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Cleaning up the nomenclatural chaos in the genus *Acinetobacter*: the effectively but not validly published names *'Acinetobacter oryzae'* Chaudhary et al. 2012, *'Acinetobacter plantarum'* De et al. 2016, *'Acinetobacter refrigeratoris'* Feng et al. 2014 and *'Acinetobacter seohaensis'* Yoon et al. 2007 are synonymous with the validly published names of well-established species.

Lenka Radolfova-Krizova, **Paul G. Higgins (Germany),** Alexandr Nemec.

Proposals of novel species names based on the description of only a single strain predominate in the current bacterial taxonomy despite the theoretical and practical limitations of this approach. Such proposals are regularly associated with taxonomically unconvincing or erroneous data. In the present study, we illustrate this problem on examples of four proposals of *Acinetobacter* species names which were effectively published outside the International Journal of Systematic and Evolutionary Microbiology. Genus-wide, whole-genome comparative analysis using the average nucleotide identity based on BLAST (ANIb) parameter revealed that the strains '*Acinetobacter* oryzae' B23, '*Acinetobacter plantarum*' KCTC 42611, '*Acinetobacter refrigeratoris*' KCTC 42011 and '*Acinetobacter seohaensis*' CCUG 56483 could be assigned to *Acinetobacter* johnsonii (ANIb of 95.4-96.0%), *Acinetobacter* junii (ANIb of 97.4-98.2%), Acinetobacter variabilis (ANIb of 95.3-96.0%) and *Acinetobacter* towneri (ANIb of 97.1%), respectively. Congruent with these assignments were the results of the genus-wide analyses of whole-cell mass fingerprints generated by matrix-assisted laser desorption/ionization-time-of-flight MS, and of metabolic and physiological features. Inspection of the original nomenclatural proposals revealed a number of inconsistencies in both genotypic and phenotypic data as compared with those obtained by us. To improve the quality of the circumscription of novel species, we emphasize the importance of using the genus-wide comparative analysis of results based on genus-targeted taxonomic methods applied on multiple strains of a novel taxon.

Developments in the taxonomy of Prokaryotes in the genomic era with implications for the genus *Acinetobacter*

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The taxonomy of the genus *Acinetobacter* has undergone a tremendous development over the past two decades. Currently, there are 54 validly named *Acinetobacter* species. Ten of these comprise one strain only, which precludes insight into their species diversity. Furthermore, the list of species names includes synonyms for four species. Considering the rapid increase of novel species over recent time, it is likely that this trend will continue, and many novel species are expected to be described in the future. This is supported by unpublished data from our laboratory based on AFLP and *rpoB* sequence analysis indicating the existence of several tens of potential novel species, many of which with one strain only.

The International Committee on Systematics of Prokaryotes (ICSP) deals with the description and nomenclature of novel species. The giving of names occurs according to the International Code of Nomenclature of Prokaryotes (the Code; Parker et al., 2016). New names appear in the International Journal of Systematic and Evolutionary Microbiology and can also be found on the Lists of Prokaryotic Names with Standing in Nomenclature (LPSN; http://www.bacterio.net/.) Description of each novel taxon is accompanied by the description of a type strain, usually a living culture that is deposited in two public culture collections. Type and reference strains of species can be found on . Uncultured organisms that are assumed to represent a novel species are usually informally named or given a so called *Candidatus* name.

The current number of described species is, as concluded from metagenomics, the tip of the iceberg of the overall species diversity in Nature. To depict this diversity, a debate is going on amongst taxonomists about how to deal with the description of novel species as inferred from genome sequences only. It has recently been proposed that gene sequences are suitable type material for the description of prokaryotic species (Whitman, 2016). A modification of the Code will be required to allow this option. The name of such a (genome) species would have priority over the eventual later description of these species based on a living strain. This debate and its outcome will have great impact on (traditional) prokaryotic systematics, including that of the genus *Acinetobacter* with its remarkable metabolic versatiliy and wide occurrence in Nature.

References

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Isolation of different *Acinetobacter* species from market meat: first identification of *Acinetobacter dijkshoorniae* in Peru.

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Introduction:
Among the 54 current species of the *Acinetobacter* genus attention is usually focused on the human pathogen A. baumannii, capable of acquiring resistance to multiple antibacterial agents. *A. baumannii* is predominantly recovered from clinical samples as a colonizer or as an infectious agent and it is also prevalent in environmental samples, including animals and food, both important sources for its dissemination. Less attention is being paid, however, to additional members of the *A. baumannii* group which are also human pathogens and whose prevalence is recently emerging worldwide.

Objective:
The aim of the present study was to analyse the phenotypic and genotypic characteristics of *Acinetobacter* spp. recovered from meat samples in Peru.

Methods:
Meat samples were obtained by random sampling of traditional markets and transported in sterile bags. Samples were homogenized and 2 g each were used to enrich the bacterial burden in overnight LB cultures. Bacterial colonies were isolated in MacConkey agar plates. Identification at the species level was performed by MALDI-TOF MS, amplified ribosomal DNA restriction analysis (ARDRA), rpoB sequencing and multilocus sequence analysis (MLSA). Clonal relatedness was assessed by multi-locus sequence typing (MLST) and pulse-field gel electrophoresis (PFGE). Sequence alignment was performed with ClustalW and phylogenetic trees were constructed using the neighbour-joining method, with genetic distances computed by Kimura's two-parameter model. Antimicrobial susceptibility was determined by E-test.

Results:
Twelve strains of *Acinetobacter* spp. were isolated from five different calves out of 138 meat samples obtained from two independent central markets in Lima, Peru. Species identification studies recognized 9 strains as A. pittii, 1 strain as *A. baumannii*, and 1 strain as the recently described novel species A. dijkshoorniae. The remaining strain could not be identified at the species level but all studies suggested close relatedness to A. bereziniae. Six strains were further selected according to either their unique PFGE profiles or if recovered from different calves. All strains were susceptible to all antibiotics tested and presented novel MLST alleles and ST types except for the *A. baumannii* isolate.

Conclusion:
Six non-redundant *Acinetobacter* spp. strains were isolated from meat samples of five different calves from two markets in Peru. Two clonally-related isolates were recovered from the meat of different animals and different markets. All but one strain were identified as member species of the clinically-relevant *A. baumannii* group, including one *A. baumannii* strain and one A. dijkshoorniae isolate, although all isolates were highly susceptible. This is the first time this novel *Acinetobacter* species is identified in Peru.

The isolation of pathogenic *Acinetobacter* species from human consumption meat may represent a risk to public health, and environmental sources such as animals and food should not be disregarded as potential reservoirs for the spread of these pathogens into community and healthcare settings.

Comparative genomics of *Acinetobacter indicus* revealed potential host and environment adaptation.

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Acinetobacter species is now recognized as a potential reservoir for antimicrobial resistance gene and a threat for debilitated patient in Intensive care unit. Many *Acinetobacter* sp. were discovered over the past decades. Among these species, *Acinetobacter indicus* was recovered from polluted dumpsite in india. Clinical A. indicus-like isolates from was recovered from a french patient and genomic analysis of this clinical isolates with other genoms of A. indicus has been performed.

Matherial and Methods

A. indicus RAB1 was recovered form urine sample. CIP53.82 and CIP110367 (also known as A648T) belong to Pasteur's Institute Collection. MICs were performed on the three isolate. Growth curve and culture with minimal media at different temperature was performed. Whole genome sequencing of A. indicus RAB1, CIP53.82 and CIP110367 was obtained using Illumina's technology. Comparative genomic was performed with all available A. indicus from Genbank. Genome assembly and analysis was performed using CLC genomic workbench, RAST server and Center for genomic epidemiology.

Results

A. indicus RAB1 was resistant to penicillins and carbapenems in accordance with the presence of the blaOXA-23 gene. Genome analysis of all available genomes showed the diversity of this species. This species can grow using a large variety of substrate as carbon source including propionic acid, malonate, d-glucose, citrate and histidine. Comparative genomic based on gene function revealed some specific features of A. indicus RAB1 compared to the environmental strains CIP110367. These features included an uncharacterized new CRISPR and a new type VI secretion system. The new CRISPR system, belonging to RAMP family, seems to be active since DNA fragments were detected between repeated elements. In the environmental strain CIP 110367, several gene involved in metabolism were identified including Sarcosine oxidase or gene involved in nitrogen metabolism.

Conclusion

This comparative genomic revealed the versatility of this species and gave new insights in the plasticity of *Acinetobacter* genomes to evolve with their environment.

Generation and characterization of unmarked single and double heme utilization gene cluster knockout mutants of *Acinetobacter baumannii* LAC-4.

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During the past several decades *Acinetobacter baumannii* has become increasingly multi-drug resistant (MDR). Additionally, the possession and acquisition of genetic elements encoding numerous drug resistance and virulence factors have made it an exceptionally problematic bacterial pathogen. We previously showed that a hospital outbreak MDR strain (LAC-4) of *A. baumannii* is hypervirulent in mice, exhibits high serum resistance and expresses a highly efficient heme utilization system. We also found a heme utilization gene cluster (named HUT for heme utilization 1) ubiquitous to all strains of *A. baumannii*, while only some strains, including LAC-4, harbor a second gene cluster encoding additional heme uptake proteins and a heme oxygenase enzyme (termed hemO cluster, HOC). Heme from host has been found to be a successful alternative source of iron for pathogens when free iron is sequestered in the circulatory system. Previously we generated an unmarked knockout (KO) mutant of LAC-4 in which the 9.5-kb eight-gene HOC was deleted. This HOC cluster KO mutant showed significant reduction in virulence, and reduced bacterial burden in all tissues of mice. In this communication we describe the generation of a single gene cluster KO mutant in which the 16.4-kb 12-gene HUT cluster was deleted and a double gene cluster KO mutant in which both the HOC and HUT clusters were deleted. We also present preliminary results of relative infectivity of these three KO mutants in the Galleria mellonela model.

Both the single cluster HUT mutant and the double cluster KO were constructed via homologous recombination of a KO cassette cloned into the suicide vector pMo130. The KO cassette was designed with an apramycin (Apr) resistance gene linked outside two fused flanking regions of the 16.4-kb heme utilization cluster via overlap extension PCR. The recombinant plasmid containing the KO cassette was then introduced via electrophoration into the wildtype LAC-4 strain and the previously generated LAC-4 ΔHOC mutant strain to generate the single cluster HUT mutant and double cluster KO mutant, respectively. After isolating a single cross-over integration of the suicide plasmid with KO construct, both an unmarked HUT mutant and a double cluster KO mutant were obtained via a second homologous recombination mediated by sacB counter selection leveraging the use of the xylE reporter gene on the suicide vector backbone. The virulence of the single cluster HUT mutant and the double mutant was then evaluated in a Galleria mellonella infection model.

The Galleria mellonella infection assays compared the sequence-confirmed double cluster KO mutant to the individual cluster KO mutants. The HUT KO mutant is not attenuated as compared to the wild-type strain in the Galleria model. While the double mutant was found to be less virulent than the wild-type and the HUT KO mutant, the single cluster HOC KO mutant still demonstrated less virulence than the double cluster KO mutant. Unexpectedly, the deletion of the HUT cluster in the single HOC KO mutant resulted in a significant increase in infectivity, suggesting a complex interaction between these two gene clusters.

Global gene expression profiling of *Acinetobacter baumannii* during a life-threatening mammalian infection.

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Acinetobacter baumannii is a pathogen of major importance in intensive care units worldwide, with the potential to cause problematic outbreaks and high-level resistance to antibiotics. There is an urgent need to understand the mechanisms of *A. baumannii* pathogenesis for the future development of novel targeted therapies. In this study we performed, for the first time, an assessment of *A. baumannii* gene expression during a life-threatening mammalian infection using in vivo RNA sequencing analysis.

Methods
A disseminated *A. baumannii* infection model in mice was used that involved an intraperitoneal injection with subsequent bacteremia and multi-organ involvement. Blood was extracted at 8 h post-infection to purify bacterial RNA for RNA-Seq using an Illumina platform.

Results
Approximately one quarter of *A. baumannii* protein coding genes were differentially expressed in vivo compared to in vitro (false discovery rate of ≤ 0.001, 2-fold change) with 557 showing decreased, and 329 increased expression. Gene groups with functions relating to translation and RNA processing were over represented in genes with increased expression, while those relating to chaperone and protein turnover were over represented in the genes with decreased expression. The most strongly up-regulated genes corresponded to the three recognised siderophore iron uptake clusters, reflecting the iron-restrictive environment in vivo. Metabolic changes in vivo included reduced expression of genes involved in amino acid and fatty acid transport and catabolism, indicating metabolic adaptation to a different nutritional environment. Genes encoding type I and type IV pili, quorum sensing components, and proteins involved in biofilm formation all showed reduced expression. Many genes that have been reported as essential for virulence showed reduced or unchanged expression in vivo, however previously unstudied metabolic pathways were identified that appear adapted for an in vivo mammalian infection.

Conclusion
This study provides the first insight into *A. baumannii* gene expression profiles during a life-threatening mammalian infection. Analysis of differentially regulated genes highlights numerous potential targets for the design of novel therapeutics, and this approach provides a significant advance to the study of this exciting yet troublesome pathogen.

The effect of metal stress on *Acinetobacter baumannii* oxidate stress tolerance.

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First-row transition metal ions are essential to the survival of all organisms, however, in excess, metals are also associated with significant toxicity. The importance of maintaining cellular metal ion homeostasis is highlighted by the fact that metals play opposing roles in the detoxification of reactive oxygen species and the induction of oxidative stress. Here, we examine the impact of metal and oxidative stress on *Acinetobacter baumannii* and the mechanisms used by the bacterium to combat these adverse conditions.

Our studies of metal ion toxicity have revealed that extracellular zinc stress induces a specific copper depletion phenotype and supplementation with copper not only fails to rescue this phenotype, but further exacerbates the copper depletion. To gain insight into the transporters involved in reducing cellular metal intoxication, we performed extensive genome and transcriptional analyses, which identified 13 putative efflux pumps, of which 4 were responsive to zinc stress, 5 to copper stress, and 7 to the combination of zinc and copper stress. Through subsequent mutant analyses we have characterised the primary zinc and copper efflux systems of *A. baumannii*. Our thorough examination of the impact of metal starvation and intoxication upon oxidative stress tolerance has revealed the critical role of transition metals in superoxide dismutase-mediated oxidative stress resistance. Collectively, this study supports the targeting of metal ion homeostatic mechanisms as an effective antimicrobial strategy against multi-drug resistant bacterial pathogens, through altering the susceptibility of bacteria to oxidative stress.

Part of this work has been published in BMC Microbiology (2017).

A high-frequency epigenetic switch controls virulence in *Acinetobacter baumannii* strain AB5075.

Chui-Yoke Chin, Kyle A. Tipton, Marjan Farokhyfar, David S. Weiss**, Philip N Rather (USA).**

Acinetobacter baumannii strain AB5075 can rapidly switch between two phenotypically distinct subpoulations distinguished by their opaque or translucent colony phenotypes under oblique lighting. The rate of interconversion between opaque and translucent variants can approach 30% under certain conditions, indicating that an epigenetic mechanism controls this switch. Multiple phenotypic differences exist between opaque and translucent variants, including biofilm formation, motility, quorum-sensing signal production and capsule thickness. We demonstrate that the opaque variant is highly virulent in a mouse lung model of infection. In contrast, the translucent variant is rapidly killed in-vivo and highly susceptible to host defenses such as cationic antimicrobial peptides (CRAMP, LL-37), lysozyme and hydrogen peroxide. RNA-Seq analysis has demonstrated that over 150 genes are differentially regulated between opaque and translucent variants. The most highly upregulated gene in the translucent variant was a TetR-type transcriptional regulator and constitutive expression of this regulator in the opaque variant converted all cells to the translucent state and rendered them unable to switch back to opaque. In addition, constitutive expression of this TetR regulator in the opaque variant abrogated virulence. Therefore, this regulator serves as a central switch to drive the opaque variant to the translucent state. In human bloodstream infections, only the opaque variant was isolated, further confirming the importance of this variant in virulence. The ability to switch between opaque and translucent variants was also observed in a variety of clinical isolates and common laboratory strains, thus underscoring the importance of separating these variants for studies involving virulence.

Fluoroquinolone resistance arising via chromosomal replacement in GC1 *Acinetobacter baumannii.*

Mohammad Hamidian (Australia), Ruth M. Hall.

Acinetobacter baumannii is an important nosocomial pathogen that often complicates treatment because of its high level of resistance to antibiotics. Resistance to the 3rd generation cephalosporins occurs mainly via activation of the intrinsic ampC gene by ISAba1 or ISAba125 providing a strong promoter. However, we previously showed that resistance to the 3rd generation cephalosporins could also occur by acquisition of a chromosomal segment that already contains an ISAba1/ISAba125-activated ampC gene from a different strain. Resistance to the fluoroquinolone antibiotics usually requires mutations in the chromosomal gyrA and parC genes that cause changes (e.g. Ser-81-Leu and Ser-84-Leu) in GyrA (DNA gyrase) and ParC (topoisomerase IV) enzymes, respectively. Here, we used whole genome sequencing data to examine how resistance to fluoroquinolones has occurred within a clonal cluster. Fifty-seven GC1 strains, 25 sequenced using Illumina HiSeq and 32 found in GenBank, were analysed. BLAST was used for sequence comparisons. All fluoroquinolones susceptible isolates carried the same gyrA and parC alleles. All fluoroquinolone resistant strains contained the mutations causing GyrA (Ser-81-Leu) and ParC (Ser-84-Leu or Ser-84-Trp) changes. In most fluoroquinolones resistant isolates the resistance determining mutation arose in the original allele. However, three groups of strains were found to include alleles of gyrA or parC or both, that are different from those in early fluoroquinolone sensitive GC1 strains. In these, sequences of the novel gyrA and parC alleles and surrounds differed from the original sequences by 1.3% to 2% nucleotide identity. In GC1 lineage 1, one group of 22 strains have imported chromosomal segments of 6.5 and 14.8 kb that include the gyrA and parC genes, respectively. The second group contained a different 13.6 kb sequence patch including the parC gene and had a mutation in the original gyrA gene. In lineage 2, another group of 3 strains contained a third chromosomal import of about 12.5 kb introducing a different gyrA gene.

This provides evidence for horizontal transfer of DNA segments that contain gyrA and parC genes followed by incorporation by homologous recombination. This also highlights the significance of the horizontal transfer of chromosomal DNA segments in GC1 *A. baumannii*.

A monooxygenase enzyme from *Acinetobacter radioresistens* confers resistance to Imipenem.

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Giorgio Gribaudo,Gianfranco Gilardi, **Sheila J. Sadeghi (Italy).**

Acinetobacter radioresistens has a Baeyer-Villiger monooxygenase (Ar-BVMO) with 100% amino acid sequence identity to a monooxygenase found in the multidrug resistant *Acinetobacter baumannii*. Ar-BVMO is not only capable of oxidizing anticancer drugs but can also oxidize other synthetic drugs such as imipenem. The latter is a member of carbapenems, a clinically important antibiotic family used in the treatment of MDR bacterial infections. In silico analysis of the 3D structural model of the Ar-BVMO, generated by homology modelling and subsequent molecular docking experiments support the productive binding of these drugs and imipenem to the active site of this enzyme (Figure below shows the docking of imipenem (cyan) in the active site of Ar-BVMO (FAD in yellow and NADP in magenta). Moreover, susceptibility tests performed by the Kirby-Bauer disk diffusion method demonstrate that imipenem sensitive E. coli BL21 cells overexpressing Ar-BVMO become resistant to this antibiotic. In order to get insights into the inactivation mechanism of imipenem by Ar-BVMO, its gene was cloned in an expression vector and subsequently expressed in bacteria and the resulting recombinant protein purified by affinity chromatography. Biochemical tests such as NADPH consumption assay with the purified Ar-BVMO demonstrate that imipenem is indeed a substrate of this enzyme and therefore its reaction product(s) were identified by liquid chromatography-mass spectrometry. Two different metabolites, as the result of the transformation of the carbonyl moiety of the beta-lactam ring, were identified by the LC-HRMS analysis.

In conclusion, this is the first report of an antibiotic-inactivating BVMO enzyme belonging to the *Acinetobacter* spp. which operates by an unprecedented mechanism resulting in carbapenem resistance. The presence of an identical monooxygenase in a MDR bacterium strongly suggests that this enzyme could be used by *A. baumannii* to metabolize and/or detoxify different types of synthetic drugs, including antibiotics, in exactly the same way as Ar-BVMO.

LN-1-255, An effective? - Lactamase inhibitor against oxa carbapenemases from *Acinetobacter baumannii.*

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Introduction

In recent years, the number of infections caused by Gram-negative pathogens carrying beta-lactamase carbapenemases has been increasing. Of these, the group of carbapenem-hydrolyzing class D beta-lactamases (CHDLs) is especially problematic. Several CHDLs have been identified in *A. baumannii*, including the plasmid encoded OXA-23, OXA-24/40, OXA-58, OXA-143, and OXA-235, and the chromosomally encoded OXA-51. Carbapenems have been successfully utilized as last resort antibiotics for the treatment of multi-drug-resistant A. baumannii infections; however, the spread of OXA carbapenemases is compromising the continued use of these antimicrobials. The aim of this work is to characterize the inhibition efficacy of LN-1-255 (a 6-alkylidene-2-substituted penicillin sulfone) and compare it to that of two established inhibitors (avibactam and tazobactam) against the most relevant enzymes of each group of class D carbapenemases in *A. baumannii*.

Material/methods

For microbiological studies blaOXA-23, blaOXA-24, blaOXA-51, blaOXA-58, blaOXA-143 and blaOXA-235 genes were cloned into the pETRA plasmid for expression in the *A. baumannii* ATCC 17978 strain. MICs for imipenem and meropenem were determined by microdilution in clinical isolates and transformants. For kinetic studies, blaOXA genes were cloned into the p-GEX-6p-1 plasmid for expression and purification. Proteins were purified by affinity chromatography from E. coli BL21, and confirmed by SDS-PAGE. Measurements of inhibition parameters were performed in the presence of LN-1-255, tazobactam and avibactam using nitrocefin as reporter substrate. IC50 and Ki (the apparent Km for inhibitors) were determined. In order to know the inactivation efficiency, kinact (the inhibitor complex inactivation rate) and KI (inhibitor concentration resulting in 50% of maximum). Also, tn (ratio of inhibitor/enzyme necessary for >90% inhibition) was estimated.

Results

Tazobactam did not significantly decrease the MIC of carbapenems in any ATCC17978 transformant expressing an OXA enzyme. Avibactam, at 16 mg/L, decreased the MIC of both carbapenems of OXA-143, decreased by 8-fold, OXA-58 decreased the MIC of imipenem by 8-fold, and OXA-24/40 decreased the MIC of meropenem by 4-fold. At 4 mg/L, avibactam did not decrease the MIC against carbapenems. In contrast, LN-1-255 at the same concentration drastically decreased the MICs of carbapenems, with LN-1-255 co-treated transformants displaying the basal level of susceptibility to carbapenems.

Kinetic assays showed low IC50 for LN-1-255 to all carbapenemases (0.008-0.040 μM) and higher for avibactam (1-35 μM) and tazobactam (2-28 μM), except the OXA-51 like enzyme, which was high for all inhibitors. This was in agreement with Ki (0.054-0.289 μM for LN-1-255, 9-154 μM for avibactam and 11-172 μM for tazobactam). The OXA-51-like enzyme had a low affinity for all inhibitors. While kinact values were similar between LN-1-255, tazobactam and avibactam, big differences were observed in the affinity of the three inhibitors, being the affinity of LN-1-255 65-6045-fold higher than tazobactam and 14-1126-fold higher than avibactam. In consequence, the inactivation efficiencies kinact/KI were also higher for LN-1-255 than tazobactam (117-3847-fold) and avibactam (8-614-fold). The turnover numbers (tn) were very similar for all CDHLs tested with LN-1-255 (2-6), except for OXA-51-like enzyme (520). The turnover numbers for avibactam and tazobactam were significantly higher, with 17-202 for avibactam and 51-4180 for tazobactam, and even higher for OXA-51-like enzyme.

Conclusions

The beta-lactamase inhibitor LN-1-255 had excellent microbiological synergy and inhibition kinetics parameters against all tested CHDLs, and a significantly higher efficacy than tazobactam and avibactam. A combination of carbapenems and LN-1-255 was effective against *A. baumannii* class D carbapenemases. Kinetic assays demonstrated that LN-1-255 had a better efficacy of inhibition than tazobactam or avibactam. LN-1-255 therefore represents a potential new therapeutic option, which can be used to treat infections by CHDL carrying A. baumannii isolates.

Lipidation of the *Acinetobacter baumannii* OXA-58 CHDL promotes its secretion in association to outer membrane vesicles.

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Objectives. The emergence of multidrug-resistant, carbapenem-resistant *Acinetobacter baumannii* constitutes a worrisome problem in health-care institutions worldwide. Carbapenem resistance results mainly from the overproduction of acquired carbapenemases such as OXA-type class-D beta-lactamases (CHDLs) with minor contributions of other factors including decreased outer membrane (OM) permeability (1). *A. baumannii* CHDLs have been characterized at the kinetic and structural levels and the genetic context of blaOXA genes characterized (2), but less attention has been given to the distinct aspects of their biogenesis pathway which also offers potential targets for antimicrobial design. Here, we report that the OXA-58 CHDL undergoes lipidation during transit to the *A. baumannii* periplasm, and that this post-translational modification is essential for membrane attachment and association to outer membrane vesicles (OMVs).

Methods

As a model we employed *A. baumannii* ATCC 17978 (Ab17978) transformed with plasmid pOXA-58 directing high-level expression of the blaOXA-58 gene (3). Lipidation of OXA-58 was analysed by [3H]palmitate labelling of the cells followed by protein analysis by SDS-PAGE. Presence of OXA-58 in different cell fractions (periplasm, membranes, secreted OMVs) was analyzed with specific antibodies and by spectrophotometric determination of imipenemase activity. Imipenem (IPM) resistance levels in susceptible cells were evaluated by Kirby-Bauer agar disk diffusion assays (1) and by the Triton-Hodge test.

Results

We predicted the presence of lipoboxes containing the Cys target residue at the N-terminal regions of A. baumannii CHDLs. Differential [3H]palmitate cell labelling followed by SDS-PAGE indicated specific lipidation of OXA-58 in Ab17978/pOXA-58 cells. Cell fractionation analysis indicated that lipidation was necessary for both OXA-58 membrane attachment and stability, as judged by the recovery of the wild-type enzyme mainly in association to membrane fractions and that of a mutant enzyme lacking the target Cys mainly in soluble periplasmic fractions in largely reduced amounts. Lipidation was found essential for OXA-58 recruitment as cargo into OMVs. The enzyme was associated to the inner side of the vesicles as judged by protease treatment, a result supported by the significant increments of imipenemase activity of the vesicles after Triton X-100 permeabilization. This secreted OXA-58 conferred protection from IPM killing to susceptible strains of *A. baumannii* and Escherichia coli after surfactant treatment of the OMVs.

Conclusions

OXA-58 is a membrane-anchored lipoprotein in *A. baumannii*, and this post-translational mechanism offers to this enzyme stability and the capability to be secreted as an OMV cargo. The observation that the secreted enzyme is capable of conferring carbapenem protection to accompanying susceptible bacteria suggests roles of this mechanism in the progression of polymicrobial infections.

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The lytic murein transglycosylases MTLB contributes to membrane homeostasis in multi-drug resistant *Acinetobacter baumannii.*

Sebastien Crepin (USA), Elizabeth Ottosen, Sara N. Smith, Harry L.T. Mobley.

Acinetobacter baumannii is rapidly emerging as a leading nosocomial pathogen, particularly among immunocompromised individuals and for patients in intensive care units. *A. baumannii* infects a wide range of anatomic sites including the respiratory tract, bloodstream, and wounds. Accordingly, the World Health Organization positions *A. baumannii* as priority number one among bacteria for which new antimicrobials are urgently needed. Indeed, little is known about the molecular basis of *A. baumannii* pathogenesis. Using the transposon-directed insertion-site sequencing (TraDIS) technique in the neutropenic mouse model, we recently identified the full set of genes required during bloodstream infection. The lytic murein transglycosylase mltB was identified as an important fitness factor. MltB encodes a lysosyme-like enzyme involved in the degradation and recycling of the peptidoglycan. Accordingly, we hypothesized that in the mltB mutant, peptidoglycan is not processed properly, which may impair the homeostasis of the bacterial membrane and consequently, resistance to stresses and colonization of the host. Indeed, the mltB mutant is more susceptible to stresses encountered during bloodstream infection, such as acid, oxidative stress, and susceptibility to serum complement and cationic antimicrobial peptides. In addition, the mutant cannot grow under osmotic shock (500 mM NaCl). Since these stresses are associated with membrane perturbation, we tested whether the genes involved in the envelope stress response are induced in the mltB mutant. Among them, degP, rstA and baeR are induced 4.4-, 4.0- and 2.6-fold, respectively. In addition, work is in progress to determine the membrane permeability using the propiodium iodide assay. Finally, in the murine model of bloodstream infection, the mltB mutant had a fitness defect of 185-, 4- and 5,557-fold in the spleen, liver and kidneys, respectively. In summary, in this study, we showed that the mltB gene is part of a complex network connecting membrane homeostasis, resistance to stresses and virulence. In addition, these results contribute to our understanding of the pathogenesis of *A. baumannii* and, in the long term, will allow us to formulate strategies to manage or prevent A. baumannii infections.

Crystal structure of phosphopantetheine adenylyltransferase from *Acinetobacter baumannii* AT 1.76 a resolution.

Akshita Gupta (India), Naseer Iqbal, Punit Kaur, Sujata Sharma, T.P. Singh.

Acinetobacter baumannii is a non motile, aerobic gram negative bacterium responsible for hospital acquired infections; mostly affect immunocompromised patients admitted in ICU. Wide spread use of antibiotics against bacterial infections has lead to the development of drug resistance in this bacteria. Therefore, new drugs are required to combat the infections caused by *Acinetobacter baumannii.* Coenzyme A serves as a cofactor in many essential biochemical reactions such as fatty acid metabolism and Krebs cycle in all organisms. Thus inhibiting Coenzyme A biosynthesis lowers the level of CoA in cell, resulting in cell death which makes this pathway an attractive one for drug designing. Phosphopantetheine adenylyltransferase (PPAT) is an essential enzyme in bacteria that catalyzes the rate-limiting step in coenzyme A (CoA) biosynthesis by transferring an adenylyl group from ATP to 4'-phosphopantetheine (Ppant), yielding 3'-dephospho-CoA (dPCoA). PPATs are the potential target for developing antibiotics because bacterial and mammalian PPATs share little sequence homology. Here PPAT from *Acinetobacter baumannii* (AbPPAT) was cloned, expressed and purified to homogeneity. AbPPAT has been crystallized using 1.5M trisodium citrate as precipitant in 0.1M HEPES buffer at pH-7.6. The crystal structure of AbPPAT complexed with citrate has been determined by the molecular-replacement method at 1.76Å resolution. The crystals belong to orthorhombic space group P212121 with cell dimensions, a = 78.26, b = 109.38, c = 121.27Å. The structure was solved with molecular replacement method and refined to Rcryst/Rfree factors of 0.211/0.262. The structures of 6 crystallographically independent molecules in the asymmetry unit were identical with r.m.s shifts for the Ca atoms ranging from 0.3 Å to 0.8 Å. They formed a hexamer with a dimer of trimers kind of arrangement.

One citrate ion in each monomer was observed in the active site which interacts with residues of the binding site via hydrogen bonds and non bonded interactions. On superimposing the the AbPPAT structure bound to citrate with MtPPAT bound to ATP, it was found citrate occupies the same position as the α, β and γ phosphates of ATP residue thus implying that citrate binds and interacts with similar residues as the α, β and γ phosphates of ATP.The new structural information reported in this study including citrate as a potent inhibitor of PPAT will supplement the existing structural data and should be useful for structure based antibacterial discovery
against PPATs.

Probing natural competence of multidrug-resistant *Acinetobacter baumannii* via acquisition of the gentiase catabolic pathway.

Gottfried Wilharm (Germany), Ulrike Blaschke, Evelyn Skiebe.

Objectives
Acinetobacter baumannii is a gram-negative nosocomial pathogen notorious for its potential to develop multidrug-resistance. Horizontal gene transfer (HGT) is critically shaping evolution of this pathogen, however the relevant pathways of HGT are not well-defined. Recently, natural competence was identified as a principle pathway of DNA uptake in *A. baumannii* but these studies were restricted to antibiotic-sensitive isolates due to the lack of suitable markers to tackle multidrug-resistant (MDR) isolates. Methods: Here, we introduce a simple transformation assay based on the acquisition of the gentisate catabolic pathway. The ability for growth on mineral medium with gentisate as the sole source of carbon is rare among members of the species *A. baumannii*. Results: We identified strains capable of gentisate utilization which can serve as donors of DNA to transform gentisate non-utilizing strains. In this way we could demonstrate that natural competence is a typical feature of MDR strains belonging to international clone 1 (IC 1). Some MDR strains representing IC 2, IC 3, IC 4 and IC 5 could also be transformed. We found IC 7 to be not amenable to this novel assay due to its intrinsic capability to utilize gentisate.

Conclusions
This method offers a tool to study natural transformation in many multidrug-resistant isolates of *A. baumannii* without introducing an antibiotic resistance gene. Natural competence is a common trait of the IC 1 lineage and also detectable in strains belonging to other IC lineages suggesting a major impact of transformation competence on the evolution of this nosocomial pathogen.

Compatible solutes in *Acinetobacter baumannii.*

Sabine Zeidler (Germany), Volker Müller

Acinetobacter baumannii is an opportunistic human pathogen emerging in intensive care units in hospitals worldwide with recent outbreaks also in Germany. *A. baumannii* has the extraordinary capacity to survive on dry surfaces, which enables the bacterium to persist in the hospital environment[1]. Desiccation has the same physicochemical consequence for a cell as the addition of salt: loss of cellular water. Osmotic stress-induced loss of water is counterbalanced by the uptake or synthesis of compatible solutes, small molecules that are tolerated by the cell in up to molar concentrations[2]. Compatible solutes are also known to protect cells from desiccation[3]. To study the molecular basis of desiccation resistance in *A. baumannii*, we address the nature and role of compatible solutes in this bacterium. *A. baumannii*, as *Acinetobacter* baylyi, synthesizes mannitol and glutamate as compatible solutes[4] but trehalose in addition. A markerless deletion mutant missing the mannitol-1-phosphate dehydrogenase / phosphatase (MtlD) does not accumulate mannitol and shows severe growth defects at high osmolarities. Both MtlD as well as the mtlD promoter are activated by the presence of salt. The same could be shown for the promoter in front of otsB, the gene for the trehalose-6-phosphate phosphatase involved in trehalose biosynthesis. Trehalose plays an even more important role in *A. baumannii* than mannitol. A mutant defect in trehalose biosynthesis is no longer able to infect Galleria mellonella larvae[5]. We could show that an otsB mutant has lower tolerance against osmotic stress. Furthermore, first experiments indicate a possible role for trehalose in desiccation tolerance. Our results give an insight into the role of compatible solutes in the pathogen *A. baumannii* beyond tolerance of salt stress.

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Clonal map of *Acinetobacter baumannii* resistant to carbapenems in Andalusia 2016 (PIRASOA Program).

Felipe Fernández Cuenca (Spain), Lorena López Cerero, Inmaculada López Hernández, María Carmen Serrano Martino, Miriam Valverde Troya, Inmaculada López Rodríguez, Fátima Galán Sánchez, María del Pilar Luzón García, Alvaro Pascual.

A. baumannii (Ab) resistant to carbapenems (Ab-CP) is an emerging nosocomial pathogen. This microorganism has a great capacity of dissemination producing large outbreaks that are usually very difficult to control. In these outbreaks a small number of oxacillinase producing clones (OXA-23, OXA-58 or OXA-24/40) are usually involved. In Andalusia there are no global data on the actual epidemiological situation or dissemination of Ab-CP clones. The objective of the study is to determine the current situation in Andalusia in relation to the dissemination of Ab-CP. Material y Methods: The program PIRASOA is the program of surveillance and control of infections associated with health care and proper use of antimicrobials in Andalusia. 77 Ab-CP isolates were voluntarily sent to the reference laboratory of the PIRASOA program (Hospital U. Virgen Macarena, Seville) during 2016. Isolates were identified by MALDI-TOF mass spectrometry and by the presence of blaOXA-51 gene. Antimicrobial susceptibility testing was performed using MicroScan panels. The presence of carbapenemase (CPase) genes was performed by PCR with specific primers for class A (KPC), B (IMP, VIM and NDM) and D (OXA-23, OXA-48, OXA-58 and OXA-24/40) CPase genes. Analysis of the clonal relationship of the isolates was performed using ApaI-PFGE and MLST (scheme of the Pasteur institute). Results: No Class A or Class B CPases were detected. OXA type CPases were detected in all isolates: 59.7% (OXA-58), 15.6% (OXA-24/40) and 24.6% (OXA-23) of isolates. Two major clones (ST2; n=54 isolates and ST745; n=22 isolates) and 1 sporadic clone (ST32; n=1 isolate) were observed. The ST2 clone contained isolates producing OXA-58 (ST2/OXA-58; n=25 isolates), OXA-23 (ST2/OXA-23; n=18 isolates) and OXA-24/40 (ST2/OXA-24/40; n=11 isolates). In contrast, the ST745 clone only contained isolates producing OXA-58 (ST745/OXA-58; n=22 isolates). The ST32 clone was represented by an OXA-58 (ST32/OXA-58) producing isolate. The distribution of clones per hospitals was: ST2/OXA-58 (6 hospitals), ST2/OXA-23 (4 hospitals), ST2/OXA-24/40 (1 hospital), ST745/OXA-58 (5 hospitals) and ST32/OXA-58 (1 hospital). Coexistence of different clones was observed with ST2/OXA-58 and ST745/OXA-58 (2 hospitals), ST2/OXA-58 and ST32/OXA-58 (1 hospital), and ST2/OXA-58 and ST2/OXA-24/40 (1 hospital).Transmission of isolates with the same pulsotype was observed for ST2/OXA-23 clone (3 hospitals) and ST745/OXA-58 clone (2 hospitals). Conclusions: 1) OXA-58 is the most frequently detected CPase and ST2 is the major clone. 2) Of the five Ab-CP clones detected in 2016, the most prevalent and the one that has shown a greater capacity of dissemination has been the clone ST2/OXA-58.

European prospective cohort study on *Acinetobacter baumannii* showing resistance to carbapenems (EURECA-CRAB substudy).

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Acinetobacter baumannii is a major nosocomial pathogen with a high potential to spread among patients in healthcare settings. *A. baumannii* also has a propensity to acquire resistance to various antibiotic classes. During the past decades, carbapenem-resistant *A. baumannii* (CRAB) has spread across different countries. The therapeutic options available against CRAB are very limited; the most frequent active drugs are so-called "second-line" agents, such as colistin, tigecycline and occasionally the aminoglycosides. The best available therapy (BAT) against these infections is unknown; identification of BAT is important for the management of patients and for the design of clinical trials.

Objetives

To characterise the features, clinical management and outcomes of hospitalised patients with bloodstream infection (BSI) caused by CRAB in order to (1) identify the predictors for negative outcomes; and (2), specifically, to analyse the impact of clinical management and different antibiotic regimens with identification of the best available therapy (BAT). Also, to develop a prospective cohort that could be used as a historic cohort for comparison of efficacy of new drugs when randomised trials are not feasible.

Methods and design

A prospective cohort study of patients with BSI due to CRAB will be performed in 50 hospitals throughout countries in southern Europe including Albania, Croatia, Greece, Italy, Kosovo, Montenegro, Romania, Serbia, Spain and Turkey. The base-population will be all patients with BSI due to CRAB during a recruitment period of 17 months. A sample size of 201 patients was estimated. All patients will be followed for 30 days. The main outcome will be all-cause mortality at day 30; secondary outcomes are clinical and microbiological response at test of cure, hospital stay and adverse events. The main exposures are empirical active antibiotic therapy, early targeted optimized therapy, and early source control. A summary of patient visits and the variables collected during follow-up is shown in Table 1. The statistical analyses will be performed using Cox regression and logistic regression. Propensity score will be used to control for confounders. Sensitivity analyses will be done using different definitions for variables and in subgroups.

Ethics and funding

Approval has been granted from appropriate regulatory agencies and local Ethics Committees of Research or Institutional Review Boards (IRBs). This is an observational study and therefore no intervention in the diagnosis, management or treatment of the patients will be required on behalf of the investigation. The project received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement nº 115620 resources of which are composed of financial contribution from the European Union Seventh Framework Programme (FP7/2007-2013) and EFPIA companies in kind contribution. The project is registered at ClinicalTrials.gov.

Results

Preliminary results will be available and reported at the meeting.

Variables	Day 0	From day 0 to day 21	Day 21 (Test of cure)	Day 30 (end of follow-up)
Selection criteria	√			
Demographics	√			
Risk factors	√			
Comorbidities	√			
Clinical features	√	√	√	
Microbiology	√			
Antibiotic therapy	√	√	√	√
Non-antibiotic treatment	√	√	√	√
Outcome			√	√
Other analytical results	√	√	√	
Safety of drugs	√	√	√	√

Table 1. Patient visits and variables collected at key timepoints.

Whole-genome comparison of OXA-23- and OXA-58-producing carbapenem-resistant International Clone 2 *Acinetobacter baumannii* clinical isolates.

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Spyros Pournaras (Greece)

Objectives

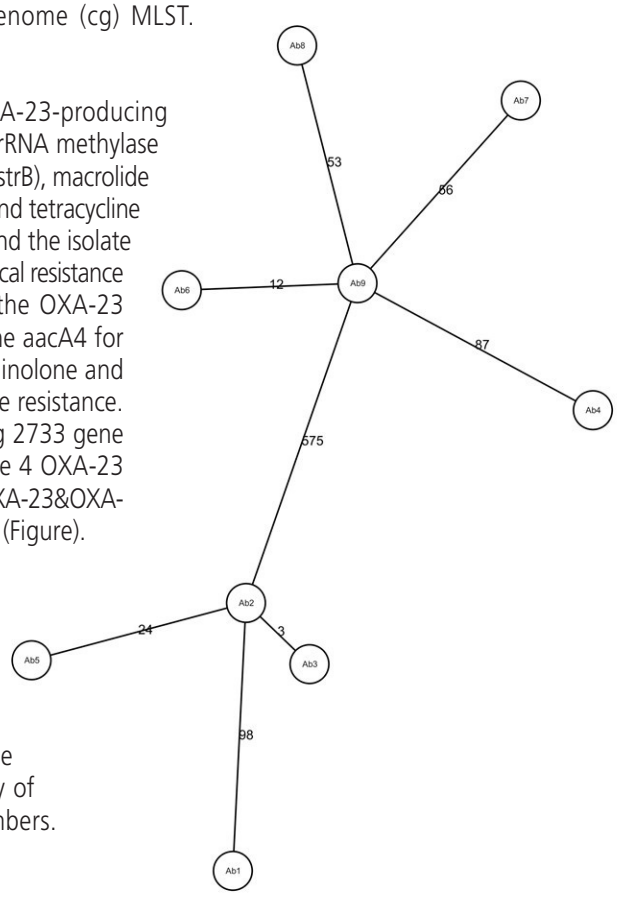
During the last few years carbapenem-resistant Acinetobacter baumannii (CRAB) are increasingly disseminated around the world. Currently, CRAB isolates mainly belong to International Clone (IC) 2 and produce OXA-23 carbapenemase, having gradually replaced isolates of IC2 that produced OXA-58. The aim of this study was to reveal the phylogenetic relationship and genome-wide differences between OXA-23- and OXA-58-producing A. baumannii clinical isolates from Greece that are assigned to IC2. The current observations are preliminary findings in the context of a larger multicenter study that will compare isolates from at least three countries.

