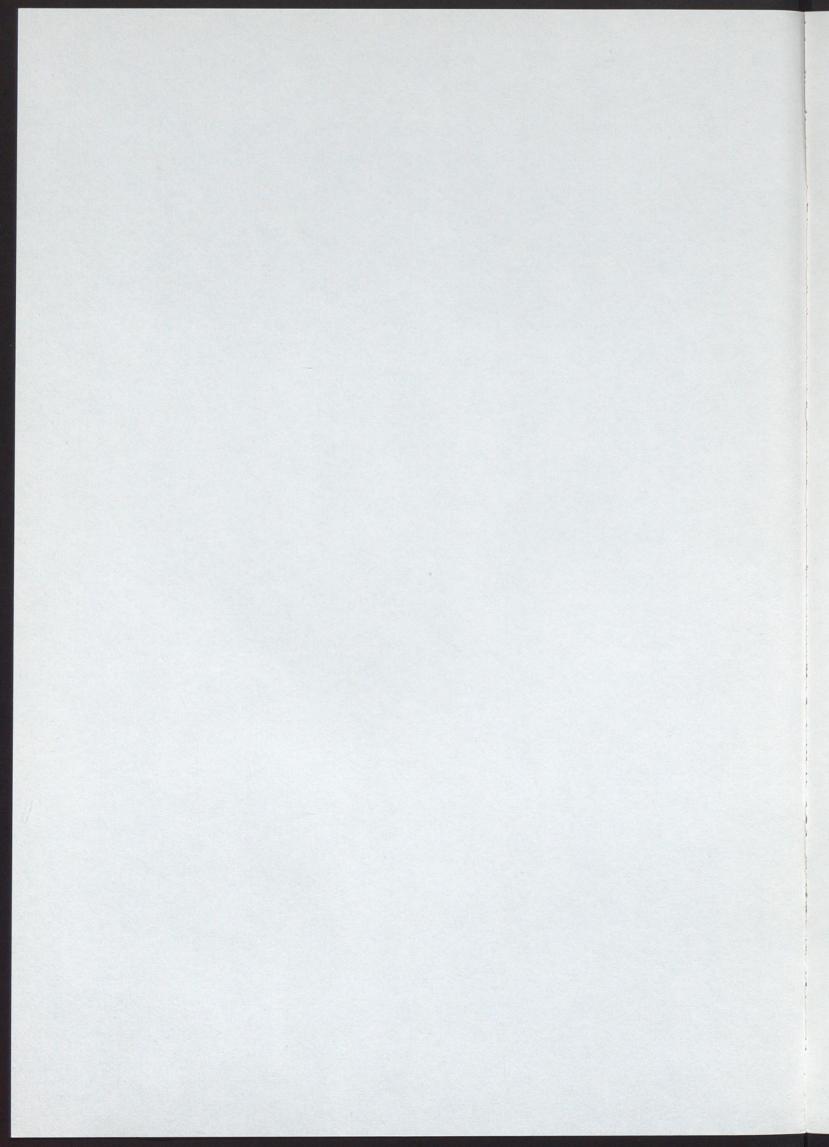
#### D. W. Wareham" and D. C. Bean"