Methods

Four OXA-23 (Ab1, Ab2, Ab3, Ab5), four OXA-58 (Ab6, Ab7, Ab8, Ab9), and one OXA-23&OXA-58 (Ab4) A. baumannii isolates, collected in four tertiary hospitals through Greece were studied. All nine isolates belonged to ST2 (IC2) according to the Pasteur MLST scheme. Antibiotic susceptibility profiles of the isolates were determined using VITEK2 system (bioMerieux, Marcy l'Etoile, France) following CLSI guidelines. The isolates were subjected to NGS using an Illumina platform. The phylogenetic relationship of the isolates was determined by NGS-based core-genome (cg) MLST.

Results

Whole genome comparison revealed that all OXA-23-producing isolates carried resistance genes encoding the 16S rRNA methylase (armA), aminoglycoside modification enzymes (strA, strB), macrolide efflux protein and phosphotranferase (msrE, mphE) and tetracycline efflux pump (tetB). All OXA-58-producing isolates and the isolate coproducing OXA-23&OXA-58 harbored almost identical resistance determinants that were different from those of the OXA-23 producers: the beta-lactamase gene blaOXA-20, the aacA4 for aminoglycoside resistance, aac(6')Ib-cr for fluoroquinolone and aminoglycoside resistance, and sul1 for sulfonamide resistance. The cg phylogenetic tree was constructed including 2733 gene targets, which were shared by all nine isolates. The 4 OXA-23 producers clustered together and the 5 OXA-58 or OXA-23&OXA-58 isolates grouped together in a different cluster (Figure).



Conclusions

Our results indicate that the 4 OXA-23 IC2 CRAB were phylogenetically related and distinct from the 4 OXA-23 IC2 CRAB that were also similar. The OXA-23&OXA-58 isolate probably derived from an OXA-58 isolate that acquired OXA-23. The resistome of each of the two lineages included a multiplicity of resistance genes, which were shared by all members.

Antibiotic usage as a driving force for spread of carbapenem resistant *Acinetobacter baumannii* in a tertiary-care hospital.

Gábor Kardos (Hungary), Hajnalka Tóth, Idan Blum, Julianna Mózes, Bence Balázs.

The incidence of *Acinetobacter baumannii* isolations as well as occurrence of carbapenem resistance in A. baumannii steadily increased since 2009 from cca. 0.2 cases/1000 occupied bed-days (OBDs) or lower between 2005-2009 to >0.9 cases/1000 OBDs in 2015 and 2016 in the tertiary-care center of the University of Debrecen. The study investigates the link between this increase and the changes in consumption of different antibiotic groups using time-series analysis.

We collected monthly data between October 2004 and August 2016 representing 143 months. Resistance of A. baumannii isolates from inpatients to carbapenems, amikacin, gentamicin, tobramycin and ciprofloxacin was recorded as monthly incidence densities of isolations of resistant bacteria per 1000 bed-days (ID). IDs of resistant A. baumannii were analysed against consumption of cephalosporins, carbapenems, aminoglycosides and fluoroquinolones expressed as defined daily dose per 100 OBDs (DDD). Association between resistance and consumption time-series were analysed by means of dynamic regression using the Eviews software and vector autoregression (VAR) models interpreted using impulse-response functions (IRFs) in R statistical environment. ID of carbapenem resistant *A. baumannii* isolations were first regressed or modelled pairwise with consumption of each single antibiotic group, then composite models initially containing all consumption series were built.

In pairwise dynamic regressions, only cephalosporin and carbapenem consumption showed significant relationship to resistance, a weaker (sum of coefficients at significant lags -3 and -4: 0.051) and a stronger (sum of coefficients at significant lags -3, -4 and -6: 0.228) association, respectively. The only VAR model showing significant relationship was the one with carbapenem consumption (IRF significant at lag -2, but not at any other with a coefficient of 0.058).

In the dynamic regression composite model consumption of carbapenems (sum of coefficients at significant lags 0, -4 and -6: 0.204), fluoroquinolones (at lag -4 with a coefficient of 0.018) and cephalosporins (at lag -4 with a coefficient of 0.035) was positively associated with the occurrence of carbapenem resistant *A. baumannii*, while aminoglycoside usage was negatively associated (sum of coefficients at significant lags 0 and -3: 0.184), suggesting that aminoglycoside usage may be associated with decreasing of prevalence. In composite VAR models an effect of carbapenem consumption was found (IRF significant at lags 0 to -5 with a sum of coefficients of 0.354), but significant effect of consumption of any other drug was absent. Curiously, aminoglycoside usage was negatively influenced by carbapenem resistance of *A. baumannii*, indicating that increasing prevalence of carbapenem resistant *A. baumannii* is linked with a decrease in aminoglycoside consumption, and not vice versa.

In conclusion, carbapenem usage was strongly associated with increasing prevalence of carbapenem resistant A. baumannii in all models. The effect of other studied drug groups was not consistently demonstrated. This suggests that carbapenem usage is probably a major provoker of increasing prevalence of carbapenem resistant A. baumannii, while usage of other drug groups seem to have little effect.

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Integration of Omics and bioinformatics tools to identify new therapeutic targets for *Acinetobacter baumannii*.

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Introduction and purpose

Acinetobacter baumannii is an important nosocomial pathogen that poses a serious health threat to immune-compromised patients. Some studies suggest that the virulence of this pathogen is strain-dependent. In this context, the aim of this study was to identify new virulence factors of bacteremic *A. baumannii* strains that could explain the different clinical outcome of a cuasi-experimental group of critically ill patients infected with these pathogens.

Methods

Six multidrug-resistant *A. baumannii* clinical strains isolated from blood samples of six mechanically ventilated individual adult patients admitted to the ICU of a tertiary hospital in 2011. All these patients presented pneumonia and bacteremia, both caused by these strains, with no co-infection. They had similar ages, Charlson index of 0 (no associated comorbidities) and an APACHE II index higher than 15 (severe patients) in the day of admission. All the patients received an optimal empiric treatment since the beginning with colistin. However, three patients died. Whole-genome sequencing (WGS) of these six strains was performed using Illumina MiSeq platform (paired-end 2 x 300 bp reads). Trimmomatic was used to filter the read sequences for quality, and AbySS was selected to assemble the results into contigs due to its high accuracy with short reads. Then, protein-coding genes was predicted by Prodigal, a widely used gene finder for bacteria, and they were functionally annotated by Sma3s v2 using the bacterial taxonomic division of UniProt database. A differential gene analysis using the Blast similarity tool was performed to detect sequence variants, including single amino acid variants, between both strain groups (isolates from the patients who died vs. from the patients who did not). Additionally, outer membrane proteins (OMPs) extracted from all the strains were analyzed by SDS-PAGE gradient gel (4-15%). Actually, a differential band found with SDS-PAGE technique is being analyzed by liquid chromatography-tandem mass spectrometry.

Results

From the differential gene analysis of the WGS data, one gene, called CarO, was found in all the isolates from the patients who died and in none of the isolates from the patients who did not die. This result was corroborated with SDS-PAGE, founding only a differential band (29 kDa) in the isolates from the patients who died. The band appeared to have the same molecular weight as the protein CarO according to several experimental studies. Conclusions: Based on WGS-differential gene analysis and SDS-PAGE, the OMP CarO could be a virulence factor associated with the clinical outcome of these six pneumonic and bacteremic patients. Further studies should be performed in order to confirm this result.

Central stress response elements in *Acinetobacter baumannii* using an integrative network biology approach.

Sonika Bhatnagar (India).

The emergence of multiple drug resistance in the opportunistic pathogen *Acinetobacter baumannii* poses a serious concern in the hospital setting. Apart from antibiotics, this gram negative bacterium also resists nutrient deprivation, injury and antiseptics. In the human host and the medical environment, it can resist a variety of stressors that allow it to pose a serious threat. We have previously established the presence of certain central stress response elements common to all stressors in the model organism Escherichia coli using a network biology approach. Integrating multiple stress response gene expression datasets and available protein-protein interactions, we constructed a protein protein interaction network to represent stress response in A. baumanii. Topological analysis of the network showed the central regulatory elements common to stress response under all stress conditions. Gene ontology analysis also identified the main pathways and biological processes responsible for the stress response. Further, our approach allowed us to detect the genes common to central stress response, biofilm formation, motility and virulence. Disrupting the central stress response will present new avenues for prevention and treatment of *A. baumannii* infections.

Construction of new shuttle-vectors for gene cloning and expression in *Acinetobacter baumannii*.

Paolo Visca (Italy), Massimiliano Lucidi, Federica Runci, Giordano Rampioni, Emanuela Frangipani, Livia Leoni.

Acinetobacter baumannii is an opportunistic nosocomial pathogen mainly associated with hospital-acquired infections. Few tools are available for gene cloning and expression in multidrug-resistant *A. baumannii*. Here, we report the generation of new multi-purpose shuttle plasmids (pVRL series) which can efficiently replicate in both A. baumannii and Escherichia coli. The pVRL1 plasmid has been constructed by combining: i) the origin of replication from the *Acinetobacter* calcoaceticus plasmid pWH1266 (a derivative of the natural cryptic plasmid of pWH1277 [Hunger et al., 1990, Gene 87:45]); ii) a gentamicin (Gm) resistance cassette (aacC1) for antibiotic selection; iii) a ColE1-like origin of replication for maintenance in E. coli; iv) a multiple cloning site (MCS) from pBluescript II KS. The replication origin of pWH1277 was fully sequenced and characterized in silico, to gain new insights into the stability properties and partitioning mechanisms of the plasmid. Then, the pVRL1 scaffold was modified to obtain the pVRL2 plasmid, which allows arabinose-inducible gene expression under control of the araC-PBAD regulation system. Both pVRL1 and pVRL2 are stable in E. coli and *A. baumannii* ATCC19606T, consistent with the presence of a putative toxin-antitoxin system, and confer Gm resistance up to 128 and >512 μg/ml, respectively. The pVRL plasmids exhibit variable transformation efficiency in *A. baumannii* (6.6 × 10^2 and 1.86 × 10^5 CFU/μg plasmid DNA in *A. baumannii* strains ATCC19606T and ACICU, respectively). pVRL1 carrying the trpE gene with its native promoter was successfully used to complement tryptophan auxotrophy in *Acinetobacter* baylyi BD4 trpE27. Expression of the promoterless trpE or lacZ genes was under tight control by the araC-PBAD promoter in pVRL2, being gradually induced by arabinose (0.25-to-8.0%) in A. baylyi BD4 (trpE27) and *A. baumannii* ATCC19606T, respectively. Thus, pVRL plasmids are promising tools for gene cloning and expression in A. baumannii, and could facilitate future genetic studies of this species.

Bifunctional Quaternary Amine Antibiotics.

Gregory A. Knauf (USA), Ashley L. Cunningham, Misha I. Kazi, Ian M. Riddington, Alexander A. Crofts, Vincent Cattoir, M. Stephen Trent, Bryan W. Davies.

Novel antibiotics for the treatment of *Acinetobacter baumannii* infections are in dire need, as antibiotic resistance continues to plague patients and doctors. Quaternary ammonium compounds (QACs) are extremely diverse and are often used in the prevention of the spread of *A. baumannii* in the clinic but, their antimicrobial mode of action is not fully defined. It is well documented that QAC biocides act through membrane disruption. However, this does not fully explain their activity. In addition to membrane activity, we show that the classic QAC biocide benzalkonium chloride (BZK) targets the *A. baumannii* ribosome, triggering proteome aggregation and oxidation, resulting in cell death. Resistance to BZK develops through spontaneous ribosomal protein mutations that decrease proteome damage and show specific cross-resistance to the ribosome-acting antibiotic erythromycin. This dual action suggests that QACs could be used as a novel class of ribosomal antibiotics for the treatment of *A. baumannii* infections. We demonstrate that the therapeutic QAC otilonium bromide has potent antimicrobial activity against both *A. baumannii* and Gram-positive multidrug-resistant pathogens in vitro and in vivo. Our discovery of the bifunctional activity of QACs and the breadth of potential QAC structures reveal the broader potential of QACs as potent antibiotics to fight multidrug-resistant infections, including *A. baumannii*.

Matrix Metalloproteinase-9 (MMP-9) Plays an Important Role in Host Innate Defense against Respiratory Infection with *Acinetobacter baumannii* in Mice.

Greg Harris, Rhonda KuoLee, Sheng Hou, Hu Susan Jiang, H. Howard Xu,
Wangxue Chen (Canada).

We and others have previously shown that rapid, local recruitment and accumulation of innate immune cells (neutrophils and macrophages) are critical in determining the clinical outcome of respiratory *A. baumannii* infection. Since MMPs play an essential role in modulating tissue leukocyte recruitment, we investigated whether MMP-9, an important member of this proteinase family, is critical in the host defense against respiratory *A. baumannii* infection in a mouse model of intranasal *A. baumannii* infection. We found that intranasal *A. baumannii* infection induced high expression and production of MMP-9 in the lungs and sera of infected mice and the neutrophil is one of main cellular sources for it. Moreover, MMP-9-/- mice were significantly more susceptible to *A. baumannii* infection with a decreased survival rate (60% vs 0%) and increased lung bacterial burden, as compared with infected wild-type mice. Histologic analysis of the lung and assessment of leukocytes in the bronchoalveolar lavage fluid revealed a significant reduction in the proportion of neutrophil infiltrates in MMP-9-/- mice, which was accompanied with a reduced production of CXCL15, a potent neutrophil chemotactic molecule released in the infected site through the action of MMP-9. Collectively, our results identified MMP-9 as a new contributor to host defense against respiratory *A. baumannii* infection through its modulation of CXCL15 and neutrophil recruitment axis at the site of infections. The potential implication of our findings in relation to the development of novel vaccine and immunotherapeutics will be discussed.

Structural and functional studies of 3-dehydroquinate Dehydratase from *Acinetobacter baumannii*.

Naseer Iqbal (India), Mukesh Kumar, Pradeep Sharma, Satya Prakash Yadav, Punit Kaur, Sujata Sharma, Tej P. Sing.

One of the most common infections in patients because of their long stays in the hospitals is caused by a bacterium, *Acinetobacter baumannii*. There are also reports that this Gram-negative bacterium has developed resistance against the currently available antibiotics. Therefore, new drugs are required to control the infections caused by *Acinetobacter baumannii*. In bacteria, the shikimate pathway is responsible for the production of a compound, chorismate which is an essential precursor for the synthesis of aromatic compounds. Such a pathway is not present in humans. In this pathway, there are seven steps which are catalysed by different enzymes. One of the steps is catalysed by an enzyme, type II 3-dehydroquinate dehydratase (DHQD). 3-dehydroquinate dehydratase (DHQD, EC 4.2.1.10) from *Acinetobacter baumannii* (AbDHQD) has been cloned, expressed and purified to homogeneity. The binding studies showed that compounds, quinic acid and citrazinic acid bound to AbDHQD at 10-6 and 10-5 molar concentrations respectively. AbDHQD has been crystallised using 30% PEG-3350, 50mM tris-HCl and 1.0M MgSO4 at PH 8.0 as a precipitating agent. The suitable crystals of AbDHQD were stabilised by placing crystals in the reservoir solution to which 25% glycerol was added for data collection at 100K. The X-ray intensity data were collected to 2.0Å resolution. The crystals belong to monoclinic space group P21 with cell dimensions, a = 82.3, b = 95.3, c = 132.3Å and beta = 95.7°. These data indicated the presence of 12 protein molecules which corresponded to a solvent content of 53%. The structure was solved with molecular replacement method and refined to Rcryst/Rfree factors of 0.200/0.232. The structures of 12 crystallographically independent molecules in the asymmetry unit were identical with r.m.s shifts for the Ca atoms ranging from 0.3Å to 0.8Å. They formed a dodecamer with four trimers arranged in a tetrahedral manner. The conformation of the classical lid indicated an open conformation of the substrate binding site although a sulfate ion was observed in the substrate binding site.

Evaluation of recombinant protein BamA from *Acinetobacter baumannii* as a potential immunogenic target.

Anna Erika Vieira de Araujo (Brasil), Luis Vidal Conde, Ana Paula D'Allincourt Carvalho-Assef, Jose Procopio Moreno Senna.

Acinetobacter baumanni outer membrane proteins (OMPs) have been studied as immunogenic targets for many years. Their formation and organization in the bacterial membrane of Gram-negative bacteria occurs mainly through the performance of Bam (beta-Barrel Assembly Machinery) protein complexes, which are essential for bacterial survival. Among these proteins, BamA represents a potential target, primarily due to the fact it is anchored to the cell membrane via a small extracellular portion, which could generate immunogenic epitopes. In silico analysis reported in the literature demonstrates that *A. baumannii* BamA represents a robust target for generating an immune response. Thus, this study aims to obtain the recombinant protein BamA from *A. baumannii* and evaluate its immunogenic response. The DNA sequence corresponding to recombinant protein BamA (rBamA) of *A. baumannii* ATCC19606 was cloned into the pET28a system, expressed in the Escherichia coli BL21 strain and purified through IMAC in HisTrap columns. Immunizations were performed through intramuscular inoculation of 25ìg rBamA adsorbed in 250ìg of Al(OH)3 with a two-dose protocol in C57/Bl6 (n=5) mice and the obtained serum submitted to immunoassays and fluorescence microscopy in order to evaluate the immune response. rBamA was expressed in inclusion bodies and, after purification and refolding, SDS-PAGE analysis of this protein yielded a homogenous fraction with a major band of 96kDa. ELISA assays demonstrated the ability of rBamA to generate good antibody titers (up to 1:256.000) in a murine model, and western blot showed antibody recognition to both rBamA and to a protein with a similar molecular weight in an *A. baumannii* lysate. Immunofluorescence images exhibited low antibody recognition to a target on the bacterial surface after immunization with rBamA, suggesting that the dominant immunogenic region is probably hidden in the membrane. In summary, it was possible to obtain rBamA from A. baumannii and this protein demonstrated high immunogenicity in a murine immunization model, inducing the production of antibodies capable of recognizing BamA protein in both bacterial lysate and surface membrane. In general, BamA proteins have orthologous sequences between Gram-negative bacteria, which could suggest a specific recognition to *A. baumannii* strains. Such results show that rBamA has significant potential to be used as a target for numerous approaches, including immunodiagnostics.

Efficient immunization with a live vaccine confers long-term protection against *Acinetobacter baumannii* -acute infection in mice.

Maria Clara Póvoa Cabral (Spain), Patricia García Fernández, Alejandro Beceiro Casas, Carlos Rumbo Lorenzo, Astrid Pérez Gómez, Miriam Moscoso Naya, Germán Bou Arévalo.

Nosocomial infections caused by *Acinetobacter baumannii* are a serious health problem. In this context, the implementation of prophylactic vaccination, both active and passive, may represent a cost-effective approach for reducing the clinical and economic burden of the infectious caused by this pathogen, specially for MDR strains. Live attenuated vaccines are highly immunogenic and effective; however, they require particular attention regarding their safety. As a part of a new platform, we previously described the first experimental live vaccine against *A. baumannii* composed of a D-glutamate (D-Glu) auxotrophic strain with inactivated Glutamate racemase (MurI), a key enzyme in gram-negative bacteria that provides the D-Glu required for peptidoglycan biosynthesis. Now, this study aims at identifying whether this D-Glu auxotroph holds the potential to induce long-term memory responses through functional antibodies and cellular-mediated immunity, which can ultimately provide long-term protection against *A. baumannii* acute lethal infections.

Materials and Methods

A. baumannii ATCC 17978 ΔmurI1 ΔmurI2 (anti-Ab vaccine) was previously obtained by eliminating the MurI coding genes - murI1 and murI2. BALB/c mice were immunized intraperitoneally with anti-Ab vaccine (5×107 CFU) on days 0 and 14. Control mice were administered saline. Serum levels of IgM, IgG, IgG1, IgG2a, IgG2b and IgG3 against ATCC 17978 were determined using indirect ELISA, from sera obtained at 1, 3 and 9 months after immunization. The cross-reactivity of the IgG's was determined against *A. baumannii* ATCC 19606, AbH12O-A2 (MDR) and Ab307-0294 (encapsulated, highly virulent) strains. Anti-Ab serum obtained at 1 month after immunization and generated with the anti-Ab vaccine was tested to measure the functionality of antibodies by in vitro opsonophagocytic killing activity assays (OPKA) against ATCC 17978, ATCC 19606, AbH12O-A2 and Ab307-0294 strains, using RAW 264.7 as phagocytic cells. To explore the nature of the T-cell responses, IFN-γ, IL-4 and IL-17-secreting splenocytes were measured after ex vivo restimulation, using ELISpot, 2 months after vaccination.

These cytokines were selected as markers of Th1, Th2 and Th17 T-cell subsets, respectively. Finally, the protective effect of vaccination was evaluated by determining mice survival after intraperitoneal infection with a lethal dose of ATCC 19606 (1×108 CFU), ATCC 17978 (3×108 CFU) and AbH12O-A2 (4×107 CFU) strains at 1 and 3 months after vaccination.

Results

Immunization of BALB/c mice with live anti-Ab vaccine resulted in significant IgM, IgG, IgG1, IgG2a, IgG2b and IgG3 levels, at 1 month after vaccination. Moreover, high antibody titres persisted without booster vaccinations until the 9th month. IgG1 was the predominant antibody isotype revealing a consistent activation of Th2 lymphocytes. IgG's obtained were cross-reactive against ATCC 19606, Abh12O-A2 and Ab307-0294 strains. Using OPKA assays, effective killing of anti-Ab serum was observed in the presence of murine macrophages against ATCC 17978, ATCC 19606, AbH12O-A2 and Ab307-0294 strains, whether minimal killing was observed with naïve serum. The anti-Ab serum also presented direct bactericidal activity in the absence of macrophages. Considering the cellular immunity, a significant increase in IL-4- and IL-17-secreting splenocytes was observed at 2 months after vaccination (fig. 1), which is consistent with a Th2/Th17 response.

Finally, immunization with the live anti-Ab vaccine protected mice from challenge with ATCC 19606 at 1 month after vaccination, whereas all control mice died within 14 hours. Similarly, survival rates of vaccinated mice were 100% after challenge with ATCC 17978 and AbH12O-A2, 3 months after vaccination. These results showing mid- and long-term vaccine efficacy.

Conclusions

Immunization with a live vaccine against *A. baumannii* composed of a D-Glu auxotrophic strain elicits functional and long-term antibody memory, cellular-immunity and effective protection against parental and heterologous strains. This approach constitutes a more broadly applicable strategy to combat other serious antibiotic-resistant bacterial pathogens.

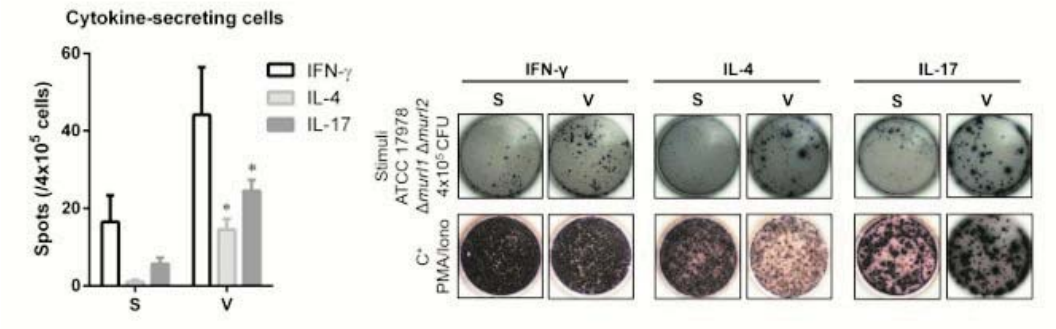


Figure 1. Number of spot forming cells per 4´105 splenocytes collected at 2 months after immunization with A. baumannii ATCC 17978 Δmurl1 Δmurl2 (anti-Ab vaccine) (n = 6) and control mice (n = 7), after being stimulated ex vivo with the anti-Ab vaccine. Representative wells for each cytokine, obtained from saline (S) and vaccinated (V) mice after incubation with stimuli or PMA (phorbol 12-myristate 13-acetate)/Ionomycin used as positive control.

Potent restoration of sulbactam activity by the novel, broad spectrum beta-lactamase inhibitor ETX2514 against multidrug resistant *Acinetobacter baumannii*.

Sarah McLeod (USA), Ken Lawrence, Alita Miller (USA).

ETX2514 is a novel diazabicyclooctenone beta-lactamase inhibitor (BLI) with broad-spectrum activity vs. classes A, C and D enzymes. ETX2514 restores the activity of multiple beta-lactam partners against a number of multidrug resistant (MDR) Gram-negative pathogens. A recent study of 848 globally diverse *A. baumannii* clinical isolates in 2016 showed that addition of 4 mg/L ETX2514 decreased the sulbactam MIC90 from >32 to 2 mg/L, consistent with previous surveillance studies. This level of activity was found to be consistent across time, regions, sources of infection and subsets of resistance phenotypes. The potent antibacterial activity of sulbactam-ETX2514 combined with robust in vivo efficacy against MDR *A. baumannii* infections and promising preclinical safety support its potential to address this significant unmet medical need. This novel combination is currently in clinical development for the treatment of MDR *A. baumannii* infections.

Clinical implications for environmental dissemination and decontamination of carbapenem-resistant *Acinetobacter baumannii* in an intensive care unit: a prospective cohort study.

Young Kyung Yoon (Korea), Hyeon Jeong Kim, Kyung Sook Yang, Byung Chul Chun, Jang Wook Sohn, Min Ja Kim.

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) poses the significant infection-control challenge in intensive care units (ICUs). We characterized environmental dissemination of CRAB in an ICU, and evaluated the effect of structured environmental cleansing methodology using a disinfectant wipes and a quantitative contamination monitoring instrument in controlling the clonal spread of CRAB.

Methods

A prospective cohort study was performed in a 23-bed medical ICU of a teaching hospital in the Republic of Korea from May 2012 to December 2013. Multi-faceted infection control measures was introduced, including CRAB surveillance cultures for pharyngeal colonization and environmental surfaces, environmental cleansing with disinfectant wipes (TriGene) twice a day and monthly environmental monitoring using the adenosine triphosphate (ATP) bioluminescence assay (Clean-traceâ"‡). CRAB (genomo-species 2) was identified by 16S-rRNA gene PCR sequencing and multiplex PCR for detection of metallo-b-lactamase and OXA-carbapenemases. Molecular analysis was performed by pulsed-field gel electrophoresis (PFGE).

Results

During the study period, a total of 2,199 patients (12,814 patient-days) were admitted to the medical ICU. Carbapenem-resistant ACB complex (CR-ACB) were isolated from the 220 patients (9.5%) from either weekly active pharyngeal surveillance cultures or clinical cultures, resulting in clinical infections in 58 patients (2.6%). Contamination rate of environmental surfaces for CR-ACB was 8.7% (52/595). Particularly, the major sites were bedside high-touch surfaces surrounding the colonized or infected-patient: T-connector (9/40, 22.5%), bedrails (9/60, 15.0%), bed sheets (8/60, 13.3%), blood pressure cuffs (7/59, 11.8%), over bed tables (7/60, 11.7%), nurse station counters (1/13, 7.7%), intra-venous pole with infusion pump (6/112, 5.4%). Among the 133 CR-ACB isolates analyzed, the proportion of CRAB (genomospecies 2) accounted for 85.5%. All the 53 CRAB clinical and environmental isolates analyzed harbored both carbapenemase genes of blaOXA-51 like and blaOXA-23 like, and were divided into 2 PFGE types (A1~A4 and B1) with a predominant A1 subtype (79.2%). In the spatiotemporal analysis, patients exposed to CRAB contaminated environmental surfaces had 2.1 times the risk acquiring CRAB in comparison to unexposed patients (relative risk, 2.1; 95% confidence interval, 1.4-3.1). After the application of structured environmental cleansing methodology, the contamination indicators in all parts of environmental surface have improved; the contamination rate environmental surfaces [18.2% (37/203) to 3.6% (7/193), P <0.001] and the median APT levels from monthly monitoring [11008.5 to 1682.7, P =0.006]. The monthly incidence rate of CRAB isolates showed a tendency to decline, but not significant for the study period (coefficient=-0.05, P=0.776).

Conclusion

This study indicates that bedside environmental surfaces of an ICU patient with CRAB are extensively contaminated, and can play as an important reservoir to facilitate dissemination. Enhanced environmental decontamination control measures might have limited role in reducing the CRAB dissemination. Furthermore, we suggest that single room isolation for a CRAB patient should be included as an important control measures.

Poster Sessions

Using is locations to track sub-lineages of the *Acinetobacter baumannii* GC2 clonal complex.

Ruth M. Hall (Australia), Steven j. Nigro, Grace A. Balckwell.

IS mapping via hybridization to digested genomic DNA is a well established epidemiological tool. More recently, tracking the evolution of bacterial clones has usually been achieved using single nucleotide polymorphisms in WGS data. However, IS locations can serve to reveal common ancestry and track the evolution of bacterial clones. This approach was used to track the relationships among multiply and extensively antibiotic resistant GC2 isolates from several hospitals on the East Coast of Australia. The isolates were known to be closely related because they all carry the oxa23 gene in a characteristic location. The positions of all copies of ISAba1, which is common in GC2 isolates, were examined using ISmapper. ISAba1 copies in positions upstream of or flanking resistance genes were set aside as they were universally present. Twelve ISAba1 locations, all confirmed by PCR for the oldest isolate, WM99c, were found to be shared by the majority of isolates in a collection of 151 GC2 isolates which includes 62 isolates that we have previously examined for resistance gene content. A major sub lineage was identifiable by the presence of ISAba1 in 3 further locations. Several further subdivisions were also found, some in only one hospital, consistent with an outbreak, and others in more than one, consistent with inter-hospital spread. Some ISAba1 positions were found only in the Australian East Coast lineage, and isolates carrying only some of the IS copies in different combinations were also identified allowing the evolution to be traced. In isolates carrying the armA aminoglycoside resistance gene from Australia and Singapore, only 3 ISAba1 locations were shared with isolates in the Australian East Coast lineage, and 2 further ISAba1 locations were characteristic of the armA-carrying sub-lineage. Hence, ISAba1 locations should provide useful, easy to use epidemiological markers.

Validation of an automated assay for *Acinetobacter baumannii* biofilm inhibitors from a microbial natural products collection.

Mercedes de la Cruz (Spain), José Carlos Martos, Pilar Sánchez, Ángeles Melguizo, Caridad Díaz, Francisca Vicente.

Acinetobacter baumannii is known for its ability to develop resistance to multiple classes of antibiotics, its ability to develop biofilm, its persistence in healthcare facilities and its inherent hardiness. Furthermore, the increasing prevalence of drug-resistant pathogens and the lack of effective treatments represent one of the most urgent medical needs at present, and it is currently estimated that more than 65% of bacterial infections and 80% of chronic infections are caused by biofilm-forming bacteria. *Acinetobacter* spp. are emerging as a major cause of severe nosocomial infections, and *Acinetobacter baumannii* is the predominant species associated with this kind of infections. Therefore, there is an urgent need to find successful anti-biofilm compounds to treat this pathogen, which requires good, consistent and well-suited high-throughput methods for determining drug susceptibility of bacterial biofilms.

The aim of this work was to set up a rapid, accurate, sensitive, reproducible and straightforward screening method to identify microbial natural product extracts in the Fundación MEDINA collection with activity against *Acinetobacter baumannii* biofilm formation. Among the several techniques employed to evaluate biofilm formation, both a colorimetric method (crystal violet assay) and a viable cells method (resazurin assay) were considered as candidates for the screening.

The results and conclusions obtained from the comparison of both methodologies are shown. This study also describes the parameter optimization of the chosen assay required for a statistically robust high-throughput screening as well as data from a preliminary screening of 320 extracts.

Comparative genomic analyses of *Acinetobacter pittii* clinical strains.

Itziar Chapartegui-González, María Lázaro-Díez, Santiago Redondo-Salvo, Laura Alted-Pérez, Jesús Navas, **José Ramos-Vivas (Spain).**

Acinetobacter baumannii has been extensively studied because infections caused by this pathogen have been associated with high morbidity and mortality rates. Among *Acinetobacter* genus, A. pittii is another clinically relevant species. The significant role of A. pittii in human infections and the emergence of resistant strains have also become a great medical concern. The whole genome sequences of three clinical strains A. pittii HUMV-4336, HUMV-6207 and HUMV-6483 are reported here.

Objectives:
To investigate the mechanisms of A. pittii pathogenesis and antimicrobial resistance this study sequenced and comparatively analyzed the whole genomes of three human isolates.

Methods:
Total genomic samples were extracted and purified using the GeneJET Genomic DNA isolation kit. The genomic DNA was submitted to Macrogen (Macrogen, South Korea) for PacBio single-molecule real-time (SMRT) sequencing. A single library was prepared for each A. pittii and run on one SMRT cell. With a genome size of approximately 3.99 Mb, 3.94 Mb and 4.07 Mb, PacBio SMRT sequencing provided approximately 100% coverage of the entire A. pittii genomes. For comparative genomic analyses we have also included the known completely sequenced strains, AP_882, IEC338SC, and YMC2010/8/T346. Comparative genome visualization software tools for this study include MEGA, DDH, ANI, RAST, EggNOG and PanOCT.

Results and conclusions:
A total of 3.678, 3.648 and 3.821 proteins-coding sequences were predicted for each clinical isolate. The final complete genome of each strain resulted in circular chromosomes with a total GC content of 38.9%, 38.9% and 39%, with additional circular contigs (plasmids) of 100,374 bp and 112,604 bp in strains HUMV-6207 and HUMV-6483. The RAST server predicted coding sequences belonging to 454, 453 and 456 subsystems, including 92, 108, and 81 involved in virulence. This deep analysis will help us to better understand the species and possibly aid in the development of better therapeutic strategies for A.pittii-mediated infections.

Detection of horizontal gene transfer events in drug resistant *Acinetobacter baumannii.*

Maria-Halima Laaberki (France), Sophie Godeux, Simon Guette-Marquet, Agnese Lupo, Marisa Haenni, Jean-Yves Madec, Xavier Charpentier.

Objectives

Natural transformation is a horizontal gene transfer (HGT) mechanism widespread in bacteria. Naturally transformable bacteria can actively take up exogenous DNA and integrate it in their genome by homologous recombination. Natural transformation is often transient and its triggering signals are elusive and species-specific. In the *Acinetobacter* genus, A. baylyi was described competent for natural transformation in 1969 but this HGT mechanism was only recently (2013) highlighted in *A. baumannii* and A. nosocomialis. Our understanding of natural transformation's contribution to pathogenic *Acinetobacter* spp. evolution and its role in the rapid acquisition of antibiotic resistance genes is still limited. We aim at understanding the conditions stimulating natural transformation of *A. baumannii* and its prevalence among clinical isolates.

Methods

To study natural transformation in bacteria, the most prevalent method uses selection for the acquisition of an antibiotic resistance marker in a target chromosomal locus by the recipient cell. Most clinical isolates of A. baumannii are resistant to multiple antibiotics, and such method appears both experimentally difficult and ethically questionable. We have developed an alternative method based on flow cytometry to detect transformation events and detect natural transformation in MDR *A. baumannii* clinical isolates. Using the strain AB5075, we benchmarked our method against the conventional antibiotic-based method.

Results and conclusion

Using this new method, we succeeded in detecting transformation events in the MDR and pathogenic *A. baumannii* AB5075 strain. We then establish for this strain the incidence of DNA form and physico-chemical conditions (pH, cationic ions) on natural transformation efficiency. Using optimized transformation conditions, we probed natural transformation in a set of MDR animal clinical *A. baumannii* isolates and found that 30% were transformable in these experimental conditions. Our new method will facilitate the study of natural transformation in *A. baumannii*. More broadly, it could allow experimental detection and quantification of any HGT events in MDR *A. baumannii*.

Acinetobacter spp. From livestock husbandry and its transfer through biogas plants.

Peter Kaempfer (Germany), Stefanie P. Glaeser.

The clinical relevance of pathogenic multidrug resistant (MDR) *Acinetobacter* has strongly increased in the last years. One source of resistant *Acinetobacter* spp. is livestock where these bacteria are highly exposed to several antibiotics. The transmission of *Acinetobacter* spp. from livestock husbandry has not been studied so far. *Acinetobacter* spp. may be released from livestock husbandry via manure into the environment. The application of manure as input material of biogas plants is often discussed as biotechnological barrier to prevent the release of these bacteria. The aim of this study was to determine the presence of *Acinetobacter* spp. in input (manure) and output samples of 15 German biogas plants to determine whether or not the processing of the manure in biogas plants effects the transmission of *Acinetobacter* species into the environment. A cultivation independent analysis of bacterial communities in input and output samples of biogas plants was performed by 16S rRNA gene 454 Pyrotaq sequencing and revealed a low relative abundance of *Acinetobacter* in most of the input samples (up to 1% of the total analyzed bacterial sequences) and with a very low abundance in only two of the investigated 15 biogas plants indicating a low abundance within those samples. However, *Acinetobacter* spp. were isolated from input and output samples in the presence of a carbapenem (studied for two biogas plants). The obtained isolates represented phylogenetically diverse strains including those assigned by 16S rRNA gene sequence phylogeny to several described species including *Acinetobacter baumanii* and a potential novel species which carried several resistance genes. Here we will give a more detailed insight in the antibiotic resistance pattern and the physiological properties of the isolates with a focus on the genome sequenced potential novel species. In summary the data indicate that MDR *Acinetobacter* are released into the environment after the transfer of biogas plants which can only be detected after isolation and characterization.

Comparative genomic analysis of *Acinetobacter bereziniae* strains revealed an overall proximity between two geographically-separated carbapenem-resistant trains containing plasmids bearing blandm-1.

Marco Brovedan, Guillermo Daniel Repizo (Argentina), Martín Espariz, Patricia Marchiaro, Alejandro Miguel Viale, Adriana S. Limansky.

Acinetobacter spp. have emerged as multi drug resistant, opportunistic, nosocomial pathogens causing global outbreaks. They have an armamentarium of mobile genetic elements responsible for horizontal transfer of antibiotic resistance genes. Studies on factors responsible for pathogenesis of *Acinetobacter* are gaining momentum. However, there is paucity of data available on effects of virulence factors on the immune system. This study has primarily focused on the extracellular virulence factors secreted by *Acinetobacter* spp. Cell free supernatants (CFS) of *Acinetobacter baumannii* AIIMS7 were found to be highly cytotoxic to human monocyte cell line THP-1. Partially purified cell free supernatants decreased the lysosomal acid phosphatase activity and increased the release of nitric oxide and induced necrotic cell death of the monocytes. Phospholipase was the major virulence factor secreted by AIIMS7. We propose that the secreted virulence factors of AIIMS7 are cytotoxic to monocytes causing these cells to lose their innate functions thus enabling the pathogen to cause deep-seeded infections in the immunocompromised host.

A genomic analysis of the *Acinetobacter baumannii* straints circulating in Mexico and Honduras.

Abraham David Salgado-Camargo (Mexico), Julio César Zuniga-Moy, Semiramis Castro-Jaimes, Oscar Taboada-Fernández, Ángeles Pérez-Oseguera, Lucía Graña-Miraglia, Suyapa Aurora Bejarano, Annabelle Ferrera, Santiago Castillo-Ramírez.

Carbapenem-resistant *Acinetobacter baumannii* is, also, an increasing problem in Mexico and in Honduras. In a previous study, 192 carbapenem-resistant *A. baumannii* clinical isolates were collected from 16 hospitals between 2006 and 2013. The initial taxonomical identification was made with API 20 (bioMérieux) and confirmed by rpoB sequencing. The collection was characterized by PFGE and the MLST profile of one pulsotype of each hospital was obtained. Then, the complete genomic sequences (chromosome and plasmids), of 13 strains, with different sequence-type, were obtained using Illumina (Miseq 2x300) and PacBio. Only a small proportion of the sequenced strains have a close relationship with the international clones. However, we were able to detect, in our collection, plasmids with DNA sequences that are almost identical to other plasmids isolated from members of the international clones. We will show and the molecular structure of the plasmids that are frequent in our collection.

We have now a full description of antibiotic resistant genes present in our strain set and to which mobile element are linked. Some strains are XDR, possessing genes conferring resistance to 5 antibiotic families. The most prevalent genes conferring resistance to beta-lactamics in the sequenced strains are: blaACD-25, blaTEM-1D, blaOXA-65, blaOXA-58, blaOXA -64, blaOXA-66, blaOXA-72 and blaOXA-100.

Regarding to the *A. baumannii* strains isolated from Honduras, they were collected from five different Hospitals, between 2015 and 2016. The genomes of sixteen strains, with different plasmid profiles, were sequenced using Illumina Myseq (2 x 300), with at least 46x coverage. Analysis of the draft sequences shows the presence of five new sequence types (following Bartual scheme). Similar to Mexico, only a small fraction of the sequenced strains show a close relationship with International Clones. In these strains the most common genes involves in resistance to beta-lactamics are: blaCTX-M-15, blaACD-25, blaCARB-8, blaOXA-23, blaOXA-64, blaOXA-65, blaOXA-132, blaOXA-180, blaOXA-359, blaTEM-1B and also blaNDM-1.

We will show a phylogenetic analysis made, using the core genes of our strains and those reported as complete or almost complete, in NCBI.

Analysis on identification of *Acinetobacter* by MALDI-TOF MS using direct smear pretreatment.

Xiuyuan Li (China), Yanyan Tang, Xinxin Li.

Object
To integratively evaluate the accuracy of *Acinetobacter* identification using MALDI-TOF MS in the clinical laboratory, especially pretreated by direct smear method, and reveal the actual causes of misidentification.

Methods
A total of 787 *Acinetobacter* strains were collected from January 2014 to December 2016 in Beijing Tongren Hospital of Capital Medical University, China. All the strains were confirmed at species level by 16S rDNA and rpoB sequencing and identified by MALDI-TOF MS using two pretreatments of direct smear and bacterial extraction. And then the evaluation was implied by analysing and comparing between the results of MALDI-TOF MS and sequencing.

Results
(1) A total of 18 *Acinetobacter* species were confirmed by sequencing, including 637 A.baumannii, 56 A.pittii, 23 A.johnsonii, 15 A.junii, 13 A.lwoffii, 8 A.ursinqii, 5 A.nosocomialis, 4 A.parvus, 2 A.calcoaceticus, 2 A.haemolyticus, 1 A.schindleri and 1 A.radioresistens that were contained in Biotyper 3.0 database, and 7 A.soli, 5 A.proteolyticus, 4 A.bereziniae, 1 A.gyllenbergii, 1 A.seifertii and 1 A.variabilis that were not contained in database. In addition, one novel species temporarily named A.corallinus was screened out and preliminarily certified by whole 16S rRNA gene sequencing, GenBank: KY828975. (2) The total accuracy of *Acinetobacter* species identification using MALDI-TOF MS was 97.1%(764/787), regardless of direct smear or bacterial extraction pretreatment was applied. Besides, if the *Acinetobacter* species excluded in Biotyper 3.0 database were neglected, the accuracy of identification by both direct smear and extraction pretreatment would be 99.6%(764/767). The misidentification only occurred on A.parvus, with an accuracy of 25%(1/4). (3) The accuracy of identification whose score was less than 2.000 was only 80.0%(88/110) and 43.2%(16/37) by two methods respectively, due to the inaccessible species in the database. (4) The mass spectra using extraction pretreatment had more primary peaks or/and less impure peaks than that of direct smear. However, all the mass spectra of *Acinetobacter* species were qualified whether or not they are able to be precisely identified.

Conclusion
The identification of *Acinetobacter* species using MALDI-TOF MS was reliable in the clinical laboratory even pretreated by direct smear. Misidentification occurred occasionally due to the lack of reference mass spectra in Biotyper 3.0 database rather than extraction failure. And we suggested 16S rDNA or rpoB sequencing should be applied when the identification score was under 2.000.

Cleaning up the nomenclatural chaos in the genus *Acinetobacter*: the effectively but not validly published names '*Acinetobacter oryzae*' Chaudhary et al. 2012, '*Acinetobacter plantarum*' De et al. 2016, '*Acinetobacter refrigeratoris*' Feng et al. 2014 and '*Acinetobacter seohaensis*' Yoon et al. 2007 are synonymous with the validly published names of well-established species.

Lenka Radolfova-Krizova, **Paul G. Higgins (Germany),** Alexandr Nemec.

Acinetobacter baumannii represents nowadays an important nosocomial opportunistic pathogen whose reservoirs outside the clinical setting are obscure. Here we traced the origins of the collection strain *A. baumannii* DSM30011 to an isolate first reported in 1944, obtained from the enriched microbiota responsible of the aerobic decomposition of the resinous desert shrub guayule. Whole-genome sequencing and phylogenetic analyses based on core genes confirmed DSM30011 affiliation to *A. baumannii*. Comparative genomic studies with 32 complete *A. baumannii* revealed the presence of 12 unique accessory chromosomal regions in DSM30011, among which five encompassed phage-related genes, five contained toxin genes associated to the type 6 secretion system and one bore an atypical CRISPRs/cas cluster. No antimicrobial resistance islands were identified in agreement with its general antimicrobials susceptibility including the folate synthesis inhibitors sulfamethoxazole/trimethoprim. Marginal ampicillin resistance was detected in DSM30011, most likely due to chromosomal ADC-type ampC and blaOXA-51-type genes. Searching for catabolic pathways genes revealed several clusters involved in the degradation of plant defenses including woody tissues and a previously unreported atu locus responsible of aliphatic terpenes degradation, suggesting that resinous plants may provide effective niches for this organism. DSM30011 also harboured most genes and regulatory mechanisms linked to persistence and virulence in pathogenic *Acinetobacter* species. This strain, which was isolated from environmental sources before the introduction of massive antibiotic use last century, may thus provides important clues into the genomic diversity, virulence potential, and evolutionary processes that led to the leap of A. baumannii towards an opportunistic pathogen of humans.

Structure of nucleoside diphosphate kinase and its inhibition studies from *Acinetobacter baumannii.*

Juhi Sikarwar (India), Punit Kaur, Sujata Sharma, T.P. Singh.

The incidences of infections caused by an aerobic Gram-negative bacterium, *Acinetobacter baumannii* are very common in hospital environments. It usually causes soft tissue infections including urinary tract infections and pneumonia. It is difficult to treat the patients who suffer from the infection of bacterium due to acquired resistance to available antibiotics is well known. In order to design specific inhibitors against Nucleoside diphosphate kinase from *Acinetobacter baumannii*, we have determined three-dimensional structures of Nucleoside diphosphate kinase. Ndk was cloned and expressed and crystallized. In order to investigate the presence of 12kDa form in *Acinetobacter baumannii* as well as observed in Pseudomonas Aeruginosa responsible for biofilm formation a major cause for its pathogenicity. We have successfully cloned expressed and purified the 12 kDa form and was as well purified by digestion of native Ndk by elastase. We have further subjected the above three proteins for inhibition studies in presence of antimicrobial protein lactoferrin and its fragments .The inhibitions have shown maximum values for the 12 kDa forms which was further confirmed by fluorescence spectroscopy. This study reveals the possibility of presence of 12 kDa form of Ndk in *Acinetobacter baumannii* as well and that lactoferrin and its fragments can be used as potent inhibitors against it.

Potential role of phospholipase C from *Acinetobacter baumannii* in suppression of innate immune response.

Karishma Pardesi (India), Rashmi Nair, Riddhi Shah, Urvi Doshi.

Acinetobacter spp. have emerged as multi drug resistant, opportunistic, nosocomial pathogens causing global outbreaks. They have an armamentarium of mobile genetic elements responsible for horizontal transfer of antibiotic resistance genes. Studies on factors responsible for pathogenesis of *Acinetobacter* are gaining momentum. However, there is paucity of data available on effects of virulence factors on the immune system. This study has primarily focused on the extracellular virulence factors secreted by *Acinetobacter* spp. Cell free supernatants (CFS) of *Acinetobacter baumannii* AIIMS7 were found to be highly cytotoxic to human monocyte cell line THP-1. Partially purified cell free supernatants decreased the lysosomal acid phosphatase activity and increased the release of nitric oxide and induced necrotic cell death of the monocytes. Phospholipase was the major virulence factor secreted by AIIMS7. We propose that the secreted virulence factors of AIIMS7 are cytotoxic to monocytes causing these cells to lose their innate functions thus enabling the pathogen to cause deep-seeded infections in the immunocompromised host.

High prevalence of *Acinetobacter baumannii* and of *Acinetobacter indicus* carrying Oxa23/58-Like genes in hessian cattle.

Klotz Peter (Germany), Semmler Torsten, Göttig Stephan, Leidner Ursula, Ewers, Christa.

To unveil a putative bovine *Acinetobacter* spp. reservoir we screened German cattle for clinical relevant *Acinetobacter* (A.) species and investigated their virulence and resistance features.

From 01/2015 to 02/2016, 422 cattle in 353 Hessian farms were sampled. *Acinetobacter* spp. were identified by MALDI-TOF MS and gyrB-PCR - most frequently *A. baumannii* and A. indicus (19% and 25% of the farms, respectively). MLST revealed 50 STs (40 new) among 80 *A. baumannii* isolates; 17 isolates belonged to international clone II; none of the isolates was resistant to carbapenems. Seven and five of 36 A. indicus isolates carried blaOXA-23-like and blaOXA-58-like genes on their chromosome, respectively. One isolate showed resistance to imipenem (VITEK2®, CLSI interpretation). Virulence in the Galleria mellonella model was comparable to human isolates for the bovine *A. baumannii* and reduced for the bovine A. indicus isolates. The number of virulence associated genes was seven to eightfold higher in bovine *A. baumannii* compared to A. indicus isolates, as determined by WGS.

A. baumannii and A. indicus were highly prevalent in cattle. *A. baumannii* STs displayed a wide variety, four STs were previously associated with human infections. Additionally, minor virulent A. indicus deserves awareness because of the carriage of OXA genes.

Punctual *rpob* mutations impairing in motility and virulence of *Acinetobacter baumannii.*

Jordi Corral Sábado (Spain), María Pérez Varela, Daniel Quiñones Celdran, Juan Andrés Vallejo Vidal, Soraya Rumbo Feal, Germán Bou Arévalo, Jesús Aranda Rodríguez.

Acinetobacter baumannii is a major cause of antibiotic-resistant nosocomial infections worldwide. Motility allows bacteria to move along the surfaces and it has been linked to the infective capacity of several pathogens. However, despite the increasing clinical importance of *A. baumannii*, little is known about the contribution of motility to its pathogenesis.

In this study, spontaneous *A. baumannii* rifampin-resistant (RifR) mutants lacking surface-associated motility were isolated. Motility was evaluated throughout semisolid agar plates. The point mutations conferring rifampin resistance were identified by sequencing the rpoB gene, encoding the beta-subunit of the RNA polymerase, the site of frequent RifR-inducing base-pair substitutions in this species. The plasmid pBAV1K-T5-gfp was used to complement the mutants. Finally, the virulence was analyzed by using the nematode Caenorhabditis elegans fertility assay. The results reported in this work showed that 60% of the RifR-isolated clones were impaired in surface-associated motility presenting an amino acid substitution at either Gln522 or Ser540 of the wild-type RpoB protein. Wild-type motility (40% of the isolates) was detected in rifampin-resistant mutants harboring an amino acid change at position Asp525 of RpoB. In several additional experiments, independent rpoB mutants were also isolated that contained an amino acid substitution at either Gln522 or Ser540; all of these mutants were similarly impaired in their surface-associated motility. Furthermore, the virulence of the RifR mutants with impaired motility but not of the RifR mutant with wild-type motility was significantly lower than that of the wild-type strain. Since the growth kinetics of all the RifR mutants were comparable to those of the wild-type strain, the defects in the motility and virulence of these two mutants cannot be attributed to any delay in the bacterial growth rates.

As expected, the introduction of the wild-type rpoB gene in an expression plasmid was able to restore rifampin susceptibility, the surface-associated motility and the virulence in the RifR mutants lacking motility. Likewise, the introduction in the wild-type strain of the expression vector containing the rpoB gene amplified from any of the RifR mutants conferred rifampin resistance, whereas only the constructions containing the rpoB genes amplified from the mutants lacking motility but not from those presenting wild-type motility were able to impair both motility and virulence.

Together, these findings demonstrated that the punctual rpoB gene mutations of *A. baumannii* strains were responsible for the lack of both surface-associated motility and virulence, probably due to a conformational change in the RpoB protein affecting substantial changes in the transcriptional profile of bacterial cells. Results obtained in this work provide strong evidence of the connection between motility and virulence in this multi-resistant nosocomial pathogen.

Transcriptional profile of *Acinetobacter baumannii* mutants lacking motility and virulence.

María Pérez Varela (Spain), Jordi Corral Sábado, Daniel Quiñones Celdran, German Bou Arevalo, Jesús Aranda Rodríguez, Jordi Barbé García.

Acinetobacter baumannii is a gram-negative bacterium often responsible for nosocomial infections, based on its capacity to acquire and develop antimicrobial resistance. Despite the importance of motility in the pathogenesis of a number of bacterial species, the implication of motility in the virulence of *A. baumannii* remains unclear. In this study, the transcriptional profile of two spontaneous *A. baumannii* rifampin-resistant mutants (RifR5 and RifR8) derived from ATCC 17978 strain and lacking surface-associated motility were examined in cDNA microarrays. Among the 3431 cDNA ORFs examined, the expression of 30 from strain RifR5 and 221 from strain RifR8 differed by more than 2-fold compared to the parental strain. In RifR5, 12 ORFs were up-regulated and 18 were down-regulated, while in RifR8 59 were up-regulated and 162 were down-regulated. Six down-regulated genes widespread among *A. baumannii* clinical isolates were shared by the two strains: two encoding putative membrane transporters and four encoding metabolic related enzymes (Table 1). Expression decrease of these genes was validated by reverse transcription-quantitative real-time PCR (RT-qPCR).

To determine the effect of these genes on the motility and virulence each of them was inactivated and the behaviour of the resulting knockout mutants with respect to motility and virulence was studied by using semisolid agar plates and the nematode Caenorhabditis elegans fertility assay, respectively. The construction of knockout mutants in each of the six down-regulated genes revealed a significant reduction in the surface-associated motility and virulence of four of them (RS14730, RS16805, RS17040, and RS17045) in the *A. baumannii* ATCC 17978 strain as well as in the virulent clinical isolate MAR002. The plasmid pBAV1K-T5-gfp was used to complement the mutants. The introduction of the corresponding wild-type gene in this expression plasmid was able to restore both surface-associated motility and virulence in the four knockout mutants.

Taken together, our results provide strong evidence of the connection between motility and virulence in this multi-resistant nosocomial pathogen. The data obtained in this work provide the basis for further investigations into the cellular components and mechanisms involved in the motility of this multi-resistant microorganism.

Gene	Product description	RifR5 a	RifR8 a
RS14730	Taurine ATP-binding transport system component	-2.7	-2.2
RS16805	Aldehyde dehydrogenase	-5.6	-4.7
RS17030	Aldehyde dehydrogenase	-5.6	-5.6
RS17035	Fumarylacetoacetate hydrolase	-6.6	-5.4
RS17040	Major facilitator superfamily permease	-4.7	-4.3
RS17045	Dihydroxy-acid dehydratase	-5.4	-5.5

Table 1. Down-regulated genes in the RifR5 and RifR8 mutants of Acinetobacter baumannii a Fold change in gene expression in the indicated mutants with respect to the wild-type parental strain (ATCC 17978).

A pathogenic role of the A1S_3412 gene of *Acinetobacter baumannii* ATCC 17978.

Se Yeon Kim, Man Hwan Oh, Seok Hyeon Na, Hyejin Jeon, Hyo Il Kwon, Min Sang Shin, **Je Chul Lee (Korea).**

Acinetobacter baumannii is a notorious nosocomial pathogen that commonly infects severely ill patients in intensive care units worldwide, but pathogenesis of *A. baumannii* has not yet been fully characterized. Using a random transposon mutagenesis to identify virulence-associated genes, we identified a mutant strain that disrupted the A1S_3412 gene, a zinc uptake regulator-regulated gene, showed a significant reduction in biofilm production compared with the wild-type *A. baumannii* ATCC 17978. This study investigated the pathogenic role of the A1S_3412 gene in *A. baumannii* ATCC 17978 using the ΔA1S_3412 mutant strain and the single copy of A1S_3412 gene-complemented strain. The A1S_3412 gene was highly conserved among the *A. baumannii* strains with 98-100% homology. Bacterial growth was not different between the wild-type and ΔA1S_3412 mutant strains under the zinc-replete and -deplete conditions. Biofilm production, surface motility in agar plate, and adherence to host cells were significantly different between the wild-type and ΔA1S_3412 mutant strains in vitro, whereas host cell death was not different between the two *A. baumannii* strains. The ΔA1S_3412 mutant strain was more susceptibility to gentamicin and colistin than the wild-type strain. Bacterial numbers in the blood were significantly different between the wild-type and ΔA1S_3412 mutant strains in mouse pneumonia model. Complementation of the A1S_3412 gene in the mutant strain rescued the pathogenic traits both in vitro and in vivo. These observations indicate that the A1S_3412 gene plays an important role in the pathogenesis of *A. baumannii*, but its regulation and interaction with other molecules should be determined.

Host- derived antimicrobial fatty acids target *Acinetobacter baumannii* lipid homeostasis.

Marjan Khorvash, Karl A. Hassan, Natalya Plakhova, Stephanie L. Begg, Victoria G. Pederick, James C. Paton, Ian T. Paulsen, Christopher A. McDevitt, **Bart A. Eijkelkamp (Australia).**

Free fatty acids hold important immune-modulatory roles during infection. However, the host's long chain polyunsaturated fatty acids, not commonly found in the membranes of bacterial pathogens, also have significant antibacterial potential. Of these, the omega-6 fatty acid arachidonic acid (AA) and the omega-3 fatty acid decosahexaenoic acid (DHA) are highly abundant and their serum concentrations increase upon infection, hence, we investigated their molecular effects on *Acinetobacter baumannii*.

Our analyses of a range of highly distinct *A. baumannii* clinical isolates revealed that AA and DHA have significant antimicrobial potential across the species. Through transcriptional profiling we demonstrate that AA and DHA dysregulate the *A. baumannii* fatty acid biosynthesis and degradation pathways. Furthermore, multiple putative fatty acid desaturases were found to be up-regulated upon treatment, highlighting that AA and DHA both target A. baumannii lipid homeostasis, as confirmed by lipidomic analyses. To identify potential AA and DHA resistance mechanisms, we examined the transcriptional responsiveness of the resistance-nodulation-cell division efflux systems and examined growth of the relevant mutants. These experiments identified the AdeIJK efflux system as the primary pathway for export of AA and DHA, thereby revealing an important role for AdeIJK during infection.

This is the first study to comprehensively examine the effects of host fatty acids on *A. baumannii* and highlights the outstanding potential of AA and DHA as antimicrobials to combat *A. baumannii* infections. The fundamental findings on the *A. baumannii* lipid homeostasis mechanisms will be of broad interest to many different aspects of *Acinetobacter* biology.

Identification and Characterization of the Thioredoxin A protein as an *Acinetobacter baumannii* Virulence Factor.

Patrick M. Ketter, Sarah Ainsworth, Jieh-Juen Yu, Holly C. May, Andrew P. Cap, M. Neal Guentzel, **Bernard P. Arulanandam (USA).**

The bacterial thioredoxin system, consisting of thioredoxin, thioredoxin reductase and NADPH, plays an important role in redox regulation and oxidative stress defense. Similar to other Gram-negative bacteria, the thioredoxin A (TrxA) protein is the primary thioredoxin expressed in Acinetobacter baumannii. We have found that secreted bacterial TrxA reduced and dissociated the secretory component from sIgA to evade immunity to establish gastrointestinal (GI) tract colonization. SIgA is the primary immunoglobulin associated with mucosal surfaces and an important mediator of mucosal immunity against microbial infection. To further define the role of TrxA in contributing to pathogenesis, we generated a TrxA-null mutant (ΔtrxA) from the WT parental Ci79 strain, a multi-drug resistant (MDR) *A. baumannii* clinical isolate. Multiple routes of infection in a murine model were utilized to asses TrxA-mediated pathogenesis. (A) In a GI infection model, whole body imaging of mice challenged with fluorophore (PSVue-794) labeled ΔtrxA revealed a statistical decrease in bacterial colonization of the GI tract through 24 hr as evidenced by the diminished fluorescent intensity. Fecal shedding also was significantly reduced at 24 hr after ΔtrxA challenge compared to mice challenged with either WT or the trxA complemented strain ΔtrxAC. (B) Using a pneumonic infection model, lung bacterial burdens and bacterial dissemination were greatly reduced in intranasally ΔtrxA challenged mice compared to WT Ci79 challenged mice. (C) In a sepsis model, mice challenged intraperitoneally (i.p.) with ΔtrxA survived doses in excess of 2 x 10^7 CFU, nearly 100-fold higher than the LD50 associated with the WT strain. These collective results indicate that (1) *A. baumannii* thioredoxin is a virulence factor and associated with bacterial pathogenesis, and (2) the ΔtrxA strain is highly attenuated and can be utilized as a putative live attenuated vaccine to control MDR *A. baumannii* infection. Indeed, subcutaneous vaccination with live attenuated ΔtrxA induced a robust humoral immune response and significantly protected mice against (otherwise) lethal Ci79 i.p. challenge. The protection was correlated with reduced tissue pathology and serum pentraxin-3 levels, a biomarker of severe sepsis. Collectively, our study has revealed that TrxA is a defined-virulence factor, and can be an ideal drug target to control MDR *A. baumannii* infection. Funding: This work was supported by funding from the UTSA Center for Excellence in Infection Genomics training grant (DOD #W911NF-11-1-0136) and the NIH (AI124021).

The mode of inhibitor binding of ligands to peptidyl-tRNA hydrolase: Binding studies and structure analysis of unbound and bound peptidyl-tRNA hydrolase from *Acinetobacter baumannii.*

Sujata Sharma (India), Naseer Khan, Punit Kaur, Tej P. Singh.

There are increasing incidences of infections caused by an aerobic Gram-negative bacterium, *Acinetobacter baumannii* are commonly happening in hospital environments. The treatments of such infections are not very successful due to acquired resistance to almost all available antibiotics. In order to design specific inhibitors against an important enzyme, peptidyl-tRNA hydrolase from *Acinetobacter baumannii* (AbPth), we have determined its three-dimensional structure. Peptidyl-tRNA hydrolase (AbPth) is involved in recycling of peptidyl-tRNAs which are produced in the cell as a result of premature termination of translation process. We have also determined the structures of two complexes of AbPth with cytidine and uridine. AbPth has been cloned, expressed and crystallized in unbound and in two bound states with cytidine and uridine. The binding studies carried out using fluorescence spectroscopic and surface plasmon resonance techniques revealed that both cytidine and uridine bind to AbPth at nanomolar concentrations. The structure determinations of the complexes revealed that both ligands were located in the active site cleft of AbPth. The introduction of ligands to AbPth caused a significant widening of the entrance gate to the active site region and in the process of binding, it expelled several water molecules from the active site. As a result of interactions with protein atoms, the ligands caused conformational changes in several active site residues to attain the induced tight fittings. Such a binding capability of this protein makes it a versatile molecule for hydrolysis of peptidyl-tRNAs having variable peptide sequences. These are the first studies that revealed the mode of inhibitor binding in Peptidyl-tRNA hydrolases which will facilitate the structure based ligand design.

Overexpression of genes in the acetoin cluster associated with Quorum Sensing in airborne *Acinetobacter* sp. 5-2Ac0.

Laura Fernández-García (Spain), Lucia Blasco, Felipe Fernández-Cuenca, Rafael López-Rojas, Maria Lopez, Luis Martinez-Martinez, Álvaro Pascual, German Bou, Maria Tomás.

In a recent study, *Acinetobacter baumannii* was detected in 53% of air samples from a hospital environment (Munoz-Price et al. Crit Care Med 2013). To date, little is known about the mechanisms of survival of these strains. In this study, we conducted array studies with airborne Acinetobacter sp. 5-2Ac02 (closely related to A. towneri) in relation to Quorum Sensing (QS)/Quorum Quenching (QQ) systems, which are important mechanisms of bacterial persistence and tolerance under stress conditions.

Methods

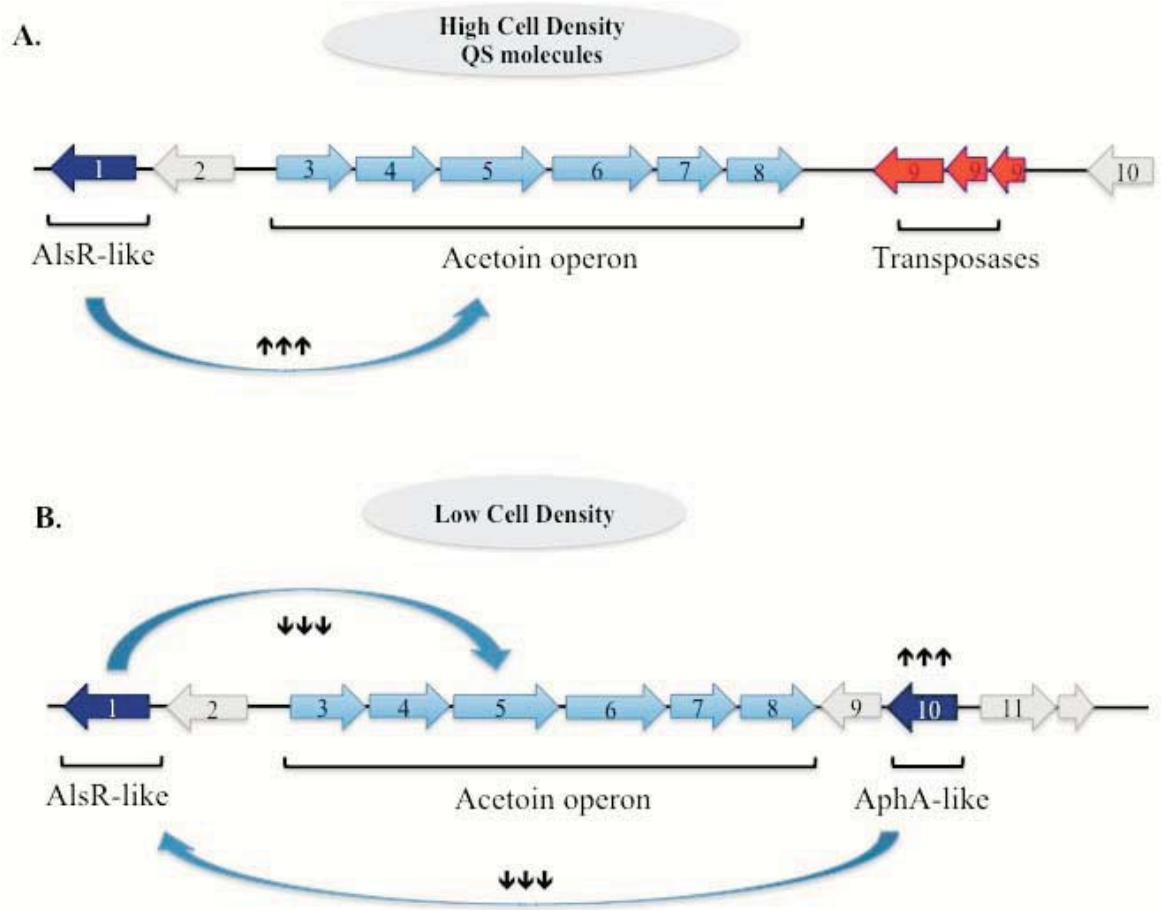
Arrays were designed using eArray (Agilent), with airborne *Acinetobacter* sp. 5-2Ac02 (Barbosa BG et al., Genome Announc 2016). The experiments were performed with three biological replicates of "treated RNA" obtained from an exponential mid-phase culture (optical density at 600 nm of 0.4-0.6) in the presence of a) QS activation, a mixture of acyl homoseine lactones (AHLs) was prepared in ethyl acetate acidified with 0.1% (vol/vol) acetic acid containing N-(butyl, heptanoyl, hexanoyl, b-ketocaproyl, octanoyl and tetradecanoyl)-DL-homoserine lactones (Sigma-Aldrich, Inc), each added to a final concentration of 1mM in the medium (Ghosh et al. 2009). b) QQ activation, a 3-oxo-C12-HSL (important signalling molecule involved in QS in P. aeruginosa) was added to a final concentration of 10 ƒÊM (Lopez M et al., PloS One 2017) Statistical analyses were performed using Bioconductor (RankProd software). The genes were considered expressed when the difference between treated and untreated RNA was ≥1.5 (at P <0.05). The RAST server was used to identify protein-coding genes, rRNA and tRNA genes in order to assign functions to these genes.

Results

In QS and QQ activation, respectively 40 and 26 genes were overexpressed (two- to fourfold). In both cases, we identified genes belonging to the acetoin cluster (aphA-like, lipoate synthase, acetoin dehydrogenase E1 [alpha and beta subunits], dihydrolipoamide acetyltransferase E2 [complex], 2,3-butanediol dehydrogenase [S/R] and alsR-like), which is essential for the viability of Vibrio cholerae by preventing lethal acidification of the end-products of metabolism of the bacterium (under stress conditions). The acetoin gene cluster has previously been associated with the QS network (Kovacikova et al., Molecular Microbiology 2005). Interestingly, we detected three transposases in the regulatory negative region (upstream site) of this acetoin gene cluster in airborne *Acinetobacter* sp. 5-2Ac02, whereas the same gene cluster in *Acinetobacter baumnaniii* ATCC 17978 includes an aphA-like negative regulatory gene (Figure 1).

Conclusion

The QS network regulated expression of genes within the acetoin cluster in airborne *Acinetobacter* sp. 5-2Ac0, which did not include an aphA-like negative regulatory gene. The acetoin gene cluster is implicated in the survival under stress conditions of important bacterial pathogens such as Vibrio cholerae.


A.
High Cell Density
QS molecules
1
2
3
4
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6
7
8
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10
AlsR-like
Acetoin operon
Transposases
B.
Low Cell Density
1
2
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7
8
9
10
11
AlsR-like
Acetoin operon
AphA-like

Size variation in naturally expressed biofilm-associated protein (BAP) in clinical strains of *Acinetobacter baumannii.*

Masoumeh Douraghi (Iran), Mansoor Kodori, Fazel Shokri, Amir Aliramezani.

Aim

The capacity of biofilm formation of *Acinetobacter baumannii* is mainly attributed to BAP. To understand the extent of variation among *A. baumannii* BAPs, we analyzed expression of BAP in 143 clinical strains of *A. baumannii*. Methods. Following generation and purification of polyclonal antibody against BAP targeting B4 module (amino acid 706 to 714) in rabbit, the strains were subjected to bap gene PCR, microtiter assay for biofilm formation, and western blotting using anti-BAP.

Results

PCR of the 1113 bp region corresponding to the B4 module showed that 127 strains were positive for bap gene. Along with a 41-kDa recombinant BAP as control, BAP was detected in whole-cell lysates of 62 (43%) of bap-positive strains whereas two BAP-expressing strains were bap-neagtive. Size of expressed proteins varied from 180-254 kDa and semi-quantitative intensity of expressed proteins ranged from +1 to +4. In addition, a statistically significant association was found between BAP expression and extent of biofilm formation (p=0.033).

Conclusion

In addition to differential expression of BAP, this analysis showed that the naturally expressed BAPs were variable in size in different strains tested. This finding is consistent with the fact that BAPs are structurally polymorphic and contain repetitive elements. Moreover, discordance of gene and protein expression may, in part, related to sequence variation of bap.

Structural basis for the *Acinetobacter baumannii* biofilm formation.

Natalia Pakharukova, Minna Tuittila, Sari Paavilainen, Henri Malmi, Olena Parilova, **Anton V. Zavialov (Finland).**

Acinetobacter baumannii-a leading cause of nosocomial infections-has a remarkable capacity to persist in hospital environments and medical devices due to its ability to form biofilms. The biofilm formation is mediated by Csu pili assembling via the novel 'archaic' chaperone-usher pathway. The x-ray structure of the CsuC-CsuE chaperone-adhesin pre-assembly complex of Csu pili reveals the basis for the attachment of the bacterium to abiotic surfaces. CsuE exposes highly hydrophobic finger-like loops at the tip of the pilus. Decreasing hydrophobicity of the loops abolishes the bacterial attachment, suggesting that the pilus tip detects and binds to hydrophobic patches on the substrate surface. Anti-CsuE antibody completely blocks the biofilm formation, presenting a means to prevent the spread of the pathogen.

The feoA gene from *Acinetobacter baumannii* 17978 is involved in the course of pneumonia infection.

Laura Álvarez-Fraga (Spain), Juan C. Vázquez-Ucha, Marta Martínez-Guitián, Juan A. Vallejo, Soraya Rumbo-Feal, Germán Bou, Alejandro Beceiro, Margarita Poza.

Background

Acinetobacter baumannii has emerged in the last decade as an important nosocomial pathogen. Gene expression profiles of *A. baumannii* ATCC 17978 cells grown in LB and cells grown in infected lungs using an experimental model of pneumonia in mice were determined using RNA-seq (Illumina). This allowed us to determine a collection of genes over-expressed in the course of the lung infection that could be involved in the pathogenicity of *A. baumannii*. In the present work, we evaluated the role of one of these genes, the A1S_0242 gene (feoA), which has been described as involved in iron uptake.

Material/Methods

An experimental model of pneumonia in mice was employed through intratracheal inoculation of 5x106 CFUs/BALB/c mice. After 20 h, a bronchoalveolar lavage was performed to obtained bacteria suitable for RNA extraction (in vivo samples). RNA extracted from bacteria grown in LB in flasks was used as an experimental control (in vitro samples). Total RNA was used for Illumina analysis. The isogenic mutant derivative strain (âˆ†0242) was obtained by double crossover recombination using the plasmid pMo130. Complementation of the mutant strain was performed using the pWH1266 plasmid. The A1S_0242-0244 operon was described through RNA extraction and reverse transcription. Both the biofilm formation and the adherence to A549 human alveolar epithelial cells abilities of the isogenic mutant derivative strain were determined. Bacterial growth was monitored to study the bacterial fitness in Muller-Hinton medium (MH), in presence and absence of the iron chelator 2,2'-bipyridyl (BIP). In order to evaluate the implication of the A1S_0242 gene in iron uptake, hydrogen peroxide discs diffusion assays were performed in MH in presence and absence of the iron chelator BIP. The virulence of the wild type strain and its derivative mutant was evaluated using both Galleria mellonella survival assay and murine pneumonia experimental models.

Results

The A1S_0242 gene was found as over-expressed in bacteria isolated during the course of the lung infection compared with bacteria grown in LB medium, this assessed by Illumina (2,50-fold more) and real time RT-PCR (5.03-fold more). The A1S_0242 gene was found as part of a single operon (A1S_0242 - A1S_0243 - A1S_0244) as assessed by RNA reverse transcription. The inactivation of the A1S_0242 gene resulted in a decrease in biofilm formation (ca. 4-fold, P value < 0.0001) and in adherence to A549 cells (ca. 80%, P value = 0.0003). Complementation of the strain with the parental allele restored both phenotypes. Growth curves showed a reduced fitness in the âˆ†0242 mutant strain compared to the wild type strain ATCC 17978. When strains were subjected to reaction oxygen species (ROS), the âˆ†0242 mutant strain showed an increase of the inhibition zone compared to the wild type strain ATCC 17978. Infection of G. mellonella larvae showed that the virulence of the âˆ†0242 mutant was significantly attenuated (20% of death) when compared with the parental strain (60% of death) (P value < 0.05). The experimental pneumonia model in mice showed a significant decreased in virulence (40% of death, P value < 0.05) compared with the parental strain (90% of death).

Conclusion

Data presented in this work indicated that the feoA gene from *A. baumannii* ATCC 17978 strain, which is involved in iron uptake and that was found as over-expressed during the course of a neumonia infection, plays a role in adhesion, biofilm formation and, definitively, in virulence. Taken together, these observations indicate the potential role that this gene could play in the pathogenicity of *A. baumannii* and its value as a potential therapeutic target.

New genes of *Acinetobacter baumannii* required for virulence in mouse model of pneumonia.

Marta Martínez-Guitián (Spain), Juan C. Vázquez-Ucha, Laura Álvarez-Fraga, Soraya Rumbo-Feal, Juan Andrés Vallejo, Germán Bou, Margarita Poza, Alejandro Beceiro.

Background
Healthcare-associated infections caused by multiresistant pathogens constitute a major problem worldwide and are associated with prolonged medical care, worse outcome and costly therapies. In vivo transcriptome studies using bacterial RNA samples directly extracted from the lung confer relevant information about the biological processes occurring during the infection. In the present work, we present a global transcriptomic analysis of *A. baumannii* RNA isolated during the course of the pneumonia infection. This allowed us to determine a collection of genes over-expressed in the course of the lung infection that could be involved in the pathogenicity of *A. baumannii*.

The objectives of the present research were: i) the identification of genes over-expressed during the course of the pneumonia infection using Illumina technologies, ii) the construction of isogenic derivative mutants of *A. baumannii* ATCC17978 strain and iii) the functional study of the target genes using in vitro and in vivo assays. Material/methods: Gene expression profiles of *A. baumannii* ATCC 17978 and AbH12OA2 strains grown in LB medium (in vitro) and isolated from infected lungs using an experimental model of pneumonia in mice (in vivo) were compared. BALB/c male were intratracheally infected with 5.5 x 107 CFUs/mouse of exponentially grown cells of the ATCC 17978 and AbH12O-A2 strains. After 20 h, bronchoalveolar lavage was performed to obtained bacteria suitable for RNA extraction. Total RNA was used for Illumina analysis. Later, a collection of isogenic mutant strains was obtained by double crossover recombination using the pMo130 plasmid. The virulence of the wild type strain and its isogenic derivative mutants was evaluated using Galleria mellonella survival assays and murine pneumonia experimental models. Growth curves determinations were performed to compare the loss of fitness between the wild type and the mutant strains.

Results
Results from Illumina revealed that 220 genes were over-expressed (16-2-fold) in bacteria isolated during lung infection compared with bacteria grown in vitro. A total of 22 isogenic mutant derivatives of the ATCC17978 strain were constructed. The experimental model of murine pneumonia showed that mice infected with the mutants âˆ†0242, âˆ†3245, âˆ†2390, âˆ†3410, âˆ†2247, âˆ†1013 and âˆ†0781 showed a significant loss of virulence (50% to 60%), compared with the wild type strain, specially the âˆ†3245 mutant, that showed a 100% of virulence loss. Also, a decrease in virulence was observed in the âˆ†2390, âˆ†0242 and âˆ†2247 mutants using the Galleria mellonella model. Finally, the fitness of âˆ†0242 and âˆ†2247 mutants showed to be reduced.

Conclusions
A1S_0242, A1S_3245, A1S_2390, A1S_3410, A1S_2247, A1S_1013 and A1S_0781 genes of the A. baumannii ATCC17978 strain were found to be over-expressed during the course of the pneumonia infection. The present study highlights the implication of these genes in virulence and, therefore, these new targets could be used for the design of new antimicrobial compounds.

An *Acinetobacter baumannii* two-component system modulates multiple virulence traits.

Sarah K. Giles, Uwe H. Stroeher, Shashikanth Marri, **Melissa H. Brown (Australia).**

Multidrug resistant *Acinetobacter baumannii* is of major global concern as infections with this bacterium can result in pneumonia, septicemia and bacteremia with extremely high mortality rates. The strategy utilised by this organism to cause disease is multifactorial and likely to be under stringent genetic control. To examine these mechanisms we investigated the two-component signal transductions systems within *Acinetobacter* since they are known to play a role influencing the expression of virulence determinants in other bacterial species. We identified a novel two-component system (designated StkRS) in a clinical *A. baumannii* strain isolated from a South Australian hospital, and using homologous recombination techniques, deleted the response regulator generating a DstkR derivative. Comparative transcriptomic analysis, of the DstkR and wild-type strains grown in media to mimic a lung environment, identified many differentially expressed genes. Of note; expression of 1,165 genes changed by 2-fold or more, with 701 up-regulated and 464 down-regulated.

In vitro assessment of this DstkR mutant strain compared with its progenitor revealed that inactivation of stkR resulted in an increase in bacterial cell adherence to A549 human pneumocyte cells (2.2-fold), enhanced survival under human serum stress (20 %), and an increase in survival in macrophage-mediated killing assays (3.3-fold). These results were extended in vivo using an intranasal murine model (C57/BL6 mice) to mimic pneumonia. Confirming the in vitro studies, an increased bacterial load in the lung (15-fold), blood (4-fold), and spleen (7-fold), was seen of the ΔstkR mutant compared to the wild-type strain 24 hours post infection. To complement the murine studies and assess if there was a differential effect on the mouse innate immune response we examined the immune response of 84 selected mouse genes using an Antibacterial RT2-PCR profiler array. In lungs from mice inoculated with the wild-type strain, 75 genes were 2-fold or more differentially expressed (24 up-regulated and 51 down-regulated) compared to the naïve lung. Infection with the ΔstkR mutant strain identified 66 differentially expressed (23 up-regulated and 43 down-regulated). This implies that removal of stkR dampens the innate immune response which consequently leads to a larger bacterial load in the mouse.

Taken together, we have identified that deletion of the StkR response regulator in *A. baumannii* affects multiple factors that influence the virulence capacity and subsequently the disease impact of this organism.

Divergent Role of Toll-like Receptor (TLR) 4 in the Host Defense against Intranasal and Intraperitoneal Infections with the hypervirulent *Acinetobacter baumannii* isolate LAC-4 in Mice.

Greg Harris, Rhonda KuoLee, Xiaoling Gao, H. Howard Xu, Hongyan Zhou, Girishchandra B. Patel, **Wangxue Chen (Canada).**

A better understanding of the host defense mechanisms against the multidrug resistant bacterial pathogen *Acinetobacter baumannii* is essential for the development of alternative non-antibiotics strategies against this emerging pathogen. Previous studies by several laboratories using ATCC and clinical isolates have demonstrated the importance of *A. baumannii* LPS and host TLR4 signalling pathway in the resistance against this pathogen. However, the detailed interactions between *A. baumannii* components and host cells have not been fully elucidated. In this study, we determined the potential role of TLR4 in host defense against intranasal infection with the newly identified clinical isolate LAC-4, which is hypervirulent in mice. Surprisingly, we found that the body weight, surviving rates, histopathology, and tissue (lungs and spleen) and blood bacterial burdens were comparable between the intranasally infected TLR4-/- and TLR4+/+ mice. In contrast, TLR4-/- mice were significantly more susceptible to a sublethal intraperitoneal infection with this isolate than TLR4+/+ mice. The intraperitoneally infected TLR4-/- mice showed >3 to 4 logs higher bacterial burdens in the lung, spleen and blood than TLR4+/+ mice at 24 hours post inoculation. Moreover, TLR4-/- mice had significantly reduced numbers of neutrophils in their peritoneal cavity at this time point although the total number of peritoneal macrophages was not significantly altered. The susceptibility of TLR4-/- mice to the intraperitoneal LAC-4 infection could be partially restored by administration of chemokine MIP-2.

Furthermore, the TLR4-mediated host resistance to intraperitoneal LAC-4 infection depends on PI3K-Akt signalling cascade in that treatment of the resistance TLR4+/+ mice with wortmannin, a PI3K/Akt inhibitor, increased its susceptibility to intraperitoneal LAC-4 infection to a magnitude of the TLR4-/- mice. Taken together, our data suggest that TLR4 plays a critical role only in host resistance against systemic LAC-4 infection and that the role of TLR4 in host defense against *A. baumannii* is dependent on the bacterial strains and the route of infection. The TLR4-mediated host resistance to systemic LAC-4 infection appears to function through the PI3K-Akt signalling cascade.