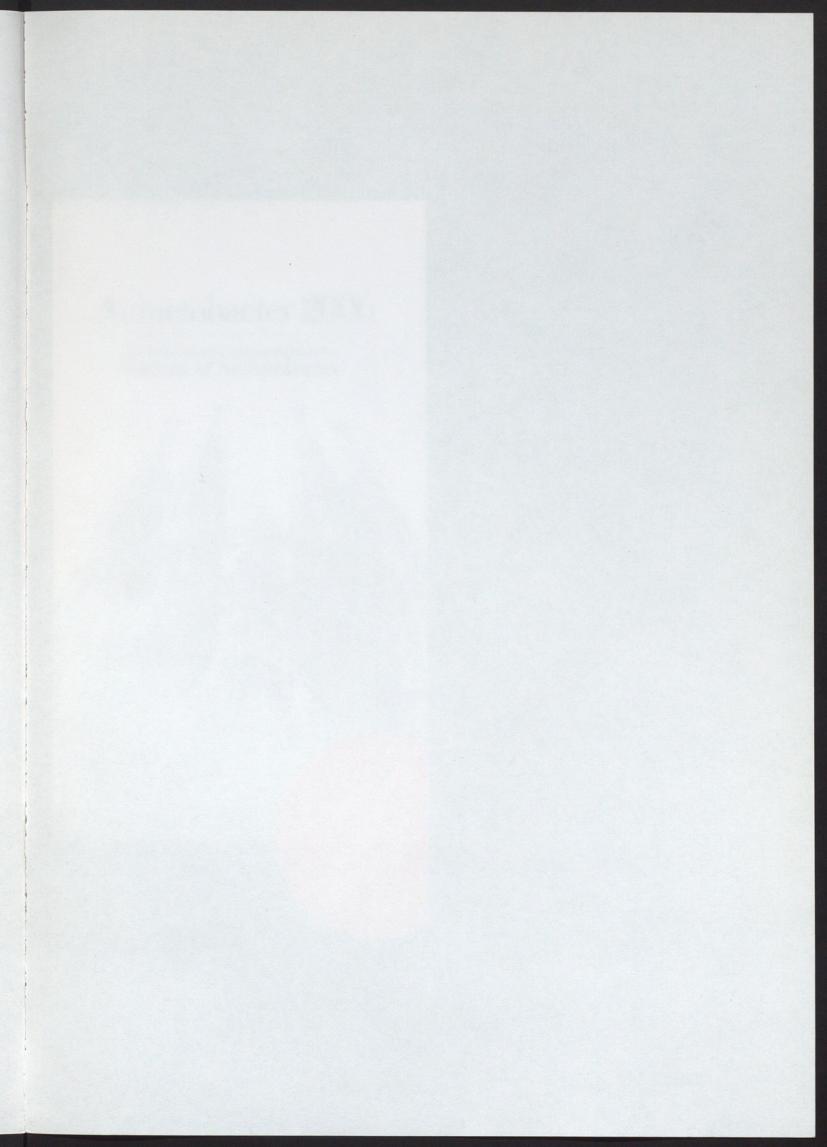
"Centre for Infectious Disease, institute of Cell and Molecular Science, Barts and The London, Queen Mary's School of Medicine and Domistry, London, UK "Department of Medical Microbiology, Division of Infection, Bans and The London NHS Trust, London, UK

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# Acinetobacter 2())6

7th International Symposium on the Biology of Acinetobacter

**Organising Committee** E Neidle, Athens, GA (USA) J Pachon, Sevilla (ES) K Towner, Nottingham (UK) J Vila, Barcelona (ES)

Secretariat Institut d'Estudis Catalans Sara Marti saramarti2@vahoo.es

Collaboration www.wveth.es farmacovigilancia@wyeth.com

# Acinetobacter 2006

7th International Symposium on the Biology of Acinetobacter

8 - 10 November 2006

Institut d'Estudis Catalans Barcelona Spain

# Acinetobacter 2006 Programme

# Wednesday 8 November 2006

16.00-18.00 Registration 18.00-21.00 Reception and Get-Together

# Thursday 9 November 2006

## Session 1

08.30-09.00 The diversity of the genus Acinetobacter: current state and emerging problems – L. Dijkshoorn

09.00-09.20 Genetic diversity of carbapenem-resistant Acinetobacter isolates in European hospitals: the ARPAC study -K. Towner

09.20-09.40 Genetic backgrounds of carbapenem resistant and -susceptible clinical isolates of Acinetobacter baumannii from two hospitals in Cape Town, South Africa -H. Segal

09.40-10.00 Evaluation of automated identification systems for identification of Acinetobacter species – H. Seifert

10.00-10.30 Coffee / Tea

## Session 2

10.30-11.00 Comparative genomics of Acinetobacter genus; from soil to multidrug resistance – V. Barbe

12.00-14.00 Poster Session – buffet lunch available at 13.00 h.