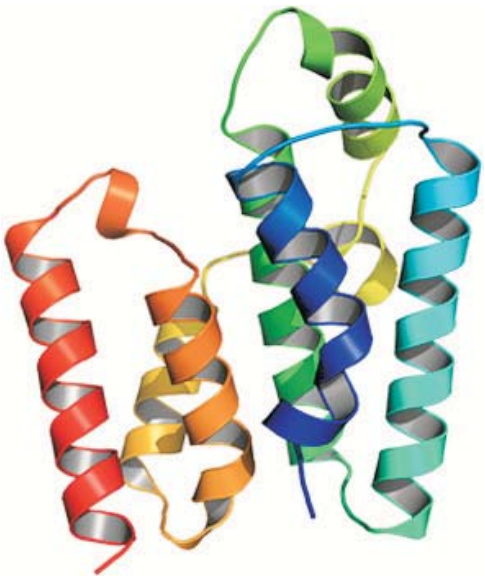
Crystal structure of the TssL cytoplasmic domain from *Acinetobacter baumannii*, a component of the T6SS.

Elena Santillana, Francisco J. Medrano, **Antonio Romero (Spain),** Federico M. Ruiz.

Secreted proteins play a major role in diseases caused by *A. baumannii*. Among the different mechanisms described in this pathogen, the Type VI secretion system (T6SS) has emerged as a versatile molecular weapon against eukaryotic host cells or bacterial competitors. This macromolecular machine is widely distributed in Gram-negative bacteria, being formed by a central tubular structure and a membrane complex. This last subassembly is responsible for the anchoring of the T6SS to the cell envelope. At least four proteins form this trans-membranes structure. Among them, TssM and TssL are the core inner-membrane proteins forming a stable inner-membrane complex. The formation of this complex has shown to be the first step in the assembly of the T6SS.

The crystal structure of TssL is known for 3 different organisms: Vibrio cholerae, Escherichia coli Enteroaggregative and Francisella novicida. Despite the relatively low sequence identity the overall structure is similar for all of them, except for subtle differences found in local structural elements.

In order to study the specific characteristics of TssL from *A. baumannii*, we have purified, crystallized and solved by X-ray crystallography the structure of the cytoplasmic domain of this key element of the T6SS. The TssL is an eight alpha-helix protein, constituted by two helix bundles connected by loops. The overall geometry resembles a hook, as previously observed in the other homologues structures. The particular geometry of long helices, the surface change and residue conservation are discussed.



Interrogating the role of *Acinetobacter baumannii* outer membrane lipoprotein, YraP.

Faye C. Morris (Australia), Rhys Grinter, Rhys Dunstan, Trevor Lithgow, Jian Li - Anton Y. Peleg.

Polymyxin therapies currently serve as our last line of defence against the scourge of multi-drug resistant A. baumannii. However, increased usage of this antibiotic in the clinic has led to the emergence of polymyxin resistant *A. baumannii* strains. In order to gain a more comprehensive understanding of the underlying cellular changes induced by polymyxin exposure and their contribution to cellular physiology, we interrogated published datasets investigating the transcriptional changes observed in *A. baumannii* after exposure to colistin. From this, we identified a conserved outer membrane lipoprotein, called YraP, which is transcriptionally upregulated in response to colistin.

Objectives
To investigate the role of YraP in *A. baumannii* physiology.

Methods and Results
Bioinformatic analysis revealed that YraP is conserved across all Gram negative bacteria and is the homolog of the E. coli protein, YraP and the Neisseria meningitidis protein, GNA2091, with the latter forming a critical component of the 4CMenB vaccine against the meningococcal serogroup B. Sequence analysis of *A. baumannii* YraP identified a lipoprotein signal peptide, N-terminal cysteine residue and two Bacterial OsmY Nodulation (BON) domains, consistent with its role as an outer membrane lipoprotein. To investigate the role of this protein we have generated defined disruption mutants in two strains of *A. baumannii*, ATCC 19606 and ATCC 17978, and have complemented these mutations by supplying the gene in trans under the control of its native promoter.

Using this panel of wildtype and isogenic mutant strains, we have shown that YraP is not required for *A. baumannii* growth in rich media under standard laboratory conditions. We have assessed the minimum inhibitory concentrations for a range of antibiotics, encompassing different classes, and confirmed the sensitivity of the mutants is not significantly different from their respective parental strains. To further elucidate the role of YraP, we will assess the strains for phenotypes more astute at highlighting differences in membrane composition, including autoaggregation, desiccation, motility and efflux activity.

Conclusions
The characterisation of this lipoprotein will enhance our understanding of its contribution to both *A. baumannii* physiology, with potential to inform future vaccine and treatment design options.

Structure of Peptidyl-tRNA hydrolase from *Acinetobacter baumannii* at 1.9 Å resolution.

Pradeep Sharma (India), Sanket Kaushik, Punit Kaur, Sujata Sharma, T P Singh.

The unwanted production of Peptidyl-tRNA molecules in the cell is a result of premature termination of translation. In order to make use of this product in the ongoing protein biosynthesis, the peptide and the tRNA parts of peptidyl-tRNA have to be separated from each other. This required recycling is done by peptidyl-tRNA hydrolase (Pth) making it an essential enzyme for the survival of bacteria. Since infections caused by bacterium *Acinetobacter baumannii* (Ab) are increasingly becoming difficult to cure with available antibiotics, the need of developing new drugs against Ab infection has become urgent. For this purpose, we have determined the structure of Pth from *Acinetobacter baumannii* (AbPth) at 1.9 Å resolution. Therefore AbPth was cloned, expressed and crystallized. The crystals belong to orthorhombic space group P21221 with cell dimensions, a = 34.5 Å, b = 59.2 Å and c = 109.2 Å. Structure determination showed that the polypeptide chain of AbPth adopts a compact α/β globular fold with a twisted β-pleated sheet in the centre that is surrounded by five α-helices. The segment Gly138 - Val150 which works like a flap on the active site is highly flexible as it lacks significant interactions with the rest of the protein molecule. Similarly, the conformations of other two segments, Met1 - Leu6, Arg52 - Arg61 and Thr109 - His115 also differ considerably in Pth molecules from different species of bacteria. The opening width to the active site in the unbound state of AbPth was found to be 6.3 Å which is similar to the corresponding distance of 6.6 Å in Escherichia coliPth (EcPth) but it is considerably more than those reported in Mycobacterium tuberculosis (MtPth) (3.3 Å) and Mycobacterium smegmatis (MsPth) (4.5 Å). The hydrogen bonded network involving residues, Asn116, His22 and Asp95 indicates a stereochemicallyfavorable arrangement for these residues to be involved in catalysis.The unwanted production of Peptidyl-tRNA molecules in the cell is a result of premature termination of translation. In order to make use of this product in the ongoing protein biosynthesis, the peptide and the tRNA parts of peptidyl-tRNA have to be separated from each other. This required recycling is done by peptidyl-tRNA hydrolase (Pth) making it an essential enzyme for the survival of bacteria. Since infections caused by bacterium *Acinetobacter baumannii* (Ab) are increasingly becoming difficult to cure with available antibiotics, the need of developing new drugs against Ab infection has become urgent. For this purpose, we have determined the structure of Pth from *Acinetobacter baumannii* (AbPth) at 1.9 Å resolution. Therefore AbPth was cloned, expressed and crystallized. The crystals belong to orthorhombic space group P21221 with cell dimensions, a = 34.5 Å, b = 59.2 Å and c = 109.2 Å. Structure determination showed that the polypeptide chain of AbPth adopts a compact α/β globular fold with a twisted beta-pleated sheet in the centre that is surrounded by five α-helices. The segment Gly138 - Val150 which works like a flap on the active site is highly flexible as it lacks significant interactions with the rest of the protein molecule. Similarly, the conformations of other two segments, Met1 - Leu6, Arg52 - Arg61 and Thr109 - His115 also differ considerably in Pth molecules from different species of bacteria. The opening width to the active site in the unbound state of AbPth was found to be 6.3 Å which is similar to the corresponding distance of 6.6 Å in Escherichia coliPth (EcPth) but it is considerably more than those reported in Mycobacterium tuberculosis (MtPth) (3.3 Å) and Mycobacterium smegmatis (MsPth) (4.5 Å). The hydrogen bonded network involving residues, Asn116, His22 and Asp95 indicates a stereochemicallyfavorable arrangement for these residues to be involved in catalysis.

Unlocking the Complete Arsenal of *Acinetobacter baumannii* Type VI Secretion System Effectors.

Jessica M. Lewis (Australia), Timothy C. Fitzsimons, Deanna Deveson Lucas, Marina Harper, John D. Boyce.

Nearly 10% of all nosocomial Gram-negative bacterial infections are caused by *Acinetobacter baumannii*, a strictly aerobic coccobacillus. *A. baumannii* expresses a type VI secretion system (T6SS), a unique surface structure resembling an inverted T4 bacteriophage that is used by *A. baumannii* to inject effectors into nearby cells. In some bacteria, the T6SS is involved in virulence or biofilm formation but in *A. baumannii* the T6SS is primarily associated with killing competitor bacteria. In *A. baumannii* strains ATCC17978 and AB307-0294, secretion of each A. baumannii effector is dependent upon a specific VgrG protein. The genes encoding the effector and its cognate VgrG protein are often co-localised on the chromosome. In order to identity novel *A. baumannii* effectors, we analysed the genomes of 41 strains representing a range of *A. baumannii* global clone lineages and sequence types. Several putative effector genes were identified in each genome, each of which were adjacent to a gene encoding a protein with a VgrG-domain. In total, 29 putative *A. baumannii* T6SS effector genes were identified and grouped based on shared domains and amino acid identity. The effectors identified included six Rhs-domain family proteins with C-terminal domains of no known function, eight predicted peptidoglycan hydrolases, and three putative nucleases. Eight of the putative effectors were novel and had no identifiable domains. Our aim is to characterise the novel effectors via heterologous expression in E. coli and in vitro functional assays. Characterisation of these novel effectors will provide an insight into bacterial killing mechanisms and potentially allow for the identification of new drug targets.

Uantification of neutrophil extracellular traps in neutrophils stimulated by *Acinetobacter*.

María Lázaro Díez (Spain), Itiziar Chapartegui González, Zaloa Bravo del Hoyo, Teresa Navascués Lejarza, Alain Ocampo Sosa, Jorge Calvo, Luis Martínez Martínez, José Ramos Vivas.

Background

Acinetobacter spp. are recognized as emerging nosocomial pathogens and a primary cause of Gram-negative infections in many parts of the world. These bacteria, mainly *A. baumannii*, are particularly difficult to trait due to its propensity to develop resistances to many groups of antibiotics. There are no detailed published reports on the interactions between *Acinetobacter* and human neutrophils. Neutrophils are the most abundant leukocytes in the blood which are rapidly recruited to the inflammatory site upon inflammation. Neutrophils can eliminate microbes using three basic strategies: phagocytosis, degranulation, and by a recently discovered mechanism called NETosis, a specific type of cell death different from both necrosis and apoptosis.

Objectives

We investigated the interaction of *A. baumannii* and A. pittii clinical isolates with neutrophils. The aim of this study was to observe and to quantify the presence of neutrophil extracellular traps (NETs) in neutrophils stimulated by *Acinetobacter baumannii* and A.pittii.

Methods

Five *Acinetobacter* strains were used in this study. Neutrophils were obtained from healthy donors by negative separation with magnetic beads. NETs were observed by confocal laser scanning microscopy after immunofluorescence staining. Also, NETs were quantified measuring microscopy in samples with SYTOX green, which stain extracellular DNA in NETs. Furthermore we measured the concentration of H3 histone citrullinated by ELISA and the activity of neutrophil elastase (NE), two proteins typically found in NETs.

Results and conclusions

Immunofluorescence staining and double-immunofluorescence performed from 30 min to 4 h demonstrated that neutrophils catch and kill bacteria continuously. This was also confirmed by time-lapse microscopy. Importantly, *Acinetobacter* strains were able to induce NETs formation by human neutrophils. Immunofluorescence analyses confirmed the co-localization of histones (H3) and NE in NETs released from these defensive cells after *Acinetobacter* infections.

DNA quantification by SYTOX green showed statistically significant differences (p-value <0.005) between unstimulated neutrophils and most of strains tested. Citrullinated histone H3 released by neutrophils range from 0.55 to 0.07 ng ml-1 and NE from 0.5 to 1.4 mU ml-1 depending on the strain used.

In this work, we demonstrate phagocytosis and killing of these two important pathogens by human neutrophils as a defense mechanism, but the induction of NETs could be also important to fight infection.

Effect of incubation temperature on virulence and antibiotic susceptibility of *Acinetobacter baumannii* ATCC17978.

P. Malaka De Silva (Canada), Patrick Chong Dinesh, M. Fernando, Garret Westmacott, Ayush Kumar.

Acinetobacter baumannii is a notorious Gram-negative coccobacillus, which poses an extremely potent threat in the modern day health care setting, as it displays multi-drug resistance and high levels of virulence. In this study, we aimed to explore the effect of incubation temperature on the antibiotic resistance and virulence traits of *A. baumannii* ATCC17978.

A. baumannii ATCC17978 was subjected to a multitude of phenotypic assays for virulence traits at both 28ËšC and 37ËšC along with determining minimum inhibitory concentrations for clinically relevant drugs at 28ËšC and 37ËšC. Furthermore, proteomic analysis was carried out using late log phase cells of freshly grown cultures of *A. baumannii* at 28ËšC and 37ËšC. We observed that 632 proteins displayed differential expression levels in the two different incubation temperatures where 367 proteins were up regulated, while 265 proteins were down regulated when cultures were grown at 28ËšC compared to 37ËšC. Among these proteins, the csu operon, responsible for pili formation and paa operon, responsible for phenyl acetic acid catabolism were respectively up regulated and down regulated. The upregulation of the proteins of the csu operon presents a correlation between the increased biofilm formation seen at 28ËšC as compared to 37ËšC. We also observed that there is no significant difference in virulence in a G. mellonella model at 28ËšC and 37ËšC. However, Resistant Nodulation Division efflux pump proteins were up regulated, which may cause the observed decreased susceptibility to aztreonam at 28 ËšC. These results suggest that incubation temperature may impact antibiotic resistance and virulence of *A. baumannii* ATCC17978

The contribution of iron uptake to *Acinetobacter baumannii* pathogenicity.

Federica Runci (Italy), Valentina Gentile, Emanuela Frangipani, Giordano Rampioni, Livia Leoni, Massimiliano Lucidi, Greg Harris, Wangxue Chen, Julia Stahl, Beate Averhoff,Paolo Visca.

Acinetobacter baumannii is an emerging bacterial pathogen. Mechanisms that allow *A. baumannii* to cause human infection are still poorly understood. Iron is an essential nutrient for bacterial growth in vivo, and the multiplicity of iron uptake systems in *A. baumannii* suggests that iron acquisition could contribute to the success of *A. baumannii* as a human pathogen. In Gram-negative bacteria, receptor-mediated ferric iron uptake is an energy-demanding process which depends on proton consumption by the conserved TonB-ExbB-ExbD energy-transducing complex. Active uptake of ferrous iron is mediated by the GTP-dependent Feo system, encoded by the feoABC operon. The A. baumannii genome invariably contains three tonB orthologs, named tonB1, tonB2 and tonB3, whose role in iron uptake remains poorly understood. Here, we used *A. baumannii* ATCC 19606T to generate knock-outs in each of the three tonB genes, as well as in feoB. We report that tonB3 is essential for *A. baumannii* growth under iron limiting conditions, as those imposed by iron-poor media or human serum, whereas tonB1 and tonB2 appear unrelated to iron uptake. Different from tonB1 and tonB2, the tonB3 gene contains a functional Fur box in its promoter and is tightly regulated by iron. We also observed that feoB markedly stimulates *A. baumannii* growth in human serum. Deletion of tonB3 caused over-production of siderophores, coherent with a severe defect in iron uptake. More significantly, we observed that TonB3 is essential for *A. baumannii* virulence, since lethality in both Galleria mellonella and mouse sepsis models was completely abrogated in the tonB3 mutant. Conversely, virulence of the feoB mutant was not attenuated, compared with the wild type. These findings demonstrate that the TonB3 energy-transducing machinery is essential for in vivo growth, and highlight the TonB3 protein as a promising target for anti-bacterial therapies.

Explaining the carbapenem-resistant *Acinetobacter baumannii* lineages dynamics by whole-genome and phenotypic analyses.

Liliana Silva (Portugal), Magdalena Ksiezarek, Filipa Grosso, Luísa Peixe.

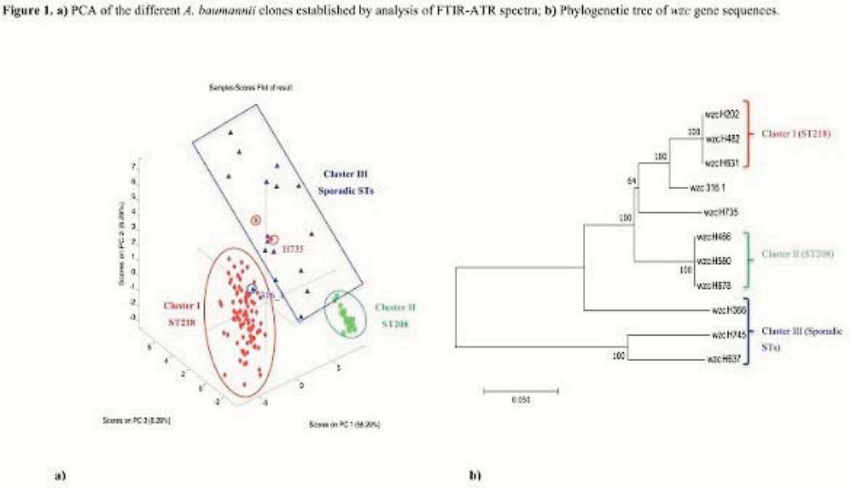
Background
During the last years, carbapenem-resistant *A. baumannii* (Ab) strains have become endemic in many European countries, with substantial effort being devoted to define their epidemiology. In Portugal, particular clones have dominated over the years (e.g. ST98 in 90's, followed by ST208 and ST218 in more recent descriptions). Despite the relevance of continuing monitoring, the main reasons behind this shift are still unknown. Objectives: To explore Ab clinical isolates features, including cell superficial molecules, to unveil reasons behind the dominance of specific clones along the years.

Methods
This study included a collection of 177 Ab isolates (2010-2015) from two hospitals in the North (H) and Centre of Portugal (CB), as well as a Long-Term Care facility (LTCF) and a community clinical laboratory (CT). Isolates characterization included susceptibility to different beta- and non-beta-lactam antibiotics and carbapenemase production (Blue-Carba). For representative isolates of ST218 (4), ST208 (3), and sporadic STs [ST234 (1), ST552 (1), STnew1 (1) and STnew2 (1)] screening for antibiotic resistance and virulence genes was performed by WGS. Ab strain typing was assessed by DNA based methods (MLST and WGS), Fourier-transformed Infrared (FTIR) spectroscopy coupled with multivariate data analysis (PCA) and capsule typing (cps locus sequencing and phylogenetic analysis performed with MEGA 7.0).

Results
ST218 was the most successfully disseminated clone (115/177; 65%), being found in two hospitals and in the community during the analyzed period, while ST208 was only observed in hospital H with a decreasing trend over the years. All ST218 isolates and almost all from ST208 were OXA-23-producers (Tn2006), Blue-Carba positive and classified as XDR. However, ST218 showed some advantages, mainly related with the higher levels of aminoglycoside resistance (justified by the presence of genes not found among ST208 isolates, e.g. aacA4, aadA1, aph(3´)-Ic and armA), and with the virulence profile (e.g. presence of hemO gene in ST218, absent in ST208). The sporadic STs were more susceptible to antibiotics (MDR corresponded only to 11%), with only one OXA-24-producer isolate (Blue-Carba positive) from LTCF. Besides, they lacked several of the putative virulence operons. By FTIR-based and PCA analysis, most of the isolates appeared in two well-delimited clusters, whereas the remaining were dispersed. As we previously demonstrated, MLST was, in general, congruent with FTIR-clustering, being the incongruences explained by the capsular types inferred by wzc sequences (Fig.1). Thus, cluster I included most of ST218 isolates (n=113), with a capsule similar to PSgc1 (legionaminic acid as main component, important for escaping the immune system). Cluster II encompassed ST208 isolates (n=44), with capsule KL2/PSgc12 (pseudaminic acid). Dispersed isolates belonging to sporadic STs harbored different capsular types (PSgc23, PSgc27, PSgc8), and ST218 (2 isolates not included in cluster I due to a different capsular type-PSgc5, α-L-frucosamine).

Conclusions
In this work genomic and phenotypic characteristics provided new data regarding the molecular evolution of Ab clones in Portugal, explaining the reasons for the exchange of ST208 by ST218. FTIR-ATR showed a high discriminatory power for the Ab population structure characterization being the assignments, however, more related with the capsular types than with STs. wzc phylogeny corroborated the FTIR-results, suggesting the potential application of this gene sequencing for Ab strain typing.

Figure 1. a) PCA of the different *A. baumannii* clones established by analysis of FTIR-ATR spectra; **b)** Phylogenetic tree of *wzc* gene sequences.



Community-acquired *Acinetobacter baumannii*: a distinct hypervirulent pathogen.

Carina Dexter (Australia), Xenia Kostoulias, Ibukun E Aibinu, Gustavo M Cerqueira, Daniel N Farrugia, Gerald L Murray, David L. Paterson, Graham Lieschke, Anton Y Peleg.

Objectives
In the community, *A. baumannii* causes a distinct and surprisingly severe infection syndrome when compared to its hospital-acquired counterpart, characterised by severe pneumonia and overwhelming sepsis with mortality up to 60%. In Australia, excess alcohol consumption is the major risk factor for community-acquired *A. baumannii* (CA-Ab) infection. Whether host or bacterial factors unique to CA-Ab are driving this predisposition to such severe disease remains unknown. The primary objective of this work was to assess genetic and phenotypic differences between a collection of CA-Ab strains against geographically paired hospital-acquired *A. baumannii* (HA-Ab) isolates. The secondary objective of this work was to assess the impact of ethanol on disease outcomes.

Methods
We collected a group of clinical CA-Ab strains from patients in northern Australia who had life-threatening infections. We also collected geographically matched HA-Ab strains. Whole genome sequencing and comparative analyses of CA-Ab, HA-Ab and publicly available *A. baumannii* genome sequences were performed. Antibiotic susceptibility, serum killing and in vivo virulence studies in a murine pneumonia model were performed. The impact of ethanol on CA-Ab disease was determined using a zebrafish (Danio rerio) infection model.

Results
CA-Ab isolates were more sensitive to antibiotics, but had greater resistance to serum killing than HA-Ab. Notably, CA-Ab strains were consistently more virulent than HA-Ab in a murine pneumonia model. At two days post infection, only 10% of CA-Ab infected mice were alive, while survival of HA-Ab infected mice was 100%. Bacterial dissemination from the lung to the blood and other organs was evident with all CA-Ab infections, whereas this was not seen with HA-Ab infection. Utilising our zebrafish model it was found that ethanol augmented disease for CA-Ab infected fish, with survival of 56% for ethanol exposed fish compared to 76% survival with no ethanol ($P < 0.01$). Importantly, we identified several ($n = 21$) unique genes shared by all CA-Ab isolates that were absent from their paired HA-Ab isolate, some of which may at least partly explain the hypervirulent syndrome associated with CA-Ab.

Conclusions
These data demonstrate that CA-Ab is genetically different from HA-Ab, and causes a distinct and more virulent infectious syndrome. Outcomes from CA-Ab infection are worse in the presence of ethanol, the most important risk factor for life-threatening CA-Ab infection. Together, our work indicates that the severe clinical presentation of CA-Ab pneumonia is likely due to a combination of both bacterial and host factors.

Generation and characterization of unmarked single and double heme utilization gene cluster knockout mutants of *Acinetobacter baumannii* LAC-4.

Peter J Ewing, Athena M Madrid, Rogelio Nuñez-Flores, Greg Harris, Wangxue Chen, **H. Howard Xu (USA).**

During the past several decades *Acinetobacter baumannii* has become increasingly multi-drug resistant (MDR). Additionally, the possession and acquisition of genetic elements encoding numerous drug resistance and virulence factors have made it an exceptionally problematic bacterial pathogen. We previously showed that a hospital outbreak MDR strain (LAC-4) of *A. baumannii* is hypervirulent in mice, exhibits high serum resistance and expresses a highly efficient heme utilization system. We also found a heme utilization gene cluster (named HUT for heme utilization 1) ubiquitous to all strains of *A. baumannii*, while only some strains, including LAC-4, harbor a second gene cluster encoding additional heme uptake proteins and a heme oxygenase enzyme (termed hemO cluster, HOC). Heme from host has been found to be a successful alternative source of iron for pathogens when free iron is sequestered in the circulatory system. Previously we generated an unmarked knockout (KO) mutant of LAC-4 in which the 9.5-kb eight-gene HOC was deleted. This HOC cluster KO mutant showed significant reduction in virulence, and reduced bacterial burden in all tissues of mice. In this communication we describe the generation of a single gene cluster KO mutant in which the 16.4-kb 12-gene HUT cluster was deleted and a double gene cluster KO mutant in which both the HOC and HUT clusters were deleted. We also present preliminary results of relative infectivity of these three KO mutants in the Galleria mellonela model.

Both the single cluster HUT mutant and the double cluster KO were constructed via homologous recombination of a KO cassette cloned into the suicide vector pMo130. The KO cassette was designed with an apramycin (Apr) resistance gene linked outside two fused flanking regions of the 16.4-kb heme utilization cluster via overlap extension PCR. The recombinant plasmid containing the KO cassette was then introduced via electrophoration into the wildtype LAC-4 strain and the previously generated LAC-4 ΔHOC mutant strain to generate the single cluster HUT mutant and double cluster KO mutant, respectively. After isolating a single cross-over integration of the suicide plasmid with KO construct, both an unmarked HUT mutant and a double cluster KO mutant were obtained via a second homologous recombination mediated by sacB counter selection leveraging the use of the xylE reporter gene on the suicide vector backbone. The virulence of the single cluster HUT mutant and the double mutant was then evaluated in a Galleria mellonella infection model.

The Galleria mellonella infection assays compared the sequence-confirmed double cluster KO mutant to the individual cluster KO mutants. The HUT KO mutant is not attenuated as compared to the wild-type strain in the Galleria model. While the double mutant was found to be less virulent than the wild-type and the HUT KO mutant, the single cluster HOC KO mutant still demonstrated less virulence than the double cluster KO mutant. Unexpectedly, the deletion of the HUT cluster in the single HOC KO mutant resulted in a significant increase in infectivity, suggesting a complex interaction between these two gene clusters.

Distribution of multidrug-resistant *Acinetobacter baumannii* in ASST "Spedali Civili" Brescia and analysis of colistin/sulbactam combination therapy by ceckerboard approach.

Bettini L., **Lorenzin G. (Italy),** Gelmi M., Fiorentini S.

A. baumannii (A.b.) is an opportunistic pathogen that has the capacity to develop resistance against a broad spectrum of antimicrobials. Carbapenem-resistant A.b. (CRAB) poses a significant threat to patients and healthcare systems in all EU/EEA countries. The problem led our department of Microbiology and Virology to monitor the dissemination of all multidrug-resistant (MDR) and extensively drug-resistant (XDR) A.b. in the ASST Spedali Civili di Brescia (Brescia, Italy) for five years, from 2012 to 2016. To cope with the new threat, all MDR and XDR A.b. coming from patients hospitalized in our hospital were analyzed and monitored in the department of Microbiology and Virology. All strains were identified by MALDI-TOF MS, using the VITEK MS system (bioMerieux). This screening identified 232 A.b. strains, and among them XDR (n=132, 57%) and MDR (n=100, 43%). Furthermore, 3 patterns of resistance identified were prevalent. They included A.b. strains susceptible only to colistin, strains susceptible only to colistin and aminoglycosides, and broadly drug-susceptible strains. Our data show that in our hospital A.b. is mainly associated with Respiratory tract infections (RTIs) (103/232, 44,4%) and it's also isolated from traumatic injuries and postsurgical wounds (83/232, 35,8%). Also, 30/232 (12,9%) A.b. were isolated from blood culture, 17 of which (56,7%) were XDR A. b. strains. In our experience Urinary tract infections (UTIs) are sporadic with an average of only 3 cases/year. Comparison analysis of aminoglycoside's susceptibility patterns showed that amikacin is the agent with a greater propability of success in empirical treatment of MDR A.b. infections. The same analysis on carbapenems highlighted that imipenem is the most effective drug against non-CRAB strains in vitro. Sulbactam is a class A beta-lactamase inhibitor that has an intrinsic whole-cell activity against certain bacterial species (Neisseria gonorrhoeae, Bacteroides fragilis) including A.b. We evaluated its activity when used alone or in combination with colistin, using two different approaches: ceckerboard and disk-diffusion. Tests were carried out on 12 A.b. strains that Vitek 2 identified as susceptibe only to colistin. We analyzed the data calculating Fractional Inhibitory Concentration (FIC) Index. Sulbactam's minimum inhibitory concentration (MIC) was reached with a dose of 4 μ/ml, with only 2 strains (16,7%) that required a higher dose of sulbactam (8 μg/ml) to reach the MIC. For the 8 strains that had a MIC of colistin > 0,125 μg/ml we investigated the presence of a synergistic effect between sulbactam and colistin. FIC index of the combination colistin/sulbactam for 50% of the A.b. tested was 0.5, the limit to define the combination as synergistic, while an other 50% showed a FIC index <2, suggesting an additive effect between the two antibiotics. Disk-diffusion approach could detect the presence of synergism in 7 out of 8 strains tested, but this wasn't always clear. What's intriguing is that it was possible to inhibit the growth of all the 8 strains tested combining the minimum dose of colistin (0,125 ƒÊg/ml) with specific sulbactam's concentrations, ranging from 0,125 ƒÊg/ml to 4 ƒÊg/ml (Figure 1). Interestingly we were able to stop the growth of all strains tested combining a dose of 0,5 ƒÊg/ml of colistin with a dose of 1 ƒÊg/ml of sulbactam. This data suggest that low doses of colistin combined with a high sulbactam/colistin ratio could lead to positive outcome with reduced side effects. Future studies are request to confirm our preliminary data that could have an immediate impact on MDR and XDR A.b. therapy.

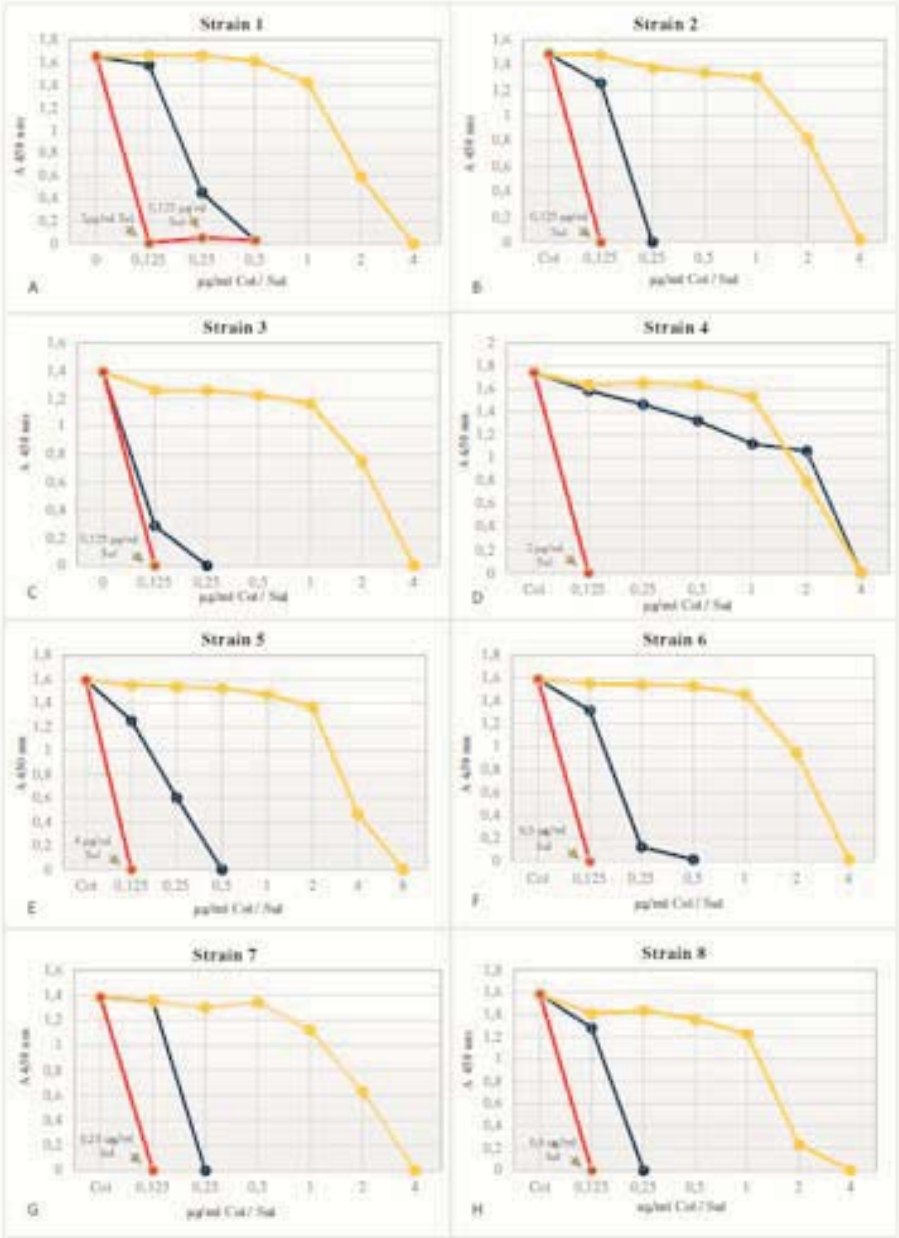


Figure 1: Variation of the absorbance at 450 nanometres (nm) in checkerboards of 8 *Acinetobacter baumannii* strains tested with: colistin alone (Col - blue lines), sulbactam alone (Sul - yellow lines) or both the antibiotics (red lines). When colistin and sulbactam are used together, the concentration reported on the x-axis refers to colistin, while the minimum concentration of sulbactam necessary to stop the bacterial growth is indicated as note (gray arrows).

New eight genes identified at the clinical multidrug-resistant *Acinetobacter baumannii* DMS0669 strain in a Vietnam hospital.

Nguyen Si-Tuan (Vietnam), Nguyen Thuy Huong, Nguyen Cuong, Hua My Ngoc, Pham Hung Van, Pham Thi Thu Hang.

Background
Acinetobacter baumannii is an important nosocomial pathogen that can develop multidrug resistance. In this study, we characterized the genome of the A.baumannii strain DMS06669 (isolated from the sputum of a male patient with hospital-acquired pneumonia) and focused on identification of genes relevant to antibiotic resistance Methods: Whole genome analysis of A.baumannii DMS0669 from hospital-acquired pneumonia patients included de novo assembly; gene prediction; functional annotation to public databases; phylogenetics tree construction and antibiotics genes identification.

Results
After sequencing the A.baumannii DMS0669 genome and performing quality control, de novo genome assembly was carried out, producing 24 scaffolds. Public databases were used for gene prediction and functional annotation to construct a phylogenetic tree of the DMS06669 strain with 21 other A.baumannii strains. A total of 18 possible antibiotic resistance genes, conferring resistance to eight distinct classes of antibiotics, were identified. Eight of these genes have not previously been reported to occur in *A. baumannii* (Figure).

Conclusion
Our obtained results in this study point out that the diverse possible mechanism of antibiotic resistance, existed in *A. baumannii* DMS06669 strain and provide a clinical advice for the therapy of *A. baumannii* infected patients.

Genomic resistance profiles and epidemiology of multidrug-resistant *Acinetobacter baumannii* strains in Colombia between 2012 and 2015.

Diego Andrés Prada Cardozo (Colombia), Verónica Del Pilar Rincón Forero, Hermes Pérez Cardona, Jhon Jairo Donato Ariza, Sandra Yamile Saavedra Rojas, Carolina Duarte Valderrama, Jaime Enrique Moreno Castañeda, María Teresa Jesús Reguero Reza, José Ramón Mantilla Anaya, Emilia María Valenzuela De Silva, Laurent J. E. FALQUET, Emiliano Barreto-Hernández.

Whole Genome Sequencing (WGS) is a promising technique to characterize outbreaks and to analyze the epidemiology of bacterial strains. The use of information from WGS of bacteria and the development of bioinformatic programs to organize and infer this information will provide in a near future a holistic tool for diagnosis in clinical laboratories (Ellington et al., 2016).

Multi-drug resistant (MDR) *Acinetobacter baumannii* has been reported with a high prevalence in Colombian clinical settings, especially in Intensive Care Units (ICU), (Hernández et al., 2014). These MDR *Acinetobacter baumannii* strains are a cause of health care-associated infections (HAI) and the costs have increased due to prolonged hospitalization and maintenance of patients at these sites. In 2005, 2008 and 2009, *A. baumannii* was reported to be resistance to carbapenems around 38%, 41% and 45.5%, respectively in Colombia (Miranda et al., 2006 and Briceño et al., 2010).

In this study, we analyzed the epidemiology and generated the genomic resistance profiles of 89 *Acinetobacter baumannii* clinical isolates of 14 Colombian states (48 hospitals) in the period 2012 - 2015. The evaluation of phenotypic resistance was performed by the Kirby Bauer test and the WGS was made by Illumina technology (Hiseq2000). The identification of resistance genes was performed by a bioinformatics workflow and the construction of genomic resistance profiles was made from a presence-absence matrix. Molecular typing was executed using Multilocus Sequence Typing (MLST-1.8 Pasteur scheme) and ribosomal Multilocus Sequence Typing (rMLST) resulting in 13 sequence types (STs), (3 novel STs) and 22 ribosomal sequence types (rSTs), (19 novel rSTs). We found that ST79 (65.2%) and ST25 (15.7%) were the dominant STs in contrast rST9802 (58.4%) and rST8921 (9%) were the dominant rSTs. The isolates showed 95.5% of MDR phenotypes where 97% were resistant to imipenem and 94% resistant to meropenem. We identified 104 resistance mediator genes, which were grouped into the following resistance mechanism: modification or degradation of antibiotics 32.7%, efflux pumps 39.4%, modification of the target site 23.1% and membrane alteration 4.8%. We found 41 Resistance Profiles, 19 were within the ST79 and 6 were within ST25. The most widespread resistance profile (13 isolates) has 52 genomic elements including 3 genes encoding enzymes that modify aminoglycosides, 3 genes encoding Beta-lactamases, 25 genes encoding for efflux pumps of the 5 families with genes that regulate the expression of bombs like adeL, adeN and adeRS, also 3 genes encoding for membrane alteration (ompW, oprD and carO), and 18 genes that modify the target site. These kinds of mechanisms make the microorganism MDR due to the action towards different families of antibiotics. The results also showed 20 genes coding for Beta-lactamases, 13 of them were OXAs, which OXA-23 and OXA-65 were reported more often. Other Beta-lactamases such as TEM-1 (67.4), CTX-M-115 (2.2%), VEB-9 (1.1%), NDM-1 (4.5%), VIM-4 (3.4%), KPC-2 (2.2%), and CARB-16 (1.1%) were also found, one of the isolates has the VEB-9 gen which has not been reported in Colombia. Finally, we did the pan genome with Roary (Page et al., 2015) to see the core and accessories genes and the phylogenetic relation.

WGS showed a great resolution for epidemiological analysis and the understanding of the resistance profiles, which change slightly in the presence of genes mainly in degradation or modification of the antibiotic and efflux pumps mechanisms. The grouping of the genomic resistance profiles, STs and rSTs coincides with the phylogenetic relationship; we determined that the genomic resistance profiles are an important determinant because it characterizes the isolate based on the resistome and this information is relevant at the moment of understanding the dispersion and evolution of resistance in *Acinetobacter baumannii*.

Epidemiology and carbapenem resistance of *Acinetobacter* spp. clinical isolates from Azerbaijan.

Fidan Yilmaz (Azerbaijan), Ignasi Roca, Laura Muñoz, Giuseppe Gornaglia, Nigar Agayeva, Jordi Vila.

Objective

A. baumannii tops the global priority list of antibiotic-resistant bacteria published by the WHO and carbapenem-resistant *A. baumannii* is currently considered the most critical pathogen regarding public health. Our knowledge on the epidemiology and antibacterial susceptibility profiles of *A. baumannii*, however, is still incomplete in many parts of the world.

Our aim was to characterise the carbapenem susceptibility and the clonal relatedness of *Acinetobacter* spp. isolates recovered from three different hospitals in Baku, Azerbaijan.

Methods

98 *Acinetobacter* spp. isolates were recovered from pathological samples collected from three different hospitals (therapeutical, surgical and military clinic) in Baku, Azerbaijan. Bacterial identification and antibiotic susceptibility were initially performed by Vitek at the participant hospitals. Species identification was then confirmed by MALDI-TOF MS and MICs of carbapenems and colistin were determined by Etest and microdilution when necessary, following EUCAST guidelines. Detection of genes encoding OXA-type carbapenemasas (-51, -23, -24, -58 and -153) and metallo-beta-lactamases (NDM, VIM and IMP) was performed by PCR. Pulse-field gel electrophoresis was used to study the clonal relatedness of all isolates and MLST analysis was performed on selected isolates.

Results

Acinetobacter spp. were mainly isolated from sputum (52.0 %) and urine (16.3%). 50.0% were obtained from ICU patients and 19.4% from outpatients. MALDI-TOF MS identification revealed that *A. baumannii* (92.8%) was the most prevalent *Acinetobacter* species whereas A. pitti (5.1%), A. johnsonii (1.0%) and A. dijkshoorniae (1.0%) accounted for 7.1% of the isolates. Carbapenem resistance was not observed among non-baumannii isolates. Carbapenem non-susceptibility among *A. baumannii* was 74.7% and associated with the presence of OXA-type carbapenemases. The blaOXA-23 and blaOXA-24 genes were detected in 21.9% and 54.9% of *A. baumannii* isolates, respectively. blaOXA-51 was present in all *A. baumannii* isolates. The presence of MBL was not detected and all strains were susceptible to colistin. PFGE analysis identified 14 different pulsotypes (A-N).

Conclusions

Our study revealed predominance of *A. baumannii* among *Acinetobacter* spp. recovered from clinical samples in Azerbaijan. Carbapenem resistance was high among *A. baumannii* isolates and linked to the production of OXA-type enzymes. OXA-24 was associated with the clonal spread of two genetically related major clones between all three hospitals that accounted for 50% of all isolates. OXA-23 was linked to three smaller clonal groups that were more restricted to particular centres. Non-baumannii *Acinetobacter* isolates were susceptible to carbapenems. The present findings suggest an endemic nosocomial situation due to OXA-24-producing *A. baumannii* clonal lineages in Azerbaijan. Fortunately, colistin resistance was not yet detected.

Bacteria from extreme environments: analysis of *Acinetobacter* communities from the Acquarossa River (Viterbo, Italy).

Carolina Chiellini, Elisangela Miceli, Camilla Fagorzi, **Luana Presta (Italy),** Giovanni Bacci, Giovanna Bianconi, Francesco Canganella, Renato Fani.

Extreme environments represent a source of bacterial strains showing interesting characteristics related to the production of antimicrobial compounds and to resistance pattern towards toxic substances. An environment is defined extreme when shows non-standard conditions related for example to temperature, pressure, humidity, nutrients and presence of radiations. The Acquarossa river (Viterbo, Italy) can be considered an extreme environment since high iron and arsenic concentration characterize it. Along the river course, red and black biofilms grow close to each other on the rock surfaces, maintaining a well-defined borderline. Samples of both black and red epilithic biofilms were collected to characterize bacterial communities. The 40% of total isolated bacterial strains (77 out of 191) belong to *Acinetobacter* sp. and represent the 6% of red epilithon, and 56% of black epilithon. *Acinetobacter* strains were divided into 12 different RAPD haplotype; none of the detected haplotype was shared between red and black epilithon thus suggesting that the structure of the *Acinetobacter* community is different in the two kind of epilithic biofilm. Resistance patterns towards heavy metals (Ni, Cu, As, Fe, Zn and Cd), highlighted that all strains grow on arsenate zinc and cadmium (up to 25 mg/ml) and copper (up to 10 mg/ml); most of the strains grow on Arsenite (up to 2.5 mg/ml) and iron (up to 10 mg/ml). Interestingly, strains from red epilithon shows better growth on zinc respect to strains from black epilithon. Resistance patterns towards antibiotics (Kanamycin, Streptomycin, Ciprofloxacin, Tetracycline, Chloramphenicol and Rifampicin) highlighted that all strains are sensitive to Rifampicin and Ciprofloxacin, while they all grow on Chloramphenicol (up to 10 mg/ml). Most strains grow on Tetracycline (up to 1.25 mg/ml), Kanamycin (up to 1 mg/ml) and Streptomycin (up to 5 mg/ml). The cross-streak test evidenced that strains don't have any inhibitory activity both against other strains from the same samples, and against strains from different samples. Finally, only two of the isolated *Acinetobacter* strains show the presence of plasmid. Further analysis will highlight the possible interaction of *Acinetobacter* strains with the other bacterial genera, in order to understand their involvement in characterizing the two different epilithic bacterial communities.

Comparative Genomic Characterization of Whole genome sequences of Clinical and environmental Strains of Multidrug-Resistant *Acinetobacter baumannii* Isolated from Morocco.

Tarek Alouane (Morocco), Jean Uwingabiye, Mostafa Elouennass, Azeddine Ibrahimi.

Acinetobacter baumannii has emerged as an important nosocomial pathogen worldwide. Here we present the whole-genome sequences of four clinical and five environmental strains of multidrug-resistant *Acinetobacter baumannii* isolated from Morocco. The library of genomic DNA was prepared using a Nextera XT DNA library preparation kit (Illumina), with dual indexing adapters and the MiSeq-Illumina platform was used for the whole genome sequencing. Analysis of annotation results using antibiotic resistance genes databases, revealed the presence of multiple genes encoding for resistance to beta-lactams, aminoglycosides, fluoroquinolone-aminoglycosides, Macrolide-Lincosamide-Streptogramin B, sulphonamide, Phenicol and tetracycline. Most of these genomes are deposited into GenBank/EMBL under BioProject ID PRJEB15065.

Outer membrane protein A contributes to antimicrobial resistance of *Acinetobacter baumannii* through the OmpA-like domain.

Mi Hyun Kim, Hyo Il Kwon, Shukho Kim, Man Hwan Oh, Yoo Jeong Kim, Young Ho Jeon, **Je Chul Lee (Korea).**

Acinetobacter baumannii outer membrane protein A (AbOmpA) is involved in the bacterial pathogenesis. However, the role of AbOmpA in the antimicrobial resistance of *A. baumannii* has not been fully elucidated. This study aimed to investigate the role of OmpA-like domain of AbOmpA in the antimicrobial resistance of *A. baumannii*. The minimum inhibitory concentrations (MICs) of antimicrobial agents against the wild-type *A. baumannii* ATCC 17978, âˆ†ompA mutant, OmpA-like domain-truncated (amino acids 223-356) AbOmpA mutant and single-copy ompA-complemented strain were determined by the E-test method. Multi-drug resistant 1656-2 strain and its isogenic âˆ†ompA mutant were used to determine the role of AbOmpA in the antimicrobial resistance. The âˆ†ompA mutant strain of the ATCC 17978 was more susceptible to trimethoprim (> 5.33-fold) and other antimicrobial agents tested (< 2-fold), except tigecycline, than the wild-type strain. The MICs of gentamicin, imipenem and nalidixic acid against the ATCC 17978 and its âˆ†ompA mutant strains were decreased in the presence of an efflux pump inhibitor. The âˆ†ompA mutant strain of 1656-2 was more susceptible to trimethoprim (> 4.0-fold) and other antimicrobial agents, including colistin, imipenem and tetracycline (< 2-fold), than the wild-type strain. A mutant strain of ATCC 17978 with the OmpA-like domain-truncated AbOmpA was more susceptible to substrates of the RND efflux pumps, including aztreonam, gentamicin, imipenem and trimethoprim, than the wild-type strain. This study demonstrates that AbOmpA contributes to the antimicrobial resistance of *A. baumannii* ATCC 17978 through the OmpA-like domain.

Dif modules in *Acinetobacter* plasmids contain antibiotic resistance and other genes flanked by inversely-oriented pdif sites.

Grace A. Blackwell (Australia), Ruth M. Hall.

A dif site in the chromosome or in a plasmid (with additional accessory sequence) is important in the resolution of dimers of their respective DNA molecules. dif sites are 28 bp long and contain 11 bp binding sites for the XerC and the XerD tyrosine recombinases, separated by a 6 bp spacer. In plasmids from *Acinetobacter*, dif-like sites, or pdif sites, have been identified, and inversely-oriented pdif sites surround the oxa24 carbapenem resistance gene in different plasmids.

We assembled pS30-1, an 18.2 kb plasmid which confers resistance to tetracycline and erythromycin, from the whole genome sequence of *A. baumannii* isolate SGH0823 from Singapore. The Rep protein of pS30-1 is 91% aa identical to RepAci3. Eight pdif sites were identified in this plasmid and the tet39 determinant and the msrE-mphE gene pair are each surrounded by two pdif sites in inverse orientation. Identical regions in different contexts were found in a number of different plasmids in GenBank, showing that the tet39 and msrE-mphE dif modules are mobile. A putative toxin/antitoxin system, a gene encoding a serine recombinase and open reading frames of unknown function were also part of dif modules in pS30-1. Many previously unnoticed pdif sites were found in published plasmids revealing that they contain multiple dif modules and that modules with internal XerC or XerD sites generally alternate. Apparently, plasmids from *Acinetobacter* are able to exploit the chromosomally-encoded XerC-XerD recombinases to mobilise DNA units (dif modules) containing antibiotic resistance and other genes, via an uncharacterised mechanism.

Two copies of ISAjo2-1 (94% identical to ISAjo2) in pS30-1 were inserted 5 bp from a XerC site. Several related IS were found adjacent to a pdif site in published plasmids, and this appears to be the preferred insertion site for this IS group.

Evaluation of the contribution of several putative efflux pumps and OMPs to antimicrobial resistance in *Acinetobacter baumannii* AB5075.

Clara Cosgaya (Spain), Maria Nieto-Rosado, Ignasi Roca, Jordi Vila.

Introduction
Intrinsic resistance due to low permeability, increased efflux of antibiotics or the interplay of both may play a substantial role in the feared multidrug-resistant phenotype of *A. baumannii*. While the contribution of RND efflux pumps to antimicrobial resistance has been deeply studied, evidence about the involvement of OMPs and other transport protein families remains scarce. *A. baumannii* AB5075 is a clinical well-characterized strain showing multidrug resistance and an exceptional genome plasticity.

In this study, we have assessed the contribution of different putative transport proteins in the multidrug resistance phenotype of AB5075 using a collection of transport protein mutants.

Methods
AB5075 mutant strains were acquired from a transposon (T26, tetracycline resistance) mutant library (http://www.gs.washington.edu/labs/manoil/baumannii.htm). T26 insertion at the desired loci was verified by PCR using external primers and a transposon-specific primer in those strains growing in LB agar plates with 5 mg/L tetracycline. Antimicrobial susceptibility was tested by broth microdilution or E-test; significant differences between wild-type (WT) and mutant strain MICs were considered when ≥3 dilution differences in at least two replicates were observed. Fitness assays were done in 96 well-plates using antibiotic-free LB broth at 37â„*f* with orbital shaking during 24 h. OD600 was measured every 15 min with a microplate spectrophotometer.

Results
61 out of 82 mutant strains grew under tetracycline pressure and presented a transposon insertion at the right locus. Of those, the T26 insertion truncated genes belonging to: OMPS (n=8), the ABC transporter family (n=11), the MATE family (n=3), the MFS family (n=24), and the RND family (5). The remaining 10 strains presented an insertion in genes with a putative transport function. 17/61 mutants showed altered susceptibility when compared to the WT strain although 4 of them had impaired fitness. We observed decreased MICs for meropenem, levofloxacin, moxifloxacin, kanamycin, amikacin, tobramycin, and gentamicin, being the MICs for the latter decreased in 10/17 mutants. Interestingly, an increase in tigecycline, moxifloxacin and amikacin's MICs was also detected for some mutants. Overall, 9/16 mutants showed altered susceptibility to more than one antibiotic, although in only 4 of them different antibiotic families were affected.

Conclusions
All transport protein families studied contributed to antimicrobial resistance in *A. baumannii* AB5075, at least one mutant from each family showed altered susceptibility. The antibiotics most commonly affected were aminoglycosides, especially gentamicin.

Despite the presence of multiple antibiotic resistance mechanisms, we could still assess the involvement of some transport proteins in the multidrug-resistant phenotype of *A. baumannii* AB5075. For instance, decreased MICs of meropenem were observed in two mutant strains, despite carriage of the blaOXA-23 gene, and were not related to truncation of RND proteins. Likewise, MIC variations of levofloxacin and moxifloxacin were observed despite the WT strain being highly resistant to quinolones due to gyrA/parC mutations.

The unexpected increase of tigecycline and moxifloxacin's MICs is currently being studied using efflux pumps inhibitors to assess if the higher resistance observed is due to a compensative overexpression of other efflux pumps. These results suggest the contribution of novel membrane transporters to the resistant phenotype of *A. baumannii*, although complementation studies are still needed to corroborate their particular role.

Overexpression of efflux pumps in persister cells from *Acinetobacter baumannii.*

Stephanie Wagner Gallo (Brazil), Antonio Frederico Michel Pinto, Diógenes Santiago Santos, Cristiano Valim Bizarro, Carlos Alexandre Sanchez Ferreira, Sílvia Dias de Oliveira.

Bacterial persistence is associated with therapy failure of several chronic infections. However, the molecular mechanisms involved in the formation and maintenance of persistence in *Acinetobacter baumannii*, an important pathogen responsible for several healthcare-associated infections, are still poorly understood. In this context, using a proteomic approach, we investigated efflux pumps proteins associated with persistence after meropenem exposure in a clinical *A. baumannii* strain (Acb1) cultured under planktonic and biofilm conditions. Acb-1 was previously characterized as susceptible to meropenem (MIC of 1 mg/mL) and to other sixteen antimicrobials, but able to form high persister levels after meropenem exposure. Nano-liquid chromatography coupled to tandem mass spectrometry was used to relatively quantify efflux pumps proteins after 48h and 96h of meropenem exposure. A control sample without drug treatment was used as the reference condition. The components of the AdeABC efflux system, AdeA and AdeC, were overexpressed in persisters from both cultures during both times analyzed. Moreover, MacB, from the MacAB-TolC efflux pump, was found to have its expression increased exclusively in biofilm persisters. Considering that Acb-1 was susceptible to a range of antimicrobials, the overexpression of efflux pumps may constitute an alternative survival strategy of the *A. baumannii* persisters in the presence of antimicrobials.

DNA uptake by clinical multidrug resistant *Acinetobacter* spp.

Sara Domingues (Portugal), Natasha Rosário, Daniela Neto, Kaare Magne Nielsen, Gabriela Jorge Da Silva.

Background
Horizontal gene transfer events provide the basis for the extensive dissemination of antimicrobial resistance traits between bacterial populations. Gene transfer by natural transformation has been described for a wide number of clinical strains; however natural competence is expected to be more prevalent than what it is known so far. The lack of laboratory protocols that can adequately reproduce competence-triggering conditions in clinical settings is considered to be the main limiting factor.

Objectives
The aim of this study was to determine to what extent multidrug resistant *Acinetobacter* spp. clinical isolates are naturally transformable using different protocols and compare their influence on the ability to detect competence.

Methods
Three established transformation protocols, two in liquid media and one associated with motility in semisolid media, were used to test the natural transformability of 22 isolates collected over a 16-year time period, from five different geographical and/or distinct Portuguese Hospitals.

Results
Liquid transformation protocols revealed between zero (0%) and nine (41%) isolates that were naturally competent for DNA uptake, while 14 isolates (64%) were competent during surface-motility on semisolid media. Overall, 16 (73%) of the tested *Acinetobacter* spp. isolates (*A. baumannii*, A. nosocomialis and one *Acinetobacter* sp.) were identified as naturally competent.

Conclusions
Naturally competent *Acinetobacter* spp. isolates were found in all tested hospitals. Our study demonstrates that the majority of the clinical strains of *Acinetobacter* spp. are naturally competent for exogenous DNA uptake, an important feature for resistance dissemination in this setting. The potential for natural transformation in clinical settings can only be meaningfully described through multiple approaches.

Expression of the AdeRS two-component system of *Acinetobacter baumannii* ATCC 17978 under different physiological conditions.

Kai Lucaßen (Germany), Stefanie Gerson, Kyriaki Xanthopoulou, Harald Seifert, Paul G. Higgins.

Objectives

Multidrug resistance is common in *A. baumannii* and is often mediated by efflux pumps, especially through resistance-nodulation-cell division (RND) efflux pump systems. The RND efflux pump AdeABC is regulated by the 2-component regulator AdeRS. Mutations or disruption of the gene has been shown to affect adeABC expression. However, regulation of adeRS expression has until now not been investigated. The objective of this study was to investigate the expression of adeRS under different physiological conditions.

Methods

Expression of adeRS was determined by beta-galactosidase reporter assays. This was performed by putting the expression of the reporter gene under the control of the adeR or adeS promoter. Physiological conditions tested were growth on solid media (LB and Mueller Hinton agar) and motility plates supplemented with X-gal. Gene expression was detected by blue colonies. The influence of different antimicrobial agents was tested by disc diffusion and by gradient plates.

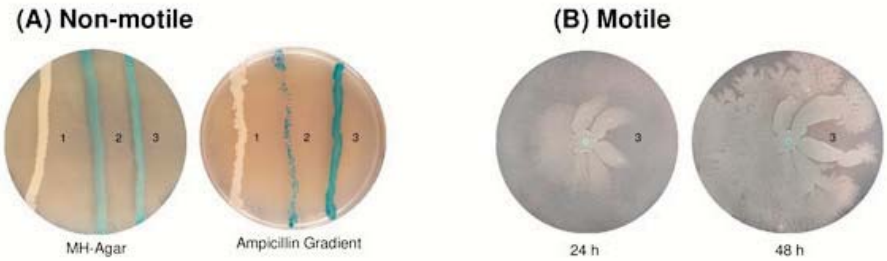
Results

Co-transcription of adeRS was demonstrated by RT-PCR. Therefor only adeR was investigated in further experiments. beta-galactosidase reporter assays revealed constitutive expression of adeR in the presence of the tested antimicrobials piperacillin, ampicillin, ampicillin/sulbactam, cefotaxime, piperacillin/tazobactam, ceftazidime, trimethroprim/sulfamethoxazole, ciprofloxacin, moxifloxacin, amikacin, gentamicin, tobramycin, cefepime, cefuroxime, meropenem, ertapenem, imipenem and kanamycin. However, growth on motility plates showed differences in expression of adeR compared to solid media. After 24 h incubation only the site of inoculation was blue while the migrating fringe was white. After 48 h incubation, the newly created migration fringe was white whereas the formerly white region turned blue (Figure 1). This indicates that motility inhibited expression of adeR. A lacZ-adeA construct also showed that the efflux pump was not expressed under motility conditions.

Conclusion

adeR was constitutively expressed under all tested growth conditions on solid media. However, growth on motility plates suggests that expression of adeR is inhibited while the cells are motile. Since the expression of adeR facilitates the expression of the adeABC efflux pump, and thereby contributes to MDR it remains to be determined how this decreased expression pattern impacts on MDR phenotype.

Figure 1:
Comparison of *adeR* expression in
1: *A. baumannii* ATCC 17978 pIG14/09 (negativ control)
2: *A. baumannii* NIPH 60 pIG14/09::*adeR*
3: *A. baumannii* ATCC 17978 pIG14/09::*adeR*
between non-motile (A) and motile (B) growth conditions. Expression is shown by blue colonies. Constitutive expression of *adeR* under tested growth conditions on solid media. Growth on motility plates (Agarose 0,5%) revealed inhibition of *adeR* expression.



Action of a resveratrol derivative against *Acinetobacter calcoaceticus-baumannii*.

Marina Silveira Gregis Monteiro, Natália da Silva Cezar, André Arigony Souto,
Sílvia Dias de Oliveira (Brazil).

Infections caused by multidrug-resistant *Acinetobacter* calcoaceticus-baumannii (ACB) have shown limited therapeutic options, highlighting the need to find alternatives to treat these infections. In this context, redresv001, a derivative of resveratrol, was synthesized and investigated with regard to its antimicrobial activity alone and associated with tobramycin. Two ACB (B98 and B137) isolates presenting minimum inhibitory concentration (MIC) of 32 and 128 μg/mL for tobramycin, respectively, were analyzed and presented MIC values for redresv001 of >24 and 12 μg/mL, respectively. Interestingly, when B137 was exposed to sub-inhibitory concentrations of redresv001 (6 and 3 μg/mL) in association with tobramycin, the MIC values for this antimicrobial reduced to 32 or 64 μg/mL, and in B98, the tobramycin MIC was changed to 16 μg/mL in association with redresv001 at 12 or 64 μg/mL. Afterwards, the minimum bactericidal concentration (MBC) was determined, and a reduction of 1 log in the MBC to tobramycin was obtained using sub-inhibitory concentrations of redresv001 in combination with this antimicrobial. The action anti-biofilm of the redresv001 was also evaluated and we found that this derivative was able to decrease the intensity of the biofilms even in sub-inhibitory concentrations. Therefore, redresv001 was able to reduce the concentration required to prevent bacterial growth in association with tobramycin, as well as the biofilm intensity formed, indicating this derivative as a possible adjuvant in the treatment of infections caused by ACB.

Influence of nutrient sources in the *Acinetobacter calcoaceticus-baumannii* persisters levels.

Bruna Kern Donamore, Stephanie Wagner Gallo, Carlos Alexandre Sanchez Ferreira,
Sílvia Dias de Oliveira (Brazil).

Acinetobacter calcoaceticus-baumannii (ACB) is a common agent of health care-associated infections, whose treatment may become challenging due to their ability to form persister cells. Although the mechanisms of formation and regulation of persisters have not been completely elucidated, the availability of different nutrient sources may be an important interference factor. Therefore, we investigated the influence of different carbon sources in persisters levels by exposing ten ACB strains to tobramycin in the presence of galactose (a metabolite that enters upper glycolysis) or sodium citrate (a metabolite that enters lower glycolysis). Fractions of surviving cells obtained with sodium citrate as sole carbon source ranged from 0.00001% to 0.11267% after 48h-exposure to tobramycin, whereas galactose promoted fractions of not detectable to 0.03184%. Interestingly, we observed that galactose promoted a significant reduction of persister cells levels in the first hours of tobramycin exposure. This reduction may have been caused by an increased proton motive force generation, which was already found to be necessary for aminoglycoside uptake and killing of these cells. Considering this, the administration of combined therapy - with specific antimicrobial-metabolite association - could be a promising strategy to eradicate these cells.

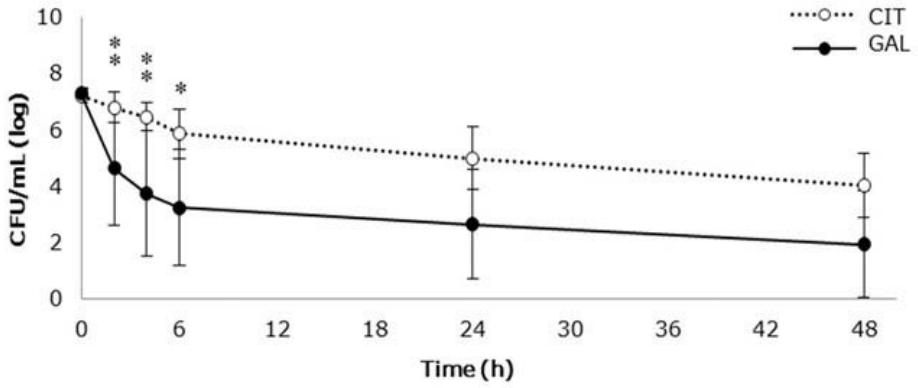


Figure. Killing curve representing the average of all ten isolates in each time evaluated after 48 h exposure to tobramycin in minimal medium broth supplemented with 10 mM of sodium citrate (CIT) or galactose (GAL) (statistically significant differences: * $p < 0.05$ and ** $p < 0.01$). Bars represent the difference between averages of all ten isolates.

Acinetobacter baumannii ability to survive and form biofilms after long periods under desiccation on hospital surfaces.

Itziar Chapartegui González (Spain), María Lázaro Díez, Zalora Bravo del Hoyo, Santiago Redondo Salvo, José Ramos Vivas.

Background
The importance of the gram-negative rod *Acinetobacter* as the cause of nosocomial infections has increased during the last years. *Acinetobacter baumannii* has been extensively studied because infections caused by this pathogen have been associated with high morbidity and mortality rates. It was recognized that at least some clinical A. baumannii strains are able to survive for long periods under dry conditions, allowing these microorganisms to survive in the hospital environment for a long time increasing thus its probability of causing infections in the hospital settings.

Objectives
The aim of the present work was to study both the survival capacity and the biofilm formation ability of five *Acinetobacter baumannii* clinical isolates under desiccation conditions, on surfaces typically found in the hospital environment (plastic, glass and lab coat) up to 2 months. Also, after those starvation conditions, we assess the sensitivity of strains to human serum as well as its infectious capacity in the animal modelGalleria mellonella.

Methods
For this work we selected 5 *Acinetobacter baumannii* clinical isolates. Viable counts present on inert surfaces under unfavorable conditions were determined up to 60 days by colony counting. Also, cell viability was determined with a Live/Dead staining and CLS Microscopy. Biofilm formation ability along time was assessed in 24-well plates by crystal violet staining. To determine human serum sensitivity, isolates were tested against different serum concentrations (25% and 50%). As animal model, Galleria mellonella, was used.

Results and conclusions
Survival and biofilm formation capacities of *A. baumannii* were strain-dependent. Although all isolates showed a significant decline of culturable count after desiccation, for some strains a viable cell subpopulation is maintained beyond 2 months. Live/Dead staining corroborated these results.

Optimization of effective disinfectant concentration against carbapenem resistant strain of *Acinetobacter baumannii.*

Deepika Biswas (India), Vishvanath Tiwari.

Acinetobacter baumannii a member of an "ESKAPE" family, has been considered as a prevalent pathogen and identified as a hospital-acquired infection described by Infectious Diseases Society of America (IDSA). Antibacterial resistance *A. baumannii* strains spread among critically ill, immunocompromised patients, and subsequent epidemics, raising concern in the medical community. Carbapenem is one of the most effective antibiotics against it. However, its increased resistance to carbapenems has been a rising global concern. Since, antibiotics such as carbapenem are unable to use on hospital setups to eradicate *A. baumannii* therefore, different concentrations of disinfectants for example phenol, sodium hypochlorite, ethanol and hydrogen peroxide; are increasingly being used. The present study highlighted that efficacy of disinfectants even in low concentration have shown better antimicrobial activity. Present result confirms that 0.5% phenol, 0.5% sodium hypochlorite, 60% ethanol and 2.5% hydrogen peroxide are effective concentration against *Acinetobacter baumannii* strains such as RS 307, RS 6694, RS 6053, 7434, 10953, 122 and ATCC strains. Bacterial proteins were extracted by sonication followed by its quantification by Bradford protein assay. Trichloroacetic acid (TCA) precipitation was performed and examined using 1-D and directly using 2-D Difference Gel Electrophoresis. We have also shown that a significant increase in the rate of reactive oxygen species (ROS) generation was observed when treated with 0.5% phenol, 1% sodium hypochlorite, 70% ethanol and 2.5% hydrogen peroxide, as compared to untreated of same all strains of A. baumannii and also confirmed by demonstrated the induced lipid peroxidation and protein carbonyl formation. These results will help in selecting effective and cost effective disinfectant and its concentrations for regular use in the hospital setups to eradicate*A. baumannii*.

Evolution towards increased carbapenemase activity in *Acinetobacter baumannii.*

Yuiko Takebayashi, Dimitri Chirgadze, Philip J. Warburton, **Benjamin A. Evans (UK).**

Introduction

The OXA-51-like beta-lactamases are a large group of enzymes intrinsic to *Acinetobacter baumannii*, with 190 variants currently represented in sequence databases. In the absence of an acquired carbapenemase, *A. baumannii* is known to be able to achieve clinical resistance to carbapenem antibiotics by increasing expression of their OXA-51-like beta-lactamase driven by a promotor provided by the insertion sequence ISAba1. However, not all A. baumannii are carbapenem-resistant, and there is evidence that different OXA-51-like enzyme variants confer different levels of resistance. This study investigated the structural basis and evolution of carbapenem resistance conferred by different OXA-51-like variants.

Methods

The enzyme variant OXA-66 was cloned and purified, and used for solving the enzyme crystal structure. The genes for the variants OXA-64, -65, -66, -69, -71, -107, -108, -110 and -111 were cloned into the plasmid pYMAb2 and inserted into E. coli DH5α, *A. baumannii* CIP70.10 and *A. baumannii* BM4547, and the MICs of the carbapenems were measured. The nucleotide sequence for OXA-66 was used to identify all OXA-51-like sequences present in the NCBI genome sequence database. These were extracted, duplicates removed, aligned, and a maximum likelihood phylogeny estimated.

Results

The crystal structure of OXA-66 was solved to a resolution of 2.1 Å and was consistent with that for OXA-51 that has recently been solved. The MICs of meropenem and imipenem showed a greater than 2-fold increase for only three enzyme variants: OXA-107, -108 and -110. These variants carry differences from consensus at positions 129 (OXA-110) and 167 (OXA-107 and -108). Phylogenetic analysis of all 190 known OXA-51-like variants suggests that substitutions at position 129 may have occurred on 10 independent occasions, and at position 167 on 8 occasions, with no enzyme carrying substitutions at both positions. Furthermore, substitutions at positions 226, 222 and 130, previously shown to be responsible for increased carbapenemase activity, may have occurred on 2, 4 and 14 separate occasions.

Conclusion

Amino acid substitutions surrounding the enzyme active site are responsible for increasing the ability of certain OXA-51-like enzyme variants to hydrolyse the carbapenems, resulting in decreases in phenotypic susceptibility. These substitutions have occurred on many separate occasions, particularly within International Clones 1, 2 and 3, representing widespread evolution towards reduced carbapenem susceptibility in *A. baumannii* independent of acquired carbapenemases.

Plasmid-Mediated-Quinolone-Resistance and blaCTX-M-group2-producing XDR *Acinetobacter baumannii* isolated from LCR of hospitalized patients in Brazil.

Anelise Stella Ballaben, Renata Galetti, Leonardo Neves de Andrade, Doroti de Oliveira Garcia, Paulo da Silva, **Ana Lucia da Costa Darini (Brazil).**

Acinetobacter baumannii is undoubtedly one of the most successful pathogens responsible for hospital-acquired nosocomial infections such as meningitis. The aim of this study was to characterized molecularly beta-lactam and quinolones resistant *A. baumannii* recovered from cerebrospinal fluid and blood of patients with meningitis in different hospitals in São Paulo state, from 2011 to 2014. These bacteria were isolated at Ribeirao Preto and Sao Paulo Adolfo Lutz Institutes, two reference laboratories. The genes conferring resistance to beta-lactams and quinolones antibiotics were investigated by PCR. The major plasmid incompatibility groups among Enterobacteriaceae as well as plasmid groups among *A. baumannii* were carried out by PCR-based replicon typing (PBRT and AB-PBRT, respectively). Furthermore, MLST analysis was performed according to Oxford scheme. Thirteen *A. baumannii* (specie confirmed by blaOXA-51 and rpoB sequencing) were studied. 10/13 isolates presented blaOXA-23-like which is widespread among this pathogen. According to MLST analysis, 9 sequence types (ST) were detected: two singletons ST407 and ST690, three CC are involved, considered responsible for the worldwide spread of antibiotic, ST225 and ST438 (CC103), ST231 and ST442 (CC109), ST227, ST233 and ST783 (CC113). Four isolates presented blaCTX-M-group 2 that has rarely been found in *A. baumannii*. CTX-M-2-producing isolates were first described in 2002 in a Japanese hospital, 2004 in Bolivian hospitals, 2006 in the USA, 2012 in Argentina and 2013 German hospitals. According to the literature, there is only one report of CTX-M-2-producing *A. baumannii* in 2004 in Brazil. Two of four CTX-M-group 2-producing isolates in this study were correlated to CC103, one to CC109 and one was classified as singleton (ST407). All isolates produced plasmid-mediated quinolone resistance genes (PMQR); qnrS and qnrB were detected among 8 and 5 isolates, respectively. Therefore, plasmid colE was detected in all isolates as well, which is commonly associated with PMQR. In 7 isolates *A. baumannii* plasmids groups (AB-GR) were identified (AB-GR2, AB-GR4, AB-GR6 and AB-GR8), being AB-GR6 the most prevalent in our study as well as in Europe countries, Australia and North Africa. Hence, those results with clinical isolates of *A. baumannii* demonstrate the ability of these pathogens acquire mobile genetic elements, such as plasmids which can strongly contribute to the widespread of resistance genes. Acknowledgements: FAPESP 2014/14494-8; FAPESP 2015/23484-9; PNPD/CAPES

Class D β-lactamase-mediated resistance to carbapenem antibiotics in *Acinetobacter baumannii.*

Sergei Vakulenko (USA), Marta Toth, Clyde Smith, Nichole Stewart.

Amongst the various β-lactamases, carbapenemases are of the greatest concern as they render bacteria resistant to the last resort carbapenem antibiotics, which have now been used extensively for the treatment of diverse life-threatening infections caused by bacteria resistant to other classes of antimicrobial agents. Of particular importance is the recent dissemination of Carbapenem-Hydrolyzing class D β-Lactamases or CHDLs, which now play a prominent role in conferring resistance to carbapenem antibiotics in clinically important bacteria, including *Acinetobacter baumannii* and Enterobacteriaceae, transforming these bacteria into deadly pathogens. The wide dissemination and clinical importance of CHDLs have rejuvenated interest in elucidating the mechanism of their carbapenemase activity. We show that in acyl-enzyme intermediates of class D β-lactamases, acylated carbapenem antibiotics entirely fill the active site of CHDLs. This results in the expulsion of water molecules, including the putative deacylating water, from positions they occupy in the active sites of apo enzymes. Since the entrance to the active site is obstructed by the acylated antibiotic, water molecules expelled from the active site upon acylation must take a different route for exit. We show that in OXA-143 the movement of a conserved hydrophobic valine residue on the surface opens an alternative channel to the active site of the enzyme. This allows for the exchange of water molecules between the active site and the milieu and creates extra space for a water molecule to position itself in the vicinity of the scissile bond of the acyl-enzyme intermediate to perform deacylation. These data complemented with structural analysis of other class D carbapenemases, strongly suggest that all CHDLs may employ a mechanism upon which the movement of conserved valine or leucine residues juxtaposed on their surface would allow a water molecule to access the active site to promote deacylation. Recognition of a universal deacylation mechanism for CHDLs provides new insights for the future development of inhibitors and novel antibiotics for these clinically important enzymes.

Characterization of OXA-143-like encoding plasmids in *Acinetobacter baumannii* clinical isolates.

Kyriaki Xanthopoulou (Germany), Julia Ertel, Alessandra Carattoli, Harald Seifert, Paul G Higgins.

Objectives
Carbapenem resistance in *Acinetobacter baumannii* is most frequently conferred by the presence of OXA-type carbapenemases which can be both chromosomally and plasmid-encoded. The present study aimed to characterize OXA-143-like encoding plasmids and the genetic environment of the *bla*OXA-143-like subclass among *A. baumannii* clinical isolates from different geographical regions.

Material/methods
Total DNA was prepared from two clinical *A. baumannii* isolates from Brazil recovered in the years 2004 and 2008, and one isolate from Honduras, recovered in 2009, using the MagAttract HMW DNA Kit (Qiagen, Germany). Sequencing libraries were prepared using the Nextera XT library prep kit for a 250bp paired-end sequencing run. Whole genome sequencing was performed on MiSeq (Illumina, USA) and de novo assembly was performed by SPAdes and Velvet. Plasmid assembly and predicted gaps were confirmed and filled by PCR-based gap closure using total DNA and/or plasmid DNA prepared with the PureYield Plasmid Midiprep System (Promega, USA). To investigate the molecular epidemiology of the isolates, a core-genome MLST (cgMLST) scheme including a core-genome of 2390 alleles was defined using the Ridom SeqSphere+ v.3.0 software using *A. baumannii* ACICU as reference genome. Genome sequences were used to determine traditional seven-loci MLST.

Results
The two isolates from Brazil were designated ST1552 (Oxford typing scheme) and did not cluster with any international clones (IC). By cgMLST these two isolates differed by 63 alleles and harbored OXA-143-like on plasmids with highly similar scaffolds. The plasmids with a size of about 4 kb encoding *bla*OXA-143 and the variant *bla*OXA-231, differing by one amino acid substitution from OXA-143, carried a replicase of the GR19 type and harbored in addition mobilization proteins. The *A. baumannii* isolate recovered from Honduras was designated ST1551 (Oxford typing scheme) and was assigned to IC5. This isolate was epidemiologically unrelated to the other OXA-143-like encoding isolates as it differed by cgMLST analysis in about 1900 alleles. The *bla*OXA-253, differing by 17 amino acid substitutions from OXA-143, was located in a 9.3 kb plasmid with a completely different scaffold, including a replicase of the GR12 type. Furthermore, the *bla*OXA-253 plasmid carried a Ton-dependent receptor involved in iron uptake, components of a toxin-antitoxin (TA) system, ensuring plasmid stability and maintenance during bacterial cell division, and a septicolysin-like gene coding for a pore-forming toxin.

Conclusions
The present study describes the plasmids involved in the dissemination of the OXA-143-like carbapenemase, a class D β-lactamase in *A. baumannii*. Our results demonstrate that OXA-143-like was found on two different plasmid types, discernable by the replicase genes. The *bla*OXA-253-encoding plasmid harbored a more complex set of genes including the TonB-dependent receptor and components of a TA system. *bla*OXA-143- and *bla*OXA-231-encoding plasmids presenting a highly similar genetic composition were found in unrelated *A. baumannii* isolates recovered within a four years' time span. This evidence suggests that these plasmids may support the spread of the OXA-143-like carbapenemase.

High diversity mechanism of carbapenem resistant *Acinetobacter baumannii* in Algerian Hospital.

Mohamed Azzedine Bachtarzi (Algeria), Fazia Djennane, Damien Fournier, Katy jeannot, Mohamed Tazir, Patrick plésiat.

Purpose

Every year, more than two hundred strains of carbapenem resistant *Acinetobacter baumannii* (CRAB)are isolated from different samples and different department in our hospital. We propose to characterize a part of them to demonstrate the high diversity mechanism of these CRAbs.

Materiel and methods

Thirty three (33) non-repetitive CRAB isolated from different department on 2015-2016 have been selected for molecular characterization. These strains have been screened by PCR for the most described Carbapenemases in *Acinetobacter baumannii* such Oxacillinases OXA23 , OXA24 and Oxa58 and class B carbapenemases mainly NDM, VIM and IMP. All molecular characterization for these strains was done on the CNR of Pseudomonas et *Acinetobacter* of Besancon; France.

Results

The carbapenemase OXA 23 was the most found in our collection, 23/33 (69, 69%) CRAB produces this carbapenemase. The NDM arrive at the second position 8/33 (24,24%). One of the NDM producers CRAB co-produced an OXA23 carbapenemase. These kinds of strain are rarely described in our region. The sequencing of intrinsic oxacillinase (OXA51) of the co-producer strain (OXA23/NDM) showed an OXA64 variant while the sequencing of the NDM producers showed an OXA94 variant. Finally, two strains (6%) produce an OXA 72 carbapenemase, a variant of OXA24 also rarely described in our region. No Oxa58 producer was found in this study.

Conclusion

This preliminary study shows the high diversity mechanism of carbapenem resistant *Acinetobacter baumannii* in our hospital. A molecular characterization on a larger sample could show other mechanisms.

Carbapenem-resistant *Acinetobacter baumannii* in Brunei Darussalam.

Muhd Haziq F Abdul Momin (UK), Dr Paul G Higgins, Dr David W Wareham.

Background

South East Asia increasingly reports a high prevalence of multi-drug resistance (MDR) in Gram-negative bacteria. *Acinetobacter baumannii* is the most common pathogen associated with nosocomial infections in this region responsible for urinary tract infections, endocarditis, surgical site infections, meningitis, septicemia, and ventilator associated pneumonia among intensive care unit (ICU) patients in hospitals. Resistance to carbapenems usually considered as drugs of last resort for treatment and it is an emerging problem which limits therapeutic options. Similarly, *A. baumannii* infections in Brunei Darussalam present a public health challenge impacting on clinical care. Here we molecularly characterized mechanisms of resistance in a collection of MDR *A. baumannii* isolates recovered from a number of hospitals in Brunei.

Material/methods

One hundred and eighty seven MDR *A. baumannii* isolates were obtained from Microbiology Laboratory Brunei, over a 4-year period (May 2012 - February 2015). The majority of isolates were from Intensive Care Units recovered from a variety of clinical samples (respiratory, urine, wound and bloodstream infections).

Bacterial identification and antimicrobial susceptibility testing (MIC) were performed using the VITEK®2XL system (bioMérieux, Marcy-l'Étoile, France). Carbapenemase production was confirmed using the Blue CARBA assay and class D beta-lactamases detected by multiplex PCR. Plasmid-mediated 16s rRNA methylase aminoglycoside resistance genes, integrons and presenceof ISAba1 were sought by PCR and sequencing. Molecular typing was performed by RAPD and semi-automated Diversilab®system (bioMérieux, Nürtingen, Germany). Whole genome sequencing (Illumina MISeq) was used to characterise the entire resistome of the clonal isolates.

Results

All isolates were resistant to cephalosporins, carbapenems, aminoglycosides, quinolones and sulfonamides. All but 3 remained susceptible to polymyxins. Five indistinguishable profiles were identified by RAPD PCR and mapped to international clones 1and 2 (IC 1/2) using the Diversilab®system. Multi-drug resistance (MDR) in both clones was mediated by several resistance genes. The aacA4, CatB8 and aadA1 genes (aminoglycoside and chloramphenicol resistance) were also contained within class1 integron similar to the structure of In634 (GenBank accession no. AY123251). ISAba1 as the promotor was detected upstream of the OXA-23 carbapenemase and armA was the most common 16s rRNA methylase aminoglycoside resistance gene in all isolates. However, no IS elements were found upstream of OXA-51-like.

Conclusions

We report a very high prevalence of MDR *A. baumannii* causing clinically significant nosocomial infections Brunei hospitals. Isolates were members of IC 1 and 2 with carbapenem-resistance mediated by the OXA-23 carbapenemase. The multiple mechanisms of resistance explains the persistence within the Brunei healthcare system. Further active surveillance, antimicrobial stewardship programs and infection control measures are crucial and need to be actively implemented within hospitals.

Diversity of GR-plasmids in carbapenemase-producing *Acinetobacter baumannii* isolated from nosocomial infections in Brazil.

Júlia da Costa Darini, Leonardo Neves Andrade, **Ana Lúcia da Costa Darini (Brazil),** Renata Galetti.

Acinetobacter baumannii is one of the most important multi drug-resistant (MDR) bacteria isolated in hospital infections. Plasmid-mediated carbapenemases genes has also been associated to *A. baumannii*, mainly OXA-carbapenemases. We studied 50 carbapenem-resistant *A. baumannii* isolated from inpatients in a tertiary hospital in Brazil. We investigated the presence of extended-spectrum beta-lactamases (ESBL) genes (*bla*CTX-M groups 1, 2, 8, 9 and 25, *bla*GES, *bla*BEL, *bla*VEB, and *bla*PER) and carbapenemase genes (*bla*KPC, *bla*SPM, *bla*NDM, *bla*IMP, *bla*VIM, and *bla*OXA). The presence of the *bla*OXA-51 gene was used to confirm the identification of *A. baumannii* (Ab) species. Zone 1 of the rpoB gene was sequenced for the isolates carrying epidemiologically important carbapenemases. In this study, all the 50 *A. baumannii* studied presented *bla*OXA-51-like as expected. 37 of the 50 *A. baumannii* also presented the *bla*OXA-23-like gene whereas 6/50 presented *bla*OXA-143-like and 1/50 presented *bla*OXA-58-like. 11 isolates presented only *bla*OXA-51-like. Moreover, the most of isolates carried more than one *bla*OXA resistance gene: 32 isolates presented *bla*OXA-51-like and *bla*OXA-23-like, 4 presented the combination of *bla*OXA-51-like, *bla*OXA-23-like and *bla*OXA-143-like, 2 presented *bla*OXA-51-like and *bla*OXA-143-like and 1 presented *bla*OXA-51-like and *bla*OXA-58-like. The investigation of *bla*OXA-51 gene facilitates efficiently the identification of *A. baumannii*. No ESBL and other carbapenemase genes investigated were identified. For the 7 *A. baumannii* carrying *bla*OXA-143-like and/or *bla*OXA-58-like we investigated the presence of 19 AB-GR plasmid groups using AB-PBRT. More than one AB-GR was found per isolate with exception of the isolate carrying *bla*OXA-58-like that presented only the GR3, being GR-3 followed by GR-8 group were the most frequently AB-GR detected. GR2, GR4, GR5 and GR13 groups were also identified. Interestingly, none of the isolates showed GR6 plasmid, which is the most widespread GR group across Europe, China and Taiwan, and most often associated with the *bla*OXA-23-like and *bla*OXA-58-like genes. The presence and wide variety of AB-GR plasmids certainly contribute to dissemination of plasmid-mediated *bla*OXA genes. The broad circulation and diversity of AB-GR plasmids could also facilitate the spread of other resistance genes beyond *A. baumannii* species. Thereby, plasmids surveillance and characterization in bacteria other than Enterobacteriaceae could improve knowledge and control of antimicrobial resistance. Acknowledgment: PIBIC/CNPq, PNPD/CAPES, FAPESP - 2014/14494-8 and2015/11728-0.