11.00-11.20 The membrane subproteomes of Acinetobacter baumannii: comparative analysis between a reference strain and a MDR strain -E. Dé

11.20-11.40 The mutS sequence in intermediate and multi-resistant clinical Acinetobacter baumannii is different from the same gene in sensitive bacteria -S. Davies

11.40-12.00 Mini-Tn7 vectors: a new tool for genetic characterisation of Acinetobacter baumannii – H. Schweizer

## Session 3

14.00-14.30 Emerging mechanisms of resistance to B-lactams in Acinetobacter baumannii - L. Poirel

14.30-14.50 Carbapenem resistance in clinical isolates of Acinetobacter baumannii: OXA-23 and CarO – G. Elisha

14.50-15.10 Clonal outbreak of multidrug-resistant Acinetobacter baumannii in a Lebanon hospital: molecular epidemiology and mechanism of carbapenem resistance – R. Zarrilli

### 2006 Programm Acinetobacter e

15.10-15.30 Instability of amikacin resistance in a carbapenem-resistant strain of Acinetobacter baumannii isolated during a hospital outbreak – A. Nemec

15.30-16.00 Tigecycline: a new alternative for the treatment of Acinetobacter infections – R. Zaragoza

16.00-16.30 Coffee / Tea

## Session 4

16.30-17.00 Structural studies of two LysR-type transcriptional activators, BenM and CatM, that explain synergistic response and transcriptional control – C. Momany

17.00-17.20 Functional comparison of two transcriptional regulators, BenM and CatM, in Acinetobacter baylyi ADP1 – E. Neidle

17.20-17.40 Analysis of carbon catabolite repression in Acinetobacter baylyi strain ADP1 - R. Fischer

17.40-18.00 ddrR and SOS-like genes in Acinetobacter baylyi strain ADP1 - L. Gregg-Jolly

20.00 Buses leave for Conference Dinner

# Friday 10 November 2006

## Session 5

08.30-09.00 Acinetobacter: a successful nosocomial opportunistic pathogen - J. Rodriguez-Baño

09.00-09.20 Comparison of ompA, csuE and blaOXA-51-like sequences in isolates of Acinetobacter baumannii from the United Kingdom reveals two highly distinct groups of outbreak strains - J. Turton

09.20-09.40 Identification of a widespread Acinetobacter baumannii strain in Portugal as belonging to European Clone II - G. Da Silva

09.40-10.00 Epidemiological surveillance of Acinetobacter baumannii colonisation and infection in ICU patients – A. Agodi

10.00-10.30 Coffee / Tea

# Session 6

10.30-11.00 The multiple-level regulation of aromatic catabolic genes includes Crcdependent RNA stability - U. Gerischer

11.00-11.20 A unique domain in esterase from Acinetobacter venetianus RAG1 mediates emulsification activity of a variety of polysaccharides – R. Furman

11.20-11.40 Auxiliary proteins for aromatic tolerance and processing, detected in an A. radioresistens S13 proteome -E. Pessione

11.40-12.00 A major outer membrane protein Omp38 of Acinetobacter baumannii is imported into the nucleus by a signaldependent pathway and degrades deoxyribonucleic acids - J. Lee

12.00-14.00 Poster Session – buffet lunch available at 13.00 h.

## Session 7

1400-1430 The natural transformation system of Acinetobacter baylyi: unique features and environmental impact -B. Averhoff

14.30-14.50 Loss of heterologous flanking DNA during introgression of an antibiotic resistance gene in mutator and non-mutator populations of Acinetobacter sp. – J. Ray

14.50-15.10 Investigation into biofilm formation and interaction with human cells to explain the clinical role of Acinetobacter baumannii versus other Acinetobacter species – A. de Breij



16.30-16.50 Molecular analysis of Acinetobacter baumannii-induced inflammatory response – C. March

16.50-17.10 The use of colistin to treat multi-resistant Acinetobacter infection in a regional burns intensive care unit - L. Teare

17.10-17.30 Cecropin A-mellittin peptides are active against clinical colistinresistant strains of Acinetobacter baumannii - L. Rivas

15.10-15.30 Gene expression patterns of respiratory epithelial cells by a major outer membrane protein Omp38 of Acinetobacter baumannii – J. Lee

15.30-16.00 Coffee / Tea

## Session 8

16.00-16.30 Treatment of nosocomial infections due to multidrug-resistant Acinetobacter baumannii – J. M. Cisneros

17.30 Final Remarks

End of Symposium