Multidisciplinary approach to understand carbapenem resistance mechanism in *Acinetobacter baumannii* and assessment of alternate therapeutics.

Vishvanath Tiwari (India).

Acinetobacter baumannii causes nosocomial infections and its prevalence in clinical setup has increased gradually. *A. baumannii* has emerged resistance against carbapenem (commonly prescribed drug), which is a significant health problem and responsible for high morbidity and mortality. We have identified the carbapenem resistance mechanism of the *Acinetobacter baumannii* using proteomics, biophysics and bioinformatics, microbiological and molecular biology techniques. Phenotyping, genotyping and quantitative proteomics studies concluded the presence of different carbapenem hydrolyzing ß-lactamase (OXA-51, AmpC and NDM), efflux pumps and upregulation of metabolism in carbapenem resistant strain. Further, the bacterium also down regulates putative OmpW and other transporter that decreases the uptake of carbapenem. OXA-51 was cloned and purified. The recombinant OXA-51 has secondary structure that is very resistant to pH and temperature change. This plays a vital role in retaining function of this β-lactamase under stress conditions. We have also signified the use of novel excitation at 305nm for monitoring the surface tryptophan of protein. Bioinformatics studies on the modeled β-lactamase showed that carbapenem is effectively hydrolyzed by OXA-51 or NDM harboring *Acinetobacter*. We have also pointed out that old ß-lactam such as penicillin might be better antibiotic for NDM-harboring *A. baumannii*. We are also trying to find the suitable nano-based, herbal-based and in-silco design alternative medicine against carbapenem resistant strain of *A. baumannii*.

XerC/D site-specific recombination mediates plasmid plasticity and dissemination of blaOXA-58 containing structures in *Acinetobacter baumannii* strains isolated in Argentina.

Maria Marcela Cameranesi, Jorgelina Moran-Barrio, Guillermo Repizo, Adriana Limansky, **Alejandro M Viale (Argentina).**

Objectives

Plasmids are considered key factors in the dissemination of multi-drug resistance determinants (MDR) among resident pathogens in the clinical setting. The presence of XerC/D-like sites flanking discrete DNA modules in plasmids carrying blaOXA genes present in clinical *A. baumannii* strains suggests that carbapenem resistance dissemination may be mediated by a XerC/D site-specific recombination mechanism (1,2). We studied this possibility in plasmids isolated from clonally-related MDR *A. baumannii* clinical strains of Argentina.

Methods

The MDR carbapenem-resistant *A. baumannii* clinical strains Ab242 and Ab825 (assigned to CC104, Oxford nomenclature) were isolated from inpatients in a public hospital of Rosario, Argentina. Plasmid DNA from each strain was extracted and subjected to 454 pyrosequencing, and the obtained sequences were characterized by database searching (BLAST), organized, and the sequence verified by PCR with specific primers. The ability of the identified plasmids to spread antimicrobial resistance was analyzed by transformation of susceptible *Acinetobacter* strains followed by imipenem selection and plasmid analysis on resistant clones.

Results

Sequencing analysis disclosed the presence of novel plasmids both in Ab242 (designated pAb242_25, pAb242_12, and pAb242_9) and Ab825 (designated pAb825_34 and pAb825_12). In silico analysis identified the presence of several XerC/D-like sites in these plasmids. In particular, in pAb242_25 XerC/D sites were found flanking a novel genetic structure carrying both blaOXA-58 and aphA6 resistance genes. Remarkably, this structure was found in an opposite orientation in pAb825_34, strongly suggesting an inversion process mediated by the identified XerC/D-sites. Moreover, pAb825_34 was found to represent a concatemer structure constituted by plasmids pAb242_9 and pAb242_25 generated by XerC/D site-specific recombination. Finally, transformation assays of susceptible *Acinetobacter* strains employing plasmid DNA from Ab242 followed by plasmid analysis of the transformants revealed a novel concatemer formed by pAb242_25 and pAb242_12, also generated by XerC/D mediated recombination. The latter result also suggested that the concatemer represented the transforming structure.

Conclusions

The overall results indicate that XerC/D site-specific mediated recombination is a relevant mechanism of plasticity among *A. baumannii* plasmids and responsible for the mobilization of blaOXA-58 resistance structures. These results add to the plethora of dissemination mechanisms of resistance genes among MDR *A. baumannii* in the clinical setting.

(1) Poirel and Nordmann. 2006. Antimicrob Agents Chemother 50:1442–1448.
(2) Merino et al. 2010. Antimicrob Agents Chemother 54:2724–2727.

Elucidating regulatory pathways controlling the expression of RND pumps in *Acinetobacter baumannii*: more pieces added to the puzzle?

Ayush Kumar (Canada).

AdeIJK and AdeFGH are two multidrug resistance efflux pumps of *Acinetobacter baumannii* that are responsible for its reduced susceptibility to a number of clinically relevant antibiotics. Expression of AdeIJK is controlled by a TetR family repressor, AdeN, and that of AdeFGH by a LysR-family repressor AdeF. Using genetic, genomic, transcriptomic, and proteomic approaches, we show that expression of both these pumps is controlled by factors other than their cognate regulators. In fact, our data suggest that both these pumps are under a two-tier regulatory control, where their cognate regulator provides a low level repression of expression whereas regulatory elements independent of their cognate regulators are responsible for much tighter repression.

We also show, using *Galleria mellonella* as a model, that both AdeN and AdeL are involved in modulating the virulence of *A. baumannii*. Intriguingly, the role of AdeN and AdeL in the virulence of *A. baumannii* is independent of the activity of AdeIJK or AdeFGH pumps, respectively.

In summary, our data suggest that both AdeIJK and AdeFGH efflux pumps of *A. baumannii* are a part of intricate regulatory networks that control the antibiotic resistance and virulence of *A. baumannii*.

Investigation of pmrB mutations and potential novel colistin resistance mechanisms in clinical *Acinetobacter baumannii* isolates.

Stefanie Gerson (Germany), Julia Ertel, Stephan Göttig, Harald Seifert, Paul G. Higgins.

The lack of antimicrobial agents effective against multidrug-resistant MDR *Acinetobacter baumannii* strains led to the reintroduction of the polymyxin antibiotic colistin. However, resistance to colistin (COL) has been reported among clinical *A. baumannii* isolates, complicating the treatment of infections significantly. This study aimed to investigate the mechanisms of colistin resistance in *A. baumannii* obtained from patients hospitalized in Germany, Spain and Greece between 2012 and 2016.

Methods

Resistance to COL was determined by agar dilution as well as microbroth dilution and interpreted as >2 mg/l using EUCAST resistance breakpoints for *Acinetobacter* spp. *A. baumannii* isolates were investigated as isolate pairs (n=4), which were defined as two isolates obtained from the same hospital, usually but not exclusively from the same patient, that displayed a phenotype shift from low-to-high COL MICs and were identical by rep-PCR. The isolate pairs were subjected to whole-genome sequencing (WGS) by MiSeq using 2x250bp paired-end run and Ridom SeqSphere+ v.3.0 software was used for de novo assembly and comparison of the assembled genomes to the reference strain *A. baumannii* ACICU. All isolates within an isolate pair displayed less than 10 allele differences. Gene expression of pmrABC was analysed by qRT-PCR.

Results

Three isolate pairs belonged to the international clonal lineage IC2 and originated from Sevilla, Spain (n=2) and Athens, Greece (n=1), while the fourth isolate pair from Frankfurt, Germany belonged to IC4. In the four isolates with high COL MICs, three amino acid substitutions (A28V, I232T and S17R) and a four amino acid deletion (âˆ†L9-G12) were found in PmrB (Table 1). Analysis of gene expression revealed that the amino acid substitutions A28V and S17R as well as the four amino acid deletion led to a significant increase (up to 70-fold) in pmrABC expression. In contrast, the amino acid substitution I232T was associated with an unchanged or decreased pmrABC expression, suggesting a different resistance mechanism in this isolate. Here, an additional allele difference was detected in the guanosine polyphosphate pyrophosphohydrolase/synthase (SpoT) (Table 1). SpoT synthesizes guanosine tetraphosphate and has been previously described to be involved in antibiotic resistance [1]. Furthermore, in isolate pair no. 4 the amino acid substitution T3I was detected in a putative membrane protein (Table 1). It has a high similarity (>95%) to the DedA family of membrane proteins, which have been suggested to be involved in resistance to cationic peptides in Salmonella enterica and Neisseria meningitidis [2]. No mutations or disruptions were found in genes encoding porins or in genes involved in the lipid A biosynthesis.

Table 1: Overview of isogenic *A. baumannii* isolate pairs. Colistin MICs were determined by microbroth dilution. Allele differences were detected by comparison of the amino acid sequences within an isolate pair.

Isolate pair no.	Isolate	Origin	Clonal lineage	COL MIC [mg/L]	Allele differences		
					PmrB	SpoT	membrane protein
1	MB_2 MB_6	Sevilla, Spain	IC2	2 256	Ã¢Ë†â€ L9-G12		
2	MB_23 MB_177	Sevilla, Spain	IC2	2 256	I232T	G575V	
3	MB_90 MB_119	Athens, Greece	IC2	32 >256	A28V		
4	SG3161 SG3166	Frankfurt, Germany	IC4	1 256	S17R		T3I

Conclusion

Two novel pmrB mutations were found to be associated with pmrABC overexpression and COL-resistance. Both the amino acid substitution in SpoT and the putative membrane protein suggest novel colistin resistance mechanisms in *A. baumannii* and further investigation will elucidate the impact in clinical isolates.

[1] Wu J, Long Q, Xie J (2010) (p)ppGpp and Drug Resistance. J. Cell. Physiol. 224:300-304
[2] Doerrler WT, Sikdar R, Kumar S, Boughner LA (2013) New Functions for the Ancient DedA Membrane Protein Family. J. Bacteriol. 195:3-11

Analysis of colistin resistant determinants in *Acinetobacter baumannii* from bacteremia using whole genome sequencing.

Saranya Vijayakumar (India), Nithin Sam, Balaji Veeraraghavan.

Objective:
The aim of this study is to characterize resistance mechanisms associated with colistin resistance in *A. baumannii* using whole genome sequencing.

Materials and Methods
A total of 8 colistin resistant clinical isolates of *A. baumannii* collected from blood stream infections between the years of January 2015 to 2016 were studied. All were selected for whole genome sequencing (WGS) to identify single nucleotide mutations that could be responsible for reduced colistin susceptibility using Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies) according to manufacturer''92s instructions. The raw sequence was assembled *de novo* using AssemblerSPAdes v5.0.0.0 embedded in Torrent suite server version 5.0.3. The annotation was performed using PATRIC database and NCBI Pipeline. The presence of acquired mcr-1 / mcr-1.2 / mcr-2 genes and the sequence type of the isolates were determined using Centre for genomic epidemiology (CGE) online tool. Targeted sequencing was performed using Genetic analyser 3500 to confirm the mutations within the genes obtained from WGS. The sequenced results were analysed using BioEdit software.

Results and discussion
All the 8 colistin resistant *A. baumannii* clinical isolates had MIC ranged between 8 – mg/ml. Mutations were identified within *pmr*B, *lpx*A and *lpx*D genes. Interestingly, 6 novel mutations were observed within the *lps*B gene. Similarly a single novel mutation was observed within *lpx*A and *lpx*D genes. No mutations were seen within *pmr*A and *lpx*C genes (Table 1). Mutations within the *lps*B gene were confirmed by targeted sequencing. Plasmid mediated mcr-1 / mcr-1.2 and mcr-2 genes were absent in all the isolates. Of the 8 colistin resistant isolates, ColR 1 to 6 belongs to ST848, ColR 7 belongs to ST451 and ColR 8 belongs to ST1305.

Table 1. Mutations observed in 8 colistin resistant clinical isolates of *Acinetobacter baumannii*

Isolate ID	Colistin MIC (µg/ml) by broth micro dilution	*lpx*A	*lpx*C	*lpx*D	*lps*B	*pmr*A	*pmr*B	Sequence Types
ColR 1	8	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 2	32	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 3	32	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 4	64	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 5	64	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 6	64	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	848
ColR 7	64	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	451
ColR 8	16	Y131H*	No mutations	K4Q* E117K	Q216K*, H218G* S219E*, L329V* I331T*, H334N*	No mutations	A444V	1305

Conclusion
Previous studies identified that, colistin resistance among clinical isolates of *A. baumannii* is associated with mutations in the two component regulatory system, *pmr*AB and loss of lipopolysaccharide by mutations in lipid A biosynthesis genes. In this study, A444V and E117K mutations within *pmr*B and *lpx*D genes respectively were found to be concordance with previous studies. Novel mutations within *lps*B gene was confirmed by targeted sequencing in all the 8 colistin resistant isolates. However, the significant contribution of this novel mutations to colistin resistance will be proved later by cloning the wild and the mutated type *lps*B gene in an expression system.

Constraint-based modeling identifies new putative targets to fight colistin-resistant *Acinetobacter baumannii* infections.

Luana Presta (Italy), Emanuele Bosi, Leila Mansouri, Lenie Dijkshoorn, Renato Fani, Marco Fondi.

Acinetobacter baumannii is a clinical threat to human health, causing major infection outbreaks worldwide. As new drugs against Gram-negative bacteria do not seem to be forthcoming, and due to the microbial capability of acquiring multi-resistance, there is an urgent need for novel therapeutic targets. Here we have derived a list of new potential targets by means of metabolic reconstruction and modelling of *A. baumannii* ATCC 19606. By integrating constraint-based modelling with gene expression data, we simulated microbial growth in normal and stressful conditions (*i.e.* following antibiotic exposure). This allowed us to describe the metabolic reprogramming that occurs in this bacterium when treated with colistin (the currently adopted last-line treatment) and identify a set of genes that are primary targets for developing new drugs against *A. baumannii*, including colistin-resistant strains. It can be anticipated that the metabolic model presented herein will represent a solid and reliable resource for the future treatment of *A. baumannii* infections.

False positive phenotypic detection of metallo-beta-lactamases in *Acinetobacter baumannii*.

Branka Bedenić (Croatia), Ranko Ladavac, Mirna Vranic-Ladavac, Nada Barišić, Natalie Karčić, Antun Ferencic, Slobodan Mihaljević, Luka Bielen, Nataša Beader, Haris Car

Background and aim
Acquired carbapenem resistance in *A. baumannii* can be mediated by acquired carbapenemases of class A (KPC), class B metallo-b-lactamases (MBLs) of IMP, VIM, SIM and NDM family and class D carbapenem-hydrolyzing oxacillinases (CHDL) (OXA-23-like, OXA-40-like, OXA-58-like, OXA-143-like and OXA-238-like. Inhibitor based test with EDTA are often used to detect MBLs in *A. baumannii*. The aim of the study was to investigate the sensitivity and specificity of inhibitor based tests with EDTA in detection of MBLs in *A. baumannii*.

Material and methods
In total 100 carbapenem-resistant *Acinetobacter baumanii* strains collected in General hospital Pula (27), University Hospital Zagreb (39), General Hospital Varaž'9edin (4), General Hospital Sisak (3), University Hospital Merkur (1), Clinic for Infectious diseases (6), General Hospital Bjelovar (1) and Godan nursing home in Zagreb (19) during 2009-2014 from various clinical specimens, were analyzed. The susceptibility to a wide range of antibiotics was determined by disk-diffusion and broth microdilution method. Phenotypic detection of metallo-b-lactamases was performed by combined disk test with addition of EDTA and by broth microdilution test with addition of fenantroline and EDTA (0.5 mM). Augmentation of inhibition zone aroung imipenem and meropenem disk for at least 5 mm in the presence of EDTA was considered as a positive results in disk diffusion while 8 fold (three dilutions) reduction of imipenem or meropenem MIC by EDTA was indicative for production of MBL in dilution test. Genes encoding metallo-b-lactamases (IMP, VIM, NDM, SIM) and carbapenem hydrolyzing oxacillinases were detected by PCR.

Results
All strains were found to be resistant to carbapenems with MICs of imipenem and meropenem ranging from 8 ->128 and from 16->128, respectively. Twenty seven strains were positive for *bla*OXA-23-like, 52 for *bla*OXA-40-like and 21 for *bla*OXA-58-like genes. Eleven of *bla*OXA-23-like positive isolates coharboured *bla*VIM-1 gene. Sequencing of representative *bla* genes revealed the presence of *bla*OXA-23, *bla*OXA-40, *bla*OXA-72, *bla*OXA-58 and *bla*VIM-1 genes.

Combined disk test with EDTA and dilution test with EDTA were positive in all 11 isolates possessing VIM-1 MBL. However, phenotypic testing was positive in 14 out of 16 isolates positive only for OXA-23 (87%), in 11 of 21 strain positive for OXA-58 (52%) and in all OXA-40 harbouring isolates (100%). The augmentation of inhibition zone in the presence of EDTA ranged from 10 to 22 mm.

Conclusions: False positive results in inhibitor based tests can occur because in the presence of EDTA oxacillinases can be converted to a less active state leading to the augmentation of the inhibition zone around the carbapenem disk or reduction of carbapenem MICs. This phenomenon was previously reported in *A. baumannii* and *P. aeruginosa*. The rate of false positive results was the highest in OXA-40 positive isolates and the lowest in OXA-58 positive isolaes which can be explained by the better hydrolysis of carbapenem substrates by OXA-40-like b-lactamases. The study showed high sensitivity but low specificity of phenotypic methods in detection of MBLs in *A. baumanii* and points out the necessity of molecular detection of MBL genes in *A. baumannii* by PCR.

Detecttion of drug resistance genes reserviores in TnAbaRs and R plasmids and study of adeRS and baeSR regulatory systems on AdeABC efflux pump among *Acinetobacter baumannii* clinical isolates with decreased susceptibility to tigecycline.

Mohammad Savari (Iran), Abbas Bahador, Alireza Ekrami.

Background

The Rise of *A. baumannii* strains with decreased susceptibility to tigecycline, recently became one of the complications of treatment. Such strains are also resistant to almost all other conventional antibiotics because of rapid acquisition of resistance genes reservoirs in genomic structures including AbaR and R plasmids. Decreased susceptibility to tigecycline is associated with increased expression of an efflux pump called AdeABC. This efflux pump be regulated by a two-component regulatory system named AdeRS. Recently, another two-component system; BaeRS also has been implicated in this affair. The aim of this study was to detecttion of drug resistance genes reserviores in TnAbaRs and R plasmids and study of AdeRS and BaeRS regulatory systems on AdeABC efflux pump among *Acinetobacter baumannii* clinical isolates with decreased susceptibility to tigecycline.

Materials and methods

One hundred and twenty isolates of *A. baumannii* isolated from burn patients, were tested for decreased susceptibility to tigecycline and then the minimum inhibitory concentration (MIC) of such isolates were determined against colistin, imipenem and meropenem. The antibiotics resistance patterns of these isolates then measured against 17 antibiotics and tested in terms of molecular epidemiology. By using PCR, more than 20 antibiotics resistance genes were tested in term of chromosomal location on AbaRs or R plasmids and the isolates were AbaR mapped. By using Real time PCR, *adeB* gene expression was evaluated in isolates with decreased susceptibility to tigecycline and were compared with a sensitive strain. The expression levels of adeRS and baeSR genes and their sequence in isolates with decreased susceptibility to tigecycline was evaluated and compared with a sensitive strains.

Results

40.8% of the studied isolates showed decreased susceptibility to tigecycline. GC2 clone was the most common clone and among the SGs; G9 was most frequent one. Isolates were clasified in 40 MLVA type, 4 clusters and 33 singleton and each of these isolates horbored 3 to 5 plasmid and they all were common in a 10kb plasmid. Genes associated with drug resistance were distributed between AbaR and R plasmid. All the isolates in this study were horboring new variants of AbaR. The *adeB*, *adeS*, *adeR*, *baeS* and *baeR* genes were overexpressed, in all isolates with decreased susceptibility to tigecycline in compare with the sensitive strain (reference). Sequencing of *adeS*, *adeR*, *baeS* and *baeR* genes in isolates with decreased susceptibility in compare to the reference strains revealed that all of them suffered multiple amino acid substitutions.

Conclusion

The majority of *A. baumannii* isolates with decreased susceptibility to tigecycline reserve the resistance gene reservoirs in AbaR and R plasmid. Both AdeRS and BaeSR two components systems in *A. baumannii* isolates with decreased susceptibility to tigecycline were overexpressed.

Comparison of three different methods for tigecyciline susceptibility testing in *Acinetobacter baumannii*.

Branka Bedenić (Croatia), Gordana Cavrić, Mirna Vranic-Ladavac, Nada Barišić, Natalie Karčić, Tatjana Tot, Aleksandra Presečki Stanko, Amarela Lukić-Grlić, Catherine Sreter, Sonja Frančkula-Zaninovć.

Background and aim

Acquired carbapenem resistance is an emerging problem in *A. baumannii* due to the production of acquired carbapenemases of class A (KPC), class B metallo-b'df-lactamases (MBLs) of IMP, VIM, SIM and NDM family and class D carbapenem-hydrolyzing oxacillinases (CHDL) (OXA-23-like, OXA-40-like, OXA-58-like, OXA-143-like and OXA-238-like. Tigecyline and colistin are often the last resort antibiotics for the treatment of infections associated with carbapenemase producing organisms. There are no guidelines established by CLSI or EUCAST for tigecycline susceptibility testing for *Acinetobacter baumannii* so far. In most studies breakpoins for Enterobacteriaecae are applied with susceptibility breakpoint of - 1 mg/L and resistance breakpoint of ³ 4 mg/L. The aim of the study was to compare the three different methods for the tigecycline susceptibility testing in *A. baumannii*: broth microdilution, E test and Vitek 2 automated method.

Material and methods

In total 74 carbapenem-resistant *Acinetobacter baumanii* strains collected in General hospital Pula, University Hospital Zagreb, General Hospital Varaž'9edin, General Hospital Sisak, University Hospital Merkur, Clinic for Infectious diseases, General Hospital Bjelovar and Godan nursing home in Zagreb during 2009-2014 from various clinical specimens, were analyzed.The susceptibility to tigecycline was determined by broth microdilution metod, E test and Vitek2 automated method. *Pseudomonas aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606 were used as quality control strains.

Results

According to EUCAST criteria for defining resistance with breakpoint of 4 mg/L, the resistance rate with Vitek 2 was 15% (11/74), with E test 23% (17/74) and with broth microdilution 46% (34/74). If broth microdilution method was considered as gold standard for antibiotic susceptibility testing, there are 23 and 17 resistant isolates classified as susceptible or intermediate susceptible by Vitek 2 and E test, respectively, demonstrating a major error of these two methods. Minor errors with susceptible isolates being identified as resistant or intermediate susceptible were not reported. MIC90 obtained by Vitek 2 and E test was 4 mg/L while broth microdilution method yielded MIC90 of 8 mg/L. MIC50 varied between 1 and 4 mg/L depending on the method.

Conclusions

Tigecycline testing by Vitek2 automated method yielded significantly lower MICs compared to broth microdilution or E test, yielding to a major error. Huge discrepancies were found between three methods, particularly between dilution method and Vitek2. According to our results Vitek2 testing does not provide reliable results and thus the results should be confirmed by E test or dilution method, particularly in the case of serious infections. Broth microdilution method is gold standard method but is laborious, time consuming and requires educated staff.

Acinetobacter baumannii survive anaerobic digestion of sewage sludge.

Jasna Hrenovic (Croatia), Marin Ganjto, Snjezana Kazazic, Blazenka Hunjak, Ana Kovacic, Svjetlana Dekic, Tomislav Ivankovic, Ivana Goic-Barisic.

Multi-drug resistant (MDR) *Acinetobacter baumannii* were found in untreated as well as in biologically or chemically treated hospital and municipal wastewaters. However, there is no evidence about the fate of *A. baumannii* in the wastewater treatment process. The aim of this study was to screen the sewage sludge after its stabilization by anaerobic mesophilic digestion for the presence of *A. baumannii.* On 9 sampling occasions at the municipal wastewater treatment plant of the City of Zagreb, 17 isolates of *A. baumannii* were recovered form digested sludge with MALDI-TOF MS score values ranging from 2.026-2.288. 3/17 isolates were sensitive to all antibiotics, while 14 MDR isolates shared the resistance to carbapenems and fluoroquinolones but sensitivity to colistin. Technology of anaerobic sludge digestion was performed at 36°'b0C, neutral pH and digestion time of 21-36 days. In laboratory controlled anaerobic conditions isolates were able to survive on Nutrient agar in anaerocultA system during 30 days, after which multiplied normally in aerobic conditions. However, isolates were not able to multiply directly in anaerocultA. The finding confirms the need of proper management and disposal of sewage sludge generated at wastewater treatment plants in order to prevent the spread of MDR *A. baumannii* in nature.

A novel genetic determination of lectin gene (lec gene) in Iraqi *Acinetobacter baumannii* isolates and using of lectin as antibiofilm agent.

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Fifty-one *Acinetobacter baumannii* isolates have been subjected to semi-quantitative and quantitative screening for detection lectin production by measuring the hemagglutination activity and found that 17/51(33.3%) isolates produced of lectin protein. A novel genetic detection for presence of lectin gene (*lec* gene) in *Acinetobacter baumannii* by designed primer and using PCR technique with the size of amplified fragment (624 bp) was done. Ten lec PCR products were sequenced and the data that were obtained were submitted to the gene bank National Center for Biotechnology Information (NCBI) under accession numbers (KX766405.1 and KX766406.1). Despite of these 17 isolates were phenotypically and genotypically positive to lectin ,but they showed different *lec* gene expression in semi-quantitative and quantitative analysis since in microtiter plate the hemagglutination activity ranged between 4-128U/ml and *Acinetobacter baumannii* w34 had higher titer 128U/ml of hemagglutination by utilizing O+ blood group.The spectrum of biofilm inhibition by the partially purified lectin for all tested bacteria revealed that in the presence of lectin the biofilm formation ability was significantly reduced to 26% for *Klebsiella pneumoniae* followed by *Pseudomonas stutzeri* and *Acinetobacter baumannii* with 46.7% and 53%.

Osmostress response of *Acinetobacter baumannii*: Role of multiple osmolyte transporter.

Jennifer Breisch (Germany), Izabela Waclawska-Krzeminski, Beate Averhoff.

Acinetobacter baumannii has become the most important nosocomial pathogen because of the increase in number of infections and emergence of multidrug-resistant strains. An extraordinary trait of *A. baumannii* is its ability to colonize almost any surface persisting desiccation and moisture. This is pointing towards very strong osmostress response mechanisms. To counteract high salt concentrations, many bacteria take up compatible solutes by betaine/choline/carnitine transporters (BCCT). Although information with respect of the role of compatible solutes in osmostress protection of *A. baumannii* is scarce, the presence of multiple potential BCC transporters suggests an impact in adaptation to osmostress.

Genome analyses of *A. baumannii* ATCC 19606 revealed four potential BCCTs, such as BetT1, BetT2, BetT3 and BetTX. Preceding functional studies of BCCTs of the non-pathogenic *A. baylyi* and sequence analyses allowed the classification of BetT1 as a osmostress independent and BetT2 as a osmostress dependent transporters specific for choline[1], which serves as precursor of the compatible solute glycine betaine, whereas BetT3 is suggested to be a betaine transporter[2]. Genome analyses of *A. baumannii* AYE, a more virulent clinical strain, revealed two additional BCCTs, designated BetTY and BetTZ.

To get insights into the role of the different BCCTs in osmostress protection we generated markerless mutants and performed mutant studies. These studies revealed that the BCCTs are important for osmostress protection. To unravel the substrate specificity of the different BCCTs the genes were heterologously expressed in *E. coli* and uptake studies were performed with radioactively labelled choline and glycine betaine. These studies revealed that BetTX is an osmostress independent choline transporter, whereas BetTY is an osmostress dependent glycine betaine transporter and BetTZ takes up glycine betaine independent of osmostress. Competition experiments suggest that the BCCTs also mediate the uptake of other substrates. These findings suggest that the BCCTs play distinct roles in osmostress protection but are also important for host adaptation.

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Transcriptional and physiological comparison of acetate catabolism between *Acinetobacter schindleri* ACE and *Escherichia coli* JM101.

Juan-Carlos Sigala (Mexico), Lucy Quiroz, Alvaro R Lara.

In nature, microorganisms are frequently exposed to dynamic changes in the availability of carbon sources and must, therefore, adapt quickly to these changing environmental conditions. A good example of this situation is when certain microorganisms first deplete acetogenic carbon sources from the environment and start to use acetate as a substrate, which is mainly considered a by-product. However, for some other microorganisms like *Acinetobacter*, acetate is a primary and common substrate. Gluconeogenic activities are required for the assimilation of acetate, which is considered a "'93poor carbon source"'94 when compared to other substrates such as hexoses and pentoses. Interestingly, *Acinetobacter* species generally grow slow on glucose, can not use pentoses but grow fast on acetate. For example, *E. coli* does not grow on acetate as fast as it does on glucose and the opposite situation occur in *Acinetobacter*.

In this work, we compare acetate catabolism of*A. schindleri* ACE and *E. coli* JM101 by quantifying the level of transcription of some relevant genes, and by means of the calculation of some cultivation parameters.

Cultivations were performed in a 1-l bioreactors containing 0.60 l of M9 media supplemented with sodium acetate and inoculated at an initial optical density of 0.1 at 600 nm. The reactor conditions were 30°'b0C for *A. schindleri* ACE and 37°'b0C *E. coli* JM101, pH of 7 and dissolved oxygen tension >20%. Commercial kits were used to isolated and purified total RNA and to synthesize cDNA. RT-qPCR was performed with the ABI Prism 7300 Real-Time PCR System (Applied Biosystems, USA) using SYBR Green Master Mix (Thermo Scientific, USA). The quantification technique used to analyze the data was the 2DDCq method described elsewhere.

Under these conditions, ACE strain had a specific growth rate of 0.93 h-1 and JM101 of 0.33 h-1. Additional cultivation parameters showed that the biomass yield of ACE on acetate was 2-fold higher than that of JM101, while the specific acetate consumption rate was 45% higher in ACE compared to that in JM101. When analyzing the relative transcrtiption levels, we found that ACE overexpressed important genes like *ackA, pta, aceA, glcB, fumA, tktA* and *talA*, while underexpressed *acsA, sfcA, ppc* and *rpiA*, compared to JM101 (fig. 1).

In conclusion, cultivation parameters showed a more efficient acetate catabolism from ACE than from JM101. In contrast to E. coli, ACE seems to form acetyl-CoA by the AckA-Pta pathway instead of Acs. Moreover, glyoxalate shunt could be more active in ACE than in JM101, which can avoid loss of carbon as CO2 in the former strain. Since ACE do not have genes of the oxidative part of the pentose phosphate pathway, ribose 5-P should be formed from trioses. According to this, talA and tktA genes are overexpressed in ACE compared to JM101.

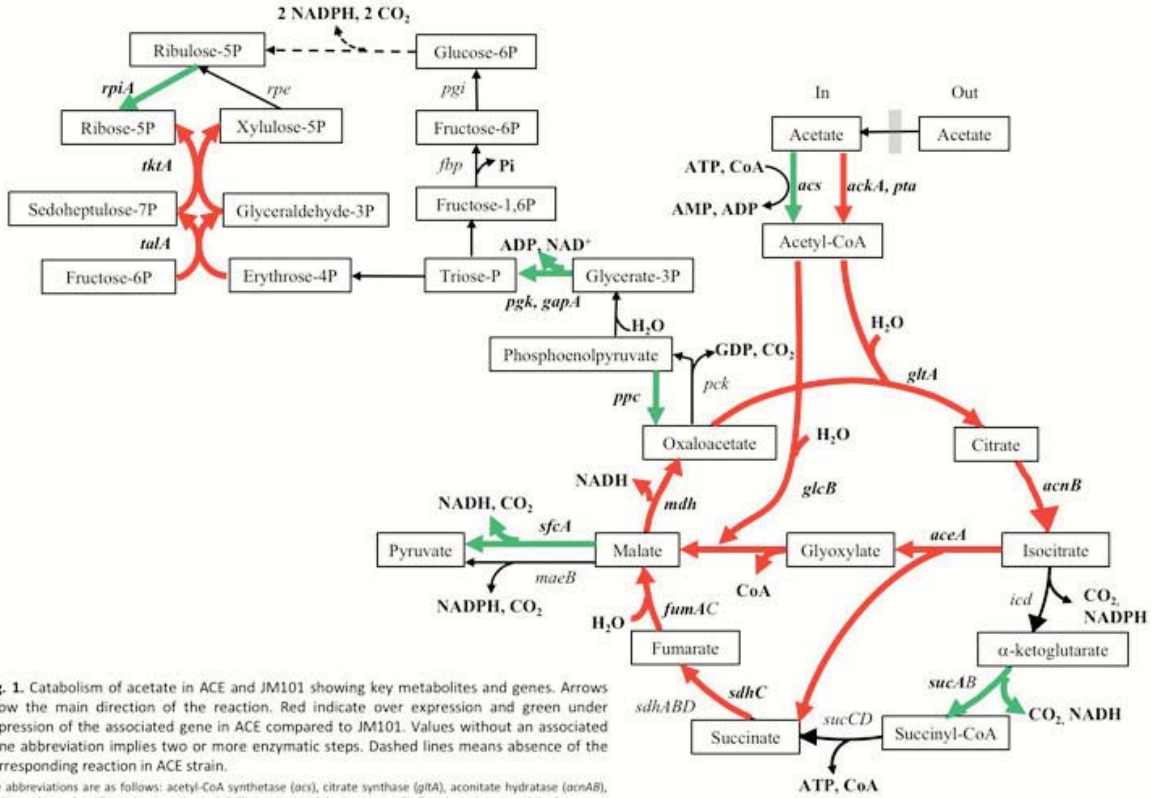

Fig. 1. Catabolism of acetate in ACE and JM101 showing key metabolites and genes. Arrows show the main direction of the reaction. Red indicate over expression and green under expression of the associated gene in ACE compared to JM101. Values without an associated gene abbreviation implies two or more enzymatic steps. Dashed lines means absence of the corresponding reaction in ACE strain.

The abbreviations are as follows: acetyl-CoA synthetase (*acs*), citrate synthase (*gltA*), aconitate hydratase (*acnAB*), isocitrate lyase (*aceA*), malate synthase (*glcB*), isocitrate dehydrogenase (*icd*), a-ketoglutarate dehydrogenase (*sucAB*), succinyl-CoA synthetase (*sucCD*), succinate dehydrogenase (*sdhABCD*), fumarate hydratase (*fumAC*), malate dehydrogenase (*mdh*), malic enzyme 1 (*sfcA*), malic enzyme 1 (*maeB*), phosphoenolpyruvate carboxykinase (*pck*), fructose-1,6-bisphosphatase (*fbp*), transaldolase (*tal*), transketolase (*tkt*), ribulose-phosphate 3-epimerase (*rpe*), ribose 5-phosphate isomerase (*rpi*).

The *Acinetobacter* DNA-(adenine N6)-methyltransferase AamA – an epigenetic regulator.

Ulrike Blaschke (Germany), Beneditta Suwono, Evelyn Skiebe, Stephan Fuchs, Gottfried Wilharm.

The *Acinetobacter baumannii* genome encodes a DNA-(adenine N6)-methyltransferase, designated A1S_0222 in strain ATCC 17978. DNA methyltransferases (MTases) transfer methyl groups from S-adenosyl-L-methionine (SAM) to adenine or cytosine bases. This process, termed DNA methylation, protects DNA against digestion from restriction endonucleases and is important for the regulation of various physiological processes such as mismatch repair and transcription with an impact on virulence as well as motility[3,4]. In bacteria the most studied epigenetic mechanism is DNA methylation which deals with heritable changes in gene expression without any changes in the DNA sequence[1,2]. We hypothesized the DNA adenine methyltransferase A1S_0222 to impose epigenetic control in *Acinetobacter baumannii* and approached its characterization.

Making use of the naturally competent *A. baumannii* strain 29D2 we inactivated the gene A1S_0222. Single-molecule real-time (SMRT) sequencing was performed on 29D2 wildtype and the A1S_0222 mutant for a comparative methylation pattern analysis. Moreover, 29D2 wildtype and the mutant were phenotypically characterized (e.g. antibiotic resistance profiles), the A1S_0222 protein was recombinantly produced and purified.

The recognition site of DNA adenine methyltransferase A1S_0222 could be identified as GAATTC. After SMRT sequencing, data were screened for m6A motifs. Inactivation of A1S_0222 resulted in the loss of the specific methylation pattern. Furthermore, the phenotypic characterization illustrates a deficiency in surface-associated motility of the mutant and an increased susceptibility to the tested antibiotics compared to the wildtype. The mutant also shows an attenuation in the *Galleria mellonella* infection model.

Taken together, the results demonstrate that A1S_0222 encodes a DNA adenine-methyltransferase which methylates the GAATTC sequence and seems to act without a corresponding endonuclease. Moreover, SMRT sequencing reveals differences in the methylation pattern of 29D2 wildtype compared to the mutant which may explain different phenotypic appearance. We propose the name AamA (Acinetobacter DNA adenine-methyltransferase A) for A1S_0222 and *aamA* for the gene.

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The search for environmental signals that trigger low water actvitiy response in *Acinetobacter baumannii.*

Josephine Hubloher (Germany), Sabine Zeidler, Beate Averhoff, Volker Müller.

The opportunistic nosocomial pathogen *Acinetobacter baumannii* is an increasing threat in hospitals worldwide. There are two main properties of *A. baumannii* which seem to be responsible for its success: multidrug resistance paired with the ability to survive for long periods of time under dry conditions [1, 2]. Our group studies the molecular basis of desiccation resistance in *Acinetobacter* and identified the compatible solutes glutamate, mannitol and trehalose that are produced in response to low water activities [3, 4, unpublished data]. The biosynthetic pathways for mannitol and trehalose in *A. baumannii* have been delineated, the enzymes involved and the encoding genes were identified [4, unpublished data]. Of special interest now is the question about the environmental signal that regulates expression of those genes. To this end, we have cloned the promoter of the mannitol-1-phosphate dehydrogenase/ phosphatase (*mtlD*) and the promoter of the trehalose-6-phosphate synthase (*otsA*) and trehalose-6-phosphate phosphatase (*otsB*) operon upstream of a promoterless b'df-glucuronidase gene on a plasmid. *A. baumannii* ATCC 19606 was transformed with the plasmids and activation of the *mtlD* and *otsAB* promoters was studied using b'df-glucuronidase as reporter enzyme. NaCl activated the *mtlD* promoter very strongly. Activation was concentration dependent with a maximum observed at around 400 mM NaCl. NaCl could be substituted by sucrose, KCl, Na-gluconate, Na-nitrate or Na-sulfate. Glycine betaine is a compatible solute taken up by *Acinetobacter* [5]. As uptake of compatible solutes is energetically preferred over *de novo* synthesis, the presence of glycine betaine in the medium usually represses the expression of genes for *de novo* synthesis. This was indeed observed for expression of *mtlD*. Furthermore, choline represses the activity of the *mtlD* promoter, but not exogenously supplied glutamate, trehalose, or mannitol. This is consistent with the observation that these compounds are not taken up by *A. baumannii*; they also do not support growth. Trehalose is only found in minor concentration in the cell, but the amount is clearly salt dependent [unpublished data]. The *otsAB* promoter was also stimulated by NaCl (or other osmolytes), but to a smaller extent. Interestingly, promoter activity was not regulated by glycine betaine, indicating a different kind of regulation of the *otsAB* promoter. This substantiates our conclusion that trehalose not only serves as a compatible solute in *A. baumannii* but also plays a role in other processes such as, for example, desiccation, persistence and infection.

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The signal peptide of KatG catalase-peroxidase directs functional folding and periplasmic localization in *Acinetobacter* sp. Ver3.

Mariana Sartorio, Alejandro Palavecino, **Néstor Cortez (Argentina).**

The *Acinetobacter* genus includes an extensive group of physiologically versatile bacteria occupying various natural ecosystems. Some species are emerging as model organisms due to their genome plasticity and the resulting adaptability to diverse environments.

Acinetobacter sp. Ver3 is a polyextremophile isolated from high-altitude Andean wetlands, able to grow under hostile environmental conditions such as intense UV radiation, high salt concentrations or extreme pH. Interestingly, total catalase activity in Ver3 cell-free extracts is about 15 times higher than those found in control collection strains. After genome pyrosequencing and gene annotation (http://rast.nmpdr.org), two related ORFs were identified, encoding a monofunctional catalase KatE and a bifunctional catalase-peroxidase KatG.

While all monofunctional catalases display a significant degree of structural conservation, Ver3-KatE exhibits one of the highest catalytic efficiencies reported to date (*k*cat = 1,29.107± 0,25.107 s-1). Hydrogen peroxide sensitivity assays in the presence of the KatE inhibitor 3-amino-1,2,4-triazole revealed a significant decrease of tolerance in Ver3 when compared to the response of several collection control strains. These results suggest a critical role of the cytosolic KatE in peroxide detoxification.

The *katG* gene encoding a bifunctional catalase-peroxidase was cloned and overexpressed as recombinant protein employing chaperone helpers in the bacterial host *E. coli* BL21 [pKJE7]. Protein sequence analysis using the algorithms JGI (img.jgi.doe.gov), PSORT (psort.hgc.jp) and SignalP (www.cbs.dtu.dk), suggests the presence of a targeting signal peptide of 19 residues. Subsequent subcellular fractionation of transformed *E. coli* cells expressing Ver3-KatG confirmed a periplasmic localization. When *katG* was expressed after removing the N-terminal leader sequence, the enzyme was unable to reach the periplasm. Moreover, the resulting protein product accumulated in the cytosol as an haem-bound inactive enzyme.

The results indicate that polyextremophile *Acinetobacter sp*. Ver3 takes advantage of both a cytosolic, highly efficient monofunctional catalase, and a bifunctional catalase-peroxidase as a periplasmic antioxidant barrier.

Identification of BfmR, a response regulator of *Acinetobacter nosocomialis*, and its roles on biofilm development and pathogenesis.

Eun Kyung Lee, Woon Young Song, **Man Hwan Oh (Korea).**

Biofilm development by bacteria is considered to be an essential stage in the bacterial infection. *Acinetobacter nosocomialis* is an important nosocomial pathogen causing a variety of human infections. However, characteristics and specific determinants of biofilm development have not been characterized in *A. nosocomialis*. In the present study, using a random transposon mutagenesis, we identified a biofilm defective mutant of *A. nosocomialis*, in which a transposon insertion inactivated an open reading frame encoding the BfmR in a two component regulatory system consisting of *bfmR* and *bfmS* encoding response regulator and sensor kinase, respectively. A mutational analysis and newly developed genomic complementation method demonstrated that BfmR is required for the initial attachment and maturation of biofilm during the biofilm development as well as the bacterial growth. Furthermore, the present study demonstrated that BfmR contributes to mouse mortality and the bacterial colonization as well as elicitation of immune response on the moue lung, suggesting that BfmR contributes to the overall success in the pathogenesis of *A. nosocomialis*. This study is the first report of BfmR associated with the biofilm development and pathogenesis of *A. nosocomialis*, and the importance as a potential drug target for *A. nosocomialis* infection.

Proteomic analysis of *Acinetobacter baumannii* SDF in biofilms: highlighting atypical adhesion-associated determinant.

Marion Nicol (France), Eun-Jeong Yoon, Annick Schaumann, Patrice Courvalin, Catherine Grillot-Courvalin, Emmanuelle De.

Acinetobacter baumannii has the natural ability to survive in hospital settings, a persistence that can be partially explained by its capacity to form biofilms. We studied, in a collection of 34 *A. baumannii* strains (1), the putative correlation between biofilm formation (biomass quantified by the crystal violet assay) and the presence in the genomes of various determinants usually associated with this phenotype, such as type 4 pilus (*pilA, pilD* & *pilT*), chaperon usher pilus (*csuA/BABCDE*), poly-N-acetylglucosamine production (*pgaABCD*), biofilm associated protein (Bap motif search) and the outer membrane protein A (*ompA*). Analysis of the data indicated that *A. baumannii* SDF, an antibiotic susceptible strain isolated from a human body louse (2), displayed an atypical profile with a high capacity to form surface-associated biofilms. This bacterium has a small genome (»'98 3050 open reading frames) with numerous mobile genetic elements which facilitate its environmental adaptation; most of its genes implicated in surface adhesion or virulence are disrupted, mutated, or absent such as several biofilm associated factors (*T4P*, *csu* and *pga*) (3). We compared by proteomic analysis the membrane protein profiles of this strain grown either in a planktonic or a biofilm state. We identified, by a label free quantitative method and nLC-MS/MS analysis, 54 proteins up-regulated in the 24-h biofilm mode of growth on glass wool. *A. baumannii* SDF adapted its cell envelope to the biofilm growth mode by overproducing proteins involved in iron acquisition, lipid storage, and phospholipids biosynthesis. It may promote its surface adhesion by synthesis of a new hemagglutinin/hemolysin protein encoded by genetic sequence absent in other *Acinetobacter* species. This suggests that molecular determinants required for *A. baumannii* biofilm formation are more diverse than proposed.

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Desiccation resistance in *Acinetobacter baumannii.*

Ngoc Dinh Ngu (Germany), Sabine Zeidler, Volker Müller.

Acinetobacter baumannii is an opportunistic human pathogen that is able to survive up to several months on dry surfaces [1]. When thriving at low water activities a cell has to combat loss of water to maintain cell turgor and function. Two strategies are known; accumulation of inorganic ions or the accumulation of compatible solutes [2]. Compatible solutes are small, highly soluble, organic molecules, that do not interfere with the central metabolism and can be accumulated up to molar concentrations. *Acinetobacter baylyi* accumulates the unusual polyol mannitol as a major compatible solute at high osmolarities [3]. The key enzyme for mannitol biosynthesis is a novel, bifunctional mannitol-1-phosphate dehydrogenase/phosphatase (MtlD) [4]. Comparison with its close relative *A.•baumannii* revealed that it also possesses a bifunctional mannitol-1-phosphate dehydrogenase/phosphatase (*mtlD*) gene. The goal of this study was to analyze whether the gene *mtlD* really encodes for a mannitol-1-phosphate dehydrogenase/phosphatase and to characterize its function, with a focus on a potential activation mechanism and its molecular basis. Therefore, the *mtlD* of *A. baumannii* ATCC 19606 was cloned into vector pET21a and heterologeously expressed in *Escherichia coli* BL21 (DE3). The enzyme was purified by affinity chromatography to apparent homogeneity. The purified enzyme indeed catalyzed the reduction of fructose-6-phosphate with NADPH to mannitol. NADPH could be substituted to some extent by NADH (19.4 %). Interestingly, enzymatic activity was completely abolished in the absence of salt. Activity was restored by addition of NaCl and increased with increasing NaCl concentrations. A maximum was observed at 0.6 M NaCl. NaCl could be substituted by KCl indicating that the nature of the cation is not important for activation. However, the nature of the anion had a pronounced effect on activity. At a total salt concentration of 0.5 M, activity was clearly chloride-dependent. At higher salt concentrations, the effect was not that strong; chloride could be substituted by gluconate and glutamate. Our data identified the *mtlD* gene of *A. baumannii* and proved that MtlD is a fructose-6-phosphate reductase (NADPH-dependent). Most important, the MtlD also acts as a sensor for high salt (low water activity). Thus, MtlD combines a sensor and a catalyst function.

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Co-regulation of DNA damage-inducible genes by UmuDAb and DdrR.

Megan Peterson, **Janelle Hare (USA).**

In many bacteria, DNA damage induces SOS genes that are normally repressed by LexA binding to a palindromic element in SOS promoters. In the genus *Acinetobacter*, multiple genes are induced by DNA damage, although the genus lacks LexA. However, a homolog of the error-prone polymerase accessory UmuD, UmuDAb, represses the transcription of multiple DNA damage-induced error-prone polymerases (*umuDC* genes), as well as *umuDAb* and *ddrR*. UmuDAb binds to a non-*E. coli*-like, palindromic SOS box that is present in the putative promoter region of the divergently transcribed *umuDAb* and *ddrR* genes. Studies in *A. baylyi* showed that mutations in one part of the SOS box abolish repression of *ddrR* and *umuDAb* transcription, while mutations in another part of the SOS box prevent transcription even under DNA damaging conditions. To understand the relationship between the UmuDAb binding site and the promoters for these two genes, their transcriptional start sites were mapped using 5''92 RACE PCR. Two primers within each gene were used, and the resultant RACE products were cloned and sequenced. Potential +1 sites were located 29-30 bp upstream of the beginning of the *umuDAb* ORF, and 40 bp upstream of the beginning of the *ddrR* ORF. These +1 sites predict adjacent -35 promoter consensus elements for *umuDAb* and *ddrR*. Given that previous data showed a loss of transcription when the bases in this -35 consensus element region were mutated, it appears that UmuDAb can co-repress both genes because its binding to DNA blocks polymerase access to both of the *umuDAb* and *ddrR* promoters. Due to this co-regulation, and a conserved, divergently transcribed chromosomal arrangement in nearly all *Acinetobactcer* species, we next investigated whether *ddrR* was also required for regulation of *umuDAb* and *ddrR*. RT-qPCR studies from both *A. baylyi* and *A. baumannii* showed that *ddrR* mutation also de-represses the expression of *umuDAb* and itself, indicating a co-regulatory action shared with UmuDAb. RNA-seq experiments are underway to examine whether this co-regulation of *umuDAb* and *ddrR* extends to the all genes of the previously identified UmuDAb regulon, as well as whether DdrR may regulate additional genes distinct from the UmuDAb regulon.

Antibiotic Treatment Modulates Protein Components of Outer Membrane Vesicles of Mutidrug-resistant Clinical Strain, *Acinetobacter baumannii* DU202.

Edmond Changkyun Park, **Sung Ho Yun (Korea),** Sang-Yeop Lee, Hayoung Lee, Chi Won Choi - Je Chul Lee, Sangmi Jun, Gun-Hwa Kim, Seung Il Kim.

The outer membrane vesicles (OMVs) of *Acinetobacter baumannii* were known to be cytotoxic and to elicit a potent innate immune response. *A. baumannii* DU202 is extensively drug-reistant clinical pathogen, from which first *A. baumannii* OMV had been reported. In this stuy, we analyzed antibiotic effects on the characteristic changes of *A. baumannii* DU202 OMV. Treatment of high concentration of antibiotics (immipenem and tetracyclin) caused *A. baumannii* DU202 OMV to be more cytotoxic effect against A549 human lung carcinoma cells. Immipenem treatment increased production of OMV more than two fold and caused the quntitative change of proteases, outer membrane proteins, and inherent prophage proteins in *A. baumannii* DU202 OMV. Major phospholipids (PA, PE, PG, PI, PC) of *A. baumannii* DU202 OMV have differernt expression patterns between immipenem- and non antibiotic-treatment. This reuslt suggest that *in vitro* stress such as antibitoic treatemnt can influence biomolecule composition and pathogenic potential of *A. baumannii* OMV.

Clonal diversity of *Acinetobacter baumannii* isolates obtained from Iranian hospitals.

Parisa Aris (Iran), Morteza Karami-Zarandi, Mohammad Rahbar, Masoumeh Douraghi.

Background
Drug-resistant *Acinetobacter baumannii* isolates have been implicated in a variety of healthcare-associated infections. Having studied the distribution of *A. baumannii*, it has been recognized as a predominant pathogen in Iranian hospitals. The aim of this study was to assess the clonal diversity of *A. baumannii* isolates obtained from patients in different hospitals.

Methods
A total of 93 isolates of *A. baumannii* were recovered from clinical samples of patients admitted to one of five targeted hospitals (I –'96 V) in Tehran between May 2012 and to August 2013. Following identification and antibiotic susceptibility testing, the isolates were subjected to pulsed field gel electrophoresis (PFGE). The band patterns were analyzed by Gel compare II software using unweighted pair group method with arithmetic mean algorithm (UPGMA) and Dice coefficient.

Results
The majority of isolates (91 out of 93) were multi drug resistant (MDR). Eleven common types (CT), here are designated CT1-CT11, and 61 single type (ST) were found among studied isolates. All CTs contained 2 isolates except CT3 and CT4 which comprised of 7 and 6 isolates, respectively. The isolates belonging to CT3 are found in hospital I and shared between ICU, Respiratory ward, and Endocrine disorders ward. In addition, the time interval between first and last isolation of these isolates was 3 months. CT4 comprised of isolates obtained from different wards of Hospital V including ICU, General, and Emergency with time interval of 4 months.

Conclusion
This study revealed the circulation of several and distinct clones in Iranian hospitals. While there was no inter-hospitals dissemination, clonal relatedness was found between isolates from different wards of the same hospital.

An outbreak caused by multiresistant *Acinetobacter baumannii* with rarely found sequence type 502 and OXA-40 carbapenemase.

Ivana Goic-Barisic (Croatia), Martina Seruga Music, Ana Kovacic, Zana Rubic, Anita Novak, Marija Tonkic, Jasna Hrenovic.

Since 2009, University Hospital of Split (UHS) in Croatia has a growing problem in number of infections caused by carbapenem-resistant isolates of *A. baumannii,* now almost endemically present in most of intensive care units inside the hospital. Here, we report a new outbreak in the Neurology Intensive Care Unit (NICU) that started in March 2017, after a transfer of patient from a hospital in a neighbouring state.

First isolate of *A. baumannii* with unusual resistant pattern (susceptible to imipenem but resistant to meropenem) was collected in the beginning of March 2017, from tracheal aspirate of the patient with brain stroke transferred from General Hospital of Livno (Bosnia and Herzegovina). This isolate was resistant to meropenem, gentamicin, amikacin, ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole, but susceptible (MIC 1.5 mg/L) to imipenem, colistin (0.19 mg /L) and ampicillin/sulbactam (2 mg/L). In the next eight weeks, 10 new isolates with same resistant pattern were isolated, also from respiratory specimens (tracheal aspirates and BALs) from NICU and Pulmonary Department inside University Hospital of Split.

Isolates were collected and subjected to further multilocus sequence typing analyses according to MLST (Oxford scheme). The MLST results have revealed that all isolates belong to the ST (sequence type) 502, within the clonal complex 92 and IC 2, very rarely described in the literature, and for the first time detected in Croatia. Multiplex polymerase chain reaction (PCR) using specific primers for *bla*OXA-51-like, *bla*OXA-40-like, *bla*OXA-23-like, *bla*OXA-58-like and *bla*OXA-143-like genes was performed and the presence of OXA-51-like and OXA-40-like oxacillinase in selected isolates was confirmed. Further sequencing of amplified fragments will show the similarity of the new sequence type of carbapenem resistant *A. baumannii* with the endemic IC 2 clone carrying OXA-72 oxacillinase.

High prevalence of extensively drug-resistant *Acinetobacter baumannii* at a Children Hospital in Bolivia.

Mónica Cerezales (Spain), Alain A Ocampo-Sosa, Laura Álvarez Montes, Catalina Díaz Ríos, Zulema Bustamante, Jazmín Santos, Luis Martínez-Martínez, Paul G Higgins, Lucía Gallego.

Objectives

The objective of this study was the analysis and characterization of all the *A. baumannii* isolates obtained from patients attended at Hospital Materno-Infantil in Cochabamba, Bolivia from April 2014 to May 2015.

Materials and methods

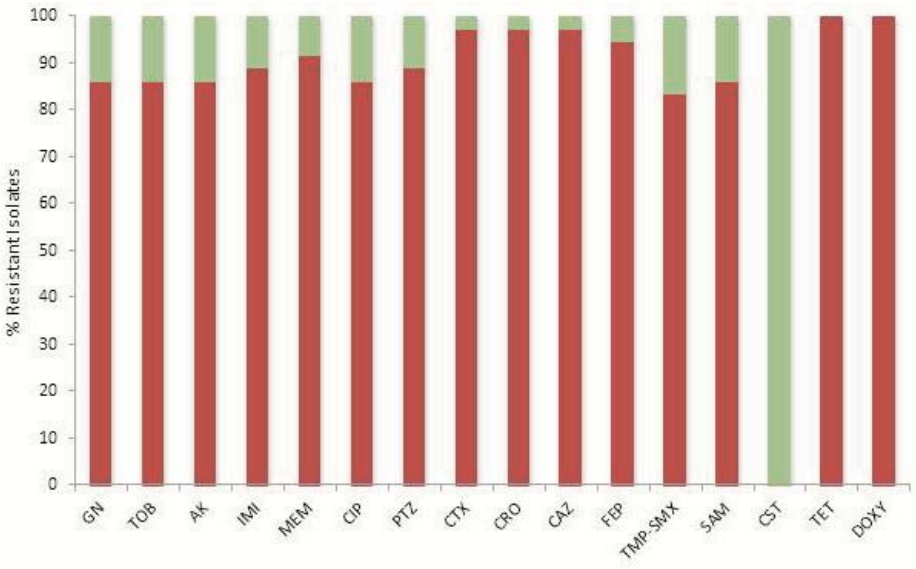
A total of 36 *A. baumannii* isolates were included in the study, they were identified by the hospital clinical laboratories and further confirmed by the presence of *bla*OXA-51-like gene. Seventeen isolates (53%) belonged to patients younger than one month old, most of them were from blood cultures (n=10) and respiratory samples (n=11). Susceptibility testing to antibiotics was carried out by disk diffusion according to EUCAST guidelines for gentamicin, tobramicin, amikacin, imipenem, meropenem, ciprofloxacin, piperacillin-tazobactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, trimetoprim-sulphametoxazole, ampicillin-sulbactam, tetracycline, doxycycline and minocycline and E-test was used for colistin according to the CLSI criteria. The isolates were screened with several multiplex PCRs for the presence of the following resistance genes: *bla*OXA-51-like, *bla*OXA-23-like, *bla*OXA-58-like, *bla*OXA-40-like and *bla*OXA-143-like; *bla*VIM, *bla*GIM, *bla*SPM-1, *bla*IMP and *bla*NDM-1; *ant(2''')94)-Ia and aac(3)-IIa; aph(3''92)-Ia, aac(3)-Ia and aph(3''92)-VIa; aac(6''92)-Ih and aac(6''92)-Ib/cr; aac(6''92)-IIa*; *rmt*B and *rmt*C. Clonal relatedness was studied by pulsed-field gel electrophoresis (PFGE) with *Apa*I and multi-locus sequence typing (MLST). The location of the *bla*OXA genes was assessed by digestion with I-Ceu-I, separated by PFGE and hybridization of the resulting fragments with a digoxigenin (DIG)-labeled probe specific for *bla*OXA-23-like.

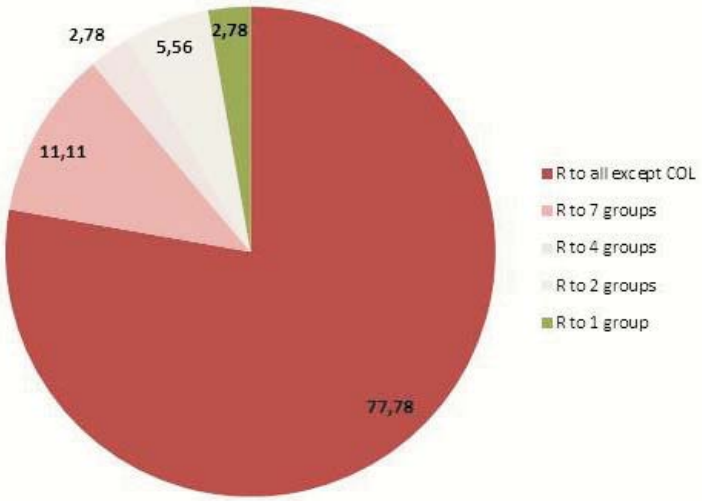
Results

Twenty-nine (80.6%) isolates were XDR, being resistant to carbapenems, fluoroquinolones, aminoglycosides, tetracyclines and other groups of antibiotics; four isolates were multidrug-resistant (MDR) (11.1%), the antimicrobial resistance rates are shown in Figure 1.. Thirty-two isolates carried the *bla*OXA-23 gene on the chromosome as detected by hybridization with a *bla*OXA-23DIG-labelled probe. By sequencing, it was found that the *bla*OXA-23 was located on a previously reported transposon (Tn*2008*) in twenty-nine of these isolates. The gene for the aminoglycoside modifying enzyme *aac(3)-IIa* was also present in thirty-one isolates (86.1%). PFGE revealed two predominant clones and some sporadic genotypes with unrelated isolates. Sequence types (STs) obtained using the Oxford scheme, clustered together the predominant clones that were obtained by PFGE in single or double locus variants of ST490, belonging to IC7. Furthermore the sporadic isolates belonged to unrelated STs.

Conclusions

The present study demonstrated the predominance and spread of closely related XDR *A. baumannii* isolates in a Children Hospital in Bolivia. The location of the *bla*OXA-23 gene in the transposon Tn*2008* could be responsible for the dissemination of this carbapenemase encoding gene among other isolates. The high prevalence of XDR *A. baumannii* clones confers increasing risk to children and is of major concern due to the kind of infections and the difficulty to treat them.


% Resistant Isolates
100
90
80
70
60
50
40
30
20
10
0
GN
TOB
AK
IMI
MEM
CIP
PTZ
CTX
CRO
CAZ
FEP
TMP-SMX
SAM
CST
TET
DOXY


2,78
5,56
2,78
11,11
77,78
R to all except COL
R to 7 groups
R to 4 groups
R to 2 groups
R to 1 group

Use of core genome MLST to identify endemic *Acinetobacter baumannii* international clone 7 isolates from two hospitals in Bolivia.

Mónica Cerezales (Spain), Kyriaki Xanthopoulou, Julia Ertel, Zulema Bustamante, Harald Seifert, Lucía Gallego, Paul G Higgins.

Objectives

The objective of this study was the analysis of the antimicrobial resistance rates and molecular epidemiology of *Acinetobacter baumannii* isolates from 2015 and 2016 in two hospitals located in the city of Cochabamba, Bolivia.

Materials and methods

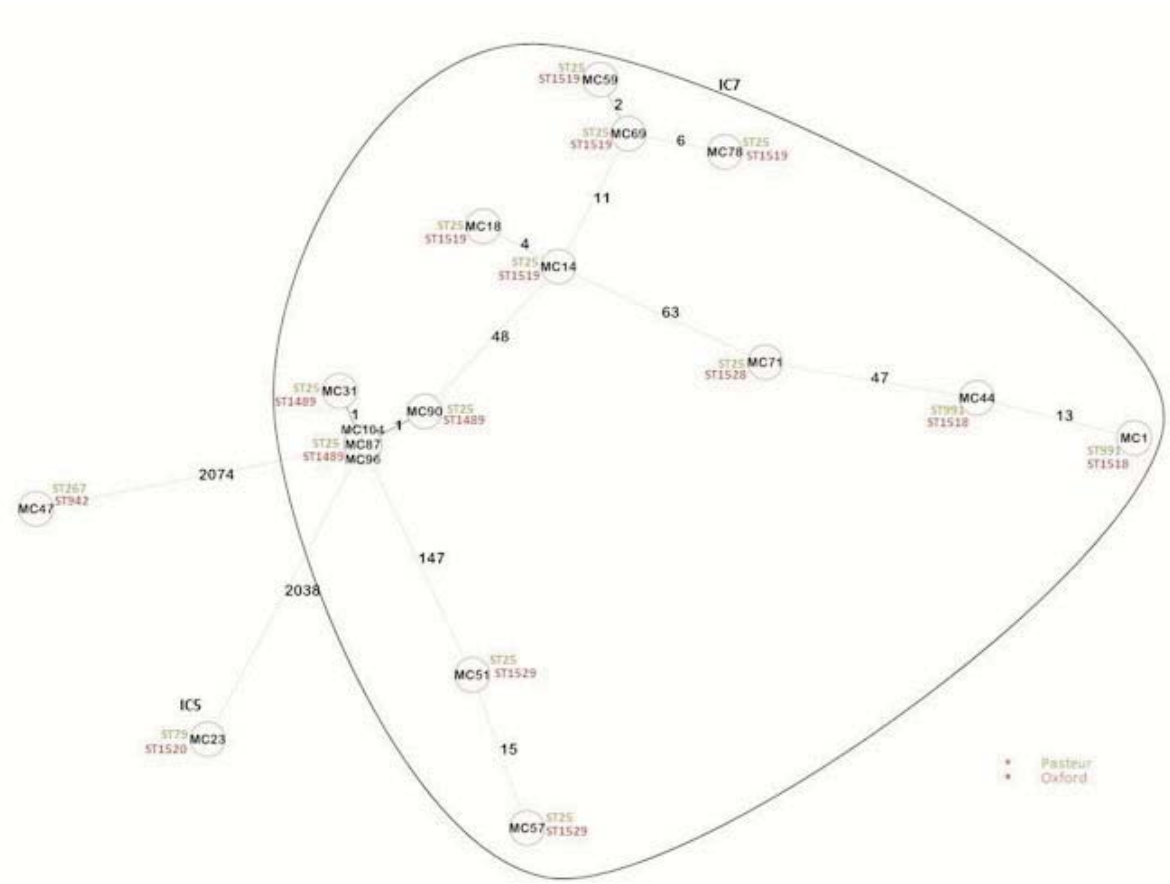
A total of 95 isolates consecutively recovered from patients in two Bolivian hospitals over an 18 month period were analysed. Species identification of *A. baumannii* was confirmed using *gyr*B multiplex PCR. The presence of carbapenemases encoding *bla*OXA (51-like, 23-like, 58-like, 40-like and 143-like) and metallo-b-lactamases (VIM, KPC, NDM, IMI, GES, GIM, IMP) was tested by three different multiplex PCRs. Minimum inhibitory concentrations (MICs) for ciprofloxacin, colistin, gentamicin, imipenem, meropenem and tigecycline were determined by agar dilution using EUCAST breakpoints for interpretation. A subset of randomly selected strains was further investigated by whole-genome sequencing (WGS). These isolates were sequenced using the MiSeq platform (250bp paired-end sequencing). A core-genome MLST (cgMLST) scheme was defined using the Ridom SeqSphere+ v.3.0 software using *A. baumannii* ACICU as reference genome. The resulting core-genome of 2390 alleles was used to investigate their molecular epidemiology. Genome sequences were also used to identify the acquired resistome by using ResFinder, and determine the traditional seven loci MLST (PubMLST). The international clone (IC) was assigned using the sequence type according to Pasteur scheme, the *bla*OXA-51-like gene and cgMLST.

Results

The resistance rates to ciprofloxacin, colistin, gentamicin, imipenem, meropenem and tigecycline were as follows 87.4%, 5%; 87.4%, 54.7%; 54.7% and 60%, respectively. All carbapenem resistant isolates carried *bla*OXA-23. WGS revealed other genes confering resistance to antibiotics such as aminoglycosides (*strA*, n=9; *strB*, n=10; *aac(3''92)-IIa*, n=9; *aac(3''92)-VIa*, n=2); b-lactams (*bla*TEM-1A, n=1; *bla*TEM-1B, n=1); sulphonamides (*sul2*, n=7); tetracyclines (*tet(B),* n=12) and trimethoprim (*dfrA*, n=1). Typing results (minimum spanning tree with the cgMLST scheme) are summarized in Figure 1. Most of the isolates belonged to ST25 (Pasteur scheme), had the characteristic *bla*oxa-51 variant *bla*oxa-64 and clustered with the IC7 control by using cgMLST. These three pieces of evidence showed that IC7 was predominant among these *A. baumannii* isolates. IC7 isolates were all ST25 by Pasteur scheme but exhibited different Oxford STs. However, cgMLST showed that most isolates were not the same clone. There were three potential transmission clusters with 0-4 allelic differences. The other CC25 isolates differed by up to 147 alleles. STs assigned by Oxford scheme were less conserved but all of them single or double locus variants of CC490. Two unrelated carbapenem susceptible isolates were also present differing in about 2050 alleles, one of them belonged to IC5 and the other one was a singleton.

Conclusions

IC7 was endemic in these two Bolivian hospitals, clustering together 15 of 17 selected isolates with different resistomes. Within the IC7 clone there were three transmision clusters and 5 unrelated isolates. Although all IC7 were ST25 by Pasteur scheme, they were further delineated into 5 STs with the Oxford scheme. IC7 has been described as one of the predominant ICs in South America and although some studies have been performed in other South American countries, the situation in Bolivia is not very well known and needs further investigation.


IC7
ST25 ST1519 MC59
2
ST25 ST1519 MC69
6
MC78 ST25 ST1519
11
ST25 ST1519 MC18
4
ST25 ST1519 MC14
63
48
ST25 ST1528 MC71
47
MC44 ST991 ST1518
13
MC1 ST991 ST1518
ST25 ST1489 MC31
1
MC90 ST25 ST1489
1
MC104
ST25 ST1489 MC87 MC96
2074
ST267 ST942 MC47
147
2038
ST25 ST1529 MC51
IC5
ST79 ST1520 MC23
15
ST25 ST1529 MC57
Pasteur
Oxford

Investigating the meropenem-resistant *Acinetobacter baumannii* outbreaks from 2003 to 2016 at Rigshospitalet using Whole Genome Sequencin.g

Tove Havnhøj Frandsen (Denmark), Leif Percival Andersen.

Objectives

Acinetobacter baumannii is an opportunistic pathogen often associated with hospital-acquired infections and outbreaks. Rigshospitalet had outbreaks with meropenem-resistant A. baumannii in 2003, 2008, 2010, 2012, 2014 and twice in 2016. For the outbreaks in 2008-2014 the isolates all belong to the same ribotype. In all outbreaks the Intensive Care Unit (ICU) and Neurology ICU (Ne-ICU) were involved, hinting that there could be a hidden reservoir in these departments. The objective of the study was to investigate the relatedness of the outbreaks to identify potential reservoirs in the hospital-settings using whole genome sequencing (WGS).

Methods

All meropenem-resistant A. baumannii isolates (only one isolate per patient) were sequenced using MiSeq with 2 x 200 bp paired-end-reads. The reads for each sample was de novo assembled and contigs over 1000 bp were used for Multi-Locus Sequence Typing (MLST) both with Oxford and Pasteur scheme an online MLST typing service was used (https://cge.cbs.dtu.dk/services/MLST/, Centre for Genomic epidemiology, Technical University of Denmark). Furthermore, a Single-nucleotide polymorphism (SNP) tree was calculated using the Velvet pipeline.

Results

Sixty-nine patients''92 isolates and ten environmental isolates were WGS**.** Twenty different MLST-types were found with the Oxford scheme and ten with the Pasteur scheme. The three most abundant types found by the Oxford scheme are ST208 (n=38), ST396 (n=8) and ST436 (n=5) and for Pasteur scheme it was ST1 (n=4), ST2 (n=64) and ST136 (n=3).

The study also shows that the Pasteur scheme is not as precise as the Oxford scheme, when comparing the typing results with the SNP-tree. Hence, the results from the Pasteur typing will not be commented further in this abstract. In the 2008-outbreak (8/8) the Oxford type was ST369, but two unrelated patient''b4s isolates have the type ST281 and ST928. The dominating Oxford MLST-type was ST208 for the outbreaks in 2010 (6/7), 2012 (14/17), 2014 (3/4) and one of the outbreaks in 2016 (3/5). The ST208 was also identified in patients from 2011 (2/7), but the SNP-tree revealed that these are not related to the ST208 causing the outbreaks.

Conclusion

There could be a hidden reservoir in the ICU or the Ne-ICU since the ST208 which was causing the outbreaks in 2010, 2012, 2012, 2014 and one of the outbreaks in 2016 are closely related.

Report of ST85 NDM-1-producing *Acinetobacter baumannii* in Tunisia: evidence of the need of continuous surveillance.

Hadhémi Ben Chikh, Sara Domingues, Eduarda Silveira, Yosr Kadri, Maha Mastouri, **Gabriela Jorge da Silva (Portugal).**

Background

Carbapenemases are emerging in the Mediterranean countries. In Tunisia, OXA-23, OXA-58-like and GES-11 *Acinetobacter baumannii* (Ab) have been reported. The metallo-beta-lactamase NDM-1 was found only in *Klebsiella pneumoniae* (1,2,3). The aim of this study was to screen and characterize the carbapenemases of imipenem-resistant Ab isolates.

Methods

A total of 101 non-duplicate imipenem-resistant Ab were recovered from patients hospitalized in different wards at the University Hospital of Monastir Tunisia (UHMT) from 2013 to 2016. Species confirmation was done by screening *bla*OXA-51-like gene by PCR and sequencing of the *rpoB* gene in a few isolates. Antibiotic susceptibility was performed by the disk diffusion method by testing 16 antibiotics. MICs of imipenem (IMP) were determined by E-test. IMP-resistant isolates were phenotypically tested for metallo-b-lactamases using EDTA. The following carbapenemase encoding genes were screened by PCR: *bla*OXA-23, *bla*OXA-24, *bla*OXA-58, *bla*OXA-143, *bla*OXA-48, *bla*VIM, *bla*IMP, *bla*NDM and *bla*KPC. Conjugation and natural transformation assays were performed using *Escherichia coli* J53 (azide resistant) and *Acinetobacter baumannii* A118 as a recipient, respectively. Genotyping was performed by MLST (Pasteur MLST scheme). Amplicons were sequenced in both senses.

Results

Most of the samples were collected from respiratory tract (63 %). The intensive care unit (ICU) was the principal ward of Ab isolation (70,3 %), followed by surgery (11,9 %). The isolates were resistant to the majority of antibiotics that include b'df–'96lactams, aminoglycosides and fluoroquinolones. Ninety and 80% were susceptible to colistin and tigecycline, respectively. IMP MICs ranged from 4 to 32 mg/L. Inhibition of carbapenemase by EDTA was observed only in one isolate, Ab51. None of the genes was detected, except for Ab51 that produced a NDM-1, and belong to ST85. The IS*Ab125* was found upstream *bla*NDM-1.The gene was not transferable by conjugation, suggesting chromosomal location. Natural transformation did not yield any NDM-1 positive transformant. The strain was isolated from a bronchial aspirate of a patient hospitalized in the ICU, a heavy smoker, with a chronic obstructive pulmonary disease and no report of travelling abroad.

Conclusion

To our knowledge, this is the first report of an Ab NDM-1 producer in Tunisia and it is a sporadic isolate (<1%). Nonetheless, a ST-85 Ab NDM-1 producer was reported in France in 2013 isolated from a Tunisian patient (4), which might raise the hypothesis that ST85 Ab is circulating in this country without being detected. Controlling the spread of multidrug resistant *A. baumannii* in North Africa countries is crucial to prevent dissemination of NDM-1.

1.Messaoudi A, et al..J Antimicrob Chemother. 2017, 72:315-6.
2.Izdebski R et al. Euro Surveill. 2015, 11; 20(23)
3.Ben Nasr A, et al. Antimicrob Agents Chemother. 2013, 57:4089-90.
4.Bonnin RA, et al. Emerg Infect Dis. 2013, 19:822-3.

NDM-1-producing *Acinetobacter baumannii* from Tunisia, spread of the ST85 clone.

Nadia Jaidane, Thierry Naas, Wejdene Mansour, Olfa Bouallegue, **Rémy A. Bonnin (France).**

Introduction
Carbapanem resistance in *Acinetobacter* species is on the rise since the beginning of the 2000''92s. The main resistance mechanism is the production of class D beta-lactamase. Metallo-beta-lactamases (MBLs) have been more rarely described in *A. baumannii*. Among them, NDM is increasingly reported from different continents. We described here the genomic analysis of NDM-producing *A. baumannii* from Tunisia.

Material and Methods
Two hundreds and fifty carbapenem-resistant *A. baumannii* isolates were studied to decipher their resistance mechanisms. Eleven NDM-producing *A. baumannii* were recovered from clinical samples between June 2013 to October 2015. Four strains were isolated from blood cultures and seven from urine culture. Antimicrobial susceptibility testings, MICs and genoyping were performed. All these isolates were sequenced using Illumina''92a technology and then genomic analysis was performed. Conjugation and transformation was performed to determine genetic support of the blaNDM-1 gene.

Results
The eleven isolates were highly resistant to carbapenems, late generation cephalosporins, fluoroquinolones, and aminoglycosides but remained susceptible to colistin. PCR revealed the presence of NDM-1 in all isolates and OXA-23 in four isolates. All these isolates belonged to ST85 according to the MLST scheme of Pasteur''92s institute. The resistome was composed of *bla*OXA-94, *bla*ADC-25-like and *aph(3')-VIa ; aadB, mph(E), msr(E), sul2 and tet(39).* Mutations in gyrases were also observed likely explaining the resistance to quinolones. Genomic analysis revealed from 80 to 200 SNPs between these isolates. A comparison with isolate isolated from France in 2013 revealed a clear link between these isolates. Transformation/conjugation failed to transfer the *bla*NDM-1 gene likely indicating its chromosomal location whereas the *bla*OXA-23 can be transferred by conjugation.

Conclusions
A particular clone of *A. baumannii* is responsible for the spread of NDM-1-producing *A. baumannii* in Tunisia. The same clone was identified in France in 2012 and carrying the blaNDM-1 gene. Further experiments is needed to understand its success.

Antibiotic resistance profiles of *Acinetobacter baumannii* complex strains isolated in the intensive care units of Gaziantep University Hospital.

Deniz Gazel (Turkey), Yasemin Zer.

Objectives
Nosocomial infections with *Acinetobacter baumannii* complex is an important global healthcare problem. We aimed to investigate the antibiotic resistance profiles of *Acinetobacter baumannii* complex strains isolated in the intensive care units (ICUs) of our university hospital in Gaziantep, Turkey.

Methods
Acinetobacter baumannii complex strains isolated in six different ICUs of our hospital from January 2016 till May 2017 were included in the study. The isolates were identified by MALDI biotyper (Bruker), antimicrobial susceptibility testings for amikacin, gentamicin, ciprofloxacin, imipenem, meropenem, colistin and trimethoprim-sulfamethoxazole were performed by Phoenix automated system (Becton-Dickinson) and evaluated according to EUCAST criteria. The antibiogram data were collected from our laboratory records retrospectively to analyze the antimicrobial resistance profiles.

Results
In total 687 *Acinetobacter baumannii* complex isolates from 276 patients were analyzed. The majority of the strains (59%) were isolated from tracheal aspirate cultures. Other isolates were from blood (25%), urine (5%), wound (4%), catheter (3%), sputum (2%), CSF (1%) and other cultures (<1%). Majority of the isolates were found resistant to imipenem (97%), ciprofloxacin (97%), meropenem (93%), gentamicin (92%), trimethoprim-sulfamethoxazole (87%) and amikacin (84%). All of the isolates were found susceptible to colistin (100%).

Conclusion
Our results indicated higher resistance rates in ICUs comparing to other previous studies in Turkey. Carbapenem resistance is still the most significant problem in ICUs. Also we observed higher rates of ciprofloxacin resistance. All isolates were found colistin susceptible. So colistin is one of the last resort antibiotics effecting on multi drug resistant *Acinetobacter baumannii* strains. Colistin drug was started to be used in Turkish healthcare system only several years ago, so this might be a reason why there is no colistin resistance in our region yet.

Genomic characterization of emerging colistin-resistant *Acinetobacter baumannii* strains belonging to ST451.

Sung Ho Yun (Korea), Edmond Changkyun Park, Hayoung Lee, Yoon-Sun Yi, Chaeuk Chung, Jae Young Moon, Gun-Hwa Kim, Seung Il Kim.

Extensively drug-resistant (XDR) *Acinetobacter baumannii* strains have emerged rapidly worldwide. The antibiotic resistance characteristics of XDR *A. baumannii* strains show regional differences; therefore, it is necessary to analyse genomic and proteomic characteristics of emerging XDR *A. baumannii* clinical strains isolated in South Korea to elucidate their multidrug resistance. Here, we isolated eight XDR Korean *A. baumannii* (KAB) clinical strains from South Korean hospitals and performed comprehensive genome analyses. Among these, two colistin-resistant strains (KAB01 and KAB03) belong to the recently emerged sequence type ST451. Genomic analysis revealed that KAB01 and KAB03 harbour two resistance islands (RIs) containing b'df-lactamases (*blaOXA-23* or *blaTEM*, respectively). In particular, the 55.6 kb RI of KAB03 harbours two copies of Tn*2009* containing *blaOXA-23*, suggesting that *blaOXA-23* plays an important role in antibiotic resistance. Other antibiotic resistance genes were scattered across the chromosome. KAB01 and KAB03 both harboured mutation A138T in PmrB, whereas KAB03 harboured additional mutations R263H in PmrB and R109H in PmrC, which are believed to confer colistin resistance. Proteomic analysis of KAB03 confirmed that b'df-lactamases (ADC-73 and OXA-23), efflux pumps (AdeIJK), outer membrane proteins (OmpA and OmpW), and colistin resistance genes (PmrAB) were major proteins responsible for antibiotic resistance.

Fast typing of carbapenem-producing *Acinetobacter baumannii* using MALDI-TOF MS.

Tove Havnhøj Frandsen (Denmark), Julie Nygaard, Rosalie Line Dahlerup Kruse, Leif Percival Andersen.

Introduction

When a high number of carbapenem-producing *Acinetobacter baumannii* infections are detected, the isolates are usually typed using molecular biological methods which are both costly and a time consuming process to confirm or reject the suspicion of an outbreak. MALDI-TOF MS can be used as a fast and economical tool for typing within a species, but it is not validated and the reproducibility is unclear. The purpose of this study was to compare the reproducibility of MALDI-TOF MS typing on a collection of 50 carbepenem-producing *A. baumannii* isolates using the Multi Locus Sequecing Typing (MLST) Pasteur scheme.

Methods

One isolate of carbapenem-producing *A. baumannii* form 47 patients and three isolates obtained from the hospital environment were all analysed by MALDI-TOF MS. The spectra were obtained from formic acid extraction of four colonies. The supernatant was transferred to the target and each spot was covered with matric solution. The spectra were analysed using FlexAnalysis software Version 3.4 (Bruker Daltonik, Germany). Peaks present in most isolates but absent and apparently replaced by another peak in a few isolates were visually detected and used to discriminate between strains.

All meropenem-resistant *A. baumannii* isolates were sequenced using MiSeq with 2 x 200 bp paired-end-reads using Nextera XT kit. DNA was extracted from overnight culture using the DNeasy blood and tissue kit (QIAGEN, Hilden, Germany). For Multi-Locus Sequence Typing (MLST) with the Pasteur scheme scheme an online MLST typing service was used (https://cge.cbs.dtu.dk/services/MLST/, Centre for Genomic epidemiology, Technical University of Denmark). The result from the MLST was blinded until the MALDI-TOF MS typing was finished.

Results

The MALDI-TOF MS typing found twelve different peaks to discriminate the isolates into subtypes. These peaks divided the isolates in four different groups (Subgroup 1 = 41 isolates, subgroup 2 = 5 isolates, subgroup 3 = 3 isolates and subgroup 4 = 1 isolate).

Subgroup 1 corresponds to the Pasteur Multi locus sequence type (MLST) ST-2, which is the international clone II. All of the isolates belonging to subgroup 1 are all clustered together in the SNP-tree, but this cluster also contains the isolate which forms subgroup 4. The subgroup 2 and 3 are forming a cluster in the bottom of the SNP-tree which are Pasteur type ST-1 and ST-136.

Conclusion

In this study we found MALDI-TOF MS typing to be capable of identifying clusters of closely related isolates of Carbapenem-producing *A, baumannii* in accordance with SNP based phylogenetic analysis and Pasteur MLST. MALDI-TOF MS typing can be done within one day, and for a very small price. So far, the method involves subjective visual assessment of peak-differences between isolates and will require standardization for the results to be reproducible. A promising usage could be an initial screening of isolates from a suspected outbreak, as the method may have the strength to determine that the isolates do not belong to the same clone, but if many isolates have the same MALDI-TOF MS pattern they will probably subsequently have to be typed with a method with a higher resolution, e.g. Whole Genome Sequencing followed by a SNP-analysis.

Faecal carriage of *Acinetobacter baumannii*: a comparison to clinical isolates from the same period.

Bence Balázs (Hungary), Julianna Mózes, Idan Blum, Gábor Kardos.

Increasing incidence of *A. baumannii* infections has been documented in a tertiary-care Hungarian hospital from 2010 to 2014, with increasing prevalence of carbapenem resistance mediated mainly by *blaOXA-23-like* carbapenemases. The study investigates whether *A. baumannii* is prevalent in faecal samples of inpatients in this relatively high incidence density setting. Characteristics of faecally carried isolates were compared to those of isolates from clinical samples between January and April 2017.

Altogether 2042 faecal samples sent for routine bacteriology examination or for pathogen screening were processed on selective medium yielding 24 *A. baumannii* isolates (1.2%). Only four faecal isolates originated from patients with A. baumannii positive clinical samples. In the same period 65 (1.7%) *A. baumannii* isolates were collected from 15752 various other clinical samples (mainly lower airway and urine samples). Isolates were identified using Microflex MALDI-TOF and the presence of *blaOXA-51-like* genes. Susceptibility tests were carried out using the EUCAST guidelines. Carbapenem resistance genes *blaOXA-23-like* and *blaOXA-24-like* as well as aminoglycoside resistance genes *aac(6''92)-Ib, aph(3'')92)-VIa* and *armA*, earlier shown to be frequent, were sought for using PCR assays. Prevalences were compared using Fisher exact test or chi square test as appropriate.

Drug resistance among the 24 faecal isolates was low; eight (33%), eight, six (25%), six and eleven (46%), were resistant to carbapenems, gentamicin, tobramycin, amikacin and ciprofloxacin, respectively. Higher resistance rate was detected in case of clinical samples, 62 (95%), 63 (97%), 16 (25%), 18 (28%) and 63 (97%) were resistant to the abovementioned drugs, respectively. The differences are significant in case of carbapenems, gentamicin and ciprofloxacin ($p<0.001$).

In the faecal *A. baumannii* isolates the dominant aminoglycoside resistance gene was *aac(6''92)-Ib* (7/24; 26%), the prevalence of *armA* was low (2/24; 7%), the gene *aph(3'')92)-VIa* was not detected at all. In the clinical isolates the occurrence of the studied aminoglycoside resistance genes were statistically comparable; we found *aac(6''92)-Ib*, *armA* and *aph(3'')92)-VIa* in 18 (28%), 6 (9%) and 4(6%) of isolates, respectively, which represent a marked decrease compared to the previous years (2010-2014).

Carbapenem resistance genes in faecal isolates were rare, *blaOXA-23-like* genes were not found, while *blaOXA-24-like* occurred in 17 isolates (63%). Notably, nine of these *blaOXA-24-like* carriers were carbapenem susceptible. In clinical isolates the prevalence of *blaOXA-23-like* genes was 12/65 (19%), representing a marked decrease compared to our last reported result (120/128; 94% in 2014). In contrast, the prevalence of the *blaOXA-24-like* genes was high (60/65; 92%). This gene was absent in the clinical isolates in 2014, which indicates a shift between dominant carbapenemase genes. Comparing faecal and clinical isolates, occurrence of both *blaOXA-23-like* and *blaOXA-24-like* was significantly higher in clinical isolates ($p=0.03$ and $p=0.01$, respectively).

In conclusion, only a minority of faecal *A. baumannii* isolates were similar to resistant isolates prevalent in the clinical samples, indicating that most isolates carried in the faecal microbiota do not originate from the hospital setting. Consequently, faecal microbiota of patients may act as reservoir, but its reservoir role seems minor.

G. Kardos was supported by a Bolyai Scholarship of the Hungarian Academy of Sciences.

Acinetobacter baumannii recovered from technosol at a dump site.

Jasna Hrenovic (Croatia), Goran Durn, Svjetlana Dekic, Blazenka Hunjak, Snjezana Kazazic.

Data on the presence of *Acinetobacter baumannii* in natural environment influenced by human solid waste are very scarce. There is only one literature report on the incidental finding of one multi-drug resistant (MDR) *A. baumannii* in palesol influenced by illegally disposed solid waste. Here we report the finding of three isolates of *A. baumannii* recovered from technosol at dump site. The dump site is situated above City of Rijeka in Croatia in a karst pit. At this dump site the hazardous industrial waste was continuously disposed from 1956-1990 and further was periodically used as an illegal dump site. Three presumptive colonies were isolated from plates inoculated with 0.01-1g of the surface part of technosol collected at the edge of dump. MALDI-TOF MS score values ranged from 2.000-2.086 for *A. baumannii*. All three isolates were MDR. They shared the resistance to carbapenems, fluoroquinolones, ticarcillin/clavulanic acid and piperacillin/tazobactam, and resistance or intermediate resistance to amikacin and ampicillin/*sulbactam. One isolate was resistant to* trimethoprim/sulfamethoxazole and intermediate resistant to minocycline. All three isolates were sensitive to tobramycin, gentamicin and colistin. The proper management and disposal of human solid waste is mandatory to prevent the spread of MDR *A. baumannii* in nature.

Global transcriptional profiling of *Acinetobacter baumannii* under microaerobiosis and normoxy.

María Luisa Gil-Marqués (Spain), Younes Smani, Michael McConnell, María Eugenia Pachón-Ibáñez, Jerónimo Pachón-Díaz.

Background

Transcriptomic techniques, such as RNA-Seq, can provide useful information for identifying bacterial targets for the development of novel antibiotics. The identification of genes that are highly expressed during infection could also point to genes that have an essential role in growth and survival during infection. There are different common medical conditions that produce hypoxemia and peripheral tissue hypoxia and they are often associated with infection and inflammation. The objective of this study was to identify genes of Acinetobacter baumannii whose expression is regulated by environmental oxygen levels to identify virulence factors and characterize their role in infection.

Materials/Methods

Characterization of the *A. baumannii* ATCC 17978 growth under microaerobiosis (<0.3% oxygen) and normoxy. Extraction of RNA from cultures of *A. baumannii* ATCC 17978 after 4 hours of growth under both conditions to perform RNA-Seq. Analysis of the generated sequence raw data was carried out using CLC Genomics Workbench 8.5.1. Expression levels were determined as Reads per Kilobase of exon model per Million mapped reads (RPKM) and a Differential Gene Expression Analysis through an Empirical Analysis of Digital Gene Expression test (DGE-test) to compare expression levels between the two conditions. Differentially expressed genes were filtered using standard conditions (a False Discovery Rate p-Value-0.05 and a fold change >2 or <-2). Results were confirmed through RT-qPCR.

Results

A. baumannii ATCC 17978 has a lower growth rate in microaerobiosis *vs.* normoxy. A total of 204 genes were identified as being differentially expressed. Among these genes, those which had the highest and the lowest fold changes (Table 1) were selected to confirm the results through RT-qPCR. 3 housekeeping genes (rpoD, recA and gyrB) were used.

Underexpressed genes in microaerobiosis *vs.* normoxy were involved in a metabolic pathway to obtain fumarate, which goes to the citrate cycle (aerobic metabolism), and acetoacetate, which participates in propanoate metabolism. Overexpressed genes in microaerobiosis *vs.* normoxy were involved in taurine transport and metabolism and alkanesulfonate transport to finally obtain sulfite, in sulfate transport and metabolism, and in pyruvate metabolism. The translocase protein TatABC and the phosphate transporter PstABCS were also overexpressed in microaerobiosis.

Table 1. Selected underexpressed and overexpressed genes in microaerobiosis vs. normoxy.

ID	Protein	Fold Change
A1S_3415	Maleylacetoacetate isomerase	-54.72
A1S_3414	Fumarylacetoacetase	-42.07
A1S_3416	Glyoxalase/bleomycin resistance protein/dioxygenase	-26.47
A1S_1443	taurine ATP-binding transport system component	10.64
A1S_2533	putative esterase	11.70
A1S_2531	sulfate transport protein	14.24

Immunization with outer membrane complexes from lipopolysaccharide-deficient *Acinetobacter baumannii* provides protective immunity to multidrug-resistant strains.

Marina R. Pulido (Spain), Meritxell García-Quintanilla, Jerónimo Pachón, Michael J. McConnell.

Background
In the context of the current multidrug-resistance in Gram-negative bacilli, non-antimicrobial approaches are needed to circumvent the lack of optimal antimicrobial therapies. Among those, active immunization is a promising alternative to prevent infections by these bacteria. In the present work, we have tested the efficacy of an *Acinetobacter baumannii* vaccine in an experimental murine model.

Methods
In the present study, we used outer membrane complexes (OMC) from the bacterial membrane of a lipopolysaccharide deficient *A. baumannii* strain (IB010) for vaccination using a mouse model of disseminated *A. baumannii* infection. Mice were immunized via intramuscular injection of 100 µl of the OMCs (250 µg/mouse) and an aluminum-based adjuvant on days 0 and 14. After immunization, the antibody response was assessed by quantifying serum IgG, IgG1, IgG2c and IgM levels by ELISA. Pro-inflammatory cytokine levels in serum and bacterial loads in spleens were measured at 12 h post-infection. Mice were carefully monitored for survival for 7 days after infection.

Results
Immunization with outer membrane complexes from IB010 produced an antibody response consisting of IgG, in particular the IgG1 subtype. After infection, immunized mice showed a 1000-fold reduction in post-infection spleen bacterial load compared to control mice ($p < 0.05$; Mann-Whitney U test) and a significant reduction in pro-inflammatory cytokine levels IL-1b'df, TNF-a and IL-6 in serum. Survival was monitored over seven days after infection. Sixty-five percent of mice that were immunized with OMCs from the LPS deficient strain survived after 48 h of infection while 100% of control mice died during the first 24h after infection ($p < 0.001$; log-rank test for vaccinated group).

Conclusions
These results suggest that prophylactic vaccination based on outer membrane complexes from a lipopolysaccharide-deficient strain could be a new approach to be developed for the prevention of infections caused by *A. baumannii*.

Cellular immune response triggered by *Acinetobacter baumannii* clinical strains in experimental pneumonia model.

Tania Cebrero Cangueiro (Spain), Gema Labrador Herrera, Younes Smani, Jerónimo Pachón, Mª Eugenia Pachón Ibañez.

Background:
Immunotherapy by enhancing the cellular immune response of the host is a promising alternative of therapy against infections caused by *Acinetobacter baumannii*. The aim of this study was to define the cellular responses elicited by non-lethal pneumonia caused by clinical strains of *A. baumannii.*

Material/methods
a) Characterization of non-lethal pneumonia models by colistin-susceptible (AbCS01) and colistin-resistant (AbCR17) *A. baumannii* clinical strains. Immunocompetent C57BL/6 male mice (7-9 weeks old) were used. Survival studies were performed (Reed and Muench Method) searching a 100% survival rate at least 14 days post-infection, and bacterial counts in tissues (spleen and lungs) and blood the first day post-infection. Bacterial concentrations in tissues and blood were assayed at different time points post-infection (1, 2, 3 , 4, 5, 6, 7, 9 and 14 days). Histopatological studies of lungs were performed according to standard methods.

b) Cellullar immune response (innata and adaptative): Monocytes, macrophages, neutrophiles and B and T (CD4+ and CD8+) lymphocites from tissues and blood were analysed by flow cytometry. At different time (30 minutes, 2, 4, 6, 24 and 48 hours for adaptative response points and 1, 3 , 7, 14, 30 days for innate response) after intratracheal instillation of the inocula above calculated for each strain, six mice (5 infected and 1 uninfected) were sacrificated (thiopental). Tissues and blood were asseptically extracted, weighed, and proccesed for bacterial concentration counts or cellullar immune response studies.

Results
A. baumannii inoculum allowing non-lethal pneumonia models were 108 and 109 log10 UFC/mL for AbCS01 and AbCR17, respectively. The strain AbCS01 showed bacterial concentration counts 14 days post-infection (1.63 ±'b1 0.21 log10 UFC/g) and the strain AbCR17 showed bacterial concentration counts 9 days post-infection (1.19 ±'b1 1.68 log10 UFC/g). Histopathological studies confirmed pneumonia in both cases.

The cell immune response was different depending on the susceptibility of the strains and the organ. Total number of macrophages in spleen increased after 24 hours of infection for both strains. Neutrophils increased in lung and blood for both strains. Briefly, in spleen, B cell response was higher in AbCS01 strain, with a marked depletion on day 7 when the microbiological infections were solved. In blood, the B cell response showed a noticeable increased, much stronger in AbCS01 strain the third day of infection, when the bacteriological counts were higher. In lungs, a progressive increase in the B cell percentages were observed, reaching its maximum by day''92s 7-14 post-infection. T cell response was smaller than B cell response, although, as with the B cell response, in lungs increased by day 7-14 in AbCS01 and by day 14 in AbCR17. The adaptive response is showed in Figure 1.

Conclusions
Macrophages and neutrophils number of cells increased after infection by colistin-susceptible (AbCS01) and colistin-resistant (AbCR17**)** *A. baumannii*. The cellular immune response triggered in pneumonia infections caused by these clinical strains showed a higher response of B cells than T cells. The immune response seems to differ depending on the strains and tissues.

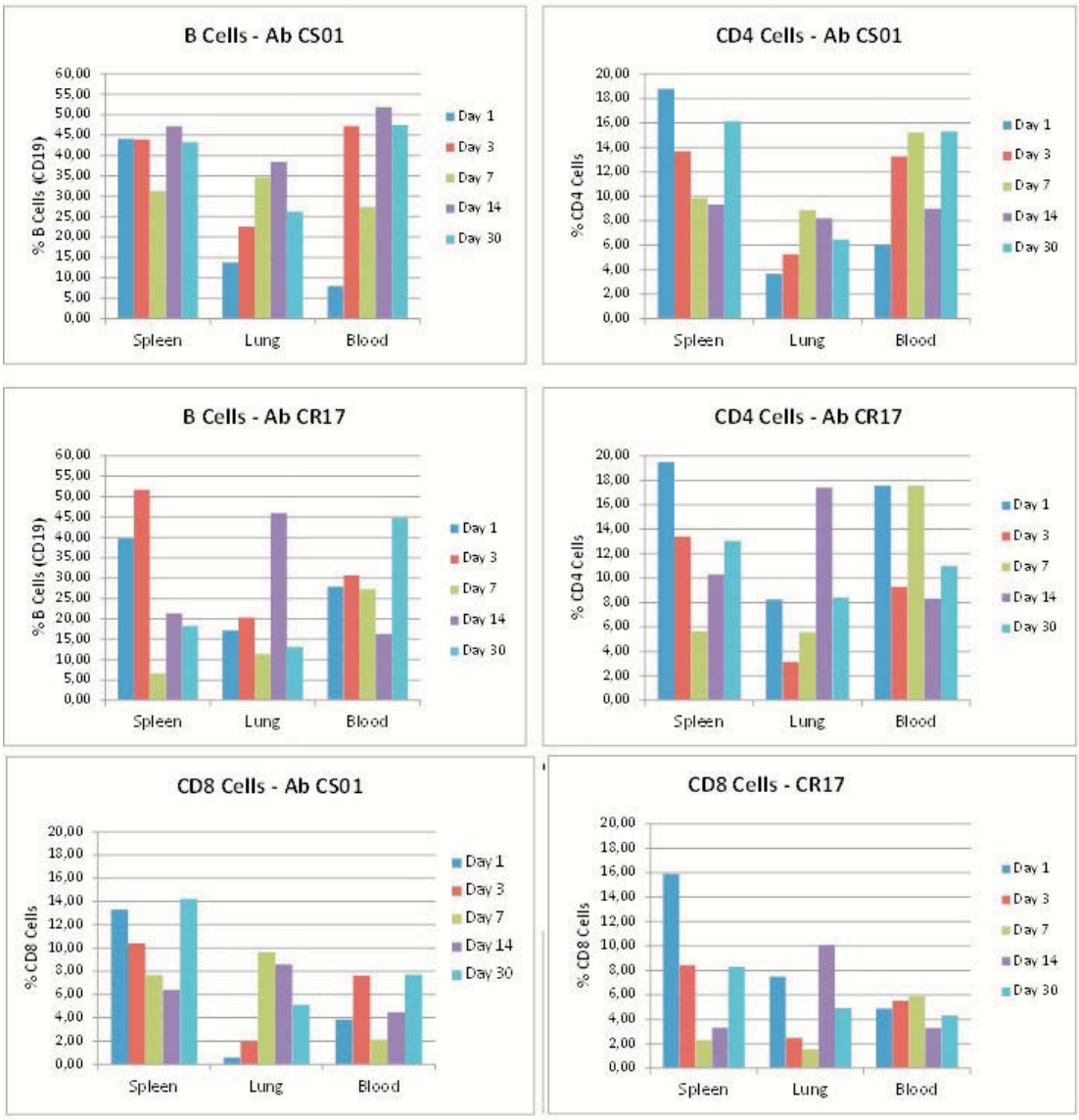


Figure 1. Percentage of B and T Cells in spleen, lung and blood in the non-lethal experimental pneumonia model by S-Col and R-Col *Acinetobacter baumannii*

Tamoxifen as repurposing drug for *Acinetobacter baumannii* infections therapy: in vitro and in vivo approaches.

Rafael Ayerbe Algaba (Spain), Andrea Miró Canturri, María Eugenia Pachón Ibáñez, Jerónimo Pachón, Younes Smani.

Objectives
The limitation of antimicrobial alternatives in the treatment of severe infections by multidrug resistant (MDR) *Acinetobacter baumannii* makes the urgent search for alternative therapeutic options. Immunotherapy is one of the promising approaches to slow the progression of infection as a preventive monotherapy or an adjuvant for the antimicrobial treatment. Tamoxifen, a selective estrogen receptor modulator, used to treat breast cancers, has been described as a drug with immunomodulator properties. A previous study has described efficacy of tamoxifen against Gram-positive bacteria. The objective of this study was to analyse the therapeutic efficacy of tamoxifen, as well as its mechanism of action, as a monotherapy against *A. baumannii* in murine peritoneal sepsis model (MPSM).

Methods
Standard ATCC 17978 and 2 clinical susceptible and MDR (Ab9 and Ab186) *A. baumannii* were used in this study. MPSM by ATCC 17978 was performed to study the efficacy of three doses of tamoxifen (80 mg/kg/24h) for three days administered to mice before bacterial inoculation, and compared with infected control group. Flow cytometry was used to study the immune cells population [myeloid origin cells (MOC), monocytes and neutrophils] in bone marrow and blood of the different treated and control groups. In order to decipher the mechanism of action of tamoxifen, ELISA assays was performed to determine the serum levels of MCP-1 and IL-18, chemoattractant factors of immune cells, at 24h post-bacterial infection in these groups of study. Additionally, mice survival was monitored in mice pretreated with three doses of tamoxifen (80 mg/kg/24h) and infected by Ab9 and Ab186.

Results
In the MPSM by ATCC 17978, treatment with tamoxifen reduced the bacterial load for ATCC 17978 in spleen, lungs and blood by difference of 6.85 and 6.13 CFU/g, and 7.17 CFU/mL, respectively, and increased mice survival by 100%. The analysis of flow cytometry data showed that in bone marrow, MOC population represent the 43.9% (5.2% of monocytes and 31.7% of neutrophils) for the non-infected control group, 12.6% (1.6% of monocytes and 1.4% of neutrophils) for the infected control group and 38.6% (2.7% of monocytes and 13.4% of neutrophils) for the treatment group with three doses of tamoxifen. On the other hand, in blood, the MOC population represent the 11.5% (1.2% of monocytes and 3.4% of neutrophils) for the non-infected control group, 39.2% (5.8% of monocytes and 12.6% of neutrophils) for the infected control group and 25.4% (0.9% of monocytes and 6.5% of neutrophils) for the treatment group with tamoxifen. Furthermore, treatment with of tamoxifen reduced the MCP-1 and the IL-18 production by difference of 3555 and 1957 pg/mL, respectively, respect to infected group without treatment. Finally, pretreatment with tamoxifen increased the mice survival to 40 and 60 % in MPSM by Ab9 and Ab186, respectively.

Conclusion
Treatment with tamoxifen prevents the mice mortality by *A. baumannii* due to the reduction of MCP-1 and IL-18 release, which consequently reduced the immune cells emigration from bone marrow to blood. These events reduced the onset of inflammatory response by *A. baumannii* infection.

AOA-2 derivatives as outer membrane protein A inhibitors for treatment of Gram-negative bacilli infections.

Rafael Ayerbe Algaba (Spain), Núria Bayó, Raquel Parra Millán, Jesús Seco, María Eugenia Pachón Ibáñez, Meritxell Teixidó, Jerónimo Pachón, Ernest Giralt, Younes Smani.

Objectives

Preventing the adhesion of pathogens to host cells provides an innovative approach to tackling MDR bacteria. In this regard, the identification of outer membrane protein A (OmpA) as a key bacterial virulence factor has been a major breakthrough. Previously, we identified that a cyclic hexapeptide AOA-2 inhibited the interaction of Gram-negative bacilli (GNB) like *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli* to host cells thereby preventing the development of infection *in vitro* and in a murine sepsis peritoneal model. In this work, we aimed to evaluate *in vitro* a library of AOA-2 derivatives in order to improve the effect of AOA-2 against GNB infections

Methods

Ten AOA-2 derivatives were synthetized for the *in vitro* assays. Their toxicities to human lung epithelial cells (A549 cells) for 24h were evaluated by determining the A549 cells viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effect of these peptide derivatives and AOA-2 at 250,125, 62.5 and 31.25 µg/mL on the attachment of *A. baumannii* ATCC 17978, *P. aeruginosa* PA01, *E. coli* ATCC 25922 strains to A549 cells was characterized by adherence assay.

Results

None of the ten derivatives showed toxicity to A549 cells. Four of them, RW01, RW02, RW03 and RW06 at 250 µg/mL reduced the bacterial attachment to A549 cells, respectively to 80, 56, 53 and 28% (for ATCC 17978 strain), to 40, 42, 20 and 31% (for PA01 strain), and to 66, 61, 29 and 44% (for ATCC 25922 strain). Two of the four peptides, RW01 (for the three strains) and RW06, (for PA01 and ATCC 25922 strains) at 125, 62.5 and 31.25 µg/mL reduced the bacterial attachment to A549 cells more than the parent peptide AOA-2. At 31.25 µg/mL RW01 reduced only the attachment of ATCC 17978 strain to A549 cells when compared with AOA-2 (to 44 vs. 48%). Meanwhile RW01 and RW06, respectively, reduced the bacterial attachment to A549 cells when compared with AOA-2 (to 50 and 46% vs. 87%, for PA01 strain), and (64 and 72% vs. 82%, for ATCC 25922 strain).

Conclusion

Four of all AOA-2 derivatives (RW01, RW02, RW03 and RW06) reduced GNB attachment to the host. Among them, RW01 and RW06 shown better results than AOA-2, being the best candidates for improving the *in vitro* OmpA inhibition against GNB.

In vitro activity of rafoxanide in combination with colistin against colistin-susceptible and colistin-resistant *Acinetobacter baumannii.*

Andrea Miró Canturri (Spain), Rafael Ayerbe Algaba, Raquel Parra Millán, María Eugenia Pachón Ibáñez, Jerónimo Pachón, Younes Smani.

Background
The number of colistin-resistant isolates of *Acinetobacter baumannii* has increased in the last years. The need of new therapeutic approaches has promoted the use of the drug repurposing, known as the research of new uses for existing drugs. Rafoxanide, an antihelmintic drug use in the veterinary, has been identified as an antibacterial drug against Gram positive bacteria. The objective of this study was to analyse the activity of rafoxanide in combination with colistin against colistin-susceptible (Col-S) and colistin-resistant (Col-R) *A. baumannii* strains.

Material and Methods
One standard Col-S ATCC 17978 and four Col-R clinical isolates of *A. baumannii* from a Spanish hospital outbreak in 2000 were used. Microdilution assay was performed to examine the activity of rafoxanide without colistin. Checkerboard and time kill curve assays were performed to determine the synergy between rafoxanide and colistin. In order to identify the mechanism of action of rafoxanide, membrane permeabilization by fluorescence impermeant indicator Ethidium Homodimer-1 (EThD-1) was determined in Col-S and Col-R *A. baumannii* strains using Thyphoon FLA-9000 laser scanner and fluorescence microscopy. Furthermore, outer membrane proteins (OMPs) profile and the zeta potential of Col-S and Col-R *A. baumannii* strains were performed and determined by SDS-PAGE and ZetaSizer, respectively.

Results
Rafoxanide alone showed MICs ³256 µg/mL for Col-S and Col-R *A. baumannii* strains. Checkerboard analysis showed that colistin in combination with 1 µg/mL of rafoxanide increased the susceptibility of colistin against Col-S and Col-R strains by 8 to >512 folds. Time kill curve analysis showed that 1 µg/mL of rafoxanide in combination with 0.125 µg/mL or 128 µg/mL of colistin, a colistin sub-MIC for Col-S ATCC 17978 and Col-R #11 strains, respectively, decreased the bacterial concentration of Col-S ATCC 17978 and Col-R #11 strains by 3.80 log10 CFU/mL and 3.05 log10 CFU/mL, respectively, respect to colistin at 8h and by 1.34 log10 CFU/mL and 4.08 log10 CFU/mL, respectively, respect to colistin at 24h. Permeabilization assays showed that Col-R #11 strain treated with rafoxanide during 24h presented higher membrane permeabilization than the Col-S ATCC 17978 strain and both non-treated strains indicating bacterial membrane disruption. Moreover, analysis of zeta potential revealed that Col-S ATCC 17978 and Col-R #11 strains in presence of rafoxanide exhibited greater negative charge (-32.14 ±'b1 0.26 and -32.38 ±'b1 0.56 mV) than both strains without treatment with rafoxanide (-29.38 ±'b1 1.17 and -26.75 ±'b1 1.58 mV). In addition, OMPs profile after 4 and 24h of incubation with rafoxanide was unchanged between Col-S ATCC 17978 and Col-R #11 strains and untreated strains suggesting that rafoxanide did not alter the expression of OMPs.

Conclusions
Rafoxanide has potentiated the colistin antibacterial effect against Col-R *A. baumannii*. This effect might be due to alterations in the structure and permeability of the membrane as well as modifications in its surface charge. Although further studies are needed, the use of rafoxanide in combination with colistin could potentially represent an effective alternative for the treatment of infections by MDR *A. baumannii*.

In vitro activity of a library of piperazine derivatives against two clinical strain of colistin resistant *Acinetobacter baumannii.*

Cebrero-Cangueiro T., Mazzotta S, **Carretero-Ledesma M. (Spain),** Iglesias-Guerra F., Jiménez-Baus A., Smani Y., Vega-Pérez JM., Pachón J. - Pachón-Ibáñez.

Objectives

The lack of therapeutical options in the treatment of colistin-resistant *A. baumannii* makes urgent the search for new alternatives in the treatment of infections caused by this kind of pathogen. The aim of this study was to evaluate the *in vitro* activity of a library of piperazine derivatives against two clinical strains of colistin-resistant *A. baumannii*.

Methods

Forty-three molecules belonging to five different piperazine derivatives families prepared in collaboration with the Department of Organic Chemistry (Faculty of Pharmacy, University of Seville) were screened to test inhibition of growth at 50µM concentration against two colistin-resistant *A. baumannii* clinical strains 21R (Ab21R) and 22P (Ab22P). Colistin (COL) Minimal inhibitory concentrations (MICS) were 2048, and 4096 µg/ml, respectively. Fifty µL of Ab21R and Ab22P culture (5x105 UFC/mL) was dispensed into the wells containing the piperazines. MICs were calculated for the molecules which presented inhibitory growth activity. The cytotoxicity of these molecules was calculated, which was analysed by commercial kit AlamarBlue®'ae with A549 epithelial using decreasing concentrations of each molecule. The cytotoxic concentration 50 (CC50) value was obtained using a statistical package GraphPad Prism. Synergistic activity of the 692 piperazine derivate together with COL was assayed by checkerboard method. The range of concentrations tested for COL and the molecule were: 1xMIC, 1/2xMIC, 1/4xMIC and 1/8xMIC. The fractional inhibitory concentration index (FICI) was calculated for each combination using the following formula: FICA+FICB=FICI, where FICA=MIC of COL in combination the molecule/MIC of COL alone, and FICB=MIC of molecule in combination with COL/MIC of molecule alone. The FICI was interpreted as follow: synergism, FICI-1; additive, FICI=1; antagonism, FICI>1. The bactericidal activity of the 692 piperazine derivative was measured by time-kill curves at concentrations of 1xMIC, 2xMIC, 4xMIC during 24 h.

Result

The 67.5% of piperazines derivatives presented inhibitory growth activity at 50 µM against Ab21R and Ab22P strains. MICs of the piperazine derivatives that inhibited the bacterial growth are showed in table 1. None of molecules tested was cytotoxic (table 1). For both strains, the combination of the 692 piperazine derivative with COL showed synergistic activity (FICI=0.375 in both strains). Bactericidal activity was found with a concentration of 4xMIC of the 692 derivate at 4 h against Ab21R.

Conclusion

Piperazine derivatives presented *in vitro* activity against two clinical colistin resistant *A. baumannii* strains. Bactericidal activity was found in one of the tested strains and synergism with colistin was found for both tested strains with the 692 piperazine derivate. These piperazine derivates seems as good adjuvants alternatives in combination with colistin to treat these kinds of pathogens. *In vivo* studies should be performed in order to confirm these results.

Table 1. Minimal inhibitory concentration (MICs) and cytotoxicity concentration 50 (CC50) of twenty-nine piperazines derivatives against colistin-resistant A. baumannii clinical strains.

CC50: cytotoxicity concentration 50, MIC: Minimal inhibitory concentration, Ab: Acinetobacter baumannii.

COMPOUND	FAMILY	MIC (µg/ml) Ab 21R	Ab 22P	CC50 (µM)
499	1ª	15	7.5	175
580	1ª	15	15	167.12
584	1ª	15	15	175
604	1ª	7.5	7.5	175
591	1ª	15	-	158.246
R-592	1ª	0.94	-	98.603
S-592	1ª	0.94	3.75	156.95
598	2ª	15	15	29.589
600	2ª	15	-	202.704
609	2ª	7.5	7.5	334.466
618	2ª	-	15	133.814
610	2ª	1.87	1.87	73.247
R-610	2ª	1.87	3.75	62.058
S-610	2ª	1.87	1.87	150
611	3ª	25	3.75	148.1
612	3ª	3.75	3.75	200
613	3ª	7.5	15	193.04
616	3ª	15	7.5	143.36
614	3ª	25	1.87	142.15
621	3ª	-	15	85.714
619	3ª	15	7.5	122.21
615	3ª	0.94	1.56	39.183
629	4ª	15	-	175
635	4ª	15	-	174.69
636	4ª	7.5	-	124.366
634	4ª	0.94	-	129.74
674	5ª	0.94	1.87	124.55
679	5ª	0.94	0.94	190.79
692	5ª	1.87	3.75	108.61

A unique vaccine candidate against *Acinetobacter baumannii* based on lipopolysaccharide-deficient whole cells.

Juan Domínguez-Herrera (Spain), Marta Carretero-Ledesma, Pilar Pérez-Romero, Jerónimo Pachón, Michael J. McConnell and Juan José Infante.

Background
The global emergence and dissemination of antibiotic resistant *Acinetobacter baumannii* require the development of novel treatment and prevention approaches. The biotechnology company Vaxdyn has developed a novel prophylactic vaccine based on its patented endotoxin-free whole cell technology (VXD-001). This vaccine has been shown to be highly immunogenic and provides protective immunity against multidrug resistant clinical isolates in preclinical murine models of infection. In order to continue with the preclinical and clinical development of VXD-001, the development of a scalable, GMP-compatible production process is necessary.

Methods and Results
VXD-001 is a suspension of inactivated, endotoxin-free whole *A. baumannii* cells combined with an aluminum-based adjuvant. In collaboration with the contract manufacturing organizations 3P Biopharmaceuticals (Spain) and Biodextris Inc. (Canada) a scalable, GMP-compatible fermentation process was developed for VXD-001. A production procedure was developed at 10-liter scale. Four fermentation runs were performed, yielding two lots of drug substance. Upstream processes, fermentation parameters, downstream processing and in-process controls were optimized, resulting in an increase in efficiency of more than 16-fold compared to laboratory-scale production at 2-liter scale. Additionally, a GMP-friendly heat inactivation process was developed for the drug substance. Molecular and physiochemical characterization of the drug substance indicated that all pre-determined criteria were achieved. Immunogenicity and efficacy studies were performed with the drug substance in preclinical models of *A. baumannii* infection.

Conclusions
A scalable GMP-compatible production process was successfully developed for the *A. baumannii* vaccine candidate VXD-001 at 10-liter scale. This process will permit the continued preclinical and clinical development of VXD-001.

To describe the temporary evolution of *Acinetobacter* spp isolates in an intensive care unit.

Mª Luisa Cantón Bulnes (Spain), Irene García Barrero, Mª de Gracia Gómez Prieto, José Garnacho Montero.

Objectives
To describe the epidemiology of MRAB over a 7-year period in a National Spinal Injuries Unit.

Methods
All admissions to the Unit are screened for MRAB, and, in addition, ongoing monthly screening is undertaken. Strains are sent to the UK Reference Laboratory for typing. Root cause analysis of outbreaks was performed.

Results
70 cases were detected in total, 51 of which were probably acquired on the Unit. Acquisition remained steady for the first 5 years, with an average of 9 cases being acquired each year. However, there has been a sharp fall in the number of cases in the latter 2 years (2015 and 2016), with 4 cases being acquired in 2015, and 2 acquired in 2016. Typing of strains was performed at the Antimicrobial Reistance and Healthcare Asssociated Infections Reference Unit (AMRHAI) at PHE Colindale, London. Clonal spread of 2 main strains was found on Pulsed Field Gel Electrophoresis. Two outbreaks were reported in the first 5 years of this period, with little spread in 2015 and 2016. A focus on lessons learned from the earlier Outbreaks, especially in relation to improved hand hygiene and environmental cleaning, has lead to a reduction in the risk of acquisition of MRAB in recent years.

Conclusion
There has been a marked reduction in MRAB in the past 2 years on this High-Risk Unit due to improved standards of Infection Prevention and Control.

	2010		2011		2012		2013	
	LOCAL	NATIONAL	LOCAL	NATIONAL	LOCAL	NATIONAL	LOCAL	NATIONAL
Previous ICU admission	**0,08**	0,26	**0,08**	0,33	**0,46**	0,27	**0,30**	0,24
During ICU admission	**0,15**	0,99	**0,38**	1,14	**0,46**	0,89	**0,15**	0,71

2014		2015		2016	
LOCAL	NATIONAL	LOCAL	NATIONAL	LOCAL	NATIONAL
0	0,24	**0,51**	0,24	**0,23**	0,19
0,08	0,54	**0,44**	0,40	**0**	0,34

Acinetobacter baumannii infection in an intensive care unit.

Mª Luisa Cantón Bulnes (Spain), Mª de Gracia Gómez Prieto, Irene García Barrero, José Garnacho Montero.

Introduction

Acinetobacter baumannii (AB) is one of the main causes of morbidity and mortality in critically ill condition patients. The objective of this study was to describe the characteristics and evolution of patients diagnosed with an AB infection during admission to the ICU.

Methods

Descriptive, observational and prospective study of all cases of AB infection diagnosed in admitted to a polyvalent 30- beds ICU with no coronary income over a period of 10 years (2007-2016). Demographic variables, reasons for admission to the unit, risk factors of infection, inICU and hospital mortality, infection focus, clinical response, AB antibiogram pattern of susceptibility and adequacy of antibiotic therapy. In Spain, the monitoring of infections acquired in the ICU is fundamentally carried out by means of the ENVIN-HELICS registry, developed by the Infectious Diseases Working Group of the Spanish Society of Intensive and Critical Care Medicine and Coronary Units (SEMICYUC). For the data collection, the local ENVIN database was used where the data are entered prospectively.
We defined multiresistant AB as those strains resistant to carbapenems.

Statistical Analysis

Continuous variables are expressed as medians (interquartile range) and categorical variables as percentages.

Results

During the study period, 12057 patients were admitted in ICU and 26 AB infections were identified. The incidence of AB infection has declined over the years, not identifying any cases in 2016. Table 1 shows characteristics of patients.

Conclusions

In the series analyzed, AB infections occur in patients with APACHE II and high prolonged average stays and have a high mortality. The most prevalent source of infection is respiratory.

Table 1	**n= 26**
Age, years, median (p25-p75)	65,50 (57-72)
Sex (man), n (%)	17/26 (65.4%)
APACHE II score at ICU admission, median (p25-p75)	20,50 (17-27)
Type of patient, n (%)	
Medical	14 (53,8%)
Scheduled surgery	3 (11,5%)
Urgent surgery	9 (34,6%)
Underlying diseases, n (%)	
Diabetes	9 (34,6%)
Cirrhosis	4 (15,4%)
Immunosuppression	3 (11,5%)
CHF	2 (7,7%)
CKD	6(23,1%)
Malnutrition	9 (34,6%)
Previous antibiotic therapy (48 hs) n (%)	17 (65,4%)
ICU nosocomial infection	19 (73,1%)
Source of infection, n (%)	
Pulmonary	15 (57,7%)
Urological	1 (3,8%)
Abdomen	3 (11,5%)
Soft tissues	5 (19,2%)
Catheter	1 (3,8%)
Other	1 (3,8%)
Bacteremic infection, n (%)	12 (46,2%)
Septic shock during infection, n (%)	15 (57,7%)
Multiresistant AB, n (%)	11 (42,3%)
Adequate antibiotic therapy (< 48 h), n (%)	17 (65,4%)
Length of ICU stay, median (p25-p75)	21 (13-43)
Days of stay from admission to infection, median (p25-p75)	10 (9-19)
ICU mortality, n (%)	17 (65,4%)

In vitro assessment of the susceptibility of clinical and environmental *Acinetobacter baumannii* isolates to antiseptics and disinfectants.

Jean Uwingabiye (Marocco), Sanae Lanjri, Mohammed Frikh, Jalal Kasouati, Tarek Alouane, Adil Maleb, Abdelouahed Bait, Azzedine Ibrahimi, Abdelhay Lemnouer, Mostafa Elouennass

Introduction
This study aims to assess the susceptibility of *Acinetobacter baumannii* isolates to the antiseptics and disinfectants commonly used, and to the non-approved product.

Materials and methods
This is a prospective study carried out from February to August 2015, in the Bacteriology department of Mohammed V Military Teaching hospital of Rabat on *A.baumannii* isolates collected from colonized and/or infected patients and environmental samples. The antiseptics and disinfectants susceptibility testing was assessed using the micromethod validated in our department. The antiseptics and disinfectants studied were: 70% ethyl alcohol, chlorhexidine, povidone-iodine, didecyldimethylammonium chloride and a commercial product which was presented as a hospital disinfectant (non-registered product).

Results
Povidone-iodine, 0.5% chlorhexidine digluconate, 70% ethyl alcohol and didecyl dimethyl ammonium chloride in combination with N- (3-aminopropyl) -N-dodecylpropane-1, 3-diamine were effective against all the 81 *A.baumannii* isolates tested, and their logarithmic reduction ³5 were observed in 100% of the isolates in their undiluted form. The strains isolated from patients were more resistant than environmental strains: at a dilution of ½'bd for 70% ethyl alcohol (37.77% vs 11.11%, $p=0.007$) and at a dilution of 1/10 (100% vs 69.44%, $p<0.001$) for povidone iodine. The non-registered product was ineffective with a resistance rate of 96.29% at a dilution of 1/50, 45.67% at a dilution of 1/10 and 13.58% in its purest form.

Conclusion
Our study revealed the effectiveness of the main disinfectants and antiseptics used in Morocco; three antiseptics tested were effective in their purest form against the 81 *A.baumannii* isolates. Regarding disinfectants, our results showed an efficacy of didecyl dimethyl ammonium at the recommended use concentration and in its purest form. This study emphasizes the need for using disinfectants and antiseptics in dilutions recommended by the manufacturer because the insufficient dilutions of these products are not effective. Our findings also demonstrated an inefficiency of the non-registered product against *A.baumanii* isolates. However, the non-registered products should be prohibited.

Surveillance of Multi-resistant *Acinetobacter baumannii* (MRAB) over 7 years in a National Spinal Injuries Centre in the UK.

Jean O'Driscoll (UK).

Introduction
Acinetobacter baumannii (AB) is one of the main causes of morbidity and mortality in critically ill condition patients. The objective of this study was to describe the characteristics and evolution of patients diagnosed with an AB infection during admission to the ICU.

Methods
Descriptive, observational and prospective study of all cases of AB infection diagnosed in admitted to a polyvalent 30- beds ICU with no coronary income over a period of 10 years (2007-2016). Demographic variables, reasons for admission to the unit, risk factors of infection, inICU and hospital mortality, infection focus, clinical response, AB antibiogram pattern of susceptibility and adequacy of antibiotic therapy. In Spain, the monitoring of infections acquired in the ICU is fundamentally carried out by means of the ENVIN-HELICS registry, developed by the Infectious Diseases Working Group of the Spanish Society of Intensive and Critical Care Medicine and Coronary Units (SEMICYUC). For the data collection, the local ENVIN database was used where the data are entered prospectively. We defined multiresistant AB as those strains resistant to carbapenems.

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Continuous variables are expressed as medians (interquartile range) and categorical variables as percentages.

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Conclusions
In the series analyzed, AB infections occur in patients with APACHE II and high prolonged average stays and have a high mortality. The most prevalent source of infection is respiratory.

Comparative in vitro antimicrobial activities of the second generation ceragenins CSA-142 and CSA-192, with CSA-13, against carbapenem-resistant *Acinetobacter baumannii.*

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WHO recently published the list of antibiotic-resistant "'93priority pathogen"'94 that pose the greatest threat to human health. The most critical group includes carbapenem-resistant *Acinetobacter baumannii* which is an important cause of nosocomial infections. As these infections have very limited treatment options, attempts have been made to discover new antimicrobials. Ceragenins (cationic steroidal antimicrobial-CSA), are a new class of antimicrobial agents. They act as antimicrobial peptide (AMP) mimics, permeabilizing the outer membrane of Gram-negative bacteria. These agents have a number of advantages over AMPs; most importantly they are resistant to cleavage by proteolytic enzymes. In this study, the in vitro activities of first generation CSA-13, the second generation ones CSA-142 and CSA-192 were investigated using 20 carbapenem-resistant *A. baumannii* strains and a standard strain. MICs and MBCs were determined by microbroth dilution technique. Time kill curves were performed to observe the behaviours of ceragenins.

The MIC50 values (mg/L) of CSA-13, CSA-142 and CSA-192 were 8, 16 and 16 respectively. The MIC90 (mg/L) were 8, 32 and 32, respectively. The MBCs were equal to or twice greater than those of the MICs. 4xMIC of CSA-142 and CSA-192 were bactericidal along the curve. However, even at 4xMIC, CSA-13 wasn''92t bactericidal. Overall, we found that ceragenins have good activity against carbapenem-resistant *A. baumannii* strains, highlighting the second generation ones have almost the same MICs and better bactericidal activity in time kill curve studies. The good MIC results and better bactericidal activities of CSA-142 and CSA-192, make the second generation of ceragenins to take a good place between cationic steroid antibiotics in terms of antimicrobial activity not only against the standard strain, but also against the problematic clinical *A. baumannii* strains.

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